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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	01/02/2018	9855302	UCHI-34458/US-3/ORD	8885

72960 7590 12/13/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site http://pair.uspto.gov for additional applicants):

Thomas F. Gajewski, Chicago, IL;
The University of Chicago, Chicago, IL;
Ayelet Sivan, Chicago, IL;
Leticia Corrales, Chicago, IL;

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

72960 7590 11/22/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
HINES, JANA A

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
11/22/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Notice of Allowability	Application No. 15/170,284	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to November 14, 2017 .
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____ .

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 1-26 and 28-30 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file areply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. 11/14/17 .
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____ .

/JANA A HINES/
Primary Examiner, Art Unit 1645

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR/ PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
15/170,284	06/01/2016	Gajewski et al.	UCHI-34458/US-3/ ORD

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
JA-NA A HINES	
ART UNIT	PAPER
1645	20171114

DATE MAILED: _____

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

Called David Staple on Nov. 14, 2017 for approval to change the dependency of claim 9 to now depend on claim 8.

/JANA A HINES/
Primary Examiner, Art Unit 1645

DETAILED CORRESPONDENCE

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claim Amendments

2. Claims 1-26 and 28-30 are under consideration in this Office Action.

EXAMINER'S AMENDMENT

3. Authorization for this examiner's amendment was given in an interview with David Staple on November 14, 2017.

The application has been amended as follows: The following is an examiner's statement of reasons for allowance:

Claim 9. (Currently Amended) The method of claim ~~9~~ 8, wherein the administration of the two or more doses are separated by at least 1 week.

4. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Reasons for Allowance

6. The following is an examiner's statement of reasons for allowance: The art does not teach or fairly suggest a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Bifidobacterium*.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

7. Claims 1-26 and 28-30 are allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to JA-NA A HINES whose telephone number is (571)272-0859. The examiner can normally be reached on M-Th 8:30am-6pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/JANA A HINES/
Primary Examiner, Art Unit 1645

Examiner-Initiated Interview Summary	Application No. 15/170,284	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status Yes

All participants (applicant, applicant's representative, PTO personnel):

(1) JA-NA A. HINES. (3) _____.

(2) David Staple. (4) _____.

Date of Interview: 14 November 2017.

Type: ☑ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☑ No.
If Yes, brief description: _____.

Issues Discussed ☐ 101 ☐ 112 ☐ 102 ☐ 103 ☑ Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: 9.

Identification of prior art discussed: _____.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Called David Staple on Nov. 14, 2017 for approval to change the dependency of claim 9 to now depend on claim 8..

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/JANA A HINES/ Primary Examiner, Art Unit 1645	

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

72960 7590 11/09/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
HINES, JANA A	

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
11/09/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Response to Rule 312 Communication	Application No. 15/170,284	Applicant(s)
	Examiner	Art Unit

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address –

1. ☒ The amendment filed on *02 November 2017* under 37 CFR 1.312 has been considered, and has been:

a) ☒ entered.

b) ☐ entered as directed to matters of form not affecting the scope of the invention.

c) ☐ disapproved because the amendment was filed after the payment of the issue fee.
Any amendment filed after the date the issue fee is paid must be accompanied by a petition under 37 CFR 1.313(c)(1) and the required fee to withdraw the application from issue.

d) ☐ disapproved. See explanation below.

e) ☐ entered in part. See explanation below.

Publishing Division

mn

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	8885
Serial No.:	15/170,284	Art Unit:	1645
Filed:	01-JUNE-2016	Examiner:	Hines, Jana A.
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS MAILED NOVEMBER 1, 2017

After Notice of Allowance Mailed

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Examiner Hines:

This communication is responsive the Notice to File Corrected Application Papers mailed November 1, 2017. Applicant requests that the amendments provided herein be entered under 37 C.F.R. 1.312.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-3/ORD. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning on page 9, lines 28-31, through page 10, lines 1-7, the following amended paragraph.

-- Fig. 1A-[[H]]I. Differences in melanoma outgrowth and tumor-specific immune responses between C57BL/6 JAX and TAC mice are eliminated upon cohousing. (A) B16.SIY tumor growth kinetics in newly arrived JAX and TAC mice. (B) IFN-γ ELISPOT in tumor-bearing JAX and TAC mice 7 days following tumor inoculation. (C) Mean size of IFN-γ spots (10^{-3} mm^2). (D) Flow cytometry of SIY^+ T cells from total $CD8^+$ T cells within the tumor of JAX and TAC mice as determined by flow cytometry 21 days post-tumor inoculation. ~~Percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of JAX and TAC mice as determined by flow cytometry 21 days post-tumor inoculation. Representative plots (left), quantification (right).~~ (E) Percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of JAX and TAC mice as determined by flow cytometry 21 days post-tumor inoculation. ([[E]]F) B16.SIY tumor growth kinetics in JAX and TAC mice cohoused for 3 weeks prior to tumor inoculation. ([[F]]G) Number of IFN-γ spots/10^6 splenocytes in tumor-bearing JAX and TAC mice cohoused for 3 weeks prior to tumor inoculation. ([[G]]H) Mean size of IFN-γ spots (10^{-3} mm^2). ([[H]]I) Percentage of SIY^+ T cells of total $CD8^+$ T cells within the tumor of JAX and TAC mice cohoused for 3 weeks prior to tumor inoculation.--

AMENDMENTS TO THE FIGURES

Please replace sheet 3 and 4 of the figures with the replacement sheet filed herewith.

REMARKS

In the Notice to File Corrected Application Papers mailed November 1, 2017, the Applicant was notified that the FIG. 1D continued onto a second page without proper labeling. The Applicant files herewith a replacement sheet of the second page of FIG. 1D now relabeled FIG. 1E in compliance with 37 C.F.R. 1.84(u)(1). Additionally, the Applicant submits herewith a replacement sheet of page 4 of the figures with FIGS. 1E-H relabeled FIGS. 1F-I. No new matter is added.

The Applicant has also amended the Brief Description of the Drawings to include reference to relabeled figures and amended the figures descriptions for Figures 1D and 1E. No new matter is added by this amendment.

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

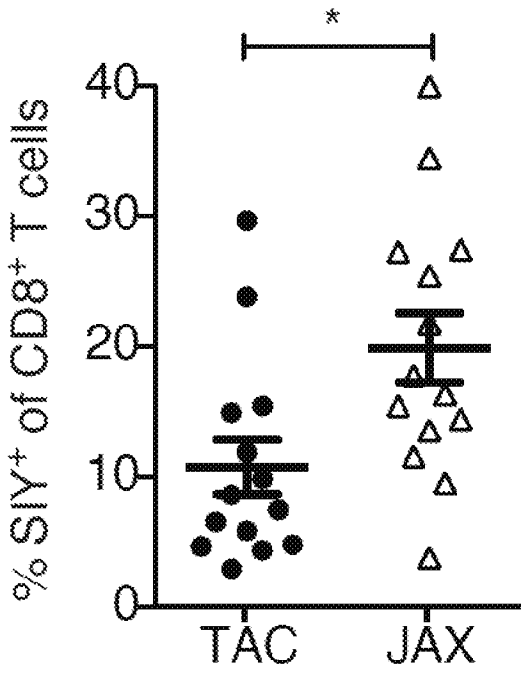
Respectfully submitted,

Date: November 2, 2017

/David W. Staple/

David W. Staple
Registration No. 65,903
Casimir Jones S.C.
2275 Deming Way
Suite 310
Middleton, WI 53562
Tel.: 608-662-1277
Fax.: 608-662-1276

FIG. 1E



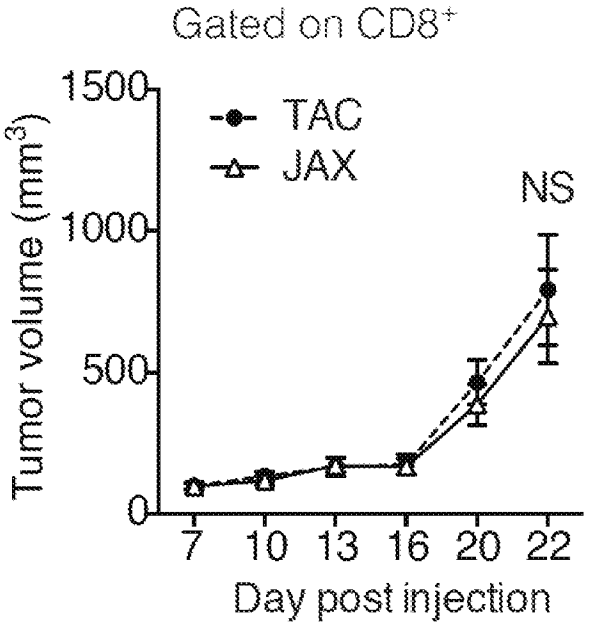


FIG. 1F

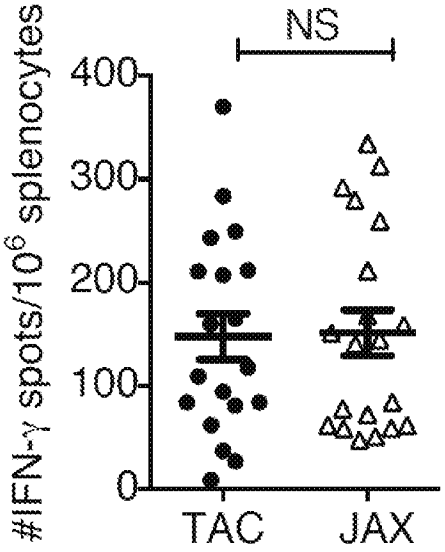


FIG. 1G

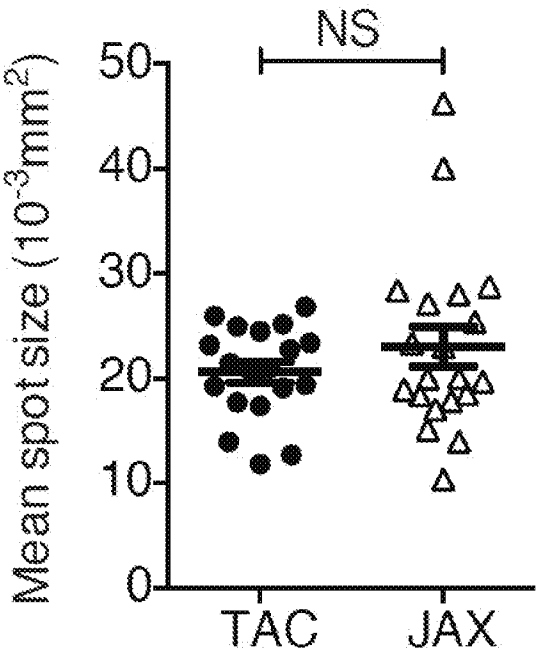


FIG. 1H

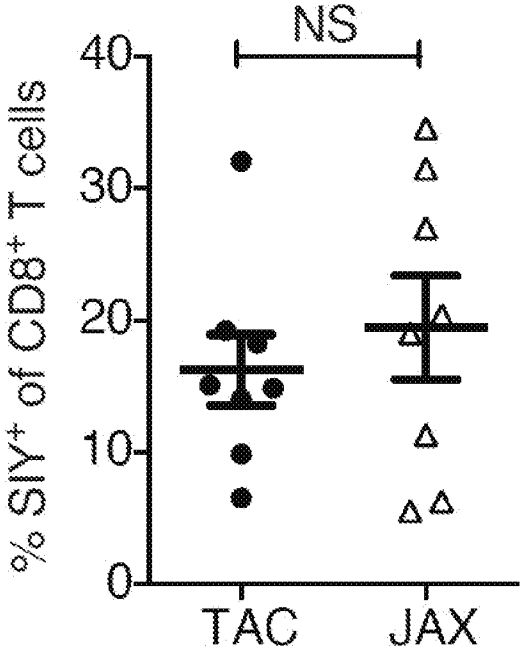


FIG. 1I

Electronic Acknowledgement Receipt

EFS ID:	30834653
Application Number:	15170284
International Application Number:	
Confirmation Number:	8885
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Stephanie Filandrinos
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-3/ORD
Receipt Date:	02-NOV-2017
Filing Date:	01-JUN-2016
Time Stamp:	16:13:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		34458US3ORD_RNCAP_11-2-17.pdf	115257 0b23e905f89ac31ca4cbf00204d76ceb6866fd8a	yes	4

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Amendment after Notice of Allowance (Rule 312)	1	1
	Specification	2	2
	Drawings-only black and white line drawings	3	3
	Applicant Arguments/Remarks Made in an Amendment	4	4

Warnings:					
Information:					
2	Drawings-only black and white line drawings	34458-ORD-REPLACEMENT-FIGURES1E-I.pdf	337236 fad54ee970c6c4a2ea93f28b3a71546bbb9d356b	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	452493		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Document Description: Issue Fee Payment (PTO-85B)

Issue Fee Transmittal Form

Application Number	Filing Date	First Named Inventor	Atty. Docket No.	Confirmation No.
15170284	01-Jun-2016	Thomas Gajewski	UCHI-34458/US-3/ORD	8885

TITLE OF INVENTION :

TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

Entity Status	Application Type	Art Unit	Class - Subclass	EXAMINER
Small	Utility under 35 USC 111(a)	1645	007320	JANA HINES

Issue Fee Due	Publication Due	Total Fee(s) Due	Date Due	Prev. Paid Fee
$480	$0	$480	29-Dec-2017	$0

1.Change of Correspondence Address and/or Indication Of Fee Address (37 CFR 1.33 & 1.363)

Current Correspondence Address:	Current Indicated Fee Address :
72960 Casimir Jones, S.C. 2275 DEMING WAY, SUITE 310 MIDDLETON WI 53562 UNITED STATES 608-662-1277 docketing@casimirjones.com	
☐ Change of correspondence address requested, system generated AIA/122-EFS form attached	☐ Fee Address indication requested, system generated SB/47-EFS form attached

2.Entity Status

Change in Entity Status

○ Applicant certifying micro entity status; system generated Micro Entity certification form attached. See 37 CFR 1.29.
Note: Absent a valid certification of micro entity status, issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment. If this box is checked, you will be prompted to choose a micro entity status on the gross income basis (37 CFR 1.29(a)) or the institution of higher education basis (37 CFR 1.29(d)), and make the applicable certification online.

◉ Applicant asserting small entity status. See 37 CFR 1.27.
Note: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

○ Applicant changing to regular undiscounted fee status.
Note: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

Doc Code: IFEE PTOL/85B-EFS

Document Description: Issue Fee Payment (PTO-85B)

3.The Following Fee(s) Are Submitted:

☒ Issue Fee

☐ I authorize USPTO to apply my previously paid issue fee to the current fees due

☐ Publication Fee

☐ The Director is hereby authorized to apply my previously paid issue fee to the current fee due and to charge deficient fees to Deposit Account Number _____

☐ Advance Order - # of copies _____

☒ If **in addition to** the payment of the issue fee amount submitted with this form, there are any discrepancies in any amount(s) due, the Director is authorized to charge any deficiency, or credit any overpayment, to Deposit Account Number 504302 . The **issue fee must be submitted** with this form. **If payment of the issue fee does not accompany this form, checking this box and providing a deposit account number will NOT be effective to satisfy full payment of the fee(s) due.**

4.Firm and/or Attorney Names To Be Printed

NOTE: If no name is listed, no name will be printed
For printing on the patent front page, list to be displayed as entered

1. CASIMIR JONES SC
2. David W. Staple
3.

5.Assignee Name(s) and Residence Data To Be Printed

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

Name	City	State	Country	Category
The University of Chicago	Chicago	ILLINOIS	united states	other

6.Signature

I certify, in accordance with 37 CFR 1.4(d)(4) that I am an attorney or agent registered to practice before the Patent and Trademark Office who has filed and has been granted power of attorney in this application. I also certify that this Fee(s) Transmittal form is being transmitted to the USPTO via EFS-WEB on the date indicated below.

Signature	/David W. Staple/	**Date**	11-02-2017
Name	David William Staple	**Registration Number**	65903

Electronic Patent Application Fee Transmittal

Application Number:	15170284
Filing Date:	01-Jun-2016
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Filer:	David William Staple/Stephanie Filandrinos
Attorney Docket Number:	UCHI-34458/US-3/ORD

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
UTILITY APPL ISSUE FEE	2501	1	480	480
PUBL. FEE- EARLY, VOLUNTARY, OR NORMAL	1504	1	0	0
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**480**

Electronic Acknowledgement Receipt

EFS ID:	30834693
Application Number:	15170284
International Application Number:	
Confirmation Number:	8885
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Stephanie Filandrinos
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-3/ORD
Receipt Date:	02-NOV-2017
Filing Date:	01-JUN-2016
Time Stamp:	16:14:35
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$480
RAM confirmation Number	110317INTEFSW16143400
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	Web85b.pdf	46162 946b911e4ff12c065d5091f0a7dc96e566038b49	no	2
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	32421 9258dd58908c45563b4144323d3687eaae89013d	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	78583		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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Address: COMMISSIONER FOR PATENTS
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

72960 7590 11/01/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
HINES, JANA A

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
11/01/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Application No. : 15170284
Applicant : Gajewski
Filing Date : 06/01/2016
Date Mailed : 11/01/2017

NOTICE TO FILE CORRECTED APPLICATION PAPERS

Notice of Allowance Mailed

This application has been accorded an Allowance Date and is being prepared for issuance. The application, however, is incomplete for the reasons below.

Applicant is given two (2) months from the mail date of this Notice within which to respond. This time period for reply is extendable under 37 CFR 1.136(a) for only TWO additional MONTHS.

The informalities requiring correction are indicated in the attachment(s). If the informality pertains to the abstract, specification (including claims) or drawings, the informality must be corrected with an amendment in compliance with 37 CFR 1.121 (or, if the application is a reissue application, 37 CFR 1.173). Such an amendment may be filed after payment of the issue fee if limited to correction of informalities noted herein. See Waiver of 37 CFR 1.312 for Documents Required by the Office of Patent Publication, 1280 Off. Gaz. Patent Office 918 (March 23, 2004). In addition, if the informality is not corrected until after payment of the issue fee, for purposes of 35 U.S.C. 154(b)(1)(iv), "all outstanding requirements" will be considered to have been satisfied when the informality has been corrected. A failure to respond within the above-identified time period will result in the application being ABANDONED.

See attachment(s).

A copy of this notice MUST be returned with the reply. Please address response to "Mail Stop Issue Fee, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450".

/Russell Lucas/
Publication Branch
Office of Data Management
(571) 272-4200

Application No. 15170284 **Drawings filed: 08/17/2016**

DRAWINGS AND BRIEF DESCRIPTION OF DRAWINGS IN NON-COMPLIANCE WITH 37 CFR 1.84(u)(1)

37 CFR 1.84(u)(1) states, "The different views must be numbered in consecutive Arabic numerals, starting with 1 ... Partial views intended to form one complete view, on one or several sheets, must be identified by the same number followed by a capital letter. View numbers must be preceded by the abbreviation 'FIG.' "

Other methods of identification are improper, including Roman numerals (FIG. I, FIG, II, etc.) and alphabetic characters (FIG. A, FIG. B, etc.). Abbreviations and words other than "FIG." ("Illustration, 1" "Diagram 2," "Photo 3," etc.) are improper.

☐ Applicant must submit drawings that number the views using consecutive Arabic numerals.

☐ Applicant must amend the brief description of the drawings on Page(s) of the specification to show consecutive Arabic numerals.

NOTE: Applicant is advised that the detailed description may voluntarily be amended to identify the drawings by Arabic numerals.

☒ The drawing is continued onto a second page (or more) without proper labeling under 37 CFR 1.84(u)(1). FIG(s) **1D**

☐ Applicant must submit drawings on which the view numbers are preceded by the abbreviation "FIG."

☐ Applicant must amend the brief description of the drawings on Page(s) of the specification to show view numbers preceded by the abbreviation "FIG."

NOTE: Applicant is advised that the detailed description may voluntarily be amended to show view numbers preceded by the abbreviation "FIG."

Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/170,284	Gajewski et al.
	Examiner	Art Unit
	JA-NA A HINES	1645

CPC - Searched*		
Symbol	**Date**	**Examiner**
A61K39/02; A61P1/00; A61P1/02; A61P1/04; A61P1/12; A61P1/16; A61P3/04 ; A61P3/10; A61P5/00; A61P7/02; A61P7/06; A61P9/00; A61P9/10; A61P11/ 00; A61P11/06; A61P13/12; A61P15/00; A61P17/00; A61P17/02; A61	3/2017	jah
updated searches	09/22/2017	jah

CPC Combination Sets - Searched*		
Symbol	**Date**	**Examiner**

US Classification - Searched*			
Class	**Subclass**	**Date**	**Examiner**
424	234.1	09/22/2017	jah
435	252.1		

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	**Date**	**Examiner**
searched inventors, applications, patents. Commerical database search of claim text	10/2016	jah
search updated based upon claim text	09/22/2017	jah

Interference Search			
US Class/CPC Symbol	**US Subclass/CPC Group**	**Date**	**Examiner**

/JANA A HINES/ Primary Examiner, Art Unit 1645	

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NOTICE OF ALLOWANCE AND FEE(S) DUE

72960 7590 09/29/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
HINES, JANA A	
ART UNIT	PAPER NUMBER
1645	

DATE MAILED: 09/29/2017

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

TITLE OF INVENTION: TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	12/29/2017

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

72960 7590 09/29/2017
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

TITLE OF INVENTION: TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	SMALL	$480	$0	$0	$480	12/29/2017

EXAMINER	ART UNIT	CLASS-SUBCLASS
HINES, JANA A	1645	435-007320

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. The following fee(s) are submitted:
- ❑ Issue Fee
- ❑ Publication Fee (No small entity discount permitted)
- ❑ Advance Order - # of Copies _____

4b. Payment of Fee(s): **(Please first reapply any previously paid issue fee shown above)**
- ❑ A check is enclosed.
- ❑ Payment by credit card. Form PTO-2038 is attached.
- ❑ The director is hereby authorized to charge the required fee(s), any deficiency, or credits any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ❑ Applicant certifying micro entity status. See 37 CFR 1.29 — NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
- ❑ Applicant asserting small entity status. See 37 CFR 1.27 — NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
- ❑ Applicant changing to regular undiscounted fee status. — NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

72960 7590 09/29/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER	
HINES, JANA A	

ART UNIT	PAPER NUMBER
1645	

DATE MAILED: 09/29/2017

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 15/170,284	Applicant(s) Gajewski et al.	
	Examiner JA-NA A HINES	Art Unit 1645	AIA Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☑ This communication is responsive to 9/18/17 .
   ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____ .

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

3. ☑ The allowed claim(s) is/are 1-26 and 28-30 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information , please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

   Certified copies:

   a) ☐ All b) ☐ Some *c) ☐ None of the:

   1. ☐ Certified copies of the priority documents have been received.
   2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
   3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file areply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____ .

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☑ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 3/28/17
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____ .
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____ .

/JANA A HINES/
Primary Examiner, Art Unit 1645

DETAILED CORRESPONDENCE

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claim Amendments

2. Claim 27 is cancelled. Claims 1-26 and 28-30 are under consideration in this Office Action.

EXAMINER'S AMENDMENT

3. Authorization for this examiner's amendment was given in an interview with David Staple on September 22, 2017.

The application has been amended as follows: The following is an examiner's statement of reasons for allowance:

Claim 1. (Currently Amended) A method of treating cancer in a human subject comprising co-administering ~~administering~~ to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Bifidobacterium*.

4. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Withdrawal of Rejections

5. The rejection of clams 1-30 under pre-AIA 35 USC 103(a) as being unpatentable over Sharon et al., in view of O'Mahoney et al., have been withdrawn in view of applicants' amendments and arguments.

Reasons for Allowance

6. The following is an examiner's statement of reasons for allowance: The art does not teach or fairly suggest a method of treating cancer in a human subject comprising co-administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Bifidobacterium*.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

7. Claims 1-26 and 28-30 are allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to JA-NA A HINES whose telephone number is (571)272-0859. The examiner can normally be reached on M-Th 8:30am-6pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Nickol can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/JANA A HINES/
Primary Examiner, Art Unit 1645

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/170,284	Gajewski et al.
	Examiner	**Art Unit**
	JA-NA A HINES	1645

CPC						
Symbol					**Type**	**Version**
A61K	/	35	/	741	F	2013-01-01
A61K	/	45	/	06	I	2013-01-01
A61K	/	35	/	742	I	2013-01-01
A61K	/	35	/	745	I	2013-01-01
A61K	/	35	/	747	I	2013-01-01
A61K	/	39	/	39558	I	2013-01-01
C07K	/	16	/	2827	I	2013-01-01
A61K	/	2039	/	505	A	2013-01-01
C07K	/	2317	/	76	A	2013-01-01

CPC Combination Sets								
Symbol					**Type**	**Set**	**Ranking**	**Version**
A61K	/	39	/	39558	I	1	1	2013-01-01
A61K	/	2300	/	00	A	1	2	2013-01-01

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	29	
/JANA A HINES/ Primary Examiner, Art Unit 1645	22 September 2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
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	JA-NA A HINES	1645

INTERNATIONAL CLASSIFICATION				
CLAIMED				
424	/	234.1	/	

NON-CLAIMED				
	/		/	

US ORIGINAL CLASSIFICATION	
CLASS	**SUBCLASS**
424	234.1

CROSS REFERENCES(S)						
CLASS	**SUBCLASS (ONE SUBCLASS PER BLOCK)**					
435	252.1					

NONE (Assistant Examiner) (Date)		**Total Claims Allowed:** 29	
/JANA A HINES/ Primary Examiner, Art Unit 1645 (Primary Examiner)	22 September 2017 (Date)	O.G. Print Claim(s) 1	O.G. Print Figure 1

Issue Classification	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15/170,284	Gajewski et al.
	Examiner	**Art Unit**
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☑ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIMS

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	1	10	10	19	19	28	29								
2	2	11	11	20	20	29	30								
3	3	12	12	21	21										
4	4	13	13	22	22										
5	5	14	14	23	23										
6	6	15	15	24	24										
7	7	16	16	25	25										
8	8	17	17	26	26										
9	9	18	18	27	28										

NONE		**Total Claims Allowed:**	
(Assistant Examiner)	(Date)	29	
/JANA A HINES/ Primary Examiner, Art Unit 1645	22 September 2017	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	1

OK TO ENTER: /J.A.H/

CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

1. (currently amended) A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the ~~genera~~ genus *Bifidobacterium*.

2. (currently amended) The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

3. (currently amended) The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

4. (original) The method of claim 1, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. (original) The method of claim 1, wherein the bacterial formulation is administered by oral administration or rectal administration.

6. (original) The method of claim 5, wherein the bacterial formulation is administered by oral administration.

7. (currently amended) The method of claim 1, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the ~~genera~~ genus *Bifidobacterium*.

8. (original) The method of claim 1, wherein the bacterial formulation is administered to the subject in two or more doses.

9. (original) The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. (original) The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

11. (original) The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

12. (original) The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. (original) The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. (original) The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. (original) The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. (original) The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. (original) The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. (original) The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. (original) The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

20. (currently amended) [[A]] The method of claim 1, wherein ~~treating cancer in a human subject comprising administering to the subject a~~ the bacterial formulation ~~comprising~~ comprises at least $5x10^6$ CFU of bacteria of the ~~genera~~ genus *Bifidobacterium,* and wherein at least 50% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

21. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

22. (original) The method of claim 20, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

23. (original) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

24. (original) The method of claim 23, wherein the bacterial formulation is administered by oral administration.

25. (original) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

26. (original) The method of claim 20, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

27. (cancelled)

28. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

29. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

30. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

1. (currently amended) A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the ~~genera~~ genus *Bifidobacterium*.

2. (currently amended) The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

3. (currently amended) The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

4. (original) The method of claim 1, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. (original) The method of claim 1, wherein the bacterial formulation is administered by oral administration or rectal administration.

6. (original) The method of claim 5, wherein the bacterial formulation is administered by oral administration.

7. (currently amended) The method of claim 1, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the ~~genera~~ genus *Bifidobacterium*.

8. (original) The method of claim 1, wherein the bacterial formulation is administered to the subject in two or more doses.

9. (original) The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. (original) The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

11. (original) The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

12. (original) The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. (original) The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. (original) The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. (original) The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. (original) The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. (original) The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. (original) The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. (original) The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

20. (currently amended) [[A]] The method of claim 1, wherein ~~treating cancer in a human subject comprising administering to the subject a~~ the bacterial formulation ~~comprising~~ comprises at least $5x10^6$ CFU of bacteria of the ~~genera~~ genus *Bifidobacterium,* and wherein at least 50% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

21. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

22. (original) The method of claim 20, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

23. (original) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

24. (original) The method of claim 23, wherein the bacterial formulation is administered by oral administration.

25. (original) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

26. (original) The method of claim 20, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

27. (cancelled)

28. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

29. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

30. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/170,284
				Filing Date	06/01/2016
				First Named Inventor	Gajewski
				Art Unit	1645
				Examiner Name	Hines
Sheet	1	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

U.S. PATENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4,816,567	1989-03-28	CABILLY et al.	
		4,946,778	1990-08-07	LADNER et al.	
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		7,195,906	2007-03-27	COLLINS et al.	
		8,449,878	2013-05-28	YONAK et al.	

U.S. PUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] (if known)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		EP 2876167	2015-05-27	INSTITUT GUSTAVE ROUSSY		
		WO 1988/01649	1988-03-10	GENEX CORPORATION		
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		WO 2014/145958	2014-09-18	SERES HEALTH, CIN.		
		WO 2015/061372	2015-04-30	HEMOSHEAR, LLC		

Examiner Signature	/JANA A HINES/ (03/22/2017)	Date Considered	03/22/2017

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /JAH/

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Sheet	2	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

NONPATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]
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		International Search Report and Written Opinion for PCT/US2016/035228, mailed August 30, 2016, 15 pages	

Examiner Signature	/JANA A HINES/ (03/22/2017)	Date Considered	03/22/2017

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /JAH/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/170,284
				Filing Date	06/01/2016
				First Named Inventor	Gajewski
				Art Unit	1645
				Examiner Name	Hines
Sheet	5	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/David W. Staple/	Date (YYYY-MM-DD)	2017-02-06
Name/Print	David W. Staple	Registration Number	65903

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[7] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible.
[8] Applicant is to place a check mark here if English language Translation is attached.
This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**.

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CONFIRMATION NO. 8885

SERIAL NUMBER	FILING or 371(c) DATE RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
15/170,284	06/01/2016	435	1645	UCHI-34458/US-3/ORD

APPLICANTS
The University of Chicago, Chicago, IL;

INVENTORS
Thomas F. Gajewski, Chicago, IL;
Ayelet Sivan, Chicago, IL;
Leticia Corrales, Chicago, IL;

**** CONTINUING DATA ***************************
This appln claims benefit of 62/169,112 06/01/2015
and claims benefit of 62/248,741 10/30/2015

**** FOREIGN APPLICATIONS ***************************

**** IF REQUIRED, FOREIGN FILING LICENSE GRANTED **** ** SMALL ENTITY **
06/15/2016

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /JA'NA A HINES/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		IL	38	30	2

ADDRESS
Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562
UNITED STATES

TITLE
TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA

FILING FEE RECEIVED		
1200	FEES: Authority has been given in Paper No._____ to charge/credit DEPOSIT ACCOUNT No._____ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	8885
Serial No.:	15/170,284	Art Unit:	1645
Filed:	01-JUNE-2016	Examiner:	Hines, Jana A.
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO ADVISORY ACTION MAILED JULY 19, 2017

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Examiner Hines:

This communication is responsive the Advisory Action mailed July 19, 2017. A three month extension of time from June 28, 2017 to September 28, 2017 is hereby requested.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-3/ORD. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

1. (currently amended) A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the ~~genera~~ genus *Bifidobacterium*.

2. (currently amended) The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

3. (currently amended) The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

4. (original) The method of claim 1, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. (original) The method of claim 1, wherein the bacterial formulation is administered by oral administration or rectal administration.

6. (original) The method of claim 5, wherein the bacterial formulation is administered by oral administration.

7. (currently amended) The method of claim 1, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the ~~genera~~ genus *Bifidobacterium*.

8. (original) The method of claim 1, wherein the bacterial formulation is administered to the subject in two or more doses.

9. (original) The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. (original) The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

11. (original) The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

12. (original) The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. (original) The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. (original) The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. (original) The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. (original) The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. (original) The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. (original) The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. (original) The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

20. (currently amended) [[A]] The method of claim 1, wherein ~~treating cancer in a human subject comprising administering to the subject a~~ the bacterial formulation ~~comprising~~ comprises at least $5x10^6$ CFU of bacteria of the ~~genera~~ genus *Bifidobacterium,* and wherein at least 50% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

21. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the ~~genera~~ genus *Bifidobacterium*.

22. (original) The method of claim 20, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

23. (original) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

24. (original) The method of claim 23, wherein the bacterial formulation is administered by oral administration.

25. (original) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

26. (original) The method of claim 20, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

27. (cancelled)

28. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

29. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

30. (currently amended) The method of claim [[27]] 20, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

REMARKS

In the Advisory Action mailed July 19, 2017, the amendments proposed in the Response Communication filed June 28, 2017 were not entered. Applicants withdraw the unentered amendments.

In the present communication, original claims 1-3, 7 and 20-21 have been amended to correct a typographical error ("~~genera~~ genus"); claim 20 has been amended to be dependent upon claim 1, in order to be consistent with the arguments made herein; claim 27 has been cancelled; and claims 28-30 have been amended to correct dependency following the cancellation of claim 27. Amendments and cancellations are made without acquiescing to the Examiner's arguments, in order to advance prosecution, and while retaining the right to pursue the original claims in the future. Amendments find support throughout the specification; no new matter is added.

Interview Summary

On September 6, 2017, Examiner Hines, Applicant's representative David Staple, and a representative for the licensee of the application, William DeVaul, conducted a telephone interview. Applicant presented arguments for the patentability of the original claims based on case law that had not previously been considered. Examiner Hines agreed to consider the arguments if filed in a Response to the Advisory Action, and agreed that the current rejection should not be maintained in light of the case law. The amendments made herein are consistent with the claims discussed with the Examiner, and are made to better place the application in condition for allowance. The rejection raised in the Final Office Action and the arguments and case law presented in the telephone interview are presented below.

Nonobviousness

Claims 1-30 are rejected under 35 U.S.C. 103 as allegedly being unpatentable over Sharon et al. (Chin. J. Cancer, 2014, 33(9):434-444) in view of O'Mahoney et al. (U.S. Pat. Pub. 2012/0276143). Applicants respectfully disagree.

The claims recite a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genus *Bifidobacterium*.

The rejection states that "it would have been *prima facie* obvious at the time of applicants' invention to incorporate O'Mahoney's Bifidobacterium to Sharon's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor when

O'Mahoney et al., already it was known to treat cancer in a human subject comprising administering to the subject Bifidobacterium in combination with other therapies that also treat cancer." (Final Office Action mailed March 28, 2017, page 5).

Applicant submits that the above combination would not have been obvious, for at least the reason that Sharon and O'Mahoney describe different and incompatible effects on immune response. O'Mahoney describes suppression of immune response. For example, O'Mahoney describes:

- [A] *Bifidobacterium* strain or a formulation as described herein for use in the prophylaxis and/or treatment of autoimmune disorders due to undesirable inflammatory activity. (O'Mahoney, paragraph [0015]);

- [A] Bifidobacterium strain or a formulation as described herein for use in the preparation of anti-inflammatory biotherapeutic agents for reducing the levels of pro inflammatory cytokines. (O'Mahoney, paragraph [0023]); and

- The invention is therefore of major potential therapeutic value in the prophylaxis or treatment of dysregulated immune responses, such as undesirable inflammatory reactions for example asthma. (O'Mahoney, paragraph [0039]).

Conversely, Sharon is cited for the teaching of inhibitors of immune checkpoints; such immune checkpoints are responsible for "reduced cytokine production, cytolytic activity, and lymphocyte proliferation" (Sharon, page 437, column 2), and therefore inhibitors of these immune checkpoints have the opposite effect, promoting immune response.

As support for the alleged combination, the rejection cites the case of *In re Kerkhoven*, which holds "[i]t is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose." (MPEP §2144.06). Applicant submits that the present claims are distinguishable from the facts of *Kerkhoven*, and point instead to *Ex Parte Bokisa*. In *Bokisa,* the Examiner had argued that:

> [I]t would be [sic: have been] obvious to one of ordinary skill in the art at the time the invention was made to use both alkane or alkanol sulfonic acid and fluoboric acid in tin plating baths of Nobel because it has been held to be obvious to use a mixture of two materials each of which has been used separately for the same purpose, *In re Kerkhoven*, [626 F.2d 846, 850,] 205USPQ 1269, [sic: 1069,] 1072 (CCPA 1980).

However, the BPAI majority in *Bokisa* held that:

> The combination of the plating baths suggested by the dissent does not flow from the teachings in Nobel showing a difference in the precipitation for fluoboric acid and alkyl or alkylol sulfonic acid even at low temperatures in example 1. Clearly, the purpose of Nobel's disclosure is to

> contrast the effect of the named antioxidants in a comparison of the plating baths. The effect of the comparison is to view each plating bath as having different characteristics. As pointed out by the dissent, even at low temperatures one causes more precipitation than the other. **One of ordinary skill in the art would not have combined a plating bath causing more precipitation with one causing less. *Kerkhoven* is not applicable on these facts.**

Applicant submits that the present claims are analogous to the facts of *Bokisa*, not *Kerkhoven*. In *Bokisa*, though both agents were applicable to the same general purpose (i.e., plating baths), the reasoning for combining of *Kerkhoven* was found not to apply because they had different and opposing underlying effects (i.e., amounts of precipitation). For the present claims, despite the same alleged general purpose (treatment of cancer), Sharon and O'Mahoney teach different and opposing underlying effects (inhibition vs. stimulation of immune response). In light of the teachings of opposing effects on immune response, Applicant submits that combining these references would not logically flow from the Sharon and O'Mahoney references.

In light of the teachings in Sharon and O'Mahoney of opposing effects on immune response, and the holding of *Ex Parte Bokisa*, which clearly distinguishes the present claim from the facts of *Kerkhoven*, Applicant submits that the claims are not obvious over the cited references, and respectfully requests reconsideration and withdrawal of the rejection of the claims under 35 U.S.C. 103(a).

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

Respectfully submitted,

Date: September 18, 2017

/David W. Staple/

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Electronic Patent Application Fee Transmittal

Application Number:	15170284
Filing Date:	01-Jun-2016
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Filer:	David William Staple
Attorney Docket Number:	UCHI-34458/US-3/ORD

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	2253	1	700	700
Miscellaneous:				
			Total in USD ($)	**700**

Electronic Acknowledgement Receipt

EFS ID:	30391170
Application Number:	15170284
International Application Number:	
Confirmation Number:	8885
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Stephanie Filandrinos
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-3/ORD
Receipt Date:	18-SEP-2017
Filing Date:	01-JUN-2016
Time Stamp:	14:29:28
Application Type:	Utility under 35 USC 111(a)

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Payment Type	CARD
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	Document Description	**Start**	**End**
	Response After Final Action	1	1
	Claims	2	6
	Claims	7	9

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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/170,284	Filing Date 06/01/2016	☐ To be Mailed

ENTITY: ☐ LARGE ☑ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $40 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $210 =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT	09/18/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 29	Minus	** 30	= 0		x $40 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							
							TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.	LIE
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".	marsha R richards
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".	
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.	

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

72960 7590 07/19/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
HINES, JANA A

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
07/19/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 15/170,284	**Applicant(s)** GAJEWSKI ET AL.	
	Examiner Ja'Na Hines	**Art Unit** 1645	**AIA (First Inventor to File) Status** Yes

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 28 June 2017 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

NO NOTICE OF APPEAL FILED

1. ☒ The reply was filed after a final rejection. No Notice of Appeal has been filed. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114 if this is a utility or plant application. Note that RCEs are not permitted in design applications. The reply must be filed within one of the following time periods:
   a) ☒ The period for reply expires *4* months from the mailing date of the final rejection.
   b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action; or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
   c) ☐ A prior Advisory Action was mailed more than 3 months after the mailing date of the final rejection in response to a first after-final reply filed within 2 months of the mailing date of the final rejection. The current period for reply expires months from the mailing date of *the prior Advisory Action* or SIX MONTHS from the mailing date of the final rejection, whichever is earlier.
   Examiner Note: If box 1 is checked, check either box (a), (b) or (c). ONLY CHECK BOX (b) WHEN THIS ADVISORY ACTION IS THE FIRST RESPONSE TO APPLICANT'S FIRST AFTER-FINAL REPLY WHICH WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. ONLY CHECK BOX (c) IN THE LIMITED SITUATION SET FORTH UNDER BOX (c). See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) or (c) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☒ The proposed amendments filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   a) ☒ They raise new issues that would require further consideration and/or search (see NOTE below);
   b) ☐ They raise the issue of new matter (see NOTE below);
   c) ☒ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   d) ☐ They present additional claims without canceling a corresponding number of finally rejected claims.
   NOTE: _____. (See 37 CFR 1.116 and 41.33(a)).
4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☒ For purposes of appeal, the proposed amendment(s): (a) ☒ will not be entered, or (b) ☐ will be entered, and an explanation of how the new or amended claims would be rejected is provided below or appended.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.
9. ☐ The affidavit or other evidence filed after final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
10. ☐ The affidavit or other evidence filed after the date of filing the Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing of good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
11. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

12. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: See continuation sheet..
13. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
14. ☒ Other: PTO Form 2323.

STATUS OF CLAIMS

15. The status of the claim(s) is (or will be) as follows:
    Claim(s) allowed: None.
    Claim(s) objected to: None.
    Claim(s) rejected: 1-30.
    Claim(s) withdrawn from consideration: None.

/Ja'Na Hines/
Primary Examiner, Art Unit 1645

The proposed amendments raise new issues that require further search and consideration. The proposed amendment does not simplify or reduce the issues of record. The proposed amendment would elicit a 112(b) rejection because the metes and bounds for determining enhancement of an immune response is not defined. There is no recited comparison to determine whether an immune response has been enhanced. Additionally, the claims now qualify the bacteria as being an immunostimulatory bacteria. Therefore new limitations are recited by the claims requiring further consideration and search.

Accordingly, because the proposed amendments are not being considered, the rejections of record are maintained.

DO NOT ENTER: /J.A.H/

CLAIMS

1. (currently amended) A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and an immunostimulatory bacteria of the genus ~~a bacterial formulation comprising bacteria of the genera~~ *Bifidobacterium*, such that the bacteria enhance immune response in the subject to treat the cancer.

2. (currently amended) The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

3. (currently amended) The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

4. (currently amended) The method of claim 1, wherein the bacteria of the genus ~~genera~~ *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ is administered by oral administration or rectal administration.

6. (currently amended) The method of claim 5, wherein the bacteria ~~bacterial formulation~~ is administered by oral administration.

7. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ comprises at least $5x10^6$ CFU of bacteria of the genus ~~genera~~ *Bifidobacterium*.

8. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ is administered to the subject in two or more doses.

9. (original) The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. (currently amended) The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacteria ~~bacterial formulation~~.

11. (currently amended) The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacteria ~~bacterial formulation~~ is administered to the subject.

12. (original) The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. (original) The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. (original) The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. (original) The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. (original) The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. (original) The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. (original) The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. (original) The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

20. (currently amended) A method of treating cancer in a human subject comprising administering to the subject a bacterial formulation comprising at least $5x10^6$ CFU of bacteria of the genus ~~genera~~ *Bifidobacterium,* wherein at least 50% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

21. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

22. (currently amended) The method of claim 20, wherein the bacteria of the genus ~~genera~~ *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium*

psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis or *Bifidobacterium sp.*.

23. (original) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

24. (original) The method of claim 23, wherein the bacterial formulation is administered by oral administration.

25. (original) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

26. (original) The method of claim 20, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

27. (original) The method of claim 20, further comprising administering to the subject an immune checkpoint inhibitor.

28. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

29. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

30. (original) The method of claim 27, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

AFCP 2.0 Decision	Application No.	Applicant(s)
	15/170,284	GAJEWSKI ET AL.
	Examiner	Art Unit
	Ja'Na Hines	1645

This is in response to the After Final Consideration Pilot request filed 6/28/17.

1. **Improper Request** – The AFCP 2.0 request is improper for the following reason(s) and the after final amendment submitted with the request will be treated under pre-pilot procedure.

   ☐ An AFCP 2.0 request form PTO/SB/434 (or equivalent document) was not submitted.

   ☐ A non-broadening amendment to at least one independent claim was not submitted.

   ☐ A proper AFCP 2.0 request was submitted in response to the most recent final rejection.

   ☐ Other:

2. **Proper Request**

   A. After final amendment submitted with the request will not be treated under AFCP 2.0.
   The after final amendment cannot be reviewed and a search conducted within the guidelines of the pilot program.

   ☒ The after final amendment will be treated under pre-pilot procedure.

   B. Updated search and/or completed additional consideration.
   The examiner performed an updated search and/or completed additional consideration of the after final amendment within the time authorized for the pilot program. The result(s) of the updated search and/or completed additional consideration are:

   ☐ 1. All of the rejections in the most recent final Office action are overcome and a Notice of Allowance is issued herewith.

   ☐ 2. The after final amendment would not overcome all of the rejections in the most recent final Office action. See attached interview summary for further details.

   ☐ 3. The after final amendment was reviewed, and it raises a new issue(s). See attached interview summary for further details.

   ☐ 4. The after final amendment raises new issues, but would overcome all of the rejections in the most recent final Office action. A decision on determining allowability could not be made within the guidelines of the pilot. See attached interview summary for further details, including any newly discovered prior art.

   ☐ 5. Other:

   Examiner Note: Please attach an interview summary when necessary as described above.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

72960 7590 07/14/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
HINES, JANA A

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
07/14/2017	ELECTRONIC

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docketing@casimirjones.com
pto.correspondence@casimirjones.com

Applicant-Initiated Interview Summary	Application No. 15/170,284	Applicant(s) GAJEWSKI ET AL.	
	Examiner Ja'Na Hines	Art Unit 1645	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Ja'Na Hines*. (3)_____.

(2) *David Staple*. (4)_____.

Date of Interview: *06 April 2017*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☒103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *Claims of record*.

Identification of prior art discussed: *Sharon et al and Mahoney et al.*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Applicants argued that Sharon et al., which teach the instantly claimed immune checkpoint inhibitor does not mediate immune suppression. Applicants argued that there would be no motivation to combine the two components which are both already known in the art to treat cancer in order to form a third composition useful for the same purpose of treating cancer.The examiner pointed to the Kerkhoven teaching. The examiner also asserted that Sharon et al., teach the immune checkpoint inhibitor reduced cytokine production, reduced cytolytic activity and reduced lymphocyte proliferation; while O'Mahoney's Bifidobacterium also reduces cytokine levels..

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/Ja'Na Hines/ Primary Examiner, Art Unit 1645	

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

CLAIMS

1. (currently amended) A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and an immunostimulatory bacteria of the genus ~~a bacterial formulation comprising bacteria of the genera~~ *Bifidobacterium*, such that the bacteria enhance immune response in the subject to treat the cancer.

2. (currently amended) The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

3. (currently amended) The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

4. (currently amended) The method of claim 1, wherein the bacteria of the genus ~~genera~~ *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ is administered by oral administration or rectal administration.

6. (currently amended) The method of claim 5, wherein the bacteria ~~bacterial formulation~~ is administered by oral administration.

7. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ comprises at least $5x10^6$ CFU of bacteria of the genus ~~genera~~ *Bifidobacterium*.

8. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ is administered to the subject in two or more doses.

9. (original) The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. (currently amended) The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacteria ~~bacterial formulation~~.

11. (currently amended) The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacteria ~~bacterial formulation~~ is administered to the subject.

12. (original) The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. (original) The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. (original) The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. (original) The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. (original) The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. (original) The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. (original) The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. (original) The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

20. (currently amended) A method of treating cancer in a human subject comprising administering to the subject a bacterial formulation comprising at least $5x10^6$ CFU of bacteria of the genus ~~genera~~ *Bifidobacterium,* wherein at least 50% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

21. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

22. (currently amended) The method of claim 20, wherein the bacteria of the genus ~~genera~~ *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium*

psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis or *Bifidobacterium sp.*.

23. (original) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

24. (original) The method of claim 23, wherein the bacterial formulation is administered by oral administration.

25. (original) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

26. (original) The method of claim 20, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

27. (original) The method of claim 20, further comprising administering to the subject an immune checkpoint inhibitor.

28. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

29. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

30. (original) The method of claim 27, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	8885
Serial No.:	15/170,284	Art Unit:	1645
Filed:	01-JUNE-2016	Examiner:	Hines, Jana A.
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO FINAL OFFICE ACTION MAILED MARCH 28, 2017

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Examiner Hines:

This communication is responsive the Final Office Action mailed March 28, 2017, and is filed under the After Final Consideration Pilot 2.0 program.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-3/ORD. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

CLAIMS

1. (currently amended) A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and an immunostimulatory bacteria of the genus ~~a bacterial formulation comprising bacteria of the genera~~ *Bifidobacterium*, such that the bacteria enhance immune response in the subject to treat the cancer.

2. (currently amended) The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

3. (currently amended) The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

4. (currently amended) The method of claim 1, wherein the bacteria of the genus ~~genera~~ *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ is administered by oral administration or rectal administration.

6. (currently amended) The method of claim 5, wherein the bacteria ~~bacterial formulation~~ is administered by oral administration.

7. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ comprises at least $5x10^6$ CFU of bacteria of the genus ~~genera~~ *Bifidobacterium*.

8. (currently amended) The method of claim 1, wherein the bacteria ~~bacterial formulation~~ is administered to the subject in two or more doses.

9. (original) The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. (currently amended) The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacteria ~~bacterial formulation~~.

11. (currently amended) The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacteria ~~bacterial formulation~~ is administered to the subject.

12. (original) The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. (original) The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. (original) The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. (original) The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. (original) The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. (original) The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. (original) The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. (original) The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

20. (currently amended) A method of treating cancer in a human subject comprising administering to the subject a bacterial formulation comprising at least $5x10^6$ CFU of bacteria of the genus ~~genera~~ *Bifidobacterium,* wherein at least 50% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

21. (currently amended) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genus ~~genera~~ *Bifidobacterium*.

22. (currently amended) The method of claim 20, wherein the bacteria of the genus ~~genera~~ *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium*

psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis or *Bifidobacterium sp.*.

23. (original) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

24. (original) The method of claim 23, wherein the bacterial formulation is administered by oral administration.

25. (original) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

26. (original) The method of claim 20, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

27. (original) The method of claim 20, further comprising administering to the subject an immune checkpoint inhibitor.

28. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

29. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

30. (original) The method of claim 27, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

REMARKS

Claims 1-8, 10-11, and 20-22 are amended in the present communication. Amendments are made without acquiescing to the Examiner's arguments, in order to advance prosecution, and while retaining the right to pursue the unamended or similar claims in the future. The amended claims find support throughout the Specification, for example, Example 2 and Figure 3E.

I. Interview summary

Examiner Hines and Applicant's representative David Staple conducted a telephone interview on April 6, 2017. An overview of the technology encompassed by the claims, the cited references, and potential claim amendments were discussed. Applicant thanks Examiner Hines for her time and insights.

II. Nonobviousness

Claims 1-30 are rejected under 35 U.S.C. 103 as allegedly being unpatentable over Sharon et al. (Chin. J. Cancer, 2014, 33(9):434-444) in view of O'Mahoney et al. (U.S. Pat. Pub. 2012/0276143). Applicant respectfully disagrees.

The claims recite a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*. As Applicants have argued previously, one of skill in the art would not combine Sharon et al. with O'Mahoney et al., as alleged in the rejection, for at least the reason that Sharon et al. describes promoting an immune response while O'Mahoney et al. describes the suppression of immune response (See, e.g., Response Communication filed February 26, 2017). The Examiner has responded to Applicant's arguments by alleging (1) "unlike Applicants arguments, the immune checkpoint inhibitor of Sharon et al., mediates immune suppression[1]" and (2) that the combination is allegedly obvious because both references allegedly "teach treating cancer in a human subject." Applicant respectfully disagrees with the Examiner's arguments for at least the following reasons.

[1] Applicant respectfully submits that this is a mischaracterization of immune checkpoint inhibitors and the disclosure of Sharon et al.

1. Immune checkpoint inhibitors

In response to Applicant's arguments regarding the apparent incompatibility of the effects on immune response of the techniques of Sharon et al. and O'Mahoney et al., the Examiner has alleged:

> Actually Sharon et al., teach the immune checkpoints refer to regulatory pathways in the immunome that inhibit a portion of an active immune response against a specific target. The immune checkpoints are necessary to modulate and maintain immune homeostasis (page 434, para. 1). Sharon et al., teach the soluble inhibitor CTLA4 mediate immune suppression either by down-regulating 87 expression or by blocking the potential interaction of 87 and the co-stimulatory molecule CD28 (page 436, col.1). Similar to CTLA4, PD-1 becomes expressed on CD4- and COS-positive T lymphocytes during antigenic stimulation, serving as a co-inhibitory signal. As a co-inhibitory signal, PD-1 engagement results in reduced cytokine production, cytolytic activity, and lymphocyte proliferation. Therefore, unlike Applicants arguments, the immune checkpoint inhibitor of Sharon et al., mediates immune suppression. (Office Action, page 11).

Applicants respectfully submit that this represents a mischaracterization of immune checkpoint inhibitors and the teachings of Sharon et al. This reasoning appears to conflate the function of immune checkpoints (e.g., CTLA4 and PD-1) with the function of an immune checkpoint inhibitor (e.g., anti-CTLA4, anti-PD1, etc.). As stated in the Sharon et al. reference itself, "[i]mmune checkpoints refer to regulatory pathways in the immunome that inhibit a portion of an active immune response." (page 434, para. 1). Therefore, immune checkpoint inhibitors are 'inhibitors of inhibition of the immune response' (i.e., potentiators of immune response). This is also made clear in Sharon et al., which states:

> [Immune] checkpoints serve to control or turn off the immune response when it is no longer needed to prevent tissue injury and autoimmunity. Cancer cells have learned or evolved to use these mechanisms to evade immune control and elimination. The development of a new therapeutic class of drugs that inhibit these inhibitory pathways has recently emerged as a potent strategy in oncology. Three sets of agents have emerged in clinical trials exploiting this strategy. These agents are antibody-based therapies targeting cytotoxic T-lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD-1), and programmed cell death ligand 1 (PD-L1). These inhibitors of immune inhibition have demonstrated extensive activity as single agents and in combinations. Clinical responses have been seen in melanoma, renal cell carcinoma, non-small cell lung cancer, and several other tumor types. (Abstract; *emphasis added*).

While **immune checkpoints** suppress the immune response, **immune checkpoint inhibitors** function by inhibiting immune checkpoints, thereby potentiating the immune response. In light of the above, Applicants respectfully submit that the characterization that the "immune checkpoint inhibitor of Sharon

et al., mediates immune suppression" is factually inaccurate. Therefore, Applicant respectfully request reconsideration of Applicant's argument that one of skill in the art would not have sought to combine the techniques of Sharon et al. and O'Mahoney et al. due to their opposing effects on immune response.

2. Treating cancer

The Examiner has argued that the combination of the teachings of Sharon et al. and O'Mahoney et al. is obvious, regardless of the opposite underlying effects on the immune response, because both references allegedly discuss utility in the in the treatment or prevention of cancer. Without acquiescing to the Examiner's arguments, Applicant has amended independent claim 1 to recite "[a] method of treating cancer in a human subject… such that the bacteria enhance immune response in the subject to treat the cancer." Contrary to the present claims, the O'Mahoney et al. reference is clearly directed toward suppression of immune response. O'Mahoney states:

> [A] *Bifidobacterium* strain which has been shown to have immunomodulatory effects, by modulating cytokine levels or by antagonizing and excluding pro-inflammatory micro-organisms from the gastrointestinal tract." (paragraph [0005]);
>
> The invention also provides a *Bifidobacterium* strain or a formulation as described herein for use in the prophylaxis and/or treatment of autoimmune disorders due to undesirable inflammatory activity. (paragraph [0015]); and
>
> The invention is therefore of major potential therapeutic value in the prophylaxis or treatment of dysregulated immune responses, such as undesirable inflammatory reactions for example asthma. (paragraph [0039]).

The various utilities of *Bifidobacterium* described in O'Mahoney are allegedly due to anti-inflammatory or immune-suppressive effects. Therefore, one of skill in the art would not be motivated to use the compositions or methods described in O'Mahoney to "enhance immune response," as is presently claimed. Particularly in light of the claim amendment, Applicant respectfully requests reconsideration and withdrawal of the rejection of the claims under 35 U.S.C. 103(a).

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

Respectfully submitted,

Date: June 28, 2017

/David W. Staple/

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Doc Code: A.NE.AFCP
Document Description: After Final Consideration Pilot Program Request

PTO/SB/434 (05-13)

CERTIFICATION AND REQUEST FOR CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0

Practitioner Docket No.:	Application No.:	Filing Date:
UCHI-34458/US-3/ORD	15/170,284	2016-06-01
First Named Inventor:	**Title:**	
Gajewski	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA	

APPLICANT HEREBY CERTIFIES THE FOLLOWING AND REQUESTS CONSIDERATION UNDER THE AFTER FINAL CONSIDERATION PILOT PROGRAM 2.0 (AFCP 2.0) OF THE ACCOMPANYING RESPONSE UNDER 37 CFR 1.116.

1. The above-identified application is (i) an original utility, plant, or design nonprovisional application filed under 35 U.S.C. 111(a) [a continuing application (*e.g.*, a continuation or divisional application) is filed under 35 U.S.C. 111(a) and is eligible under (i)], or (ii) an international application that has entered the national stage in compliance with 35 U.S.C. 371(c).
2. The above-identified application contains an outstanding final rejection.
3. Submitted herewith is a response under 37 CFR 1.116 to the outstanding final rejection. The response includes an amendment to at least one independent claim, and the amendment does not broaden the scope of the independent claim in any aspect.
4. This certification and request for consideration under AFCP 2.0 is the only AFCP 2.0 certification and request filed in response to the outstanding final rejection.
5. Applicant is willing and available to participate in any interview requested by the examiner concerning the present response.
6. This certification and request is being filed electronically using the Office's electronic filing system (EFS-Web).
7. Any fees that would be necessary consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, extension of time fees, are being concurrently filed herewith. [There is no additional fee required to request consideration under AFCP 2.0.]
8. By filing this certification and request, applicant acknowledges the following:
    - Reissue applications and reexamination proceedings are not eligible to participate in AFCP 2.0.
    - The examiner will verify that the AFCP 2.0 submission is compliant, *i.e.*, that the requirements of the program have been met (see items 1 to 7 above). For compliant submissions:
        - The examiner will review the response under 37 CFR 1.116 to determine if additional search and/or consideration (i) is necessitated by the amendment and (ii) could be completed within the time allotted under AFCP 2.0. If additional search and/or consideration is required but cannot be completed within the allotted time, the examiner will process the submission consistent with current practice concerning responses after final rejection under 37 CFR 1.116, *e.g.*, by mailing an advisory action.
        - If the examiner determines that the amendment does not necessitate additional search and/or consideration, or if the examiner determines that additional search and/or consideration is required and could be completed within the allotted time, then the examiner will consider whether the amendment places the application in condition for allowance (after completing the additional search and/or consideration, if required). If the examiner determines that the amendment does not place the application in condition for allowance, then the examiner will contact the applicant and request an interview.
            - The interview will be conducted by the examiner, and if the examiner does not have negotiation authority, a primary examiner and/or supervisory patent examiner will also participate.
            - If the applicant declines the interview, or if the interview cannot be scheduled within ten (10) calendar days from the date that the examiner first contacts the applicant, then the examiner will proceed consistent with current practice concerning responses after final rejection under 37 CFR 1.116.

Signature	Date
/David W. Staple/	2017-06-28
Name (Print/Typed) David W. Staple	**Practitioner Registration No.** 65903

***Note**: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.*

☑ * Total of 1 forms are submitted.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt

EFS ID:	29630006
Application Number:	15170284
International Application Number:	
Confirmation Number:	8885
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple/Stephanie Filandrinos
Filer Authorized By:	David William Staple
Attorney Docket Number:	UCHI-34458/US-3/ORD
Receipt Date:	28-JUN-2017
Filing Date:	01-JUN-2016
Time Stamp:	16:37:42
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		34458US3ORD_RespFOA.pdf	141159 8a25ecddf5113807fade169233d21d8e434a736a	yes	9

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Response After Final Action	1	1
	Claims	2	5
	Applicant Arguments/Remarks Made in an Amendment	6	9

Warnings:					
Information:					
2	After Final Consideration Program Request	34458US3ORD_AFCP_Form_6-28-17.pdf	226633 818cab1e429a6f11c2ee1079ec662c70c274fdec	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	367792		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 15/170,284	Filing Date 06/01/2016	☐ To be Mailed

ENTITY: ☐ LARGE ☒ SMALL ☐ MICRO

APPLICATION AS FILED – PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA	RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	

APPLICATION AS AMENDED – PART II

AMENDMENT	06/28/2017	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 30	Minus	** 30	= 0	X $40 =	0
	Independent (37 CFR 1.16(h))	* 2	Minus	***3	= 0	X $210 =	0
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	0

AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))						
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
						TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

SLIE
HELENA PAYTON

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
15/170,284	06/01/2016	Thomas F. Gajewski	UCHI-34458/US-3/ORD	8885

72960 7590 03/28/2017

Casimir Jones, S.C.
2275 DEMING WAY, SUITE 310
MIDDLETON, WI 53562

EXAMINER
HINES, JANA A

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
03/28/2017	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

docketing@casimirjones.com
pto.correspondence@casimirjones.com

Office Action Summary	Application No. 15/170,284	Applicant(s) GAJEWSKI ET AL.	
	Examiner Ja'Na Hines	Art Unit 1645	AIA (First Inventor to File) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *2/6/17*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1-30* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-30* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *2/6/17*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

4) ☐ Other: _____.

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

DETAILED ACTION

Claim Status

1. Claims 1-30 are under consideration in this Office Action.

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

2. Claims 1-30 are rejected under 35 U.S.C. 103 as being unpatentable over Sharon et al., (Chin. J.Cancer. 2014. 33(9):434-444) in view of O'Mahoney et al., (US Patent Application 2012/0276143 published Nov. 2012).

The claims are drawn to a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera Bifidobacterium.

Sharon et al., teach immunology-based therapy is rapidly developing into an effective treatment option for a surprising range of cancers (abstract). Sharon et al., learned over the last decade that powerful immunologic effector cells may be blocked by inhibitory regulatory pathways controlled by specific molecules often called "immune checkpoints" (page 434). These checkpoints serve to control or turn off the immune response when it is no longer needed to prevent tissue injury and autoimmunity. Cancer cells have learned or evolved to use these mechanisms to evade immune control and elimination. The development of a new therapeutic class of drugs that inhibit these inhibitory pathways has recently emerged as a potent strategy in oncology. Three sets of agents have emerged in clinical trials exploiting this strategy. These agents are antibody-based therapies targeting cytotoxic T-lymphocyte antigen 4 (CTLA4), programmed cell death 1 (PD-1), and programmed cell death ligand 1 (PD-L1)(page

434). See Table 1 for additional immune checkpoint proteins and their inhibitors, such as B7H3, Lag3, and KIR (page 435). Sharon et al., teach CTLA4 inhibition, human cancers and the inhibitor being an antibody (page 436). These inhibitors of immune inhibition have demonstrated extensive activity as single agents and in combinations. Clinical responses have been seen in melanoma, renal cell carcinoma, non-small cell lung cancer, urothelial, head and neck, ovarian cancer and various lymphomas (page 434). Sharon et al., teach PD-1 and PDL1 targeting agents (pages 439-440). Despite the autoimmune or inflammatory immune-mediated adverse effects which have been seen, the responses and overall survival benefits exhibited thus far warrant further clinical development (page 434). Sharon et al., a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor.

O'Mahoney et al., teach *Bifidobacterium* strain which has been shown to have immunomodulatory effects, by modulating cytokine levels or by antagonizing and excluding pro-inflammatory micro-organisms from the gastrointestinal tract [para. 0005]. O'Mahoney et al., teach *Bifidobacterium* strain may be significantly immunomodulatory following oral consumption in human [para. 0007]. *Bifidobacterium* strain or a formulation as described herein for use in the prophylaxis and/or treatment of gastrointestinal cancer(s) [para. 0013]. *Bifidobacterium* strain or a formulation as described herein for use in the prophylaxis and/or treatment of cancer due to undesirable inflammatory activity [para. 016]. O'Mahoney et al., teach a variety of administration protocols including daily [para. 0128] The combined administration of a probiotic strain with one or more prebiotic compounds may enhance the growth of the

administered probiotic in vivo resulting in a more pronounced health benefit, and is termed symbiotic [para. 0139]. It will be appreciated that the probiotic strains may be administered prophylactically or as a method of treatment either on its own or with other probiotic and/or prebiotic materials as described above. In addition, the bacteria may be used as part of a prophylactic or treatment regime using other active materials such as those used for treating inflammation or other disorders especially those with an immunological involvement. Such combinations may be administered in a single formulation or as separate formulations administered at the same or different times and using the same or different routes of administration [para. 0140]. The *Bifidobacterium* strain may be present in an amount of more than 10^6 cfu per gram of the formulation [para. 0008]. The formulation may further comprise a drug entity. The formulation may further comprise a biological compound. The formulation may be used for immunization and vaccination protocols [para. 008].

Therefore, it would have been *prima facie* obvious at the time of applicants' invention to incorporate O'Mahoney's Bifidobacterium to Sharon's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor when O'Mahoney et al., already it was known to treat cancer in a human subject comprising administering to the subject Bifidobacterium in combination with other therapies that also treat cancer. One of ordinary skill in the art would have a reasonable expectation of success by combining both components because the prior art combination therapy was well known to produce beneficial and even synergistic results.

It is noted, that while the references recite oral administration at the instantly claimed dosage amount, there is no specific teaching of the dosage routines and previous administration routines as recited by claims 8-11 and 25-26. Regarding the specific schemes recited in the instant claims, MPEP 2144.05 states, "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955) (Claimed process which was performed at a temperature between 40℃ and 80℃ and an acid concentration between 25% and 70% was held to be *prima facie* obvious over a reference process which differed from the claims only in that the reference process was performed at a temperature of 100℃ and an acid concentration of 10%.); see also *Peterson*, 315 F.3d at 1330, 65 USPQ2d at 1382 ("The normal desire of scientists or artisans to improve upon what is already generally known provides the motivation to determine where in a disclosed set of percentage ranges is the optimum combination of percentages."); *In re Hoeschele*, 406 F.2d 1403, 160 USPQ 809 (CCPA 1969) (Claimed elastomeric polyurethanes which fell within the broad scope of the references were held to be unpatentable thereover because, among other reasons, there was no evidence of the criticality of the claimed ranges of molecular weight or molar proportions.). For more recent cases applying this principle, see *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989); *In re Kulling*, 897 F.2d 1147, 14 USPQ2d 1056 (Fed. Cir. 1990); and *In re Geisler,* 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997)."

Additionally, *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007), discloses combining prior art elements according to known methods to yield predictable results, thus the combination is obvious unless its application is beyond that person's skill. *KSR International Co. v. Teleflex Inc.,* 127 S. Ct. 1727, 1741 (2007) also discloses that "The combination of familiar element according to known methods is likely to be obvious when it does no more than yield predictable results". It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of inhibitor or the *Bifidobacterium*; thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. The claimed invention is *prima facie* obvious in view of the teachings of the prior art, absent any convincing evidence to the contrary.

Response to Arguments

3. Applicant's arguments filed February 6, 2017 have been fully considered but they are not persuasive. The rejection of claims 1-30 under 35 U.S.C. 103 as being unpatentable over Sharon et al., (Chin. J.Cancer. 2014. 33(9):434-444) in view of O'Mahoney et al., (US Patent Application 2012/0276143 published Nov. 2012) is maintained for reasons of record.

Applicants argue that the cited references neither teach nor suggest the administration to a subject of both an immune checkpoint inhibitor and a bacterial formulation comprising Bifidobacterium. Applicant respectfully submits that when one considers the full scope of the teachings of Sharon et al. and O'Mahoney et al., rather than just their alleged applicability to cancer, it is apparent that there is no motivation to combine these references as alleged.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Sharon et al., teach a method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor. O'Mahoney et al., teach *Bifidobacterium* strain or a formulation used for the prophylaxis and/or treatment of cancer. Therefore Sharon et al., and O'Mahoney et al., teach treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera Bifidobacterium. Contrary to applicants assertion, the prior art references teach the instant claims. Thus, Applicants argument that the claims are not obvious over the cited references is not found persuasive.

Applicants urge that one of skill in the art would not have had a reasonable expectation of success because there is no actual indication in O'Mahoney that

Bifidobacterium is useful for the treatment of cancer, and cancer is listed among other maladies of overactive immune/inflammatory responses. Applicants point out that the anti-inflammatory (e.g., immune suppressive) effect of Bifidobacterium administration which O'Mahoney suggests has utility in treating/preventing cancers, particularly of the gastrointestinal and immune systems. It is position of the Office that a reasonable expectation of success would clearly exist for incorporating O'Mahoney's Bifidobacterium to Sharon's method for treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor when O'Mahoney et al., already it was known to treat cancer in a human subject comprising administering to the subject Bifidobacterium in combination with other therapies that also treat cancer. O'Mahoney et al., teach *Bifidobacterium* strain for use in the prophylaxis and/or treatment of cancer due to undesirable inflammatory activity, gastrointestinal cancers and cancer. Furthermore, both teach the components reduce cytokine production. In this case, all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded nothing more than predictable results to one of ordinary skill in the art; thereby providing a reasonable expectation of success. Therefore, Applicants argument that there was not an expectation of success because there is no actual indication in O'Mahoney that Bifidobacterium is useful for the treatment of cancer is not correct since O'Mahoney et al., repeatedly teaches using *Bifidobacterium* strain for the prophylaxis and/or treatment of cancer, cancer due to undesirable inflammatory activity, and gastrointestinal cancers.

Applicants argue that O'Mahoney et al., disclose using *Bifidobacterium* strain for the prophylaxis and/or treatment of cancer among other maladies of overactive immune/inflammatory responses. The MPEP section 2123 teaches that patents are relevant as prior art for all they contain, "The use of patents as references is not limited to what the patentees describe as their own inventions or to the problems with which they are concerned. They are part of the literature of the art, relevant for all they contain." *In re Heck*, 699 F.2d 1331, 1332-33, 216 USPQ 1038, 1039 (Fed. Cir. 1983) (quoting *In re Lemelson*, 397 F.2d 1006, 1009, 158 USPQ 275, 277 (CCPA 1968)). A reference may be relied upon for all that it would have reasonably suggested to one having ordinary skill the art, including nonpreferred embodiments. *Merck & Co. v. Biocraft Laboratories*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), cert. denied, 493 U.S. 975 (1989). See also *Celeritas Technologies Ltd. v. Rockwell International Corp.*, 150 F.3d 1354, 1361, 47 USPQ2d 1516, 1522-23 (Fed. Cir.1998) (The court held that the prior art anticipated the claims even though it taught away from the claimed invention. "The fact that a modem with a single carrier data signal is shown to be less than optimal does not vitiate the fact that it is disclosed."). Therefore applicant's argument is not persuasive especially when considering that O'Mahoney et al., clearly describes using *Bifidobacterium* strain for the prophylaxis and/or treatment of cancer, cancer due to undesirable inflammatory activity, and gastrointestinal cancers.

Applicant notes that Sharon et al. describes potentiators of immune response, while O'Mahoney et al. describes immune suppressing bacteria. Given these contrary effects on an immune response, one cannot reasonably conclude that there would be

"no change in the respective function of inhibitor or the Bifidobacterium," nor that "the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention," as alleged in the rejection. Rather, the combination of these counteracting effects (suppressing vs. promoting an immune response) would have been at the very least unpredictable based on the cited references, and more likely what would have been predicted is that the immune checkpoint inhibitors and the Bifidobacterium strain would cancel each other out, reducing treatment efficacy. There would have been no reasonable expectation of success in combining agents that the references teach as having counteracting effects.

Actually Sharon et al., teach the immune checkpoints refer to regulatory pathways in the immunome that inhibit a portion of an active immune response against a specific target. The immune checkpoints are necessary to modulate and maintain immune homeostasis (page 434, para. 1). Sharon et al., teach the soluble inhibitor CTLA4 mediate immune suppression either by down-regulating B7 expression or by blocking the potential interaction of B7 and the co-stimulatory molecule CD28 (page 436, col.1). Similar to CTLA4, PD-1 becomes expressed on CD4- and CD8-positive T lymphocytes during antigenic stimulation, serving as a co-inhibitory signal. As a co-inhibitory signal, PD-1 engagement results in reduced cytokine production, cytolytic activity, and lymphocyte proliferation. Therefore, unlike Applicants arguments, the immune checkpoint inhibitor of Sharon et al., mediates immune suppression.

Similarly, O'Mahoney et al., teach a *Bifidobacterium* strain for reducing the levels of pro inflammatory cytokines. Therefore the combination two compositions each of which is taught by the prior art to be useful for the same purpose of reducing cytokine production and treating cancer, in order to form a third composition to be used for the very same purpose of treating cancer would have been obvious. Furthermore, the idea of combining them flows logically from their having been individually taught in the prior art. Please see MPEP 2144.06.

2144.06 Art Recognized Equivalence for the Same Purpose [R-08.2012]
I. COMBINING EQUIVALENTS KNOWN FOR THE SAME PURPOSE

"It is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." *In re Kerkhoven,* 626 F.2d 846, 850, 205 USPQ 1069, 1072 (CCPA 1980) (citations omitted) (Claims to a process of preparing a spray-dried detergent by mixing together two conventional spray-dried detergents were held to be *prima facie* obvious.). See also *In re Crockett,* 279 F.2d 274, 126 USPQ 186 (CCPA 1960) (Claims directed to a method and material for treating cast iron using a mixture comprising calcium carbide and magnesium oxide were held unpatentable over prior art disclosures that the aforementioned components individually promote the formation of a nodular structure in cast iron.); and *Ex parte Quadranti,* 25 USPQ2d 1071 (Bd. Pat. App. & Inter. 1992) (mixture of two known herbicides held *prima facie* obvious).

Furthermore, each component functions as expected. Applicants have not argued that the results were greater than those which would have been expected from the prior art to an unobvious extent. Therefore, the rejection of record is maintained.

Conclusion

4. No allowed claims.

5. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ja-Na Hines whose telephone number is 571-272-0859. The examiner can normally be reached Monday thru Thursday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor Gary Nickol, can be reached on 571-272-0835. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Ja'Na Hines/
Primary Examiner, Art Unit 1645

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/170,284
				Filing Date	06/01/2016
				First Named Inventor	Gajewski
				Art Unit	1645
				Examiner Name	Hines
Sheet	1	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

U.S. PATENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4,816,567	1989-03-28	CABILLY et al.	
		4,946,778	1990-08-07	LADNER et al.	
		5,260,203	1993-11-09	LADNER et al.	
		7,195,906	2007-03-27	COLLINS et al.	
		8,449,878	2013-05-28	YONAK et al.	

U.S. PUBLISHED PATENT APPLICATIONS

Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] (if known)	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

Note: Submission of copies of U.S. Patents and published U.S. Patent Applications is not required.

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] (if known)	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		EP 2876167	2015-05-27	INSTITUT GUSTAVE ROUSSY		
		WO 1988/01649	1988-03-10	GENEX CORPORATION		
		WO 2011/068810	2011-06-09	SHIRE HUMAN GENETIC THERAPIES		
		WO 2014/145958	2014-09-18	SERES HEALTH, CIN.		
		WO 2015/061372	2015-04-30	HEMOSHEAR, LLC		

Examiner Signature	/JANA A HINES/ (03/22/2017)	Date Considered	03/22/2017

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /JAH/

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/170,284
				Filing Date	06/01/2016
				First Named Inventor	Gajewski
				Art Unit	1645
				Examiner Name	Hines
Sheet	2	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

NONPATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]
		ABT et al. Commensal bacteria calibrate the activation threshold of innate antiviral immunity. Immunity. 2012 Jul 27;37(1):158-70	
		BAK et al., Differential requirement for CD70 and CD80/CD86 in dendritic cell-mediated activation of tumor-tolerized CD8 T cells. J Immunol. 2012 Aug 15;189(4):1708-16	
		BLANK et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. Cancer Res. 2004 Feb 1;64(3):1140-5.	
		CAPORASO et al., PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics. 2010 Jan 15;26(2):266-7	
		CAPORASO et al., QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010 May;7(5):335-6	
		CLACKSON et al., Making antibody fragments using phage display libraries. Nature. 1991 Aug 15;352(6336):624-8.	
		COMPEER et al., Antigen processing and remodeling of the endosomal pathway: requirements for antigen cross-presentation. Front Immunol. 2012 Mar 7;3:37	
		DONG et al., The role of intestinal bifidobacteria on immune system development in young rats. Early Hum Dev. 2010 Jan;86(1):51-8	
		FUERTES et al., Host type I IFN signals are required for antitumor CD8+ T cell responses through CD8{alpha}+ dendritic cells. J Exp Med. 2011 Sep 26;208(10):2005-16	
		GAJEWSKI et al., Gene signature in melanoma associated with clinical activity: a potential clue to unlock cancer immunotherapy. Cancer J. 2010 Jul-Aug;16(4):399-403.	
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		GOTO et al., Segmented filamentous bacteria antigens presented by intestinal dendritic cells drive mucosal Th17 cell differentiation. Immunity. 2014 Apr 17;40(4):594-607.	
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Examiner Signature	/JANA A HINES/ (03/22/2017)	Date Considered	03/22/2017

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/170,284
				Filing Date	06/01/2016
				First Named Inventor	Gajewski
				Art Unit	1645
				Examiner Name	Hines
Sheet	3	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

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				Examiner Name	Hines
Sheet	4	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

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CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

☐ That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

☐ See attached certification statement.

☒ The fee set forth in 37 CFR 1.17(p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/David W. Staple/	Date (YYYY-MM-DD)	2017-02-06
Name/Print	David W. Staple	Registration Number	65903

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Search Notes	Application/Control No.	Applicant(s)/Patent Under Reexamination
	15170284	GAJEWSKI ET AL.
	Examiner	Art Unit
	JA'NA HINES	1645

CPC- SEARCHED		
Symbol	Date	Examiner
A61K39/02; A61P1/00; A61P1/02; A61P1/04; A61P1/12; A61P1/16; A61P3/04; A61P3/10; A61P5/00; A61P7/02; A61P7/06; A61P9/00; A61P9/10; A61P11/00; A61P11/06; A61P13/12; A61P15/00; A61P17/00; A61P17/02; A61	3/2017	jah

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
searched inventors, applications, patents. Commerical database search of claim text	10/2016	jah

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	University of Chicago	Confirmation No.:	8885
Serial No.:	15/170,284	Art Unit:	1645
Filed:	01-JUNE-2016	Examiner:	Hines, Jana A.
Title:	**TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA**		

RESPONSE TO OFFICE ACTION MAILED NOVEMBER 4, 2016

VIA EFS-WEB
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Examiner Hines:

This communication is responsive the Office Action mailed November 4, 2016.

The Commissioner is authorized by this paper to charge any fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 that may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-4302, referencing Attorney Docket No.: UCHI-34458/US-3/ORD. This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).

CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

1. (original) A method of treating cancer in a human subject comprising administering to the subject an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*.

2. (original) The method of claim 1, wherein at least 50% of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*.

3. (original) The method of claim 1, wherein at least 90% of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*.

4. (original) The method of claim 1, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

5. (original) The method of claim 1, wherein the bacterial formulation is administered by oral administration or rectal administration.

6. (original) The method of claim 5, wherein the bacterial formulation is administered by oral administration.

7. (original) The method of claim 1, wherein the bacterial formulation comprises at least $5x10^6$ CFU of bacteria of the genera *Bifidobacterium*.

8. (original) The method of claim 1, wherein the bacterial formulation is administered to the subject in two or more doses.

9. (original) The method of claim 9, wherein the administration of the two or more doses are separated by at least 1 week.

10. (original) The method of claim 1, further comprising administering to the subject an antibiotic prior to the administration of the bacterial formulation.

11. (original) The method of claim 10, wherein the antibiotic is administered to the subject at least 1 day before the bacterial formulation is administered to the subject.

12. (original) The method of claim 1, wherein the immune checkpoint inhibitor is a protein or polypeptide that binds to an immune checkpoint protein.

13. (original) The method of claim 12, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

14. (original) The method of claim 13, wherein the immune checkpoint protein is PD-1 or PD-L1.

15. (original) The method of claim 1, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to an immune checkpoint protein.

16. (original) The method of claim 15, wherein the immune checkpoint protein is CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

17. (original) The method of claim 16, wherein the immune checkpoint protein is PD-1 or PD-L1.

18. (original) The method of claim 1, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

19. (original) The method of claim 1, wherein the immune checkpoint inhibitor is administered by intravenous injection, intramuscular injection, intratumoral injection or subcutaneous injection.

20. (original) A method of treating cancer in a human subject comprising administering to the subject a bacterial formulation comprising at least $5x10^6$ CFU of bacteria of the genera *Bifidobacterium,* wherein at least 50% of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*.

21. (original) The method of claim 20, wherein at least 90% of the bacteria in the bacterial formulation are of the genera *Bifidobacterium*.

22. (original) The method of claim 20, wherein the bacteria of the genus *Bifidobacterium* comprise bacteria of the species *Bifidobacterium lactis, Bifidobacterium bifidium, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium catenulatum, Bifidobacterium pseudocatenulatum, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium asteroides, Bifidobacterium boum, Bifidobacterium choerinum, Bifidobacterium coryneforme, Bifidobacterium cuniculi, Bifidobacterium denticolens, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium gallinarum, Bifidobacterium indicum, Bifidobacterium inopinatum, Bifidobacterium magnum, Bifidobacterium merycicum, Bifidobacterium minimum, Bifidobacterium pseudolongum, Bifidobacterium pullorum, Bifidobacterium psychraerophilum, Bifidobacterium ruminantium, Bifidobacterium saeculare, Bifidobacterium scardovii, Bifidobacterium simiae, Bifidobacterium subtile, Bifidobacterium therammcidophilum, Bifidobacterium thermophilum, Bifidobacterium tsurumiense, Bifidobacterium urinalis* or *Bifidobacterium sp.*.

23. (original) The method of claim 20, wherein the bacterial formulation is administered by oral administration or rectal administration.

24. (original) The method of claim 23, wherein the bacterial formulation is administered by oral administration.

25. (original) The method of claim 20, wherein the bacterial formulation is administered to the subject in two or more doses.

26. (original) The method of claim 20, further comprising administering to the subject an antibiotic before the bacterial formulation is administered to the subject.

27. (original) The method of claim 20, further comprising administering to the subject an immune checkpoint inhibitor.

28. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to CTLA4, PD-1, PD-L1, PD-L2, A2AR, B7-H3, B7-H4, BTLA, KIR, LAG3, TIM-3 or VISTA.

29. (original) The method of claim 27, wherein the immune checkpoint inhibitor is an antibody or antigen binding fragment thereof that binds to PD-1 or PD-L1.

30. (original) The method of claim 27, wherein the immune checkpoint inhibitor is nivolumab, pembrolizumab, pidilizumab, AMP-224, AMP-514, STI-A1110, TSR-042, RG-7446, BMS-936559, BMS-936558, MK-3475, CT Ol l, MPDL3280A, MEDI-4736, MSB-0020718C, AUR-012 and STI-A1010.

REMARKS

No claims are amended, cancelled, or added in the present communication. The rejections raised in the Office Action are addressed below.

Claims 1-30 are rejected under 35 U.S.C. 103 as allegedly being unpatentable over Sharon et al. (Chin. J. Cancer, 2014, 33(9):434-444) in view of O'Mahoney et al. (U.S. Pat. Pub. 2012/0276143). Applicants respectfully disagree.

The cited references neither teach nor suggest the administration to a subject of both an immune checkpoint inhibitor and a bacterial formulation comprising *Bifidobacterium.* Rather, Sharon et al. is cited as allegedly describing "inhibitors of immune inhibition" and O'Mahoney is cited as allegedly describing administration of *Bifidobacterium.,* As described below, one of skill in the art would not have been motivated to co-administer an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium*, as recited by the instant claims. The arguments set forth in the pending office action in support of the rejection do not identify any motivation to combine the treatments, other than the description in each reference that the techniques/agents described therein might be useful for the treatment of cancer. The rejection alleges that it would have been obvious to combine these references, and use an immune checkpoint inhibitor and a bacterial formulation comprising *Bifidobacterium* together for the treatment of cancer, because the references allegedly describe their use separately for the treatment of cancer. Applicant respectfully submits that when one considers the full scope of the teachings of Sharon et al. and O'Mahoney et al., rather than just their alleged applicability to cancer, it is apparent (A) that there is no motivation to combine these references as alleged, (B) that one of skill in the art would not have had a reasonable expectation of success in combining the references, and therefore (C) that the claims are not obvious over the cited references.

A. No motivation to combine

The test for *prima facie* obviousness is consistent with legal principles enunciated in *KSR Int'l Co. v. Teleflex Inc.,* 127 S. Ct. 1727 (2007). The Federal Circuit summarized the Supreme Court's holding in *KSR* that "While the *KSR* Court rejected a rigid application of the teaching, suggestion, or motivation ("TSM") test, the Court acknowledged the importance of identifying 'a ***reason*** that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does' in an obviousness determination." *Takeda Chem. Indus., Ltd. v. Alphapharma Pty., Ltd., 06-1329, slip op. (Fed. Cir. June 28, 2007)*, at 13-14 (quoting *KSR,* 127 S. Ct.

at 1731) (emphasis added). Although the TSM test should not be applied in a rigid manner, it can provide helpful insight to an obviousness inquiry. *KSR,* 127 S. Ct. at 1731. Applicant respectfully submits that upon consideration of the full disclosures of the cited references, one of skill in the art would not have been motivated, or had any other such reason, to combine these references as alleged in the rejection.

The Sharon et al. reference describes immune checkpoints, inhibitors thereof, and their applicability to cancer, stating:

> These checkpoints serve to control or turn off the immune response when it is no longer needed to prevent tissue injury and autoimmunity. Cancer cells have learned or evolved to use these mechanisms to evade immune control and elimination. The development of a new therapeutic class of drugs that inhibit these inhibitory pathways has recently emerged as a potent strategy in oncology… These inhibitors of immune inhibition have demonstrated extensive activity as single agents and in combinations. Clinical responses have been seen in melanoma, renal cell carcinoma, non-small cell lung cancer, and several other tumor types. Despite the autoimmune or inflammatory immune-mediated adverse effects which have been seen, the responses and overall survival benefits exhibited thus far warrant further clinical development. (Sharon et al., Abstract).

Sharon et al. therefore describes the inhibition of these controls on the immune response in order to promote an immune response for the treatment of cancer.

O'Mahoney et al. describes a strategy that is completely converse to that of Sharon et al. Rather than being directly related to the treatment of cancer, O'Mahoney describes the use of "a *Bifidobacterium* strain which has been shown to have immunomodulatory effects, by modulating cytokine levels or by antagonizing and excluding pro-inflammatory micro-organisms from the gastrointestinal tract." (O'Mahoney, paragraph [0005]). The reference repeatedly notes that the *Bifidobacterium* is useful in the suppression of immune responses, for example:

> The invention also provides a *Bifidobacterium* strain or a formulation as described herein for use in the prophylaxis and/or treatment of autoimmune disorders due to undesirable inflammatory activity. (paragraph [0015]); and
>
> The invention is therefore of major potential therapeutic value in the prophylaxis or treatment of dysregulated immune responses, such as undesirable inflammatory reactions for example asthma. (paragraph [0039]).

It is due to that alleged immunosuppressive effect of *Bifidobacterium* that O'Mahoney describes the administration of probiotics containing *Bifidobacterium* as allegedly useful for prevention and/or the treatment of:

> [I]nflammatory disorders, immunodeficiency, inflammatory bowel disease, irritable bowel syndrome, cancer (particularly of the gastrointestinal and immune systems), diarrhoeal disease, antibiotic associated diarrhoea, paediatric diarrhoea, appendicitis, autoimmune disorders, multiple sclerosis, Alzheimer's disease, rheumatoid arthritis, coeliac disease, diabetes mellitus, organ transplantation, bacterial infections, viral infections, fungal infections, periodontal disease, urogenital disease, sexually transmitted disease, HIV infection, HIV replication, HIV associated diarrhoea, surgical associated trauma, surgical-induced metastatic disease, sepsis, weight loss, anorexia, fever control, cachexia, wound healing, ulcers, gut barrier function, allergy, asthma, respiratory disorders, circulatory disorders, coronary heart disease, anaemia, disorders of the blood coagulation system, renal disease, disorders of the central nervous system, hepatic disease, ischaemia, nutritional disorders, osteoporosis, endocrine disorders, epidermal disorders, psoriasis, acne vulgaris, panic disorder, behavioral disorder and/or post traumatic stress disorders. (paragraph [0021]).

O'Mahoney et al. reasons that *Bifidobacterium* suppresses immune responses, particularly in the gut, and therefore may find use in the treatment of the above laundry list of conditions. There is no actual indication in O'Mahoney that *Bifidobacterium* is useful for the treatment of cancer, and cancer is listed among other maladies of overactive immune/inflammatory responses. O'Mahoney et al. notes that:

> The production of multifunctional cytokines across a wide spectrum of tumour types suggests that significant inflammatory responses are ongoing in patients with cancer. It is currently unclear what protective effect this response has against the growth and development of tumour cells in vivo. However, these inflammatory responses could adversely affect the tumour-bearing host… For a tumour to grow and spread it must induce the formation of new blood vessels and degrade the extracellular matrix. The inflammatory response may have significant roles to play in the above mechanisms, thus contributing to the decline of the host and progression of the tumour. Due to the anti-inflammatory properties of *Bifidobacterium longum infantis* these bacterial strains they may reduce the rate of malignant cell transformation. (paragraph [0136]).

It is specifically because of the anti-inflammatory (e.g., immune suppressive) effect of *Bifidobacterium* administration that O'Mahoney suggests its potential utility in treating/preventing cancers, particularly of the gastrointestinal and immune systems.

The Sharon et al. and O'Mahoney et al. references therefore describe administration of therapies with directly opposing effects on immune response. For example, Sharon et al. describes inhibitors of engagement of the PD-1 checkpoint, and notes that in the absence of inhibition of this checkpoint, "PD-

1 engagement results in reduced cytokine production." On the other hand, O'Mahoney et al. describes the use of *Bifidobacterium* "for reducing the levels of pro inflammatory cytokines." Therefore, the checkpoint inhibitors of Sharon et al. prevent the very reduction in cytokine production that the *Bifidobacterium* of O'Mahoney et al. promote. Co-administration of these two therapies is counterintuitive based upon a reading of the Sharon et al. and O'Mahoney et al.

The checkpoint inhibitors of Sharon et al. prevent cancer cells from evading an anti-cancer immune response, while of O'Mahoney et al. teach that the disclosed *Bifidobacterium* strain suppresses such immune responses. One of skill in the art would not be motivated to combine therapies that have directly opposing mechanisms of action, despite the alleged applicability of each to the broad class of cancer treatments. In light of their apparent counteracting mechanisms of action, one of skill in the art would not have been motivated to combine the references as alleged.

B. No reasonable expectation of success

"Where there is a reason to modify or combine the prior art to achieve the claimed invention, the claims may be rejected as prima facie obvious **provided there is also a reasonable expectation of success**." In re Merck & Co., Inc., 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). While Applicant certainly does not concede that there was reason at the time of the present invention to combine the prior art to achieve the claimed invention (indeed, as described above, Applicant submits that there was no reason to combine), Applicant respectfully submits that one of ordinary skill in the art would not have had a reasonable expectation of success in making such a combination, based on the teachings of the Sharon et al. and O'Mahoney et al. references addressed above.

The rejection states:

> It is well known to take a method of treating cancer when each ingredient is well-known to treat cancer, and where there is no change in the respective function of inhibitor or the *Bifidobacterium;* thus the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention. Therefore, it would have been obvious to a person of ordinary skill in the art to combine prior art elements according to known methods that is ready for improvement to yield predictable results. (Office Action, page 7).

Applicant notes that Sharon et al. describes potentiators of immune response, while O'Mahoney et al. describes immune suppressing bacteria. Given these contrary effects on an immune response, one cannot reasonably conclude that there would be "no change in the respective function of inhibitor or the

Bifidobacterium," nor that "the combination would have yielded a reasonable expectation or success along with predictable results to one of ordinary skill in the art at the time of the invention," as alleged in the rejection. Rather, the combination of these counteracting effects (suppressing vs. promoting an immune response) would have been at the very least unpredictable based on the cited references, and more likely what would have been predicted is that the immune checkpoint inhibitors and the *Bifidobacterium* strain would cancel each other out, reducing treatment efficacy. There would have been no reasonable expectation of success in combining agents that the references teach as having counteracting effects.

C. Summary

The Examiner has not identified any single reference that teaches or suggests the claimed administration of an immune checkpoint inhibitor and a bacterial formulation comprising bacteria of the genera *Bifidobacterium* for the treatment of cancer. While the references separately describe the individual therapies recited in the claims, one of skill in the art would not have been motivated to combine the therapies and would not have expected such a combination to be successful. To the contrary, the mechanisms of action described in the cited references would actually motivate one or skill in the art to avoid the combination, since the references teach counteracting effects on immune response (suppression vs. potentiation). In light of the above, Applicant respectfully requests the rejection under 35 U.SC. 103 be withdrawn.

CONCLUSION

Applicant respectfully submits that the remarks herein overcome the Office's rejections and place the claims in condition for allowance. If the Examiner wishes to discuss this case, Applicants encourage the Examiner to call the undersigned at 608-662-1277 at the Examiner's convenience.

Respectfully submitted,

Date: February 6, 2017

/David W. Staple/

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Electronic Patent Application Fee Transmittal

Application Number:	15170284
Filing Date:	01-Jun-2016
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Filer:	David William Staple
Attorney Docket Number:	UCHI-34458/US-3/ORD

Filed as Small Entity

Filing Fees for Utility under 35 USC 111(a)

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	2806	1	90	90
		Total in USD ($)		**90**

Electronic Acknowledgement Receipt

EFS ID:	28268874
Application Number:	15170284
International Application Number:	
Confirmation Number:	8885
Title of Invention:	TREATMENT OF CANCER BY MANIPULATION OF COMMENSAL MICROFLORA
First Named Inventor/Applicant Name:	Thomas F. Gajewski
Customer Number:	72960
Filer:	David William Staple
Filer Authorized By:	
Attorney Docket Number:	UCHI-34458/US-3/ORD
Receipt Date:	06-FEB-2017
Filing Date:	01-JUN-2016
Time Stamp:	16:19:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$90
RAM confirmation Number	020717INTEFSW00003767504302
Deposit Account	
Authorized User	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	34458US3ORD_IDS_SB08.pdf	175599 a6b447bd6302982c3df7152c404b685c7c16ab56	no	6
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Information:					
This is not an USPTO supplied IDS fillable form					
2	Foreign Reference	EP2876167.pdf	5757251 168f6ee1ef6442eb8369257aa1c1ffe5eb50b581	no	78
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Information:					
3	Foreign Reference	WO2011068810A1.pdf	2486474 1b98747c672c9bb8bcb2efa4e9cce30ae2381c3c	no	73
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Information:					
4	Foreign Reference	WO2014145958A2.pdf	17191530 fb73e001d629cbbf4abf72e5a553ab10f655b2b3	no	649
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5	Foreign Reference	WO2015061372A1.pdf	14150293 a2be0b8026fed13d60d05f0476bdae35ee1713b5	no	208
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16	Non Patent Literature	HARLOW_LANE1988Antibodies.pdf	1103367 c68b5bcc473a295eab6daa0b54f37b2636a5bd30	no	9
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18	Non Patent Literature	Caporaso_2010.pdf	177164 d91ea5f10fce13cf843fb964db19ae925c95689b	no	2
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52	Other Reference-Patent/App/Search documents	34458WO1ORD_ISR_WO_8-30-2016.pdf	910376 953c96fa9c61f011d5624368a5239db7530679de	no	15
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53	Non Patent Literature	Sivan.pdf	2107668 d841ef68b28a72d2941dd5c519d022c1262bd593	no	13
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54	Non Patent Literature	Tumeh.pdf	5806951 04d4fafb1c711b5ff71db1955dfacfcf4d9933ac	no	24
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Information:					
55		34458US3ORD_Resp1.pdf	168537 728f6e729b5a6aeb1a610d3cd6c0a3ba332477ac	yes	12
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	Claims		2	6	
	Applicant Arguments/Remarks Made in an Amendment		7	12	
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New Applications Under 35 U.S.C. 111
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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
				Application Number	15/170,284
				Filing Date	06/01/2016
				First Named Inventor	Gajewski
				Art Unit	1645
				Examiner Name	Hines
Sheet	1	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

U.S. PATENTS					
Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		4,816,567	1989-03-28	CABILLY et al.	
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U.S. PUBLISHED PATENT APPLICATIONS					
Examiner Initials*	Cite No.[3]	Document Number Number-Kind Code[4] *(if known)*	Issue or Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear

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FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[5] Number[6] Kind Code[7] *(if known)*	Publication Date YYYY-MM-DD	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[8]
		EP 2876167	2015-05-27	INSTITUT GUSTAVE ROUSSY		
		WO 1988/01649	1988-03-10	GENEX CORPORATION		
		WO 2011/068810	2011-06-09	SHIRE HUMAN GENETIC THERAPIES		
		WO 2014/145958	2014-09-18	SERES HEALTH, CIN.		
		WO 2015/061372	2015-04-30	HEMOSHEAR, LLC		

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Use as many sheets as necessary)				Complete if Known	
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				First Named Inventor	Gajewski
				Art Unit	1645
				Examiner Name	Hines
Sheet	2	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

NONPATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and-or country where published.	Translation[6]
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Sheet	3	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

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				Examiner Name	Hines
Sheet	4	of	5	Attorney Docket Number	UCHI-34458/US-3/ORD

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(54) Microbiota composition, as a marker of responsiveness to chemotherapy, and use of microbial modulators (pre-,pro- or synbiotics) for improving the efficacy of a cancer treatment

(57) The present invention provides methods for determining if a patient is likely to benefit from a cancer treatment, by determining if said patient has a gut dysbiosis with an over representation of certain bacterial species. The present invention also provides probiotic strains to improve the efficacy of a cancer treatment, especially chemotherapy, in patients in need thereof.

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to the field of anticancer treatment. In particular, the present invention concerns the role of the microbiota in the efficacy of cancer treatments, and provides methods for determining if a patient is likely to benefit from a cancer treatment, as well as probiotics to improve the efficacy of such a treatment in patients in need thereof.

BACKGROUND AND PRIOR ART

[0002] Conventional cancer treatments involve a combination of chemotherapy, surgery, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient. Cancer chemotherapy is based on the use of drugs which kill replicating cells, hopefully faster than the agents kill the patient's normal cells. Surgery is used to reduce tumor bulk, but has little impact once the cancer has metastasized. Radiation is effective only in a localized area. All of these approaches pose significant drawbacks and added risks such as increased susceptibility to infection.

[0003] A further approach to cancer therapy is to target the immune system ("immunotherapy") rather than and/or in addition to targeting the tumor itself.

[0004] However, despite advances in detection and treatment, many therapeutic protocols make only a minor contribution to survival rates, raising into question the cost-effectiveness and impact on quality of life of such treatments.

[0005] Recently, the important contribution of the innate and adaptive immune systems to the antitumour effects of conventional chemotherapy-based and radiotherapy-based cancer treatments has been described (Kroemer et al., ; Zitvogel et al., 2008).

[0006] It is now well established that gut commensal bacteria profoundly shape mammalian immunity (Hooper et al.). Intestinal dysbiosis, which constitutes a disequilibrium in the bacterial ecosystem, can lead to overrepresentation of some bacteria able to promote colon carcinogenesis by favoring chronic inflammation or local immunosuppression (Grivennikov et al., ; Wu et al., 2009). However, the effects of microbial dysbiosis on non-gastrointestinal cancers are unknown.

[0007] Anticancer chemotherapeutics often cause mucositis (a debilitating mucosal barrier injury associated with bacterial translocation) and neutropenia, two complications that require treatment with antibiotics, which in turn can result in dysbiosis (Ubeda et al., ; van Vliet et al.).

[0008] There is therefore a compelling need for the development of improved treatments for cancer which favor a constructive interaction, if not a synergy, between treatments such as chemotherapy and/or radiation and immunity.

SUMMARY OF THE INVENTION

[0009] In this context, the inventors observed that cyclophosphamide (CTX) alters the composition of small intestinal microbiota in mice and provokes the translocation of selected species of Gram+ bacteria into secondary lymphoid organs. There, these bacteria stimulate the generation of a specific subset of "pathogenic" T helper 17 (pTh17) cells and memory Th1 immune responses. The inventors also demonstrated that germ-free mice or hosts treated with antibiotics killing Gram+ bacteria exhibited reduced pTh17 responses and relative chemoresistance to CTX unless adoptively transferred with pTh17 cells. Moreover, dysbiosis interfered with the activity of other anticancer chemotherapeutics (such as anthracyclines and oxaliplatine) and even other anticancer treatments such as anti-CTLA4 antibodies. These results reveal a crucial role of the gut microbiota in shaping the anticancer immune response.

[0010] The present invention hence provides a method for *in vitro* determining whether a cancer patient can benefit from an antineoplastic treatment, comprising the following steps:

(i) from an appropriate biological sample from said patient, for example obtained from a biopsy of duodenum or ileum mucosae, or from a fecal sample from the patient, determining the relative abundance of "unfavorable" bacteria in the specific context of cancer treatment, for example bacteria from a group comprising or consisting of the species *Parabacteroides distasonis and Faecalibacterium prausnitzii* and the genera *Gemmiger, Alistipes* and *Clostridium* cluster IV in said patient's gut microbiota;
(ii) determining the presence or absence of an intestinal dysbiosis;

wherein an intestinal dysbiosis with an over-representation of "unfavorable" bacteria indicates that the patient will not be a good responder to the antineoplastic treatment.

[0011] The present invention also provides a method for *in vitro* determining whether an antineoplastic treatment is to be continued or stopped for a cancer patient, comprising the following steps:

(i) from a biological sample from said patient, such as a blood sample obtained 3 to 9 weeks, preferably 6-9 weeks after the beginning of said antineoplastic treatment, analyzing memory $CD4^+$ T cell response directed against at least one commensal species of bacteria, for example against *L. johnsonii, E. hirae* and/or *E. Faecalis;*
(ii) for each commensal species against which the $CD4^+$ T cell response is analyzed, classifying the response in one of the following categories:

- no memory $CD4^+$ T cell response;
- memory response of a Th 10 phenotype;
- memory response of a Th1 phenotype,

wherein if a memory response of a Th1 phenotype is observed for at least one commensal species, the antineoplastic treatment is continued, and in absence of such a response, the antineoplastic treatment is stopped.

[0012] The classification of the response can be performed, for example, by comparing pre- and post-treatment secretion of cytokines in *ex vivo* restimulation assays.

[0013] The present invention also pertains to a method for *in vitro* determining the biological effects of a neoadjuvant antineoplastic treatment which has been administered to a patient, comprising the following steps:

(i) from an appropriate biological sample from said patient, for example obtained from a biopsy of duodenum or ileum mucosae from the patient, determining the relative abundance of bacteria from a first group comprising *Lactobacillus* and *Bifidobacterium* genera in said microbiota;
(ii) from the same biological sample, determining the relative abundance of bacteria from a second group comprising *Parabacteroides distasonis, Faecalibacterium prausnitzii, Gemmiger, Alistipes* and *Clostridium* cluster IV in said gut microbiota;
(iii) calculating the ratio between the abundance of bacteria from the first group and the abundance of bacteria from the second group,

wherein if said ratio is above a predetermined threshold, the result indicates that the neoadjuvant antineoplastic treatment induced a T-bet/Th1 local and systemic immune response.

[0014] Another object of the present invention is a probiotic bacterial strain selected from the group consisting of *Lactobacillus johnsonii* (especially strain CNCM I-4823), *Enterococcus hirae* (especially strain CNCM I-4815) and *Enterococcus faecalis,* for use in combination with an antineoplastic agent for inducing a T-bet/Th1 local and systemic immune response, as well as a composition comprising the same.

[0015] The invention also pertains to adoptive cell transfer of a cell obtained by stimulating naive CD4+ T cells from a cancer patient in the presence of a mixture of IL-1β, IL-6 and IL23, in said cancer patient, in combination with an antineoplastic treatment, for treating cancer.

LEGENDS TO THE FIGURES

[0016]

Figure 1: Cyclophosphamide disrupts gut mucosal integrity.

(**A-B**). Hematoxilin-eosin staining of the small intestine epithelium at 48h post-NaCl (Co) or CTX or doxorubicin (Doxo) therapy in C57BL/6 naive mice (A). The numbers of inflammatory foci depicted/mm (B, left panel, indicated with arrowhead on A), thickness of the lamina propria reflecting edema (B, middle panel, indicated with # on A) and the reduced length of villi (B, right panel, indicated with arrowhead in A) were measured in 5 ilea on 100 villi/ileum from CTX or Doxo -treated mice. (**C**). A representative microphotograph of an ileal villus containing typical mucincontaining goblet cells is shown in vehicle- and CTX or Doxo-treated mice (left panels). The number of goblet cells/villus was enumerated in the right panel for both chemotherapy agents. (**D**). Specific staining of Paneth cells is shown in two representative immunofluorescence microphotographs (D, left panels). The quantification of Paneth cells was performed measuring the average area of the lysozyme-positive clusters in 6 ilea harvested from mice treated with NaCl (Co) or CTX at 24-48 hours. (**E**). Quantitative PCR (qPCR) analyses of Lysozyme M and RegIIIγ transcription levels in duodenum and ileum lamina propria cells from mice treated with CTX at 18 hours. Means±SEM of normalized deltaCT of 3-4 mice/group concatenated from three independent experiments. (**F**). *In vivo* intestinal permeability assays measuring 4 kDa fluorescein isothiocyanate (FITC)-dextran plasma accumulation at 18 hours post-CTX at two doses. Graph showing all data from four independent experiments, each dot representing one mouse (n=13-15). Data were analyzed with the t-test. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Figure 2: Cyclophosphamide induces mucosa-associated microbial dysbiosis and bacterial translocation in secondary lymphoid organs.

(A-B). At 48 hours post-CTX or Doxo, mesenteric lymph node (mLN) and spleen cells from naive mice were cultivated in aerobic and anaerobic conditions and colonies were enumerated (A) from each mouse treated with NaCl (Co) (n=10-16), CTX (n=12-27) or Doxo (n=3-17) (3-4 experiments) and identified by mass spectrometry (B). In NaCl controls, attempts of bacterial identification mostly failed and yielded 67% *Lactobacillus. murinus* (not shown). Data were analyzed with the t-test. (C). The microbial composition (genus level) was analyzed by 454 pyrosequencing of the 16S rRNA gene from ilea and caeca of naive mice and B16F10 tumor bearers. Principal Component Analyses (PCA) highlighted specific clustering of mice microbiota (each dot represents one mouse) depending on the treatment (NaCl: Co, grey dots; CTX-treated, black dots). A Monte Carlo rank test was applied to assess the significance of these clusterings. (**D**). Quantitative PCR (qPCR) analyses of various bacterial groups associated with small intestine mucosa were performed on CTX or NaCl (Co)-treated, naïve or MCA205 tumor-bearing mice. Absolute values were calculated for total bacteria, *Lactobacilli, Enterococci* and *Clostridium* group IV and normalized by the dilution and weight of the sample. Standard curves were generated from serial dilutions of a known concentration of genomic DNA from each bacterial group and by plotting threshold cycles (Ct) vs. bacterial quantity (CFU). Points below the dotted lines were under the detection threshold. Data were analyzed with the linear model or generalized linear model. *, $p<0.5$, **, $p<0.1$, ***, $p<0.001$, ns, non significant.

Figure 3: CTX-induced pTh17 effectors and memory Th1 responses depend on gut microbiota.

(A). Splenocytes from CTX *versus* NaCl treated animals reared in germ-free (GF) or conventional specific pathogen-free (SPF) conditions (left panel) and treated or not with ATB or vancomycin (Vanco) (right panel) were cross-linked using anti-CD3+anti-CD28 Ab for 48h. IL-17 was measured by ELISA. Two to 3 experiments containing 2-9 mice/group are presented, each dot representing one mouse. (**B**). Correlations between the quantity of specific mucosal bacterial groups and the spleen Th17 signature. Each dot represents one mouse bearing no tumor (round dots), a B16F10 melanoma (diamond dots) or a MCA205 sarcoma (square dots), open dots featuring NaCl-treated mice and full dots indicating CTX-treated animals. (**C**). Intracellular analyses of splenocytes harvested from non-tumor-bearing mice after 7 days of either NaCl or CTX treatment, under ATB or water regimen as control. Means±SEM of percentages of $IFN\gamma^+$ Th17 cells, T-bet^+ cells among $ROR\gamma t^+$ $CD4^+$ T cells and $CXCR3^+$ cells among $CCR6^+CD4^+$ T cells in 2 - 8 independent experiments, each dot representing one mouse. (**D**) Intracellular staining of total splenocytes harvested 7 days post-CTX treatment from naive mice orally-reconstituted with the indicated bacterial species after ATB treatment. (**E**). 7 days post CTX or NaCl (Co) treatment, splenic $CD4^+$ T cells were restimulated *ex vivo* with bone-marrow dendritic cells (BM-DCs) loaded with decreasing amounts of bacteria for 24 hours. $IFN\gamma$ release, monitored by ELISA, is shown. The numbers of responder mice (based on the NaCl baseline threshold) out of the total number of mice tested is indicated (n). Statistical comparisons were based on the paired t-test. Data were either analyzed with beta regression or linear model and correlation analyses from modified Kendall tau. *, $p<0.05$, ***, $p<0.001$, ns, non significant.

Figure 4: Vancomycin blunts CTX-induced pTh17 differentiation which is mandatory for the tumoricidal activity of chemotherapy.

(**A**). After a 3 week-long pretreatment with broad-spectrum ATB, DBA2 mice were inoculated with P815 mastocytomas (day 0), treated at day 6 with CTX (arrow) and tumor growth was monitored. Tumor growth kinetics are shown in Fig. 13A and percentages of tumor-free mice at sacrifice are depicted for two experiments of 11-14 mice/group. (**B**). MCA205 sarcoma were inoculated at day 0 in specific pathogen-free (SPF) or germ-free (GF) mice that were optionally mono-associated with segmented filamentous bacteria (SFB), treated with CTX (arrow) and monitored for growth kinetics (means±SEM). One representative experiment (n=5-8 mice/group) out of two to three is shown for GF mice and two pooled experiments (n=14 mice/group) for SPF mice (**C**). After a 3 week- conditioning with vancomycin or colistin, C57BL/6 mice were inoculated with MCA205 sarcomas (day 0), treated at day 12-15 with CTX (arrow) and tumor growth was monitored. Concatenated data (n=15-20 mice/group) from two independent experiments are shown for colistin treatment and one representative experiment (n=6 mice/group) for vancomycin treatment. (D). Eight week-old **KP** (*KrasLSL*-G12D/WT; $p53^{Flox/Flox}$) mice received an adenovirus expressing the Cre recombinase (AdCre) by intranasal instillation to initiate lung adenocarcinoma (d0). Vancomycin was started for a subgroup of mice ("Chemo + Vanco") on d77 post-AdCre. One week after the start of vancomycin, CTX-based chemotherapy was applied i.p. to mice that only received chemotherapy ("Chemo") or those that received in parallel vancomycin ("Chemo + Vanco"). Mice received

chemotherapy on d84, d91 and d98. A control group was left untreated ("Co"). Data show the evolution of total lung tumor volumes (mean±SEM) assessed by non invasive imaging between d73 and d100 in 6-12 mice/group. (**E**). As in Fig. 3C, the number of pTh17 cells in spleens from untreated or vancomycin treated mice bearing established (15-17 days) MCA205 tumors was determined, 7 days after CTX treatment. Each dot represents one mouse from 2 pooled experiments. (**F**). Flow cytometric analyses of $CD3^+$ and $CD4^+IFN\gamma^+$ T cells were performed by gating on $CD45^+$ live tumor-infiltrating lymphocytes (TILs) extracted from day 18 established MCA205 tumors (8 days post-CTX) in water or vancomycin-treated mice. Each dot representing one mouse from up to four pooled experiments. (**G**). MCA205 tumors established in WT mice pretreated for 3 weeks with water or vancomycin were injected with CTX (arrow), and tumor growth was monitored. At day 7 post-CTX, 3 million *of ex vivo* generated Th17 or pTh17 $CD4^+$ T cells were injected intravenously. Up to three experiments comprising 2-10 mice/group were pooled. Data were either analyzed with the t-test, linear model or generalized linear model. *, $p<0.5$, **, $p<0.1$, ***, $p<0.001$, ns, non significant.

Figure 5: Lack of dysbiosis 24 or 48 h post-CTX.

(**A**). Overall composition of the gut microbiota as assessed by high-throughput 454 pyrosequencing of the 16S rRNA gene at various time points (0, 24, 48 hours post-CTX). Each column represents data from one mouse small intestine mucosal microbiota, t0 (before CTX injection), t24 and t48 (24 and 48 hours post-CTX). The positive gradient of representativity of distinct genera (heatmap of the Log_{10}-tranfonnation) is indicated. Statistical analyses: ns between t0 and t24 or t48 hrs. (**B-C**). Detailed example of the pyrosequencing data for *Clastridium* sp. clone 40 and for *L. reuteri*. **qPCR** analysis of *Lactobacilli* amounts overtime as detailed in Materials and Methods.

Figure 6: Distribution of bacterial genera in the ileum of mice treated with CTX. Heatmap of the Log_{10}-transformation of relative abundance of genus in the small intestine from NaCl (Co) and CTX-treated animals. Prior to CTX therapy, tumors were inoculated in a subgroup of animals (TB+). Only bacterial genera representing more than 0.05% of the whole microbiota are presented. The applied log_{10}-transformation on relative abundances data has been explained in the microbiota Materials and Methods section. No specific clustering method has been applied for heatmap construction. Average delta of percentages between Co and CTX for each genus was calculated to re-order bacterial genera.

CTX induced a reduction of bacterial groups from the *Firmicutes* phylum distributed within four genera and groups (*Clostridium cluster XIVa, Roseburia, unclassified Lachnospiraceae, Coprococcus*, Table 2) in the mucosa of CTX-treated animals. CTX was also associated with a reduction in the proportion of *Spirochaetes* phylum ($p=0.016$), in particular the *Treponema* genus (0.025% in NaCl vs 0% in CTX group; $p=0.016$). At the level of species, some bacteria were either overrepresented (such as *Lactobacillus reuteri*) or underrepresented (such as *Clostridium sp.* clone 40 and several other butyrate-producers from the *Lachnospiraceae* family and from the *Clostridium* cluster XIVa) post-CTX (Fig. 2B). Segmented filamentous bacterium X77814 (SFB) did not reveal a consistent enrichment in CTX-treated mice compared with controls (7.95% SFB in CTX versus 0.83% in vehicle controls, $p=0.08$, Table 2). Wilcoxon test: *, $p<0.05$ in Co versus CTX in TB^- groups, ‡, $p<0.05$ in Co versus CTX in TB^+ groups.

Figure 7: Loss of $CD103^+CD11b^+$ and Th17 cells in the duodenal lamina propria and Th1 polarization of splenocytes correlating with small intestine bacterial species.

(A). *Dendritic cell (DC) subsets in LP of the small intestine.* Flow cytometry analyses and quantification of various DC subsets residing in small and large intestine LP at day 0, day 3 and day 7 post-CTX injection. The graph depicts means+SEM of the percentages of DC in 7 mice/time point in two concatenated experiments. Large intestine DC subsets were not affected by CTX (not shown). Data were analyzed with the Mann Whitney t-test. (B-C). *Modulations of Th17 cells seven days post-CTX.* (B) Flow cytometry analyses of lymphocytes separated from the LP of duodenum and ileum, harvested from NaCl versus CTX-treated mice. The graphs depict the concatenated data from eight independent experiments, each dot representing one experiment. Statistical comparisons were based on the Wilcoxon test. (C). Left panel: a micrograph picture of immunofluorescence staining of ileum in NaCl versus CTX-treated mice. $\gamma\delta$ TCR^+ cells were stained in green (Alexa Fluor 488) using an anti-$\gamma\delta$□ TCR Ab and $CD3^+$ T cells were stained in blue (Alexa Fluor 647) using anti-CD3 Ab. Right panel: the enumeration of positive cells was performed on 100 villi in three ilea by two independent researchers. (**D**). *Th1 polarization of splenocytes at day 7 post-CTX injection.* Splenocytes from CTX versus NaCl treated animals reared in GF or conventional SPF conditions (left panel) and treated or not with ATB or vancomycin (right panel) were cross-linked using anti-CD3±anti-CD28 Abs for 48h. The levels of IFNγ were monitored in 48 hour-supernatants by ELISA. Three experiments containing 2-9 mice/group are presented, each dot representing one

mouse. Data from (C) and (D) were analyzed with the t-test. (**E**). Idem as in Fig. 3C but correlations were analyzed between each bacterial group and IFNγ □ secretory profile as shown Fig. 6 (C) in naive, B16F10 or MCA205 tumor bearers. (**F**). Representative dot plot flow cytometry analysis of splenic pTh17 cells as enumerated in Fig. 3C at day 7 post-CTX. *, p<0.05, **, p<0.01, ns, not significant.

Figure 8: Doxorubicin failed to induce pTh17 cells in the spleen and does not require gut commensals for reducing tumor growth.

(**A-B**). Failure of doxorubicin (Doxo) to induce splenic IL-17 producing $CD4^+$ T cells. Doxo was injected i.p. into mice at the indicated doses (A) or at a fixed dose of 50 µl at 2 mM (being 3 mg/kg for a mouse weighing 20g) (B), and splenocytes were recovered 7 days later to evaluate the production of IL-17 in response to 48 hours anti-CD3/anti-CD28 cross-linking (A, B) or the frequency of cells with a $CD4^+$T-$bet^+ROR\gamma t^+$ phenotype was determined by flow cytometry (B). Cyclophosphamide (CTX) used at a dose of 100 mg/kg was used as a positive control. Optionally, regulatory T cells were depleted by injections (250 µg, 1 and 3 days before Doxo administration) of anti-CD25 Ab and an irrelevant isotype-matched control Ab was used as control. (**C**). Antitumor effects of doxorubicin against established MCA205 in specific pathogen-free (SPF), antibiotic (ATB)-treated and germ-free mice. Kinetics of tumor growth (mean size±SEM) are depicted in 2 to 3 pooled experiments including 4-6 animals/group. Data were analyzed with the t-test, linear model or generalized linear model. *, p<0.05, **, p<0.01, ***, p<0.001, ns, not significant.

Figure 9: Efficacy of broad spectrum ATB in bacterial depletion from the feces of naïve or tumor-bearing mice. Feces were freshly harvested from mice that were left untreated or were treated with broad spectrum ATB at various time points and plated onto blood agar plates for aerobic and anaerobic conditions, as well as onto DCO agar plates (BioMérieux) for the specific growth of enterococci. After 48h of culture, isolated colonies were enumerated. All the mice of each distinct experiment have been monitored and scored in this manner. One representative monitoring is shown.

Figure 10: CTX-induced pTh17 differentiation depends on Myd88 but not Nod1/2.

(**A**). Flow cytometry analyses of lymphocytes harvested from NaCl versus CTX-treated WT (as in Fig. 3C) or *Nod2*$^{-/-}$ versus *Myd88*$^{-/-}$ mice restimulated 4 hours with PMA/ionomycin (using intra-and extra-cellular stainings with anti- CD3, CD8, IFNγ and IL-17 Abs). The graph depicts the mean percentages of $IFN\gamma^+$ positive cells among $IL\text{-}17^+CD4^+$ T cells from two independent experiments, each dot representing one mouse. (**B**). *Nod1 and Nod2 are dispensable for tumor growth reduction induced by CTX.* MCA205 tumors were established in WT or *Nod1*$^{-/-}$*Nod2*$^{-/-}$ mice before administration, at day 5 and 12, of CTX. The tumor growth kinetics (means±SEM) were monitored in 5 animals/group. Two independent experiments yielded similar results. Data were analyzed with the t-test, linear model or generalized linear model. ***, p<0.001, ns, not significant

Figure 11: Immunization against commensal bacteria post-CTX.

(**A-C**). *Recovery of CBir Tg T cells in congenic mice after CTX.* One million naïve B6.$CD45.1^+$ CBir1 TCR Tg $CD4^+$ T cells were adoptively transferred i.v. in naive CD45.2 WT recipient congenic mice that were treated, one day later, with NaCl or CTX and sacrificed 7 days later for FACS analysis of splenocytes and *ex vivo* restimulation with CBir1 specific peptides. Gating of CD45.1 cells allowed to analyze the percentages of recovery or proliferation of CBir1 Tg T cells (A, means±SEM for 5 animals) and to analyze IL-17 and IFNγ production using intracellular staining after 6h PMA/ionomycin activation. A representative dot plot is shown for one animal in B. Splenocytes were restimulated for 24h with the CBir1 specific peptide or a control irrelevant peptide. Commercial ELISA monitored the concentrations of IFNγ in the supernatants (C). Three experiments were performed encompassing 4-5 animals/group. Mann Whitney t-test: **, p<0.01.

Figure 12: Translocated bacteria processed and presented by dendritic cells lead to the polarization of naïve $CD4^+$T cells *in vitro*. *Ex vivo differentiation of Th17/Th1 cells with translocated bacteria.* Cross-talk between BMDCs loaded with various bacteria and naïve $CD4^+$T for 4 days. Monitoring of IL-17 (left) or IFNγ (right) cytokine concentrations by commercial ELISA. Each dot represents one *in vitro* experiment performed in triplicate wells. Eleven experiments were performed and are depicted. t-test: *, p<0.5, **, p<0.01, ns, non significant.

Figure 13: Gut microbiota affects chemotherapy efficacy.

(**A**). *Bacterial depletion by ATB reduced chemosensitivity of established mastocytomas.* Day 6 P815 bearing DBA2 mice pretreated or not for 3 weeks with broad spectrum ATB were inoculated i.p. with 100 mg/kg of CTX and tumor growth was monitored until sacrifice. Growth kinetics are shown for each individual mouse in water versus ATB- treated mice in a representative experiment out of three. (**B**). *Vancomycin reduced the efficacy of CTX against MCA205 sarcomas.* Day 10 MCA205- bearing C57BL/6 mice pretreated or not for 3 weeks with vancomycin were inoculated i.p. with 100 mg/kg of CTX and tumor surfaces as well as tumor rejection rates were monitored over one month. Growth kinetics are shown for each individual mouse in water versus vancomycin- treated mice in a representative experiment out of two while the percentages of tumor free mice are indicated in parentheses.

Figure 14: *Parabacteroides distasonis* and chemoresistance.

(**A**). *Manoassociation with Parabacteroides distasonis induced chemoresistance of established sarcomas.* Conventionally reared mice were treated for 2 weeks with broad spectrum antibiotics (ATB), inoculated with MCA205 for 7 days and then treated with doxorubicin. In this particular experiment, feces were contaminated by one single bacterial species identified as *P. distasonis* by means of VITEK® automated system and MALDI-TOF. Tumor growth kinetics (means±SEM) revealed that ATB combined with *P. distasonis* contamination induced a cancer chemoresistance status *in vivo* (n=4-5 mice/group) (**B**). Conventionally reared mice were treated for 3-4 weeks with ATB, implanted 4 days with MCA205 and then orally inoculated with *P. distasonis* that monocolonized feces. At day 6 post-tumor inoculation, mice were treated with doxorubicin. The tumor growth kinetics between *P. distasonis* reconstituted or unreconstituted ATB treated-mice post-doxorubicin (means±SEM) were monitored in 8-12 mice/group. Data were analyzed with the linear model or generalized linear model. *p<0.05, ***p<0.001.

Figure 15: Transcriprional profiling of *ex vivo* generated Th17 and pTh17 compared with CTX-induced spleen CD4$^+$ T cells.
Naive T cells were stimulated with plate-bound antibodies against anti-CD3 and anti-CD28 Abs in the absence (Th0) or presence of either recombinant mouse IL-1β (10ng/ml)+ IL-6 (10ng/ml)+ IL-23 (20ng/ml) (as for "pTh17"cells) or with rTGF- β (2.5 ng/ml)+IL-6 (as for "Th17" cells). The transcriptional profile *of in vitro* generated pTh17, Th17 cells (**A**) as well as *ex vivo* harvested splenic derived **CD4**$^+$ T cells post-NaCl or CTX (**B**) is shown. Quantitative RT-PCR were performed with specific probes detecting transcription factors and cytokines defining Th1 versus Th17 polarization.

Figure 16: Vancomycin-resistant microbial microbiota.
Fecal commensals from tumor bearers that were left untreated or were treated with vancomycin were plated, enumerated and identified as specified in Materials and Methods to analyze the number of resistant colonies. The results of two independent experiments run in two different animal facilities (CGFL, Dijon versus IGR, Villejuif) are depicted.

Figure 17. Antibiotics affect the chemotherapy-induced accumulation of γδT17 tumor infiltrating lymphocytes. MCA205 sarcomas were treated with chemotherapy at day 10 and harvested at day 18 for flow cytometry phenotyping of tumor-infiltrating γγT cells producing IL-17. The percentages of TCRγδ$^+$IL-17$^+$ among CD45$^+$ live leukocytes are depicted in each group treated or not with vancomycin or broad spectrum ATB. Each group contained 6-21 mice. Student t' test: **, p<0.01, ***, p<0.001.

Figure 18: Primary cellular Th1 and Tc1 immune responses against chicken OVA are not affected by antibiotics regimen in wild type naïve mice. C57B1/6 mice were pre-treated for 8-10 days with various antibiotic regimens, including large spectrum antibiotics (ATB), colistin (Coli) or vancomycin (Vanco), monitored by culturing feces at various time points and then, immunized in the footpad with 1 mg of OVA admixed with 50 μg of Poly (I:C) three days after i.p. CTX administration. At day 5 post-vaccine, popliteal and inguinal draining lymph nodes were harvested and restimulated with OVA protein (left panel) or SIINFEKL peptides (right panel) at 1 mg and 10 μg/ml respectively. IFNγ release was monitored at 72 hours in the supernatants by ELISA. Each dot represents one mouse, and the means of triplicate wells of *in vitro* restimulation. The statistical analyses have been performed in a paired t test comparing with versus without antigen restimulation to capture Ag specific effector /memory responses. **, p<0.01, ***, p<0.001, ns: not significant.

Figure 19: CTLA4 blockade mediates antitumor effects and pTh17 splenic responses that can be modulated by antibiotics.

A. MCA205 tumors were established for 10 days in WT C57BL/6 mice and treated with three systemic i.p. administrations of 9D9 Ab injected every other 3 days. (A, left panel). Effects of broad spectrum sterilizing antibiotics (ATB; given for 3 weeks before 9D9 injections) in MCA205 bearing WT mice treated with 9D9 Ab (A, right panel). **B.** Idem as in A. but mice were continuously treated for 3 weeks with imipenem. **C.** CTLA4 blockade elicited pTh17 cells in the spleen after 3 injections that could be further boosted by anti-IL-10R neutralizing antibodies. Each graph depicts one representative experiment containing 5 mice/group (mean tumor size ± SEM) out of two yielding similar results. Student t' test or Anova: *, $p<0.05$, **, $p<0.01$, ns: not significant.

Figure 20: Treatment of lung adenocarcinoma-bearing KP mice using chemotherapy. Eight week-old KP (*KrasLSL*-G12D/WT; $p53^{Flox/Flox}$) mice received an adenovirus expressing Cre recombinase (Ad-*cre*) by intranasal instillation to initiate lung adenocarcinoma (d0). Mice were either left untreated («Co») or received chemotherapy (d84, d91 and d98) in absence (« Chemo ») or presence of 0.25mg/ml vancomycin (« Chemo + Vanco ») (mixed into drinking water starting on d77 post *Ad-cre* and until the end of the experiment; antibiotic-containing water was replaced biweekly). **A.** Tumor volumes were quantified on d73 and 100 (equivalent of 'pre' and 'post' chemotherapy) in anesthetized mice by nonivasive imaging as described before *(Cortez-Retamozo et al.,* Immunity, 2012). Data show absolute changes in total lung tumor volumes (mean ± SEM) between the two time points. Of note, antibiotics had no impact on the natural progression of this disease (not shown). **B.** The CD8/Treg ratio was determined by flow cytometry measurements on dissociated lung tissue samples derived from the respective groups. A group of tumor free mice (« No tumor ») was also investigated. $n \geq 6$ mice for each group; *, $p<0.05$, **, $p<0.01$ (two-tailed unpaired t test).

Figure 21: CTX-induced Th1 and Th10 immune responses directed against commensal bacteria in cancer patients.
Ex vivo restimulation assays using patients' autologous monocytes loaded with defined bacteria for 3 hours, neutralized with antibiotics, then cultured in GM-CSF+IL-4 (to differentiate into DC) and incubated for 3 days with $CD4^+CD45RO^+$ T cells (at a 1:2 ratio) purified from autologous blood at various time points (Day 0: before CTX, Day 12-46: after CTX, NSCLC: non small cell lung cancer). **A.** Cytokine release (IFNγ, TNF, IL-10) was monitored using ELISA. Numbers (A, left panel) and percentages (A, right panel) of patients exhibiting at least a 2 fold increase of IFNγ secretion between the pre-and post-CTX time points. **B.** Exemplification of 3 cases with a developing Th1 immune response; patient 3 developing a strong Th1 immunity elicited against *L. johnsonii* + *E. hirae.* **C.** One case with a strong Th1 immunity against *E. faecalis.* **D.** Two cases with a contrasting Th1/TH10 specific responses. B-D show the cytokine levels in the 40h supernatants of 250.000 memory $CD4^+$ T cells for each individual patient pre- and post-CTX administration.

Figure 22: Principal component analysis on the isolate levels of all patients' ileum after 16SrRNA pyrosequencing, comparing controls (no chemotherapy) versus post-neoadjuvant chemotherapy.

Figure 23: Random forest analysis : Main discrininative genera between patients receiving or not chemotherapy and bearing a colon cancer.
Analysis from 6 patients in neoadjuvant oxaliplatine-based chemotherapy and 7 patients prior to therapy.

Figure 24: Main genera that are significantly different between controls (no chemotherapy) versus post-neoadjuvant chemotherapy.

Figure 25: Distribution of lactobacilli, Bifidobacterium and Clostridium group IV. In the ileum among colon cancer patients treated or not with chemotherapy.

Figure 26: Scanning Electron Microscope images of *Lactobacillus johnsonii (A) and Enterococcus hirae (B)*

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0017] In the present text, the following general definitions are used:

Gut microbiota

[0018] The "gut microbiota" (formerly called gut flora or microflora) designates the population of microorganisms living in the intestine of any organism belonging to the animal kingdom (human, animal, insect, *etc.*). While each individual has a unique microbiota composition (60 to 80 bacterial species are shared by more than 50% of a sampled population

on a total of 400-500 different bacterial species/individual), it always fulfils similar main physiological functions and has a direct impact on the individual's health:

- it contributes to the digestion of certain foods that the stomach and small intestine are not able to digest (mainly non-digestible fibers);
- it contributes to the production of some vitamins (B and K);
- it protects against aggressions from other microorganisms, maintaining the integrity of the intestinal mucosa;
- it plays an important role in the development of a proper immune system;
- a healthy, diverse and balanced gut microbiota is key to ensuring proper intestinal functioning.

[0019] Taking into account the major role gut microbiota plays in the normal functioning of the body and the different functions it accomplishes, it is nowadays considered as an "organ". However, it is an "acquired" organ, as babies are born sterile; that is, intestine colonisation starts right after birth and evolves afterwards.

[0020] The development of gut microbiota starts at birth. Sterile inside the uterus, the newborn's digestive tract is quickly colonized by microorganisms from the mother (vaginal, skin, breast, *etc.*), the environment in which the delivery takes place, the air, *etc.* From the third day, the composition of the intestinal microbiota is directly dependent on how the infant is fed: breastfed babies' gut microbiota, for example, is mainly dominated by *Bifidobacteria,* compared to babies nourished with infant formulas.

[0021] The composition of the gut microbiota evolves throughout the entire life, from birth to old age, and is the result of different environmental influences. Gut microbiota's balance can be affected during the ageing process and, consequently, the elderly have substantially different microbiota than younger adults.

[0022] While the general composition of the dominant intestinal microbiota is similar in most healthy people (4 main phyla, *i.e.,* Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria), composition at a species level is highly personalised and largely determined by the individuals' genetic, environment and diet. The composition of gut microbiota may become accustomed to dietary components, either temporarily or permanently. Japanese people, for example, can digest seaweeds (part of their daily diet) thanks to specific enzymes that their microbiota has acquired from marine bacteria.

Dysbiosis

[0023] Although it can adapt to change and has a high resilience capacity, a loss of balance in gut microbiota composition may arise in some specific situations. This is called "dysbiosis", a disequilibrium between potentially "detrimental" and known "beneficial" bacteria in the gut or any deviation to what is considered a "healthy" microbiota in terms of main bacterial groups composition and diversity. Dysbiosis may be linked to health problems such as functional bowel disorders, inflammatory bowel diseases, allergies, obesity and diabetes. It can also be the consequence of a treatment, such as a cytotoxic treatment or an antibiotic treatment.

[0024] A specific dysbiosis can be highlighted depending on the pathogenic condition. For instance, patients with Crohn's disease, a chronic inflammatory bowel disease, present a microbiota with reduced percentages and diversity of bacteria belonging to the Finnicutes phylum, and mostly from the *Clostridium leptum* (cluster IV) group (Manichanh et al., 2006; Sokol et al., 2006).Generally, decreased percentages of bacteria from the Lachnospiraceae family can be observed. Moreover mucosa-associated microbiota of these patients is depleted in bacteria from the *Bifidobacterium* and *Lactobacillus* genera toward increased levels of potentially pathogenic bacteria such as specific strains of *Escherichia coli* with adherent and invasive phenotypes (AIEC) (Darfeuille-Michaud et al., 2004; Joossens et al.).

[0025] To the contrary, patients with obesity and metabolic disorders have higher proportions of bacteria belonging to the Firmicutes phylum and lower levels of *Escherichia coli* in their feces (Ley et al., 2005; Turnbaugh et al., 2009). An increased in proportions of *E. coli* in these patients has been associated with weight loss following bariatric surgery and lower levels of serum leptin (Furet et al.).

[0026] In patients with colorectal cancer (CRC), however, gut microbial dysbiosis relates to enrichment in bacterial species from the *Bacteroides* genus and decrease of *Faecalibacterium* and *Roseburia* genera belonging species (Sobhani et al., ; Wu et al.). Specifically, *Fusobacterium* and *Campylobacter genera* were found to be consistently increased in both feces and mucosa of CRC patients.

[0027] In the context of cancer, "beneficial or "favorable" bacteria are essentially *Lactobacillus* and *Bifidobacterium,* and "detrimental" or "unfavorable" bacteria are essentially the species *Parabacteroides distasonis* and *Faecalibacterium prausnitzii,* the genera *Gemmiger, Alistipes* and *Clostridium Cluster IV. (Clostridium leptum* group).

Antineoplastic treatments

[0028] "Antineoplastic treatments" herein designate any treatment for cancer except surgery. They include chemo-

therapy, hormonal and biological therapies, and radiotherapy.

Chemotherapy

[0029] "Chemotherapy" is defined herein as the treatment of cancer with one or more "chemotherapeutic agents". Chemotherapeutic agents are chemical molecules which act by killing cells that divide rapidly, one of the main properties of most cancer cells. Several categories of chemical agents exist:

- alkylating agents (further defined below);
- spindle poisons such as mebendazole, colchicine;
- mitotic inhibitors (including taxanes (paclitaxel (Taxol®), docetaxel (Taxotère®)) and vinca alkaloids (*e.g.*: vincristine, vinblastine, vinorelbine, vindesine)),
- cytotoxic/antitumor antibiotics: such as anthracyclines (*e.g.*:

  doxorubicin, daunorubicin, adriamycine, idarubicin, epirubicin and mitoxantrone, valrubicin), streptomyces (*e.g.*: actinomycin, bleomycin, mitomycin, plicamycin)

- anti-metabolites (such as pyrimidine analogues *(e.g.*:

  fluoropyrimidines analogs, 5-fluorouracil (5-FU), floxuridine (FUDR), Cytosine arabinoside (Cytarabine), Gemcitabine (Gemzar®), capecitabine; purine analogues (*e.g.*: azathioprine, mercaptopurine, thioguanine, fludarabine, pentostatin, cladribine, capecitabine, clofarabine); folic acid analogues (*e.g.*: methotrexate, folic acid, pemetrexed, aminopterin, raltitrexed, trimethoprim, pyrimethamine),

- topoisomerase inhibitors (*e.g.*: camptothecins: irinotecan, topotecan, amsacrine, etoposide, etoposide phosphate, teniposide);
- DNA methyltransferase inhibitors: 2'-deoxy-5-azacytidine (DAC), 5-azacytidine, 5-aza-2'- deoxycytidine, 1 -[beta] -D-arabinofuranosyl-5-azacytosine, dihydro-5-azacytidine;
- vascular disrupting agents, such as flavone acetic acid derivatives, 5,6-dimethylxanthenone-4- acetic acid (DMXAA) and flavone acetic acid (FAA);
- also other chemotherapeutic drugs such as aprepitant, bortezomib (Velcade®, Millenium Pharmaceuticals), imatinib mesylate (Gleevec®), carmustine (BCNU), lomustine (CCNU), tamoxifen, gefitinib, erlotinib, carboxyamidotriazole, efaproxiral, tirapazamine, xcytrin, thymalfasin, vinflunine.

Alkylating agents

[0030] "Alkylating agents" are so named because of their ability to alkylate many molecules, including proteins, RNA and DNA. This ability to bind covalently to DNA via their alkyl group is the primary cause for their anti-cancer effects, since it provokes cell apoptosis. Alkylating agents are cell cycle-independent drugs, and their effects are usually dose dependent.

[0031] The subtypes of alkylating agents are the nitrogen mustards, nitrosoureas, tetrazines, aziridines, and non-classical alkylating agents. Nitrogen mustards include mechlorethamine, cyclophosphamide, melphalan, chlorambucil, ifosfamide and busulfan. Nitrosoureas include N-Nitroso-N-methylurea (MNU), carmustine (BCNU), lomustine (CCNU) and semustine (MeCCNU), fotemustine and streptozotocin. Tetrazines include dacarbazine, mitozolomide and temozolomide. Aziridines include thiotepa, mytomycin and diaziquone (AZQ). Non-classical alkylating agents include procarbazine and hexamethylmelamine.

[0032] Throughout the present application, "alkylating-like agents", which are platinum-based chemotherapeutic drugs (also termed "platinum analogues") and act in a similar manner as alkylating agents, will be included in the category of "alkylating agents". These agents do not have an alkyl group, but nevertheless damage DNA. They permanently coordinate to DNA to interfere with DNA repair. Example of this subcategory of alkylating agents as herein defined are platinum, cisplatin, carboplatin, nedaplatin, oxaliplatin, satraplatin and triplatin tetranitrate.

Biological therapies

[0033] Anti cancer "biological therapies" involve the use of living organisms, substances derived from living organisms, or laboratory-produced versions of such substances to treat cancer, by targeting either the cancer cells directly, or by stimulating the body's immune system to act against cancer cells ("immunotherapy"). Biological therapies include monoclonal antibodies (including those targeting cancer cell surface, e.g. rituximab and alemtuzumab; anti-CTLA4 Mabs,

such as ipilimumab; targeting growth factors, e.g.: bevacizumab, cetuximab, panitumumab and trastuzumab; anti-PD-1 Mabs; anti-Tim3 Mabs; anti-ICOS Mabs), immunoconjugates (*e.g.*: ^{90}Y-ibritumomab tiuxetan, ^{131}I-tositumomab, and ado-trastuzumab emtansine), cytokines (including interferons such as IFNα; interleukins such as IL-2, IL-11, G-CSM, GM-CSF), therapeutic vaccines (*e.g.*: Sipuleucel-T (Provenge®)), the bacterium bacillus Calmette-Guérin, cancer-killing viruses, gene therapy, and adoptive T-cell transfer.

Prebiotics, probiotics and synbiotics

[0034] "Prebiotics" are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health. They usually are selectively fermented ingredients that allow specific changes, both in the composition and/or activity of the gut microbiota.

[0035] "Probiotics" are micro-organisms that have claimed health benefits when consumed. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures, such as in yogurt, soy yogurt, or as dietary supplements. Generally, probiotics help gut microbiota keep (or re-find) its balance, integrity and diversity. The effects of probiotics are usually strain-dependent.

[0036] "Synbiotics" refer to nutritional supplements combining probiotics and prebiotics in a form of synergism, hence synmbiotics. Using prebiotics and probiotics in combination is often described as synbiotic, but the United Nations Food& Agriculture Organization (FAO) recommends that the term "synbiotic" be used only if the net health benefit is synergistic.

Cancer, treatment, etc.

[0037] As used herein, "cancer" means all types of cancers. In particular, the cancers can be solid or non solid cancers. Non limitative examples of cancers are carcinomas or adenocarcinomas such as breast, prostate, ovary, lung, pancreas or colon cancer, sarcomas, lymphomas, melanomas, leukemias, germ cell cancers and blastomas.

[0038] As used herein, the terms "treat", "treatment" and "treating" refer to any reduction or amelioration of the progression, severity, and/or duration of cancer, particularly a solid tumor; for example in a breast cancer, reduction of one or more symptoms thereof that results from the administration of one or more therapies.

[0039] Other definitions will be specified below, when necessary.

[0040] According to a first embodiment, the present invention pertains to a method for *in vitro* determining whether a cancer patient can benefit from an antineoplastic treatment, comprising the following steps:

(i) from an appropriate biological sample from said patient, determining the relative abundance of "unfavorable" bacteria in the specific context of cancer and chemotherapy, for example bacteria from a group comprising or consisting of the species *Parabacteroides distasonis* and *Faecalibacterium prausnitzii,* bacteria from the genera *Gemmiger, Alistipes* and *Clostridium cluster IV* (group *Clostridium leptum;* as described in the taxonomic description of Clostridium bacteria by Collins et al) in said patient's gut microbiota; Optionnally, in the same biological sample, the relative abundance of "favorable" bacteria in the specific context of cancer and chemotherapy, for example bacteria from the genera *Lactobacillus and Bifidobacterium,* is also determined;
(ii) determining the presence or absence of an intestinal dysbiosis;
wherein an intestinal dysbiosis with an over-representation of "unfavorable" bacteria from the taxons recited in step (i) indicates that the patient will not be a good responder to antineoplastic treatment.

[0041] In what precedes, the "relative abundance" is defined as the number of bacteria of a particular taxonomic level (from phylum to species) as a percentage of the total number of bacteria in the biological sample. This relative abundance can be assessed, for example, by measuring the percentage of 16S rRNA gene sequences present in the sample which are assigned to these bacteria. It can be measured by any appropriate technique known by the skilled artisan, such as 454 pyrosequencing and quantitative PCR of these specific bacterial 16S rRNA gene markers, as described in the experimental part below, or quantitative PCR of any gene specific for a bacterial group.

[0042] In the present text, a "good responder to a treatment", also called a "responder" or "responsive" patient or in other words a patient who "benefits from" this treatment, refers to a patient who is affected with a cancer and who shows or will show a clinically significant relief in the cancer after receiving this treatment. The disease clinical data may be assessed according to the standards recognized in the art, such as immune-related response criteria (irRC), WHO or RECIST criteria.

[0043] According to a particular embodiment, the biological sample is a biofilm of a biopsy (preferably of a large biopsy) of duodenum or ileum mucosae obtained from the patient. For example, this biopsy can have been obtained during a specific surgery in pancreatic, stomach, biliary tract or colon cancers.

[0044] According to another embodiment, which concerns any type of cancer, the biological sample is a sample of feces obtained from the patient. This sample can have been collected at diagnosis, for example, or at any moment before

deciding the beginning of the treatment.

[0045] When an intestinal dysbiosis with an over-representation of "unfavorable" bacteria as defined above is observed, this shows that the patient requires a treatment to balance the gut microbiota prior to starting the antineoplastic treatment, or as an adjuvant of said treatment (*e.g.*: prebiotics or probiotics administration before/when starting a chemotherapy). Hence, decision can be made to adapt the patient's regimen (providing pre- or probiotics) during a period of time (for example, a few weeks) before beginning the antineoplastic treatment.

[0046] According to another aspect, the present invention pertains to a method for *in vitro* determining whether an antineoplastic treatment is to be continued or stopped for a cancer patient, comprising the following steps:

(i) from a biological sample from said patient, obtained at least 3 weeks after the beginning of the antineoplastic treatment, preferably 6-9 weeks after the beginning of the antineoplastic treatment (corresponding to three cycles of chemotherapy), analyzing memory $CD4^+$ T cell response directed against at least one commensal species of bacteria (preferably at least 2 and more preferably at least 3, 4 or more commensals);
(ii) for each commensal species against which the $CD4^+$ T cell response is analyzed, classifying the response in one of the following categories:

- no memory $CD4^+$ T cell response;
- memory response of a Th10:Tr1/Treg phenotype;
- memory response of a Th1 phenotype,

wherein if a memory response of a Th1 phenotype is observed for at least one commensal species, the antineoplastic treatment is continued, and in absence of such a response, the antineoplastic treatment is stopped or compensated with appropriate probiotics (see below).

[0047] This pharmacodynamic assay is particularly useful to predict, after 3-9 weeks of a chemotherapy (1-3 cycles of chemotherapy), preferably after 6-9 weeks (2-3 cycles) of chemotherapy, whether this chemotherapy is likely to trigger an adjuvant immune response and a clinical benefit.

[0048] In order to classify the responses, the secretions of IL-2, TNFα, IFNγ and IL-10 are measured in *ex vivo* restimulation assays. In a preferred embodiment, a first assay is done before the beginning of the treatment, in order to compare the cytokine secretion profile after a few weeks of treatment to that observed pre-treatment. These assays can be performed, for example, using patients' autologous monocytes loaded with defined bacteria and incubated with $CD4^+CD45RO^+$ T cells purified from autologous blood. The response will be classified in the third (favourable) category if it is of a Th1 phenotype, i.e., if restimulation triggers a significant secretion of IL-2, TNFα and IFNγ, and a low secretion of IL-10, especially when comparing the results obtained post- to pre-treatment. Typically, for a patient having a response of the Th1 phenotype, at least a 2-fold increase of IFNγ secretion is observed post-treatment (compared to pre-treatment). The first category (no memory $CD4^+$ T cell response) corresponds to the absence of significant cytokine secretion in restimulation assays post-treatment, whereas the second category corresponds to a response in which the IL-10 secretion in a restimulation assay post-treatment is superior to that observed pre-treatment.

[0049] According to a particular embodiment of the above method, the memory $CD4^+$ T cell responses directed against at least two species selected amongst *Lactobacillus johnsonii, Enterococcus hirae* and *Enterococcus faecalis* are analyzed. Preferably, the responses directed against 2 of these, and more preferably against all of these, are assessed.

[0050] One particularly advantageous aspect of this pharmacodynamic method is that it can be performed using a blood sample. Of course, it can be done for patients having any kind of cancers.

[0051] According to a third aspect, the present invention pertains to a method for *in vitro* determining the biological effects of a neoadjuvant antineoplastic treatment which has been administered to a patient, comprising the following steps:

(i) from an appropriate biological sample from said patient, determining the relative abundance of "favorable" bacteria in said microbiota;
(ii) from the same biological sample, determining the relative abundance of "unfavorable" bacteria in said gut microbiota;
(iii) calculating the ratio between the abundance of favorable bacteria and the abundance of unfavorable bacteria,

[0052] wherein if said ratio is above a predetermined threshold, the result indicates that the neoadjuvant antineoplastic treatment induced a T-bet/Th1 local and systemic immune response.

[0053] To perform the above method, the "favorable" bacteria can be those from a group comprising or consisting of the genera *Lactobacillus* and *Bifidobacterium*, and the "unfavorable" bacteria can be those from a group comprising or consisting of the species *Parabacteroides distasonis* and *Faecalibacterium prausnitzii* and the genera *Gemmiger, Alistipes* and *Clostridium Cluster IV (Clostridium leptum* group).

[0054] The skilled artisan will determine the appropriate threshold depending on the technique which is used to de-

termine the relative abundance of bacteria from each group (for example, pyrosequencing or quantitative PCR) and depending on the definition of each group of patients. Indeed, a unique threshold cannot be determined for all cancer patients, and the ratio must be appreciated having regard to several factors, including the patient's health and food habits.

[0055] For performing the above method, the biological sample preferably is a biofilm from a biopsy (preferably from a large biopsy) of duodenum or ileum mucosae obtained from the patient. For example, this biopsy can have been obtained during a specific surgery in pancreatic, stomach, biliary tract or colon cancers.

[0056] Importantly, the methods described above can be performed to prognosticate or diagnose the responsiveness of a cancer patient to any antineoplastic treatment as defined above, including chemotherapies, biological therapies, radiotherapies, hormone therapies, *etc.* In particular, these methods can be advantageously used to assess the (potential) benefit, for a cancer patient, of a chemotherapy, more particularly with an alkylating agent or a platinum salt such as any of those cited above, and/or of a biological therapy such as an immunotherapy, more particularly with an anti-CTLA4 antibody, an anti-PD-1/PDL-1 Ab, anti-Tim3, anti-ICOS, ICOS inhibitors *etc.*, or an anti-tumor vaccine. The experimental data below clearly describe the role of microbiota on the immune response induced by cyclophosphamide (Examples 1, 3 and 4), doxorubicine (see at least Fig. 14) and oxaliplatine (Example 2). Interestingly, Examples 3 to 5 show that the results obtained in mice can be extrapolated to humans. The experimental data show that a "beneficial" microbiota also has a positive impact on the efficiency of a treatment by anthracyclins (Fig. 8) and obviously, if bacterium species having an immunomodulatory role, such as *Faecalibacterium prausnitzii,* are too abundant in the gut microbiota, these bacteria will negatively impact the drug efficiency.

[0057] The present invention also relates to a probiotic bacterial strain selected from the group consisting of *Lactobacillus johnsonii, Enterococcus hirae* and *Enterococcus faecalis,* for use in combination with an antineoplastic agent for inducing a T-bet/Th1 local and systemic immune response, for treating a cancer.

[0058] Examples of probiotics according to the present invention are the *Lactobacillus johnsonii* strain LJFS001B, deposited on November 15, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4823, and the *Enterococcoccus hirae* strain EHFS001, deposited on November 7, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4815.

[0059] According to a preferred embodiment, the probiotic bacterial strain according to the invention is formulated for oral administration. The skilled artisan knows a variety of formulas which can encompass living or killed microorganisms and which can present as food supplements (*e.g.*, pills, tablets and the like) or as functional food such as drinks, fermented yoghurts, *etc.*

[0060] The present invention still relates to the use of such probiotics, in combination with an antineoplastic treatment, for treating a cancer patient.

[0061] As used herein, the term "in combination" refers to the use of more than one agents (*e.g.*, a probiotic strain and a chemotherapeutic drug). The use of the term "in combination" does not restrict the order in which therapies are administered to the patient, although it is preferable to administer the probiotic strain prior to or simultaneously with the antineoplastic treatment. For example, the probiotic strain can be administered prior to the antineoplastic agent (*e. g.*, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), either punctually or several times (for example, each day) before the antineoplastic treatment is administered.

[0062] According to a preferred embodiment, the probiotic bacterial strain according to the invention is used in combination with a chemotherapeutic agent or an biological immunotherapy, for example in combination with a treatment by an alkylating agent or by immunotherapy.

[0063] A composition comprising at least the *Lactobacillus johnsonii* strain LJFS001B, (Fig. 26A) deposited on November 15, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4823, and/or the *Enterococcoccus hirae* strain EHFS001 (Fig. 26B), deposited on November 7, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4815, is also part of the present invention. Optionally, such a composition also comprises lipopolysaccharide (LPS) to increase its immune-stimulating properties. The compositions according to the invention can be in the form of food supplements (*e.g.*, pills, tablets, syrups and the like) or in the form of functional food such as drinks, fermented yoghurts, *etc.* The probiotics are preferentially alive in these compositions.

[0064] According to another aspect, the present invention relates to adoptive cell transfer of "pathogenic" Th17 (pTh17) cells derived from CD4+ T cells from a cancer patient, preferentially in combination with an antineoplastic treatment such as a chemotherapy (*e.g.*, with an alkylating agent) or an immunotherapy (*e.g.*, anti-CTLA4 MAbs, antitumor vaccine, ...), for treating said patient. For example, CD4+ naive T cells can be obtained from blood, then amplified and stimulated *ex vivo* in the presence of cytokines favouring the pTh17 phenotype (for example in the presence of IL-1β, IL-6, IL-21 and IL-23 and optionally IL-1b+IL-9) as well as TCR cross-linking (such as beads coated with anti-CD3/anti-CD28 Ab). As described above, pTh17 cells share hallmarks of Th1 cells (nuclear expression of the transcription factor T-bet, cytoplasmic expression of IFNγ and surface exposure of the chemokine receptor CXCR3) and Th17 cells (expression of RORγt, IL-17 and CCR6). The phenotype of the cells is controlled before their transfer to the patient. If necessary, cells obtained *ex vivo* are sorted to retain only those exhibiting the pTh17 phenotype.

[0065] Other characteristics of the invention will also become apparent in the course of the description which follows of the biological assays which have been performed in the framework of the invention and which provide it with the required experimental support, without limiting its scope.

EXAMPLES

EXAMPLE 1 : THE INTESTINAL MICROBIOTA MODULATES THE ANTICANCER IMMUNE EFFECTS OF CYCLOPHOSPHAMIDE - MOUSE STUDY

Materials and Methods

[0066] *N.b.:* absent contrary indication, the materials and method which are described in the present example are those which have also been used in the other examples.

[0067] Animals and tumor models. All animal experiments were carried out in compliance with French and European laws and regulations. Mice were used between 7 and 14 weeks of age. WT SPF C57BL/6J and DBA2/J mice were obtained from Harlan, Charles River or Janvier and kept in specific pathogen-free conditions (SPF). *Nod1*$^{-/-}$*Nod2*$^{-/-}$ and *Nod2*$^{-/-}$ C57BL/6J mice were provided by I. Gomperts Boneca (Institut Pasteur, France), *Myd88*$^{-/-}$ C57BL/6J mice by B. Ryffel (CNRS, France) and C57BL/6J germ-free mice were obtained from CDTA (Orléans, France) or Institut Pasteur and maintained in sterile isolators. MCA205, B16F10 (syngeneic from C57BL/6J mice) and P815 (syngeneic from DBA2/J mice) were cultured at 37°C under 5% CO_2 in RPMI 1640 containing 10% FCS, 2 mM L-glutamine, 100 IU/ml penicillin/streptomycin, 1 mM sodium pyruvate and MEM non-essential amino acids (Invitrogen). 0.5 - 1 $\times$ 10^6 MCA205, 0.3 $\times$ 10^6 B16F10 or 0.8 $\times$ 10^6 P815 tumor cells were inoculated s.c. in the right flank. Chemotherapy was performed by intratumoral injection of doxorubicin (Doxo) (2 mM, 50 µl) or intraperitoneal inoculation of CTX (100 mg/kg of body weight) when tumors reached 35-60 mm^2.

[0068] Treatment of lung adenocarcinoma-bearing KP mice using chemotherapy. Eight week-old *KrasLSL-G12D/WT; p53*$^{Flox/Flox}$ mice received an adenovirus expressing Cre recombinase by intranasal instillation (defined as d0). The Cre recombinase system activates oncogenic *Kras (KrasG12D)* and deactivates *p53* in a few somatic cells of the lung; grade 3 and 4 adenocarcinomas become visible at ~d70 (Cortez-Retamozo et al.). Mice were either left untreated or received chemotherapy (d84, d91 and d98) in absence or presence of 0.25mg/ml vancomycin (mixed into drinking water starting on d77 and until the end of the experiment; antibiotic-containing water was replaced biweekly). Tumor volumes were quantified on d73 and 100 (equivalent of 'pre' and 'post' chemotherapy) in anesthetized mice by noninvasive imaging as described before (Cortez-Retamozo et al.). Data show absolute changes in total lung tumor volumes (means ± SEM) between the two time points.

[0069] Reagents. Cyclophosphamide (CTX) (Eudoxan, Baxter) was provided by Institut Gustave Roussy. Doxorubicin hydrochloride (D1515) and Fluorescein isothiocyanate-dextran (FITC-dextran) (46944, 4 kDa) were obtained from Sigma-Aldrich. Anti-mouse antibodies for CD3ε, CXCR3 (CXCR3-173), CD4 (GK1.5), CD8α (53-6.7), γδTCR (GL-3), IL-17 (eBio17B7), IFNγ (XMG1.2), T-bet (4B10), RORγt (AFKJS-9), CD45, CCR6 (140706) were obtained from BioLegend, eBioscience and R&D. LIVE/DEAD fixable yellow stain fluorescence for viability staining was purchased from Invitrogen/Molecular Probes. All cells were analyzed on a Cyan (Beckman Coulter) or a FACSCANTO II (BD) flow cytometer with FloJo (Tree Star) software.

[0070] Antibiotics protocols. Mice were treated with antibiotics 2-3 weeks before tumor implantation and continued until the end of the experiment. A mix of ampicillin (1 mg/ml) + streptomycin (5 mg/ml) + colistin (1 mg/ml) (Sigma-Aldrich) or vancomycin (0.25 mg/ml) or colistin alone (2.10^3U/ml) were added in sterile drinking water. Solutions and bottles were changed 2-3 times a week. Antibiotic activity was analyzed by macroscopic changes observed at the level of caecum (dilatation) and by cultivating the fecal pellets resuspended in BHI+15% glycerol on blood agar and anaerobic blood agar plates for 48h at 37°C with 5% CO_2 for aerobic conditions or in anaerobic conditions respectively. In experiments shown in Fig. 4, vancomycin biased the repertoire of commensal bacteria towards distinct commensal species (such as *E. coli* and different species of *Clostridium*, Fig. 16) whereas colistin promoted the outgrowth of *E. faecalis*.

[0071] Bacterial isolation, cultivation and identification. Mesenteric lymph nodes and spleens were aseptically removed, smashed in PBS and plated onto COS agar plates (BioMérieux), for aerobic and anaerobic growth. After 48h of culture, single colonies were isolated and stocked in glycerol at -80°C.

[0072] Serial dilutions of feces from naïve mice or tumor bearers treated with NaCl or CTX, vancomycin or broad spectrum antibiotics (ATB) (ampicillin+streptomycin+colistin), were plated onto COS agar plates and after 48h, single colonies were isolated and Gram staining was performed. The identification of specific bacteria was accomplished through the combination of morphological tests and the analysis through VITEK® automated system (BioMérieux, France) and verified in mass spectrometry (MALDI-TOF, see below) performed at Pasteur Institute, Paris, France.

[0073] *P. distasonis* used in the experiments was isolated from feces of SPF mice treated with prolonged broad spectrum ATB and identified as described above. For *in vitro* experiments, *E. hirae, E. faecalis* and *E. coli* were grown

in BHI medium (Fluka analytical), while *L. johnsonii, L. plantarum* and *L. murinus* in MRS broth (BD) at 37°C until they reach an OD_{600}=1 when the growth was exponential. *L. reuteri* was grown in anaerobic conditions onto COS agar plates for 48h at 37°C. Serial dilutions of bacteria preparations were plated so that the administered doses could be assessed. *E. coli* MC1061, *E. faecalis* JH2-2 and *L. plantarum* NCIMB8826 were kindly provided by I. Gomperts Boneca, Institut Pasteur, France. *P. distasonis* was grown onto COS agar plates in anaerobic conditions for 48h, then colonies were resuspended in PBS to reach an OD_{600}=1.

[0074] The identification of bacteria was done by MALDI-TOF analysis and 16S rRNA gene sequencing. The MALDI-TOF MS analysis was done on prepared cells as follows. Strains were grown overnight at 37°C on MRS agar. About 5 to 10 mg of cells were resuspended in 300 µl of sterile ultrapure water and 900 µl of absolute ethanol, homogenized by flicking the tubes, centrifuged for 2 min at 13000 g and the supernatant was discarded. Subsequently, 50 µl of formic acid was added to the pellet and mixed before the addition of 50 µl acetonitrile. The mixture was centrifuged again at 13000 g for 2 min. One microliter of the supernatant was spotted on the MALDI-TOF sample plate and air-dried at room temperature. Each sample was covered with 1 µl of (HCCA) matrix solution (Bruker Daltonics ref 201344: saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile-2.5% trifluoroacetic acid) and air dried at room temperature. Measurements were performed with an Autoflex mass spectrometer (Bruker Daltonik GmbH, Germany) using flexcontrol software (version 3.0). Spectra were recorded in the positive linear mode (laser frequency, 200Hz, ion source I, voltage at 20kV; ion source 2, voltage at 18.4 kV; lens voltage, 9.1 kV; mass range, 2000-20 000 Da). For automated data analysis, raw spectra were processed using the MALDI BioTyper 2.0 software (Bruker Daltonik GmbH, Germany) with default settings. The 16S rRNA gene from the strains studied was amplified by PCR using the universal primers A, 5'-AGAGTTTGATCATGGCTCAG-3' (SEQ ID No: 1) (position 8 to 27, *Escherichia coli* numbering) and H, 5'-AAGGAGGTGATCCAACCGCA-3' (SEQ ID No: 2) (position 1541 to 1522) (Bottger, 1989), in a GeneAmp® thermal cycler (Perkin-Elmer, Wellesley, MA) and the following parameters: 4 min at 94 °C, 25 cycles of 1 min at 94 °C, 25 of 1 min at 57 °C, 25 of 2 min at 72 °C with a final extension step at 72 °C for 5 min. Sequencing of PCR-generated amplicons was performed by GATC Company using primers A, H, and two other sequencing primers (*E. coli* numbering system): B, 5'-CTCCTACGGGAGGCAGCAGT-3' (SEQ ID No: 3), position 339 to 358; and G, 5'-GCATGTGGTTTAATTCGA-3' (SEQ ID No: 4), position 947 to 964. The almost-complete sequences of the gene coding for 16S rRNA were obtained after assembling using software BioNumerics version 6.6 (Applied-Maths, Belgium) and then blasted in NCBI BLAST program.

[0075] Histology and immunofluorescence of gut tissue. The whole small intestine (duodenum, jejunum and ileum) was removed, cleaned from fecal content and fixed in 4% of PFA for 1h. Re-hydratation of the tissue was performed in 15% sucrose for 1h and in 30% sucrose overnight. Depending on the experiment, the small intestine was entirely rolled, or cut into small pieces, then embedded in optimum cutting temperature (OCT) compound (Sakura), snap frozen and longitudinal or transversal 6 µm sections were prepared.

[0076] For histological analyses, the longitudinal sections were counterstained with hematoxilin and eosin. For the histological quantitative analyses, inflammatory foci, altered villi and the thickness of lamina propria were scored for each section, while the number of goblet cells was counted for each villus. For Paneth cells enumeration, the longitudinal sections were permeabilized with 0.5% triton for 15 min, and were blocked with a solution of 0.1 % triton, 5% serum and 1% BSA for 1h. Then, a rabbit polyclonal antibody against the lysozyme protein (1:500 for 1h, Thermo Scientific) and Alexa Fluor 488 Fragment of goat anti-rabbit IgG (1:300 for 1h, Molecular Probes) were used. All steps were performed at room temperature. Lysozyme-positive areas were quantified on mosaic images using the Histolab software (Microvision Instruments). The quantification of Paneth cells was performed measuring the average area of the Lysozyme-positive clusters (group of Paneth cells) as well as the emission/μm^2 of those clusters (not shown).

[0077] Immunofluorescence stainings of the gut leukocytes. For the γδ TCR $^+$ and γδ TCR$^-$ T cell quantification, transversal sections were blocked with a solution of 0.1% triton and 10% goat normal serum for 1h. Then, the sections were immunostained with hamster anti-γδ TCR (10 µg/ml O/N 4°C, BD Pharmingen) and with goat anti - hamster A488 (7.5 µg/ml for 45 min, Jackson ImmunoResearch) as secondary antibody, or with hamster anti-mouse CD3 A647 (5 µg/ml for 2h, Biolegend). For each section, the number of total cells, γδ TCR$^+$ CD3$^+$ and CD3$^+$ cells were counted to determine the percentage of γδ TCR$^+$ and γδ TCR- T cells.

[0078] *In vivo* intestinal permeability assay. Gut barrier integrity was assessed by permeability to FITC-dextran (4 kDa, Sigma Aldrich). Fourteen hours after i.p. injection with NaCl or CTX at 100 or 200 mg/kg, mice were fasted for 4 hours and then orally fed with FITC-dextran at 0.6 mg/g body weight (80 mg/ml in NaCl, 18h after NaCl/CTX treatment). After 3 to 4h, the mice were euthanized and exsanguinated by cardiac puncture. Plasma FITC levels were subsequently determined using a fluorescence spectrophotometer ($\lambda_{ex}/\lambda_{em}$=485/535 nm).

[0079] Isolation of lamina propria cells from small intestine. Whole duodenum and ileum were harvested, Peyer's patches were removed, as well as all fat residues and fecal content. Small fragments were obtained by cutting them first longitudinally along the length and then transversally into pieces of 1-2 cm length. After removing the intra-epithelial lymphocytes (IELs), the gut pieces were further cut and incubated with 0.25 mg/ml collagenase VIII and 10 U/ml DNase I for 40 min at 37 °C under shaking to isolate lamina propria cells (LPCs). After digestion, intestinal pieces were mashed on a cell strainer. For FACS analysis, cell suspensions were subjected to a percoll gradient for 20 min at 2100 RPM,

while for RNA extraction, cells were directly lysed in RLT buffer (Qiagen) and frozen at -80°C.

[0080] Analyses of dendritic cell subsets in CTX-treated small intestines. Cell suspensions from mouse spleen and lymph nodes were prepared by digestion with collagenase and DNase for 60 min and subsequently strained through a 70 μm mesh. Colonic and small intestinal lymphocytes were isolated as previously described (Schlitzer et al.). In brief colon and small intestine were digested in PBS containing 5 mM EDTA and 2 mM DTT shaking at 37°C. After initial digestion colonic and small intestinal tissue pieces were digested in collagenase/Dnase containing RPMI medium for 30 min. Tissue pieces were further strained through a 70 μm mesh. For flow cytometry analyses, cell suspensions were stained with antibodies against the following surface markers: CD11c (N418), CD11b (M1/70), Ly6c (HK1.4), MHC class II (M5/114.15.2), CD24 (M1/69), CD64 (X54-5/7.1), CD317 (ebio927), CD45 (30-F11), F4/80 (C1:A3-1), CD8α (53-6.7). DAPI was used for dead cell exclusion. Antibodies were purchased from eBiosciences, BD Biosciences or BioLegend respectively. Cell populations were gated as follows: small intestine (migratory fraction): $CD103^+$ DC ($CD45^+$ $CD11c^+$ $MHC\text{-}II^+$ $CD103^+$ $CD24^+$), $CD11b^+$ $CD103^+$ ($CD45^+$ $CD11c^+$ $MHC\text{-}II^+$ $CD103^+$ $CD11b^+$ $CD24^+$), $CD11b^+$ ($CD45^+$ $CD11c^+$ $MHC\text{-}II^+$ $CD11b^+$ $CD24^+$), inflammatory DC ($CD45^+$ $CD11c^+$ $MHC\text{-}II^+$ $CD11b^+$ $CD64^+$ $Ly6c^+$), large intestine: $CD103^+$ DC ($CD45^+$ $CD11c^+$ $MHC\text{-}II^+$ $CD103^+$ $CD24^+$), $CD11b^+$ ($CD45^+$ $CD11c^+$ $MHC\text{-}II^+$ $CD11b^+$ $CD24^+$), inflammatory DC ($CD45^+$ $CD11c^+$ $MHC\text{-}II^+$ $CD11b^+$ $CD64^+$ $Ly6c^+$).

[0081] Microbiota reconstitution. For inoculation of GF mice with SFB, fecal pellets were collected from SFB-mono-colonized mice with sterilized test tubes. Colonization was performed by oral gavage with 200 μl of suspension obtained by homogenizing the fecal pellets in water. Efficient colonization was first checked before tumor inoculation.

[0082] *E. hirae*, *L. johnsonii* and *L. plantarum* were grown in BHI (Fluka analytical) and MRS (BD) broth, respectively, overnight at 37°C. Bacteria were centrifuged, washed once and resuspended in sterile PBS at an OD(600nm) of 1, which corresponds approximately to $1x10^9$ colony-forming units (CFU)/ml. Equal volume of each bacteria suspension was mixed to give a suspension of equal proportion of each type of bacteria at $1x10^9$ bacteria/ml. *L. reuteri* was grown in anaerobic conditions onto COS agar plates for 48h at 37°C. For *P. distasonis* colonization, mice were treated with a mix of ampicillin/streptomycin/colistin (ATB) for 4 weeks and orally inoculated with 10^9 CFU in 200 μl of PBS 4 days post MCA205 inoculation. For other experiments, after 2-3 weeks of ATB, the treatment was stopped and mice were orally gavaged with 10^9 CFU of *E. hirae* + *L. johnsonii* or *L. plantarum* or *L.reuteri* one day after CTX administration and 0 to 3 days post treatment suspension.

[0083] TCR and T cell assays. For cross-linking experiment, 2×10^5 total splenocytes per well (after red cell lysis) were incubated in MaxiSorp plates (Nunc) precoated with anti-CD3ε mAb (145-2C11) (0.5 μg per well; eBioscience) and/or anti-CD28 mAb (37.51) (2 μg/ml; BD). The supernatants were assayed at 48h by ELISA for mouse IL-17A (eBioscience) and IFNγ (BD). For TIL analyses, tumors were removed, cut into small pieces and digested in Liberase TM (Roche) and DNase I for 30 min at 37°C. Single-cell suspensions were obtained by crushing the digested tissue with a syringe plunger and filtering through a 100 μM cell strainer. For intracellular, cells were incubated for 4h at 37°C with 50 ng/ml of PMA, 1 μg/ml of ionomycin and BD Golgi STOP™. After membrane staining, cells were stained with anti-IL-17A, IFNγ, T-bet and RORγt using eBioscience FoxP3/Transcription factor staining buffer set.

[0084] T cell polarization and propagation *in vitro*. *Adoptive transfer of Th17 cells (pathogenic or regulatory Th17).* Naive $CD4^+$ T cells ($CD4^+CD62L^{hi}$) were obtained from spleens and lymph nodes of C57BL/6 WT mice. Cells were then sorted by flow cytometry (BD ARIA III with FACSDiva Software) accordingly. The purity of isolated T cell populations routinely exceeded 95%. Naive T cells were stimulated with plate-bound antibodies against CD3ε (145-2C11, 2 μg/ml) and CD28 (PV-1, 2 μg/ml) in the presence of either recombinant mouse IL-1β (10 ng/ml), IL-6 (10 ng/ml), and IL-23 (20 ng/ml) (pTh17) or TGF-β (2.5 ng/ml) and IL-6 (Th17) (Miltenyi). Regulatory Th17 (Th17) resulted from a differentiation in TGF-β (2.5 ng/ml) and IL-6 while pathogenic Th17 (pTh17) resulted from incubation in IL-1β, IL-6 and IL-23. Mice were intravenously injected with 3×10^6 T cells. *Priming of T cells in vitro.* Bone marrow-derived dendritic cells (BMDCs) were generated from femurs and tibiae of C57BL/6 mice, cultured for 8 days in Iscove's medium (Sigma-Aldrich) with J558 supernatant (containing 40 ng/ml of GM-CSF), 10% FCS, 100 IU/ml penicillin/streptomycin, 2 mM L-glutamin, 50 μM 2-mercaptoethanol (Sigma-Aldrich) and split every 3-4 days. At day 8, BMDCs were infected with the isolated bacterial strains at a MOI (multiplicity of infection) 1:50 for 1 h at 37°C in the appropriate medium without antibiotics. Then, cells were washed with PBS and incubated in complete medium supplemented with gentamicin (50 mg/ml) to kill extracellular bacteria. After 24h, BMDCs were cultured together with naive $CD4^+$ $CD62L^+$ T cells, purified from spleen and lymph nodes (Miltenyi), at the ratio 1:1 for 4 days. Culture supernatants were then assayed for IL-17 and IFNγ by ELISA. *$CD4^+$ T cell memory response.* BMDCs were infected with different doses of bacteria (ratio cells:bacteria 1:2, 1:10 and 1:50) as described above and after 24h were cultured 1:1 with $CD4^+$ T cells, purified from spleens (Miltenyi) of CTX- or NaCl-treated C57BL/6 mice. After 24h culture supernatants were assayed for IL-17 and IFNγ by ELISA.

[0085] Adoptive T cell transfer. B6.CBir1 TCR transgenic (CBir1 Tg) mice (Cong et al., 2009) were generated and bred in the Animal Facility at the University of Alabama at Birmingham. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. $CD4^+$ T cells were isolated from B6.CBir1 TCR Tg mice using anti-mouse CD4 magnetic beads. Briefly, splenic cells were washed twice and incubated with anti-CD4 magnetic beads at 4°C for 30 min and then separated by magnetic field. When checked

by flow cytometry, over 95% of the cells were $CD4^+$ T cells. One million CBir1 Tg T cells ($CD45.1^+$) were adoptively transferred i.v. into CTX or NaCl treated- naïve congenic ($CD45.2^+$) mice two days after chemotherapy and spleens were harvested at day 5-7 post-transfer for flow cytometry analyses and *ex vivo* splenocyte restimulations. Flow cytometry analyses gated on $CD45.1^+$ cells to appreciate percentages of intracellular $IL\text{-}17^+$ or $IFN\gamma^+$ cells after PMA/ionomycin 5h restimulation in the presence of monensin. Other splenocytes were incubated in triplicate in 24 well flat bottom plates at 1.0 million/ml, cultured without or with CBir1- peptide 455-475 (DMATEMVKYSNANILSQAGQ) at 1 μg/ml and supernatants were analysed using anti-IFNγ specific commercial ELISA.

[0086] Quantitative RT-PCR for antimicrobial peptide determination. Lamina propria cells were isolated from duodenum, ileum and colon 18h post CTX, and total RNA extraction and genomic DNA removal were performed with the RNeasy Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Total RNA extraction and genomic DNA removal of ilea or duodena were performed with the RNeasy Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Total RNA was then reverse transcribed into cDNA with the SuperScript III Reverse Transcriptase and the RNaseOUT™ Recombinant Ribonuclease Inhibitor (Life Technologies, Saint Aubin, France), in the presence of random primers (Promega, Charbonnieres, France) and the Deoxynucleoside Triphosphate Set, PCR grade (Roche Diagnostics, Meylan, France). Expression of RegIIIγ (Mm00441127_ml) and LysM (Mm01612741_ml) -related genes was analyzed with TaqMan® Gene Expression Assays using the Universal Master Mix II on a StepOnePlus™ Real-Time PCR System (Life Technologies, France). Quantitative RT-PCR data were invariably normalized to the expression levels of the housekeeping gene peptidylprolyl isomerase A (*Ppia*) by means of the $2^{-\Delta Ct}$ method.

[0087] Microbial DNA extraction, 454 pyrosequencing and quantitative PCR on commensal bacteria. Total DNA was extracted from mucosal samples (~50-100 μg) as previously described (Lepage et al., 2005; Seksik et al., 2003) using both physical and chemical lysis. DNA concentration and integrity were determined both visually by electrophoresis on a 1% agarose gel containing ethidium bromide and spectrophotometrically by using a Nanodrop instrument (Thermo Scientific).

[0088] Microbiota composition was assessed by 454 pyrosequencing (GS FLX Ti technology) targeting the V3-V4 region of the bacterial 16S rRNA gene (V3fwd: 5'TACGGRAGGCAGCAG3', SEQ ID No: 5; V4rev: 5'GGACTACCAGGGTATCTAAT3', SEQ ID No: 6). Sequences were trimmed for barcodes, PCR primers, and binned for a minimal sequence length of 300pb, a minimal base quality threshold of 27, a maximum homopolymers length of 6. Resulting sequences were assigned to the different taxonomic levels, from phylum to genus using the RDP database (release 10, update 31) (Cole et al., 2009). Sequences were further clustered into OTUs (Operational Taxonomic Units or phylotypes) at 97% of identity using QIIME (Caporaso et al.) and cdhit (Li and Godzik, 2006). OTUs were assigned to closest taxonomic neighbors and relative bacterial species using Seqmatch (RDP) and Blastall (NCBI). Relative abundance of each OTUs and other taxonomic levels (from phylum to genus) was calculated for each sample to account for different levels of sampling across multiple individuals. After trimming, the number of sequences clustered within each OTUs (or other taxonomic levels) was converted to a fraction representing the relative contribution of each feature to each of the individuals. For heatmaps representation, $\log_{10}$-transformation was applied on the relative abundance data matrix, which allowed visualizing similarities or differences between samples that affect members of the community that may make up less than 1% of the relative abundance in a sample. Principal component analyses of the different mice microbiota were computed based on bacterial genus composition. Robustness of each clustering result was assessed using a Monte Carlo rank test (n=10 000 repetitions, $p < 0.05$) (Romesburg, 1985). To gain further insight into bacterial counts, quantitative PCR was applied. Targeted qPCR systems were applied using either Taqman technology (for systems targeting All Bacteria domain, *Clostridium leptum* group (Mayeur et al.) or SybrGreen (for systems targeting *Lactobacillus/Leuconoctoc/Pediococcus* group (Mayeur et al.), *Enterococcus* group(Furet et al., 2009), SFB (Yin et al.) and TM7 (Hugenholtz et al., 2001)). No CTX-specific modulations of the relative amounts of SFB and TM7 or Clostridium group XIV was observed at day 7 post-CTX (not shown). Quantitative PCR was performed using an ABI 7000 Sequence-Detection System with software version 1.2.3 (Applied-Biosystems). Amplification and detection were carried out with either TaqMan Universal PCR 2_MasterMix (Applied-Biosystems) or SYBR-Green PCR 2_Master Mix (Applied-Biosystems) in duplicate in a final volume of 25 μl with 10 μl of appropriate dilutions of DNA samples as previously described. Amplifications were carried out using the following ramping profile: 1 cycle at 95 °C for 10 min, followed by 40 cycles of 95°C for 30 s, 60°C for 1 min. For SYBR-Green amplification, a melting step was added (Yin et al.). For the quantification of bacterial groups, standard curves were generated from serial dilutions of a known concentration of genomic DNA from a representative of each group. Standard curves were generated by plotting threshold cycles (Ct) vs. bacterial quantity (CFU). The total number of bacteria (CFU) was interpolated from the averaged standard curves.

[0089] Characterization of adoptively transferred Th17 cells by quantitative PCR Analysis. Total RNA from T cells was extracted with Trizol (Invitrogen). 100 to 300 ng of RNA were reverse-transcribed into cDNA by M-MLV reverse transcriptase, Random Primers, and RNaseOUT inhibitor (Invitrogen). cDNA were quantified by real-time PCR with a SYBR Green Real-time PCR kit (Applied Biosystems) on a Fast7500 detection system (Applied Biosystems, France). Relative mRNA levels were determined with the ΔCt method. Values were expressed relative to cyclophilin A. The sequences of the oligonucleotides used are described below.

Table 1: oligonucleotides used for characterizing Th17 cells expression profiles

Gene	Forward	SEQ ID No	Reverse	SEQ ID No
Actin	ATGGAGGGGAATACAGCCC	7	TTCTTTGCAGCTCCTTCGTT	8
Cd3e	CCAGGATACTGAGGGCATGT	9	CTTATCAGTTGGCGTTTGGG	10
Cd4	CCTGTGCAAGAAGCAGAGTG	11	GTTCTGCTGATTCCCCTCC	12
Ppia	GGCCGATGACGAGCCC	13	TGTCTTTGGAACTTTGTCTGCAA	14
Eomes	CAGCACCACCTCTACGAACA	15	CGCCACCAAACTGAGATGAT	16
Foxp3	CTCGTCTGAAGGCAGAGTCA	17	TGGCAGAGAGGTATTGAGGG	18
Gata3	AGGATGTCCCTGCTCTCCTT	19	GCCTGCGGACTCTACCATAA	20
Ifng	TGAGCTCATTGAATGCTTGG	21	ACAGCAAGGCGAAAAAGGAT	22
Il10	TGTCAAATTCATTCATGGCCT	23	ATCGATTTCTCCCCTGTGAA	24
Il17a	TGAGCTTCCCAGATCACAGA	25	TCCAGAAGGCCCTCAGACTA	26
Rorc	GGTGATAACCCCGTAGTGGA	27	CTGCAAAGAAGACCCACACC	28
Tbx21	ATCCTGTAATGGCTTGTGGG	29	TCAACCAGCACCAGACAGAG	30
Tgfb	CAACCCAGGTCCTTCCTAAA	31	GGAGAGCCCTGGATACCAAC	32

[0090] Bioinformatics and statistics. At the exception of proportion and count data that were respectively compared by beta regression and negative binomial regression, linear modeling was applied to evaluate the impact of treatment to the parameters in their original scale or in logarithmic scale. Systematic examination of the model residuals and application of diagnostic tools respective to each method confirmed the appropriate fit of the data. The influence of tumor and CTX treatment on the bacteria content were estimated by maximum likelihood to account for non detected measurements as previously described (Helsel, 2005). Given that non-detects could appear in both parameters, Kendall's tau (Newton and Rudel, 2007) was computed for correlation studies between IL-17/IFNγ and bactena content with the regression line standard error bands estimated by bootstrapping (B=1999) Similar outcome was obtained by further validation studies including the application of the same procedure to the data were the samples containing non detects are excluded and the determination of the p-values by permutation Tumor growth modeling was carried by linear mixed effect modeling on log pre-processed tumor surfaces (Demidenko, 2006, Sugar et al.). Reported p-values are obtained from testing jointly that both tumor growth slopes and intercepts (on log scale) are the same between treatment groups of interests. For sake of clarity, the outcome of the test is only given for comparisons found significant at p<0 05. Post-hoc pairwise testing at single sampling time point confirmed the effects reported on the graphs. Note that no significant differences in tumor area were highlighted between treatment groups at time of treatment. "Tumor presence/absence of tumor growth" incidences were compared with Firth's penalized-likelihood logistic regression (Hemze, 2006). All reported tests are two-tailed and were considered significant. *, for a p-value<0.05, **, p<0 01,***, p<0 001, ns, non significant

Results

[0091] In the present example, the impact of CTX on the small intestine microbiota and its ensuing effects on the antitumor immune response are described.

[0092] The inflammatory status of the gut epithelial barrier was characterized 48 hours following therapy with non-myeloablative doses of CTX or the anthracycline doxorubicin in naive mice. Both drugs caused shortening of small intestinal villi, discontinuities of the epithelial barrier, interstitial edema and focal accumulation of mononuclear cells in the lamina propria (LP) (Fig. IA-B). Post-chemotherapy, the numbers of goblet cells and Paneth cells were increased in villi (Fig. 1C) and crypts (Fig. 1D), respectively. The antibacterial enzyme lysozyme (but not the microbiocide peptide RegIIIγ) was upregulated in the duodenum of CTX-treated mice (Fig. 1E). Orally administered fluorescein isothiocyanate (FITC)-dextran became detectable in the blood (Yang et al.) 18 h post CTX, confirming an increase in intestinal permeability (Fig. 1F). Disruption of the intestinal barrier was accompanied by a significant translocation of commensal bacteria in >50% mice into mesenteric lymph nodes and spleens that was well detectable 48 h post-CTX, less so after doxorubicin treatment (Fig. 2A). Several Gram$^+$ bacterial species, including *Lactobacillus johnsonii* (growing in >40% cases), *Lactobacillus murinus* and *Enterococcus hirae,* could be cultured from these lymphoid organs (Fig. 2B).

[0093] Next, the overall composition of the gut microbiota was analyzed by high-throughput 454 pyrosequencing, followed by quantitative PCR targeting the domain bacteria and specific bacterial groups. Although CTX failed to cause a major dysbiosis at early time points (24-48h, Fig. 5), CTX significantly altered the microbial composition of the small intestine (but not of the caecum) in mice bearing subcutaneous cancers (namely metastasizing B16F10 melanomas and non-metastasizing MCA205 sarcomas) one week after its administration (Fig. 2C, Fig. 5). Consistent with previous reports on fecal samples from patients (Zwielehner et al.), CTX induced a reduction of bacterial species from the *Firmicutes* phylum (Fig. 5) distributed within four genera and groups (*Clostridium* cluster *XIVa, Roseburia, unclassified Lachnospiraceae, Coprococcus,* Table 2) in the mucosa of CTX-treated animals.

					All Animals			Tumor Bearers		
Phylum	Genus	1st isolate sequence name	AN	S_ab	Co	CT	*pvalue*	Co	CT	*pvalue*
Firmicutes	*Unclassified_Clostridiaceae 1*	*Segmented filamentous bacterium*	X77814	1.000	0,83	7,95	0,083	0,56	2,55	
Firmicutes	*Lactobacillus*	*Lactobacillus reuteri; LU3*	AY735406	1.000	0,13	0,24		0,02	0,14	0,048
Firmicutes	*Clostridium XIVa*	*Butyrate-producing bacterium SM4/1*	AY305314	0.908	1,31	0,21	0,045	1,76	0,20	0,049
Firmicutes	*Clostridium XIVa*	*Butyrate-producing bacterium M62/1*	AY305309	0.920	0,43	0,07	0,056	0,54	0,06	0,049
Firmicutes	*Clostridium XIVa*	*Clostridium sp. Culture-41*	AB622820	0.933	0,35	0,10	0,045	0,32	0,08	0,048
Firmicutes	*Clostridium XIVa*	*Rumen bacterium NK4A66*	GU124467	0.872	0,26	0,07	0,046	0,34	0,10	0,080
Firmicutes	*Roseburia*	*Roseburia intestinalis, XB6B4*	AM059815	0.827	0,24	0,02	0,032	0,26	0,00	0,016
Firmicutes	*Roseburia*	*Roseburia faecis (T); M72/1*	AY305310	0.910	1,20	0,35		2,00	0,41	0,095
Firmicutes	*Unclassified_Lachnospiraceae*	*Clostridium sp. Clone-49*	AB622849	0.973	1,04	0,07	0,056	1,61	0,09	0,052
Firmicutes	*Unclassified_Lachnospiraceae*	*Clostridium sp. A9*	DQ789119	0.942	0,70	0,23	0,045	0,96	0,27	0,024
Firmicutes	*Unclassified_Lachnospiraceae*	*Lachnospiraceae bacterium 14-2*	DQ789124	0.859	0,57	0,05		0,95	0,02	0,028
Firmicutes	*Unclassified_Lachnospiraceae*	*Clostridium sp. Clone-40*	AB622844	0.977	0,25	0,05		0,42	0,02	0,026
Firmicutes	*Unclassified_Lachnospiraceae*	*Lachnospiraceae bacterium 607*	AB700365	0.900	0,06	0,06		0,11	0,01	0,043
Firmicutes	*Unclassified_Lachnospiraceae*	*Clostridium sp. Culture-54*	AB622823	0.968	0,09	0,03	0,064	0,13	0,04	0,049
Firmicutes	*Unclassified_Lachnospiraceae*	*Clostridium sp. ASF502*	AF157053	0.943	0,10	0,01	0,022	0,17	0,00	0,016
Firmicutes	*Unclassified_Lachnospiraceae*	*Clostridium sp. Clone-33*	AB622843	0.885	0,07	0,02	0,046	0,10	0,00	0,016
Firmicutes	*Coprococcus*	*Coprococcus catus; L8*	AB361624	0.827	0,13	0,03		0,20	0,03	0,052
Bacteroidetes	*Tannerella*	*Tannerella forsythia; OB071*	JN713185	0.561	0,06	0,02		0,10	0,02	0,026

Table 2: CTX-induced mucosal microbiota dysbiosis at a species level

[0094] Average relative abundances of bacterial species that are significantly differentially represented between CTX treated (CTX) and NaCl-treated mice (Co) are represented. Taxonomic affiliation of these sequences is also added (phylum and genus levels). Following phylotype (OTU) determination, phylotype centroids are assigned to their closest relative isolate (RDP Seqmatch database). All animals were compared together and tumor bearers were further distinguished. AN: NCBI Accession Number; Sab_Score: RDP similarity score between the centroid sequence and the referent isolate. Wilcoxon test p-values.

[0095] Quantitative PCR was applied to determine the bacterial counts of all bacteria and of targeted groups of bacteria (*Lactobacillus, Enterococcus, Clostridium leptum* cluster IV group) in the small intestine mucosa from CTX versus vehicle-treated naïve and tumor-bearing mice. In tumor bearers, the total bacterial load of the small intestine at 7 days post-CTX as well as the bacterial counts of the *Clostridium leptum* were not affected (Fig. 2D). However, CTX treatment led to a reduction in the abundance of lactobacilli and enterococci (Fig. 2D). Altogether, these data reveal the capacity of CTX to provoke the selective translocation of distinct Gram$^+$ bacterial species followed by significant changes in the small intestinal microbiome.

[0096] Coinciding with dysbiosis 7 days post-CTX, the frequencies of CD103$^+$CD11b$^+$ dendritic cells (Fig. 7A) and TCR$\alpha\beta^+$CD3$^+$ T cells expressing the transcription factor RORγt (Fig. 7B) were significantly decreased in the lamina propria (LP) of the small intestine (but not the colon), as revealed by flow cytometry of dissociated tissues (Fig. 7B) and *in situ* immunofluorescence staining (Fig. 7C). RORγt is required for the generation of Th17 cells (which produce interleukin-17, IL-17), and strong links between gut-residing and systemic Th 17 responses have been established in the context of autoimmune diseases affecting joints, the brain or the pancreas (Ghiringhelli et al., 2004; Lee et al., ; Wu et al.). Confirming previous work (Michaud et al., ; Viaud et al.), CTX induced the polarization of splenic CD4$^+$ T cells towards a Th1 (interferon-γ [IFNγ]-producing) and Th17 pattern (Fig. 3A, Fig. 7D). This effect was not found for doxorubicin (Fig. 8). The gut microbiota was indispensable for gearing the conversion of naïve CD4$^+$T cells into IL-17 producers in response to CTX. Indeed, the *ex vivo* IL-17 release by TCR-stimulated splenocytes increased upon CTX treatment of specific pathogen-free (SPF) mice, yet failed to do so in germ-free (GF) mice (Fig. 3A, left panel). Sterilization of the gut by broad-spectrum antibiotics (ATB, a combination of colistin, ampicillin and streptomycin, Fig. 9) also suppressed the CTX-stimulated secretion of IL-17 (Fig. 3A, right panel) and IFNγ by TCR-stimulated splenocytes (Fig. 7D). Treatment of mice with vancomycin, an antibiotic specific for Gram$^+$ bacteria (Rice, 2006), also reduced the CTX-induced Th17 conversion (Fig. 3A, right panel). In conventional SPF mice, the counts of lactobacilli and SFB measured in small intestine mucosa (Fig. 2D) positively correlated with the Th1 and Th17 polarization of splenocytes (Fig. 3B, Fig. 7E) whereas that of *Clostridium* cluster IV did not (Fig. 3B). Altogether, these results point to a specific association between particular

microbial components present in the gut mucosa (and occasionally in lymphoid organs) and the polarity of Th responses induced by CTX treatment.

[0097] CTX increased the frequency of "pathogenic" Th17 (pTh17) cells, which share hallmarks of Th1 cells (nuclear expression of the transcription factor T-bet, cytoplasmic expression of IFNγ and surface exposure of the chemokine receptor CXCR3) and Th17 cells (expression of RORγt, IL-17 and CCR6) (Ghoreschi et al., ; Lee et al.), within the spleen (Fig. 7F, Fig. 3C). Again, this response depended on the gut microbiota (Fig. 3C). Moreover, the increase in pTh17 cells required expression of myeloid differentiation primary response gene 88 (MyD88), which signals downstream of toll-like receptors (Fig. 10A) and is required for the therapeutic success of anticancer chemotherapies in several tumor models (Apetoh et al., 2007). In contrast, the two pattern recognition receptors, nucleotide-binding oligomerization domain-containing (Nod)1 and Nod2, were dispensable for the CTX-induced raise in splenic pTh17 cells and for the tumor growth retarding effects of CTX (Fig. 10B). These results establish the capacity of CTX to stimulate pTh17 cells through a complex circuitry that involves intestinal bacteria and MyD88, correlating with its anticancer effects. Beyond its general effect on the frequency of pTh17 cells, CTX induced TCR-restricted, antigen specific immune responses against commensal bacteria (Fig. 11). Hence, the inventors addressed whether Gram$^+$ bacterial species that translocated into secondary lymphoid organs in response to CTX (Fig. 2A) could polarize naïve CD4$^+$ T cells towards a Th1 or Th17 pattern. Both *L. johnsonii* and *E. hirae* stimulated the differentiation of naïve CD4$^+$ T cells into Th1 and Th17 cells *in vitro*, in the presence of bone marrow-derived dendritic cells, while toll-like receptor 4-activating purified bacterial lipopolysaccharide (LPS) or *E. coli* both had a minor effect (Fig. 12). Moreover, orally fed *L. johnsonii* and *E. hirae* but neither *L. plantarum* (a bacterium that was not detected in translocation experiments, Fig. 2B) nor *L. reuteri* facilitated the reconstitution of the pool of pTh 17 cells in the spleen of ATB-treated SPF mice (Fig. 3D). Th1 memory responses against *L. johnsonii* were consistently detected in 50% of mice receiving CTX (Fig. 3E) but not in control mice, after *in vitro* restimulation of CD4$^+$T cells with bone marrow-derived dendritic cells loaded with *L. johnsonii* (and to a lesser extent *E. hirae*, but not with other commensals or pathobionts). These results suggest that the translocation of a specific set of Gram$^+$ commensal bacteria can mediate the CTX-driven accumulation of pTh17 cells and Th1 bacteria- specific memory T cell responses.

[0098] Because commensal bacteria modulate intestinal and systemic immunity post-CTX, the inventors further investigated the effect of antibiotics on CTX-mediated tumor growth inhibition. Long-term treatment with broad-spectrum ATB reduced the capacity of CTX to cure P815 mastocytomas established in syngenic DBA2 mice (Fig. 4A, Fig. 13A). Moreover, the antitumor effects mediated by CTX against MCA205 sarcomas were reduced in GF compared with SPF mice (Fig. 4B, left and middle panels). Driven by the observations that CTX mostly induced the translocation of Gram$^+$ bacteria and that Gram$^+$ bacteria correlated with splenic Th1/Th17 polarization, the inventors compared the capacity of several ATB regimens, namely vancomycin (depleting Gram$^+$ bacteria) and colistin (depleting most Gram$^-$ bacteria) to interfere with the tumor growth-inhibitory effects of CTX. Vancomycin, and to a lesser extent colistin compromised the anti-tumor efficacy of CTX against MCA205 sarcoma

[0099] (Fig. 4C, Fig. 13B). Using a transgenic tumor model of autochthonous lung carcinogenesis driven by oncogenic K-Ras coupled to conditional p53 deletion (Cortez-Retamozo et al.), the inhibitory role of vancomycin on the anticancer efficacy of a CTX-based chemotherapeutic regimen was confirmed (Fig. 4D). Vancomycin also prevented the CTX-induced accumulation of pTh17 in the spleen (Fig. 4E) and reduced the frequencies of tumor-infiltrating CD3$^+$ T cells and Th1 cells (Fig. 4F).

[0100] Although the feces of most SPF mice treated with ATB usually were free of cultivable bacteria (Fig. 9), some mice occasionally experienced the outgrowth of *Parabacteroides distasonis*, a species reported to maintain part of the intestinal regulatory T cell repertoire and to mediate local anti-inflammatory effects (Geuking et al., ; Kverka et al., ; Lathrop et al.). This bacterial contamination was associated with the failure of an immunogenic chemotherapy (doxorubicin) against established MCA205 sarcomas (Fig. 14A). Moreover, experimental recolonization of ATB-sterilized mice with *P. distasonis* compromised the anticancer effects of doxorubicin (Fig. 14B), demonstrating that gut microbial dysbiosis abrogates anticancer therapy. Finally, monoassociation of tumor-bearing GF mice with SFB, which promotes Th17 cell differentiation in the LP (Hooper et al., ; Lee et al., ; Wu et al.) also had a detrimental impact on the tumor growth-inhibitory effect of CTX (Fig. 4B, right panel).

[0101] The aforementioned results highlight the association between specific CTX-induced alterations in gut microbiota, the accumulation of pTh17 cells in the spleen and the success of chemotherapy. To establish a direct causal link between these phenomena, the inventors adoptively transferred Th17 or pTh17 populations into vancomycin-treated mice and evaluated their capacity to reestablish the CTX-mediated tumor growth retardation. *Ex vivo* propagated pTh17 exhibited a pattern of gene expression similar to that expressed by CTX-induced splenic CD4$^+$ T cells *in vivo* (Fig. 15). Only pTh17 but not Th17 cells were able to rescue the negative impact of vancomycin on the CTX-mediated therapeutic effect (Fig. 4G). These results emphasize the importance of pTh17 cells for CTX-mediated anticancer immune responses.

[0102] To gain further insight into the links between gut microbiota and cellular anticancer-immunity, two distinct experimental approaches were used.

[0103] First, the inventors analyzed the impact of vancomycin on the microenvironment of auchthonous non-small cell lung cancers resulting from oncogenic activation of K-Ras and P53 and treated with CTX-based chemotherapy. They

analyzed the impact of vancomycin on the infiltration of chemotherapy-treated tumor beds by γδT17 cells, which are known to be crucial for the recruitment of antitumor CTLs post-chemotherapy (Ma et al.). In vancomycin- or broad-spectrum ATB-treated mice, tumor beds were devoid of γδT17 post-therapy in contrast to water-treated chemotherapy recipients (Fig. 17).

[0104] Secondly, they analyzed whether affecting gut microbiota with various antibiotic regimens could interfere with the elicitation of Th1 or Tc1 primary immune responses directed against a widely studied model antigen (chicken ovalbumin and its immunodominant H-2^b restricted epitope) that were combined with the TLR3 agonist poly-(I:C) and injected into the foodpad of antibiotic-treated or untreated mice that received CTX. None of the antibiotics that were used was capable of inhibiting IFNγ production by draining lymph node cells. Similarly, IFNγ secretion triggered by restimulation with the H-2^b-restricted SIINFEKL immunodominant peptide of OVA was maintained in vancomycin-treated mice, suggesting that Th1 (or Tc1) immune responses are not affected by the gut microbiota (Fig. 18). So, in this model, the antibiotic-mediated elimination of commensal bacteria causes defects at the level of innate immunity (loss of γδT17 cells in the tumor microenvironment) that result in modulations of cognate antitumor immune responses (effector memory TILs) with a reduced $CD8^+$/Foxp3 ratio (Fig. 20) and blunted Th1 responses (Fig. 4).

[0105] Although much of the detailed molecular mechanisms governing the complex interplay between epithelial cells, gut microbiota and intestinal immunity remain to be deciphered, the present study unveils the unsuspected impact of the intestinal microbiota on chemotherapy-elicited anticancer immune responses. The above data underscore new risks associated with antibiotic medication during cancer treatments as well as the potential therapeutic utility of manipulating the gut microbiota.

EXAMPLE 2 : THE INTESTINAL MICROBIOTA MODULATES THE ANTICANCER IMMUNE EFFECTS OF CANCER TREATMENT WITH ANTI-CTLA4 ANTIBODIES - MOUSE STUDY

[0106] The effects of CTLA4 blockade on the system were also characterized. Indeed, this treatment provokes major immune-related side effects such as colitis.

[0107] The antitumor effects of an antibody capable of blocking CTLA4 (9D9 hybridoma kindly provided by J. Allison) (Fig. 19A-B) were associated with pTh17 splenic responses (Fig. 19C). The tumor growth-reducing activity of anti-CTLA4 Ab was compromised in animals treated with imipenem (a penicillin derivative that kills Gram-positive and Gram-negative bacteria).

[0108] Altogether, it can be concluded that the perturbation of the barrier function of the intestine may contribute to the efficacy of different anticancer treatments.

EXAMPLE 3 : THE INTESTINAL MICROBIOTA MODULATES THE ANTICANCER IMMUNE EFFECTS OF CYCLOPHOSPHAMIDE - RESULTS ON A PRECLINICAL MODEL MIMICKING HUMAN TUMORIGENESIS

[0109] A transgenic tumor model of autochthonous NSCLC driven by oncogenic K-Ras coupled to a conditional P53 deletion (as initially described by T. Jacks, Cell 2012) was used to test the inhibitory role of vancomycin-based antibiotherapy on the anticancer efficacy of a combination of oxaliplatin plus CTX. In this preclinical model mimicking human tumorigenesis, the concept that the eradication of Gram-positive bacteria by vancomycin compromised the efficacy of CTX-based chemotherapy was validated (Fig.20A and Fig. 4D), correlating with a reduced intratumoral $CD8^+$ T effector/$Foxp3^+$ regulatory T cell ratio (Fig. 20B).

[0110] Thus, Gram-positive bacteria appear to be necessary for the optimal efficacy of the CTX-induced anticancer immune response and tumor mass reduction.

EXAMPLE 4 : HUMAN RESULTS: CYCLOPHOSPHAMIDE INDUCES TH1 AND TH10 IMMUNE RESPONSES DIRECTED AGAINST COMMENSAL BACTERIA IN CANCER PATIENTS

[0111] In order to further demonstrate that CTX induces bacterial translocation to secondary lymphoid tissues in humans as in mice, the inventors assessed memory $CD4^+$Th1 cell responses, in peripheral blood, specific for a series of bacteria in advanced cancer patients before and after treatment with metronomic cyclophosphamide (CTX). The responses that were monitored included those against enterococci (*E. hirae and E. faecalis,* both immunogenic in mice receiving CTX), lactobacilli (*L. johnsonii* and the less relevant *L. plantarum*), as well as against *E. coli.* The results were obtained from 6 patients with metastatic ovarian cancer treated with CTX+Avastin (Viaud et al.), 3 NSCLC (non small cell lung cancer) patients treated with CTX before a DC-based exosome Phase II vaccine trial (Chaput et al., 2006), and 2 melanoma patients enrolled in a Phase I trial of targeted immunotherapy preceded by CTX (Chaput et al.). From these 11 patients, 6 (54%) developed memory Th1 responses against *enterococci,* 2 against *L. johnsonii* (18%), 2 (18%) against *E. coli,* while one (9%) of them mounted a cellular immune response against *L. plantarum* (Fig. 21). Interestingly, some individuals elicited a Th10 immune response (*i.e.*, IL-10 release which is often associated with tumor progression)

against *E. faecalis* (high IL-10 & low IFNγ production, as Patients 5&6).

[0112] In summary, three patterns of cytokine release were observed in these experimental conditions: (i) no cytokine release, *i.e.*, no memory response to commensals; (ii) memory response of a Th10 phenotype; and (iii) Memory response of a Th1 phenotype.

[0113] The inventors anticipate that only pattern 3 will be proned to benefit from chemotherapy, and they now correlate this anti-commensal bacterial immune response with clinical outcome. This pharmacodynamic assay is useful to predict, after 3-6 weeks (1-2 cycles of chemotherapy) whether such a CTX-based chemotherapy would trigger an adjuvant immune response and a clinical benefit.

EXAMPLE 5 : HUMAN RESULTS: OXALIPLATINE-BASED CHEMOTHERAPY INDUCES A CHANGE IN THE DISTRIBUTION OF BACTERIAL SPECIES IN GUT MICROBIOTA AND AN INCREASE OF T-BET TRANSCRIPTION BY THE GUT MICROBIOTA

[0114] During a surgery of debulking of a primary colon cancer, or pancreatic cancer or stomach cancer, it is conceivable to access the duodenum (for stomach and pancreatic tumors) or ileum (for right colon cancer). In such cases, mucosal samples can be scratched and harvested (for 16S rRNA gene pyrosequencing analyses and description of the mucosal microbiota composition at the different taxonomic levels as described above), as well as mucosa that can be kept frozen (in RNAzol for qRT-PCR) or in paraffin-embedded tissues (for immunohistochemistry analyses).

[0115] This surgery can be performed either before chemotherapy (adjuvant chemotherapy) or after chemotherapy (neoadjuvant chemotherapy).

[0116] In the present example, ileal mucosa from patients operated for a right colon cancer (6 patients in neoadjuvant oxaliplatine-based chemotherapy and 7 patients prior to therapy) were analyzed to compare the composition of ileal microbiota and the relative loss or gain of representativity of distinct genera and species (isolates) in cases of adjuvant versus neoadjuvant chemotherapy, meaning in colon cancer bearing patients that already received (« chemo ») or did not receive (« controls ») chemotherapy.

[0117] The distribution of bacteria at a species (1st relative isolates) level was significantly different in the ileum post-chemotherapy (principal component analyses, Monte Carlo test, p=0.018) (Fig. 22).

[0118] Like in mice, chemotherapy induced the decrease of species belonging to *Clostridium cluster IV* in almost all patients, more specifically of bacteria from the genera *Dorea, Coprococcus, Lachnospiraceae, Gemmiger, Alistipes,* and bacterial species *Faecalibacterium prausnitzii* (Fig. 23 and 24, Table 3). In contrast, bacteria from the *Bifidabacterium* and Lactobacillus genera tended to increase post-chemotherapy (Table 3, Fig. 25).

Table 3: Differentially represented bacterial species (isolates) with p<0.1 between Chemo treated and non treated patients

Bacetrial species	AV Chemo	AV_Control	SD Chemo	SD_Control	T-test p value	Classification_Genus
Bacteroidescaccae_JCM9498_EU136686	3,828	0,135	4,619	0,190	0,056	Bacteroides
Flavonifractorplautii_17_GU968170	0,712	0,109	0,767	0,162	0,065	Flavonifractor
Bifidobacteriumlongum_IMAUFB091_XJ2813_JQ805709	0,530	0,029	0,653	0,076	0,067	Bifidobacterium
Bilophilawadsworthia_L35148	0,195	0,023	0,242	0,061	0,092	Bilophila
ClostridiumspAP4_JX101685	0,112	0,024	0,109	0,064	0,090	unclassified Ruminococcaceae
unidentifiedbacterium_CCCM26-AY654952	0,097	0,015	0,115	0,026	0,088	unclassified_Clostridiales
ClostridiaceaebactenumFH042_AB298771	0,053	0,000	0,070	0,000	0,068	Anaerovorax
bacteriumNLAEzlC328_JQ608041	0,027	0,000	0,038	0,000	0,079	Bacteroides
AlistipesspNML05AG04_EU189022	0,000	0,023	0,000	0,024	0,028	Alistipes
Doreaformicigenerans_SaLBHf10_JN093132	0,000	0,034	0,000	0,027	0,006	Dorea
Bacteroidesuniformis_JCM5828T_AB050110	0,009	0,090	0,024	0,104	0,068	Bacteroides
Clostridiumleptum_DSM753T_AJ305238	0,006	0,195	0,015	0,166	0,011	Clostridium IV
butyrateproducingbacteriumSR1_1_AY305321	0,115	0,318	0,207	0,215	0,097	Blautia
ClostridiaceaebacteriumDJF_LS13_EU728741	0,050	0,260	0,074	0,177	0,013	Dorea
Clostridiumruminantium_LA1_EU089964	0,043	0,294	0,077	0,325	0,070	Clostridium XI
ClostridialesbacteriumoraltaxonF32_VO026_HM099644	0,005	0,306	0,012	0,445	0,098	Acetivibrio
unidentifiedeubacteriumcloneBSV28_AJ229190	0,068	0,440	0,126	0,521	0,091	unclassified- Bacteria
butyrateproducingbacteriumA2231_AJ270484	0,047	0,438	0,087	0,438	0,039	Coprococcus
BacteroidesspdnLKV2_JF813174	0,278	0,893	0,262	0,589	0,027	Bacteroides
Gemmigerformicilis_ATCC27749_X256_GU562446	0,121	1,264	0,198	1,421	0,057	Gemmiger
Alistipesputredinis_JCM16772_AB554232	0,000	1,171	0,000	1,319	0,037	Alistipes
bacteriumIARFR194_KC153191	1,199	3,993	1,288	3,686	0,083	Uncl Lachnospiraceae
Faecalibacteriumprausnitzii_A2165_AJ270469	1,167	4,820	1,585	3,226	0,020	Faecalibacterium

[0119] The inventors also investigated, in parallel to pyrosequencing analyses of 16SrRNA of gut microbiota of ileum, the transcriptional profiling of cytokines and transcription factors detectable in mucosae of patients receiving or not chemotherapy. This investigation was done by qRT-PCR from ileal mucosa from the same patients. While RORγt and IL-17 were not very different in both groups, T-bet was upregulated post-chemotherapy and in two patients that had high levels of Bifidobacterium and Lactobacilli post-chemotherapy, T-bet transcripts were rather high compared with the other patients, suggesting that a pTh17 T cell response had been elicited by the treatment.

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<120> MICROBIOTA COMPOSITION, AS A MARKER RESPONSIVENESS TO CHEMOTHERAPY AND USE OF MICROBIAL MODULATORS (PRE-,PRO-SYMBIOTICS) FOR IMPROVING THE EFFICACY OF A CANCER TREATMENT

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Claims

1. A method for *in vitro* determining whether a cancer patient can benefit from an antineoplastic treatment, comprising the following steps:

   (i) from an appropriate biological sample from said patient, determining the relative abundance of bacteria from a group comprising or consisting of the species *Parabacteroides distasonis* and *Faecalibacterium prausnitzii* and the genera *Gemmiger, Alistipes* and *Clostridium* cluster IV in said patient's gut microbiota;
   (ii) determining the presence or absence of an intestinal dysbiosis; wherein an intestinal dysbiosis with an over-representation of bacteria from the group recited in step (i) indicates that the patient will not be a good responder to the antineoplastic treatment.

2. The method of claim 1, wherein the biological sample is obtained from a biopsy of duodenum or ileum mucosae obtained from the patient.

3. The method of claim 1, wherein the biological sample is a sample of feces obtained from the patient.

4. The method of any of claims 1 to 3, wherein step (i) is performed by pyrosequencing of 16S rRNA or by quantitative PCR of specifically targeted bacterial groups.

5. A method for *in vitro* determining whether an antineoplastic treatment is to be continued or stopped for a cancer patient, comprising the following steps:

   (i) from a biological sample from said patient, obtained 3 to 9 weeks after the beginning of said antineoplastic treatment, analyzing memory $CD4^+$ T cell response directed against at least one commensal species of bacteria;
   (ii) for each commensal species against which the $CD4^+$ T cell response is analyzed, classifying the response in one of the following categories:

   - no memory $CD4^+$ T cell response;
   - memory response of a Th10 phenotype;
   - memory response of a Th 1 phenotype,

   wherein if a memory response of a Th1 phenotype is observed for at least one commensal species, the antineoplastic treatment is continued, and in absence of such a response, the antineoplastic treatment is stopped.

6. The method of claim 5, wherein memory $CD4^+$ T cell responses directed against *L. johnsonii, E. hirae* and *E. Faecalis* are analyzed.

7. The method of claim 5 or claim 6, wherein the biological sample is a blood sample.

8. A method for *in vitro* determining the biological effects of a neoadjuvant antineoplastic treatment which has been administered to a patient, comprising the following steps:

(i) from an appropriate biological sample from said patient, determining the relative abundance of bacteria from a first group comprising *Lactobacillus* and *Bifidobacterium* genera in said microbiota;
(ii) from the same biological sample, determining the relative abundance of bacteria from a second group comprising *Parabacteroides distasonis, Faecalibacterium prausnitzii, Gemmiger, Alistipes* and *Clostridium* cluster IV in said gut microbiota;
(iii) calculating the ratio between the abundance of bacteria from the first group and the abundance of bacteria from the second group,
wherein if said ratio is above a predetermined threshold, the result indicates that the neoadjuvant antineoplastic treatment induced a T-bet/Th1 local and systemic immune response.

9. The method of claim 8, wherein the biological sample is a biopsy of duodenum or ileum mucosae obtained from the patient.

10. The method according to any of the preceding claims, wherein said antineoplastic treatment is a treatment with a chemotherapeutic agent or an anti-CTLA4 molecule.

11. A probiotic bacterial strain selected from the group consisting of *Lactobacillus johnsonii, Enterococcus hirae* and *Enterococcus faecalis,* for use in combination with an antineoplastic agent for inducing a T-bet/Th1 local and systemic immune response.

12. The probiotic bacterial strain according to claim 11, which is the *Lactobacillus johnsonii* strain LJFS001B, deposited on November 15, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4823 or the *Enterococcoccus hirae* strain EHFS001, deposited on November 7, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4815.

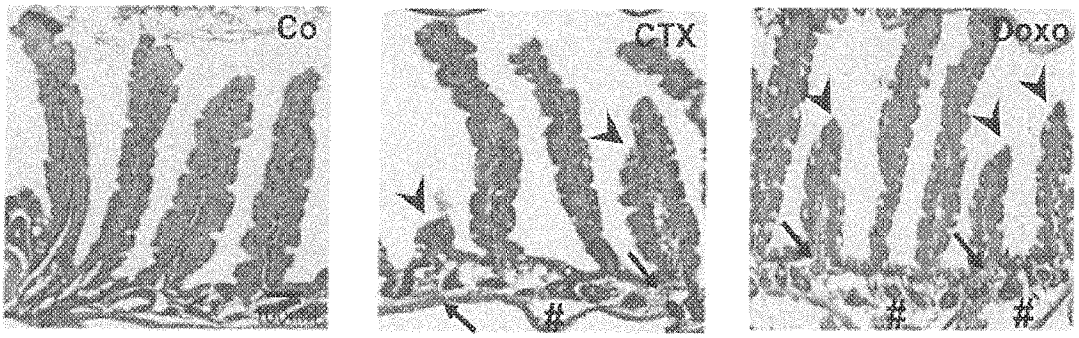
13. The probiotic bacterial strain according to claim 11 or claim 12, which is formulated for oral administration.

14. The probiotic bacterial strain according to any of claims 11 to 13, for use in combination with a chemotherapeutic agent or with an anti-CTLA4 molecule.

15. A composition comprising at least one bacterial strain selected from the group consisting of the *Lactobacillus johnsonii* strain LJFS001B, deposited on November 15, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4823 and the *Enterococcoccus hirae* strain EHFS001, deposited on November 7, 2013 at the Collection Nationale de Cultures de Microorganismes (CNCM), under the number I-4815.

16. The composition of claim 15, further comprising lipopolysaccharide (LPS).

17. A cell obtained by a process comprising stimulating naive CD4+ T cells from a cancer patient in the presence of a mixture of IL-1β, IL-6 and IL23, for use in adoptive cell transfer to said patient, in combination with an antineoplastic treatment, for treating cancer.



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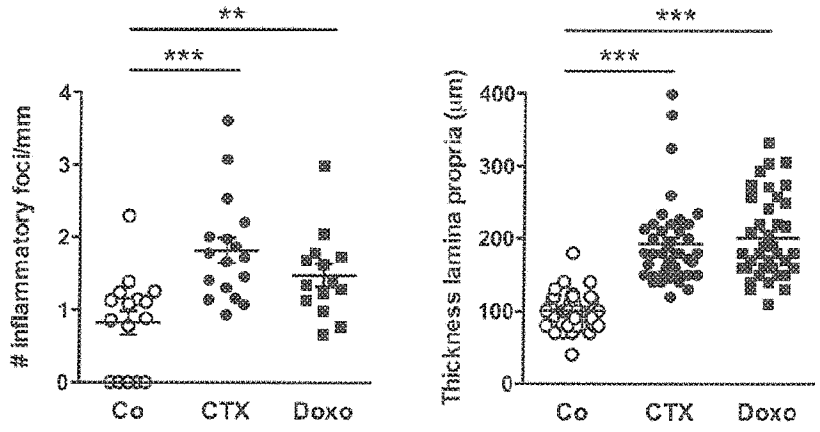

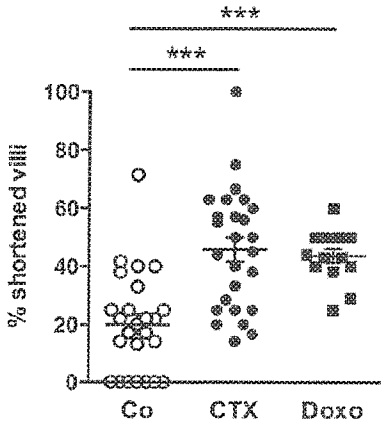

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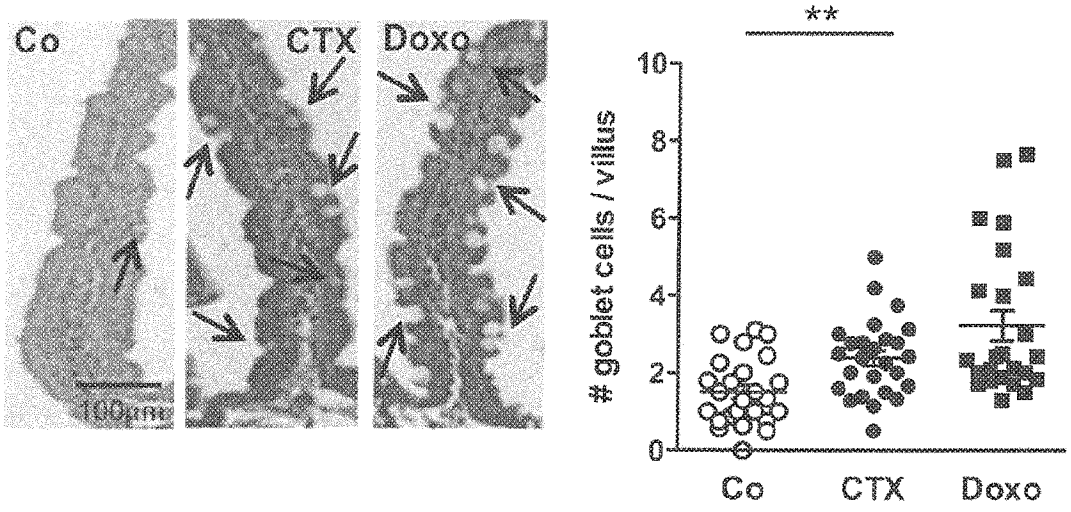

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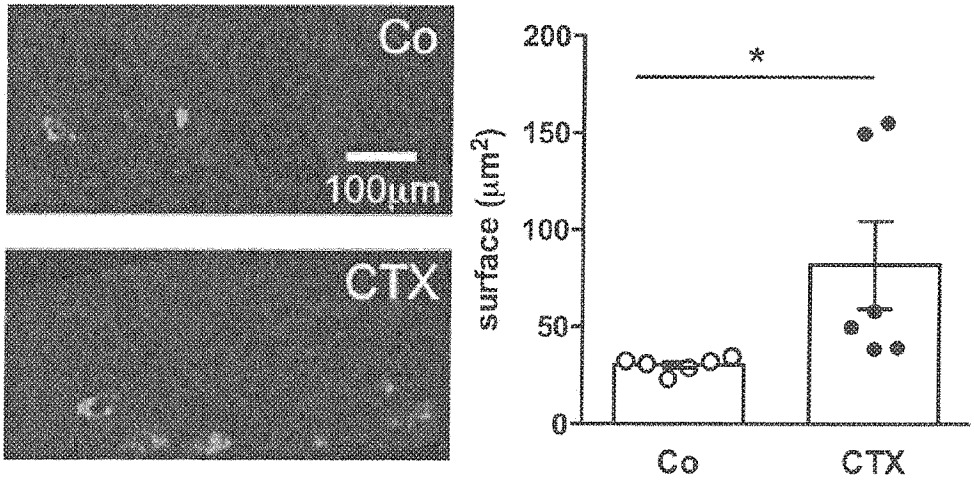

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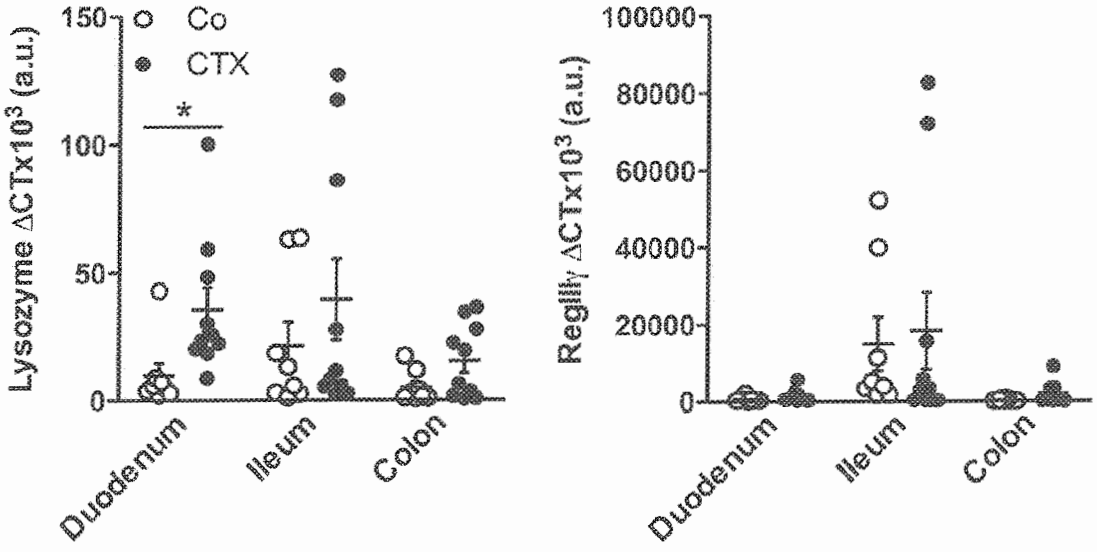

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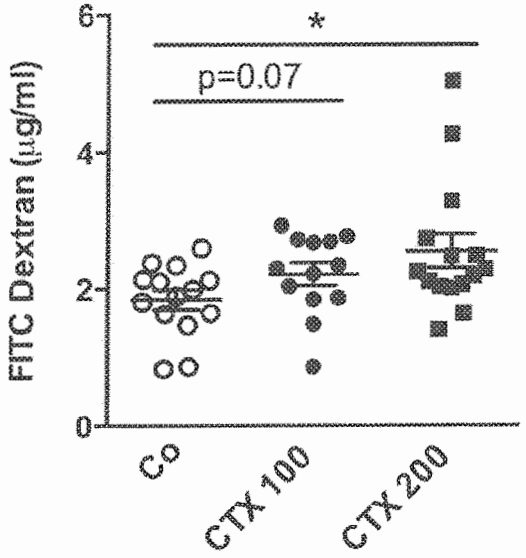

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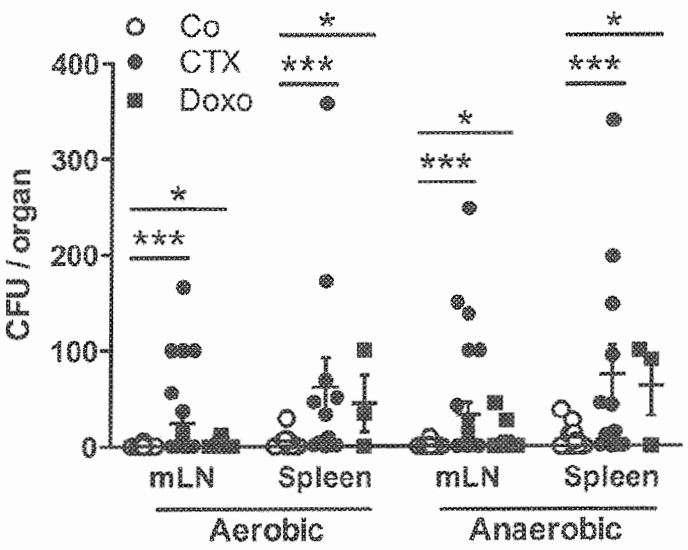

FIGURE 2A

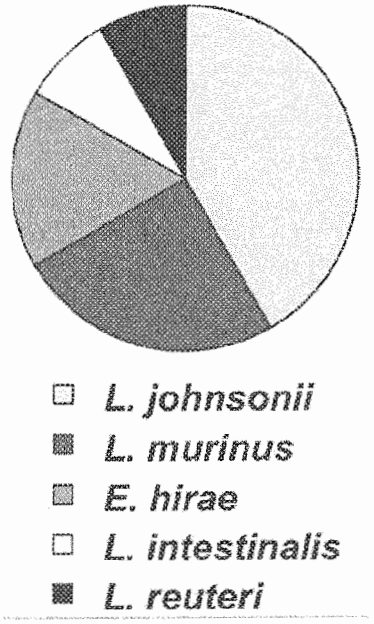

FIGURE 2B

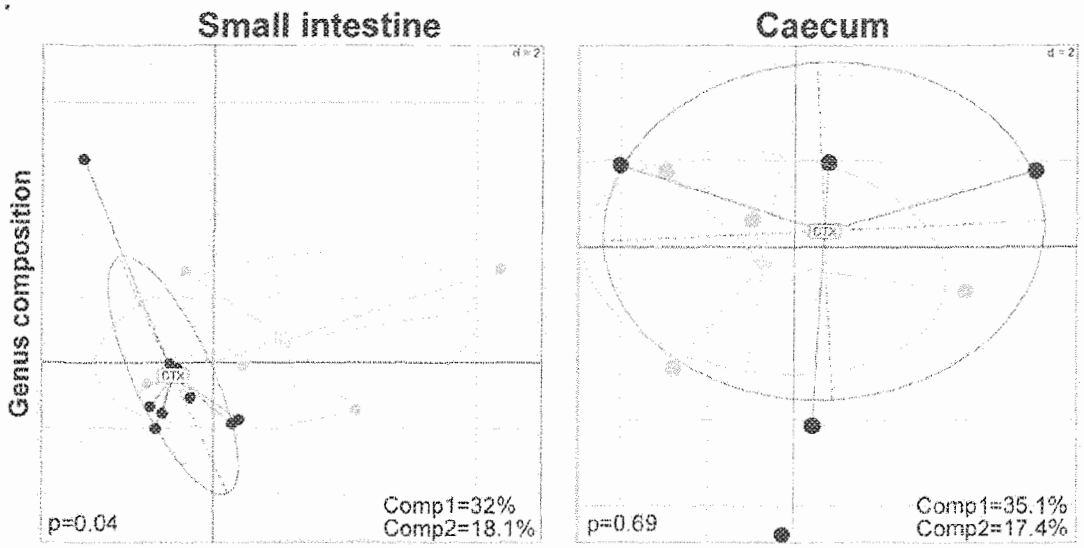

FIGURE 2C

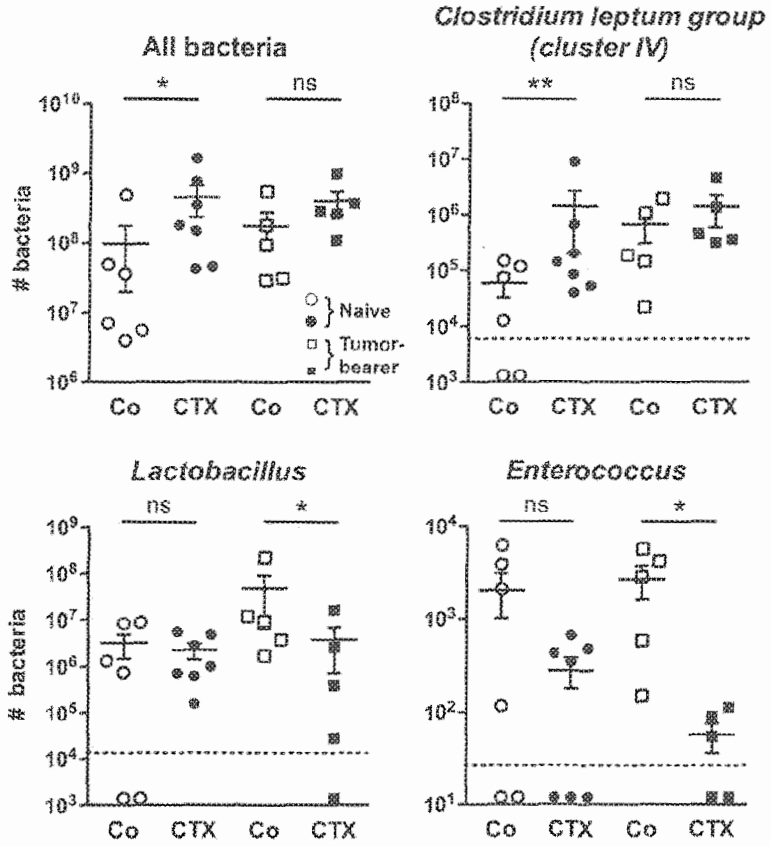

FIGURE 2D

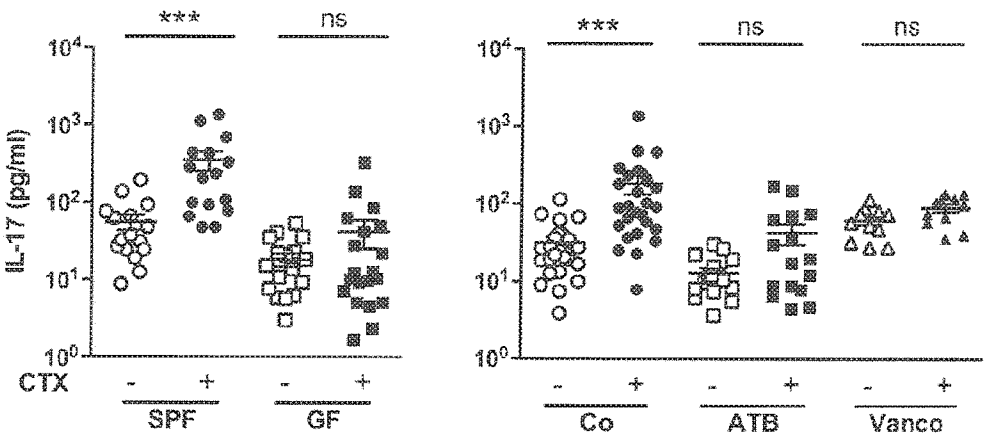

FIGURE 3A

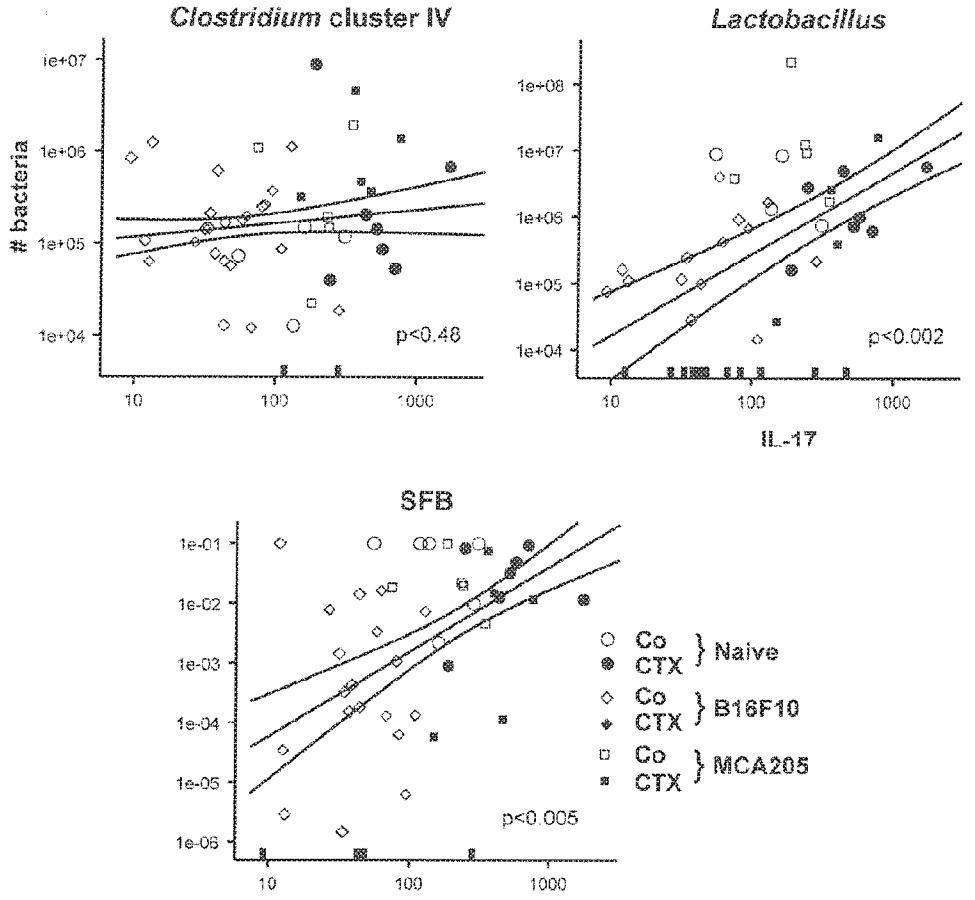

FIGURE 3B

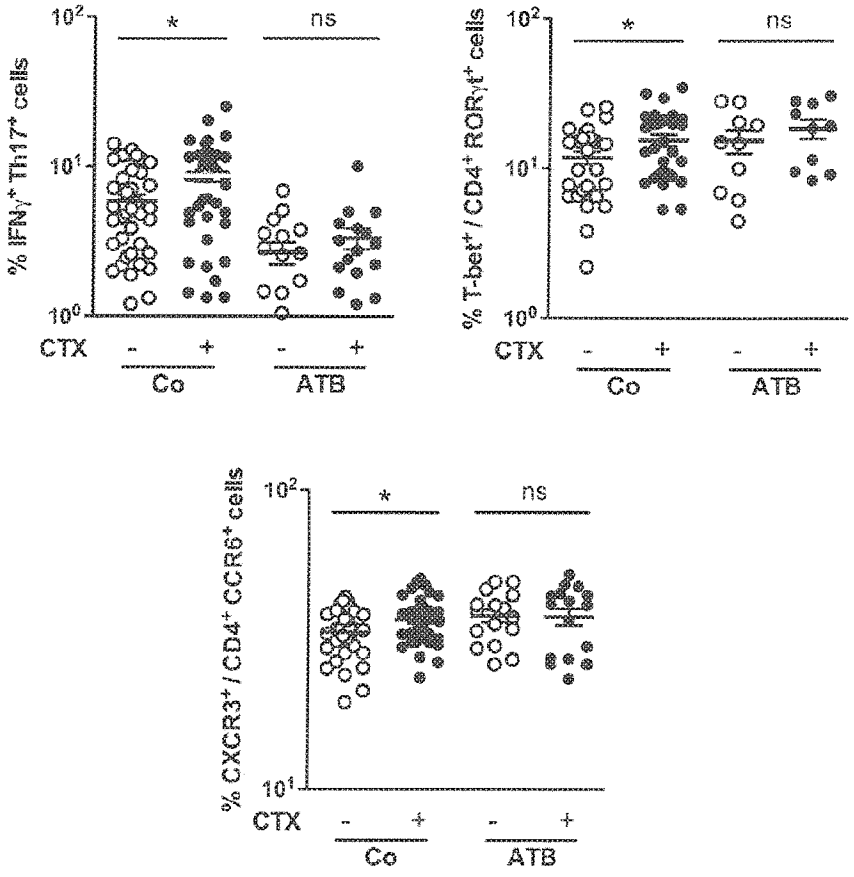

FIGURE 3C

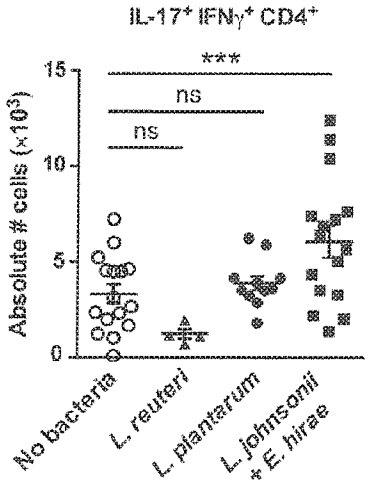

FIGURE 3D

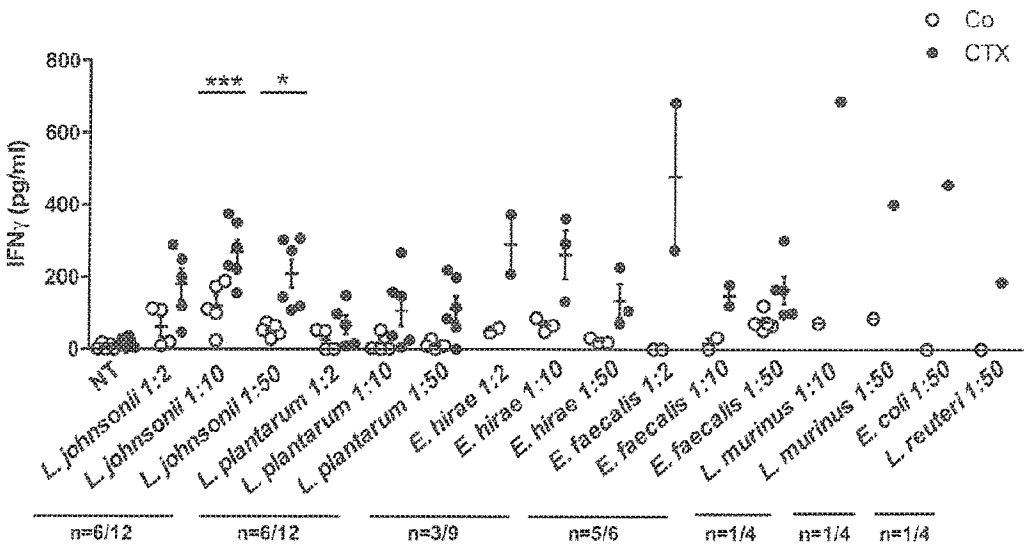

FIGURE 3E

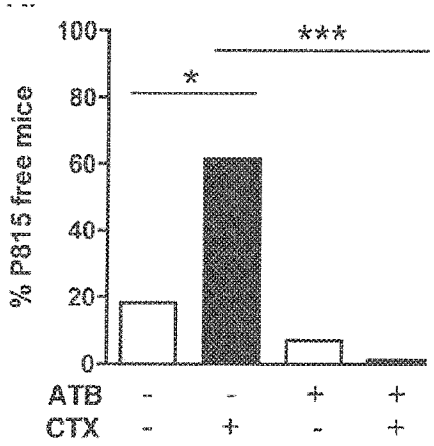

FIGURE 4A

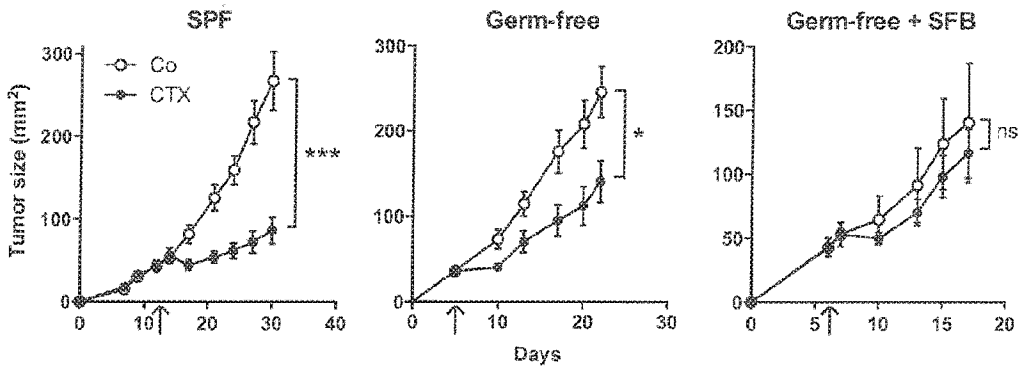

FIGURE 4B

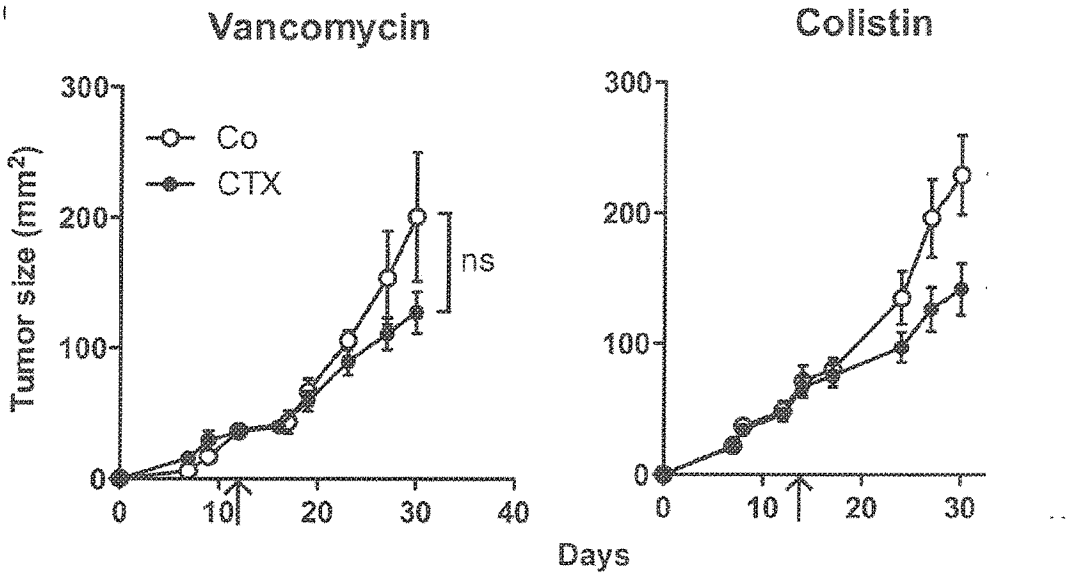

FIGURE 4C

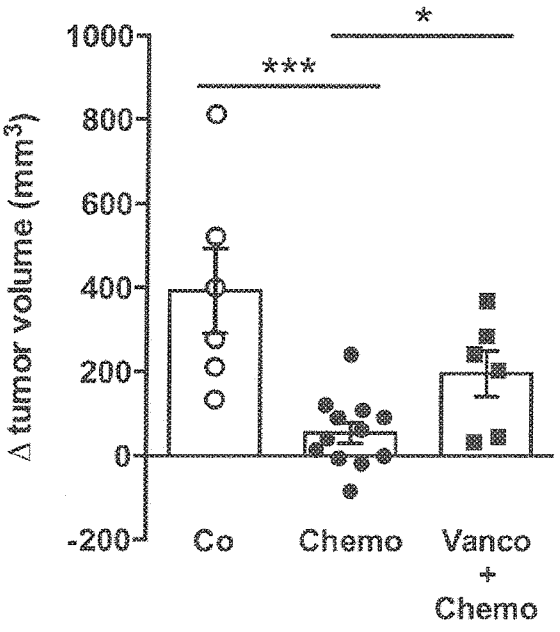

FIGURE 4D

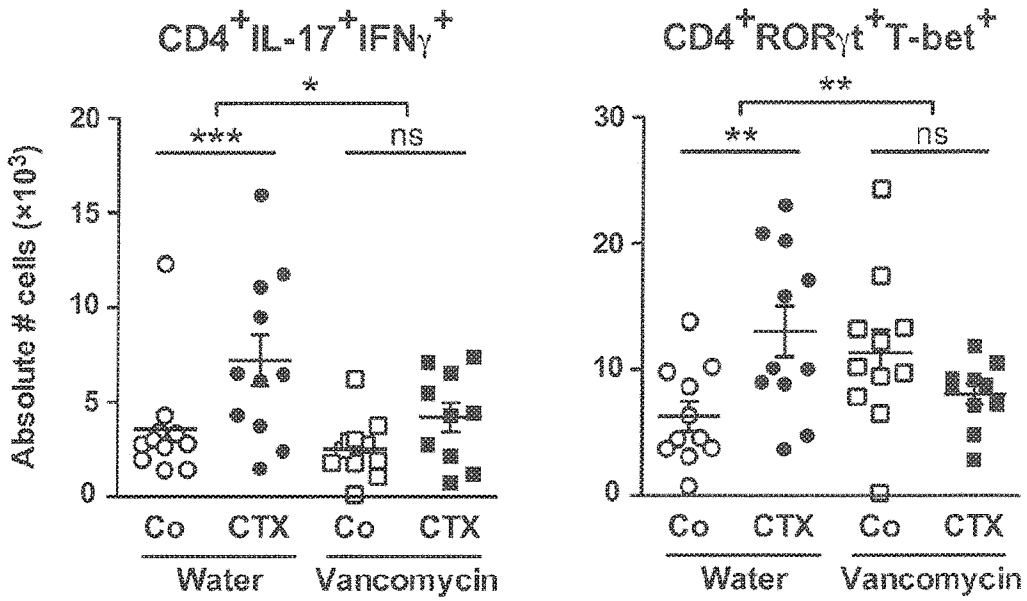

FIGURE 4E

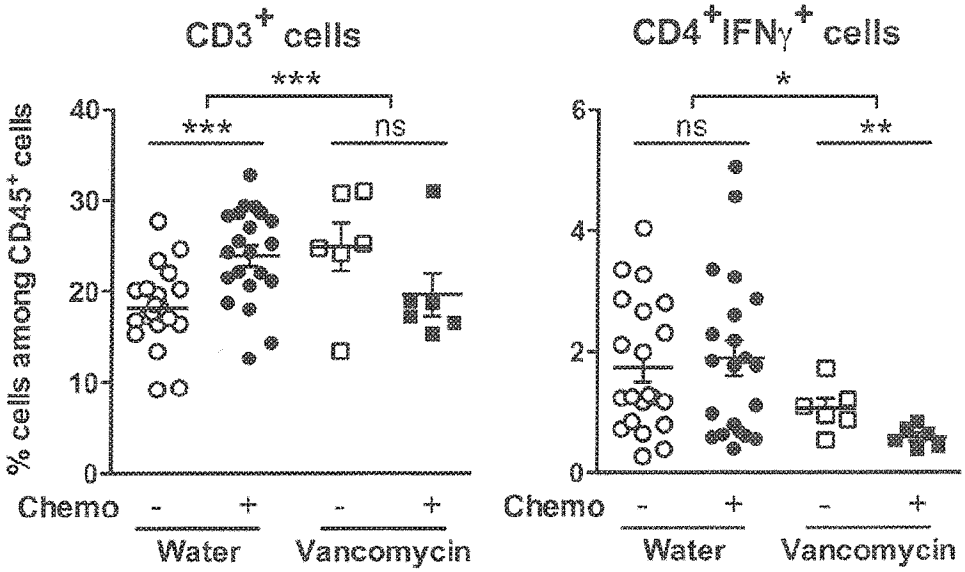

FIGURE 4F

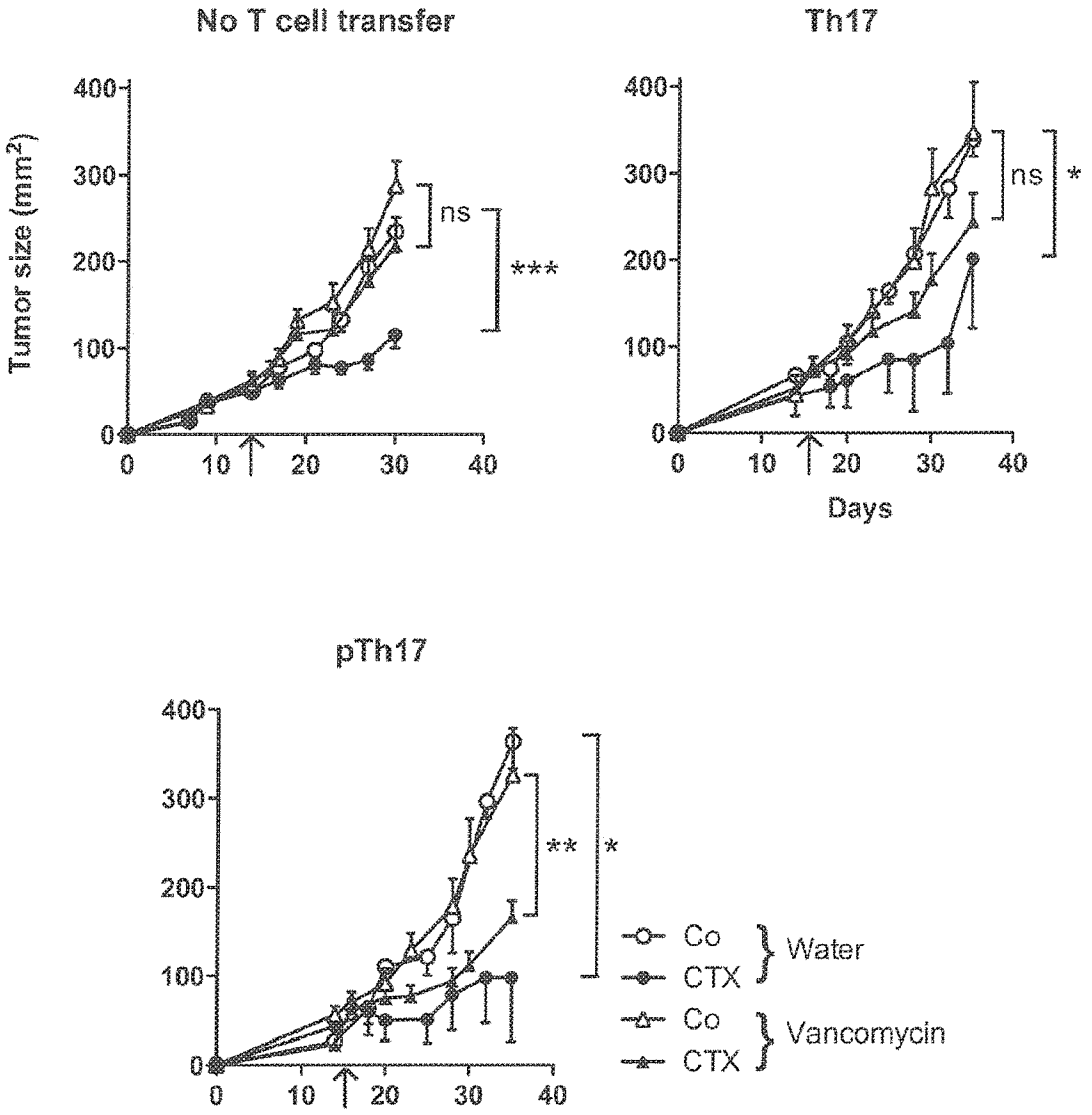

FIGURE 4G

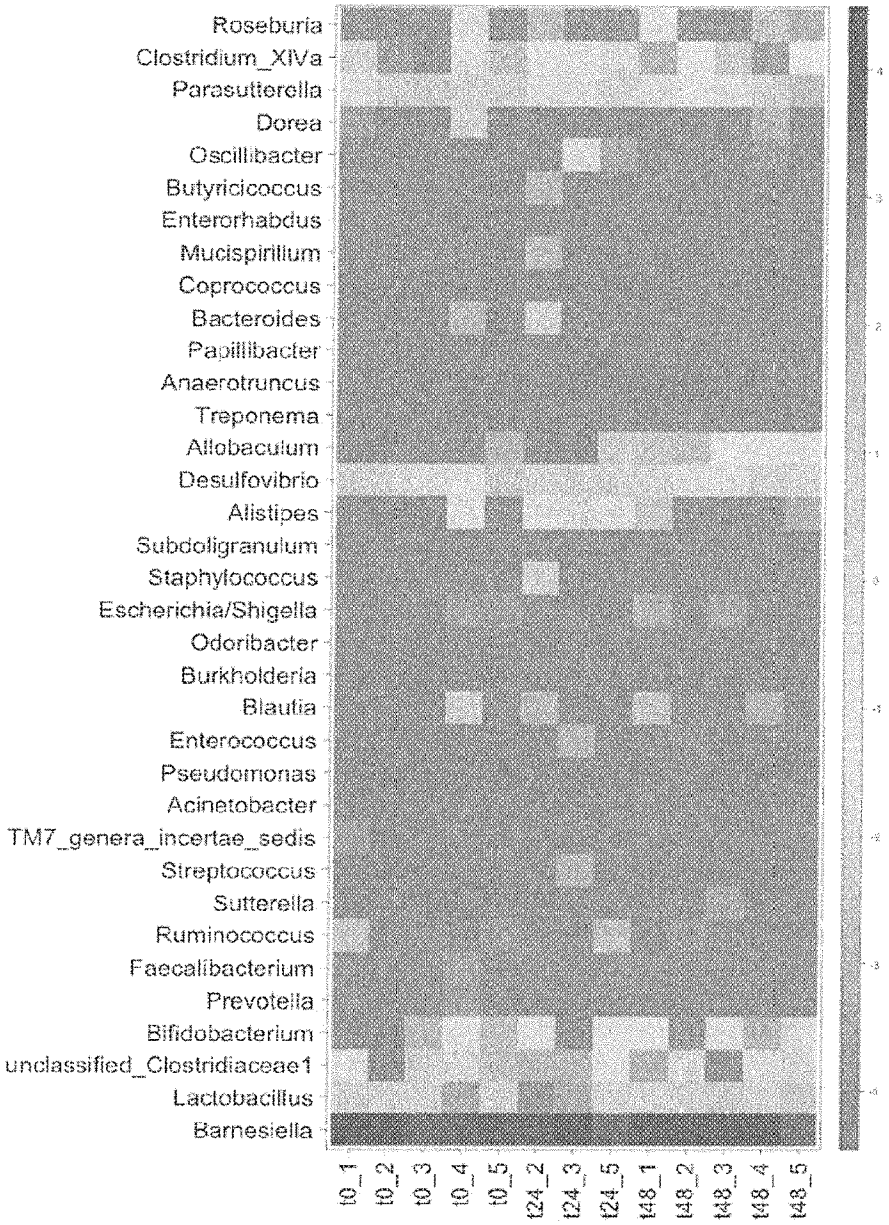

FIGURE 5A

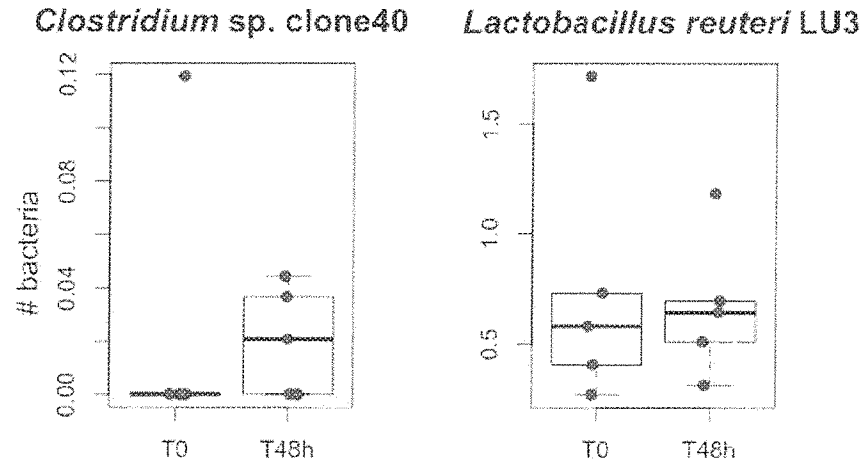

FIGURE 5B

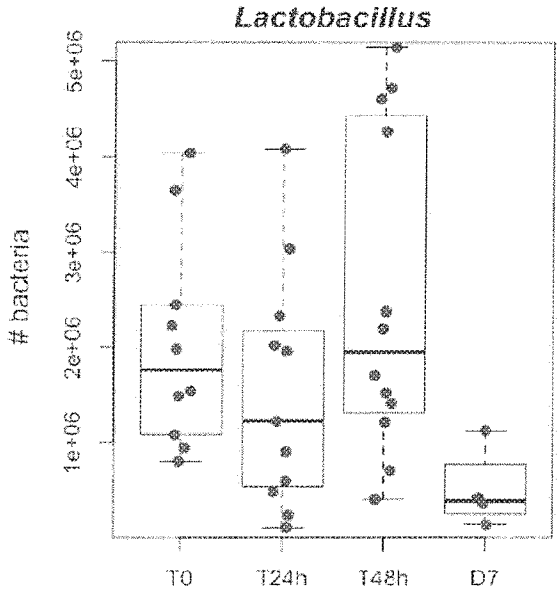

FIGURE 5C

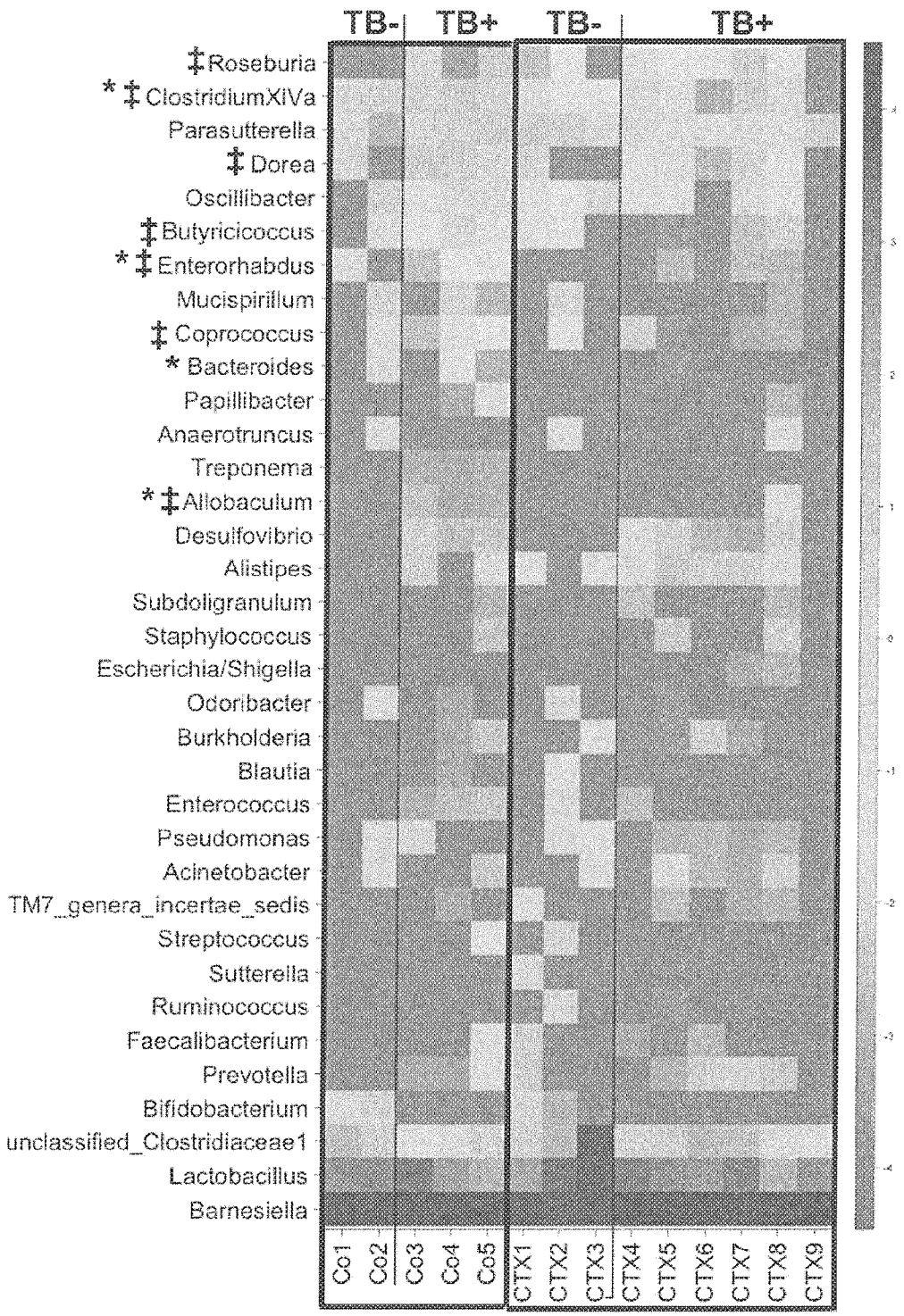

FIGURE 6

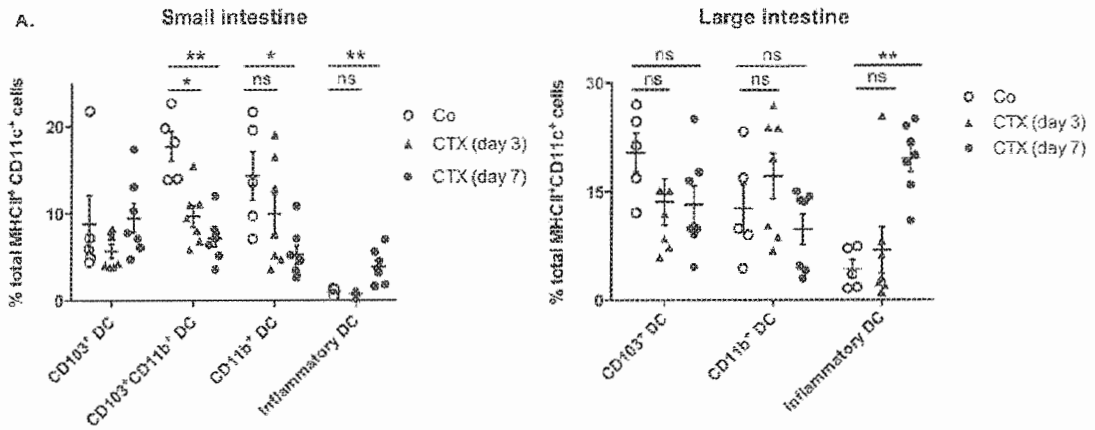

FIGURE 7A

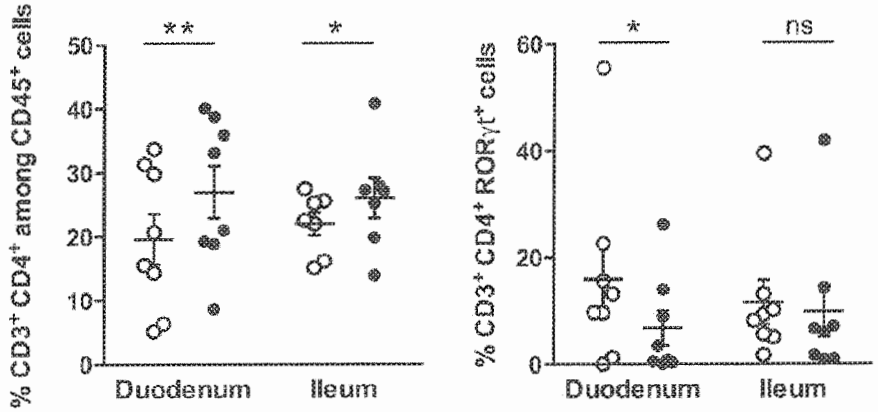

FIGURE 7B

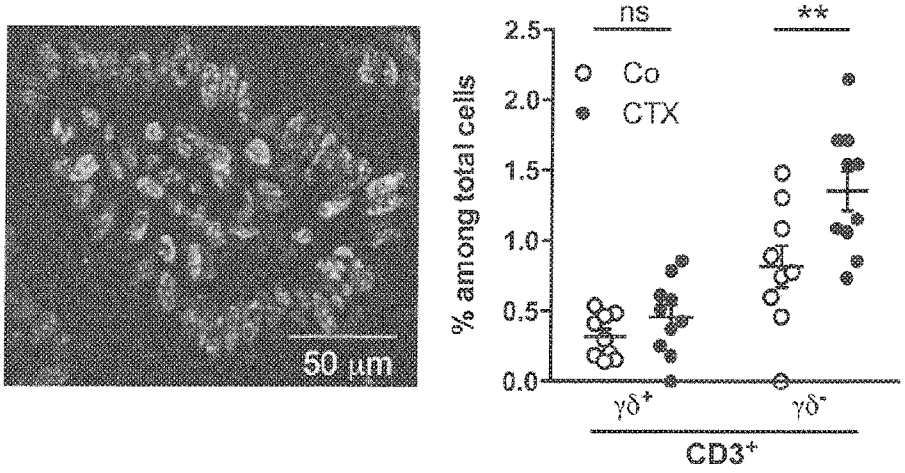

FIGURE 7C

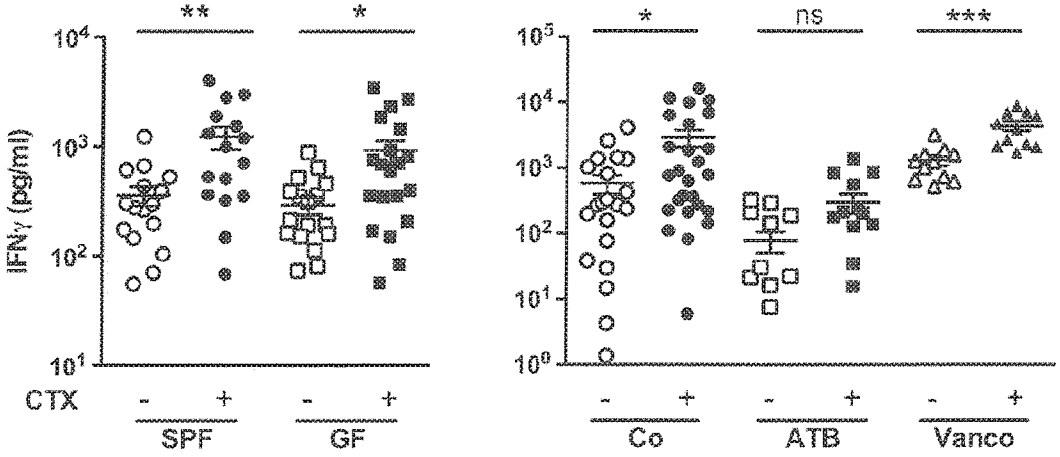

FIGURE 7D

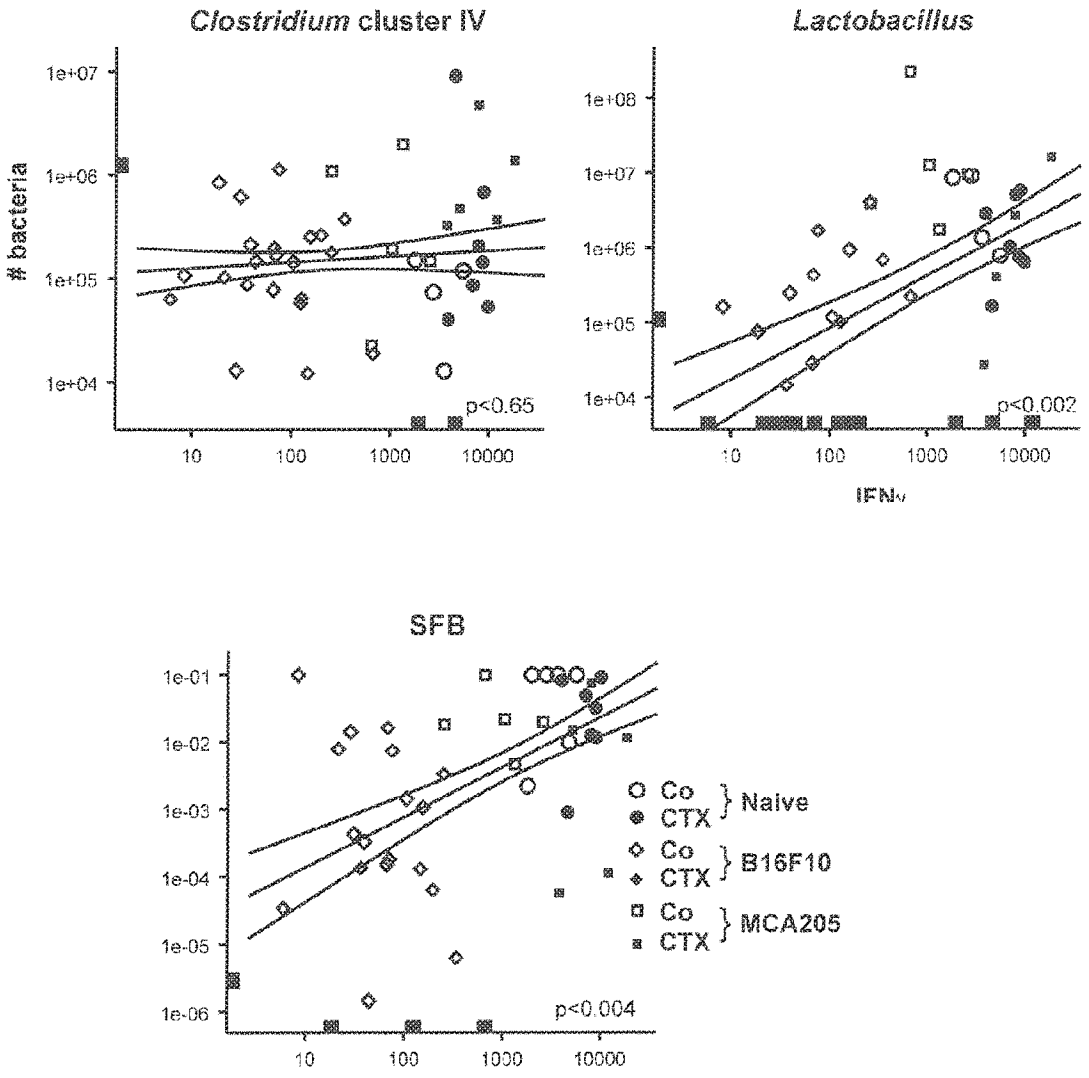

FIGURE 7E

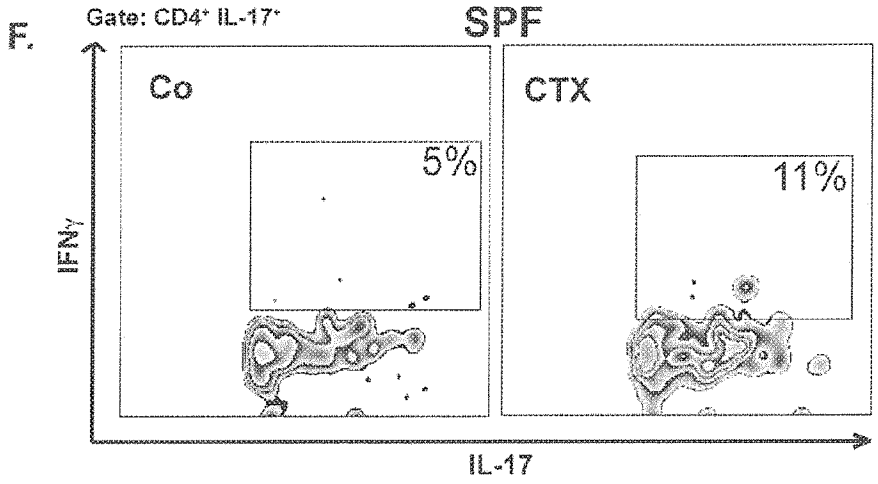

FIGURE 7F

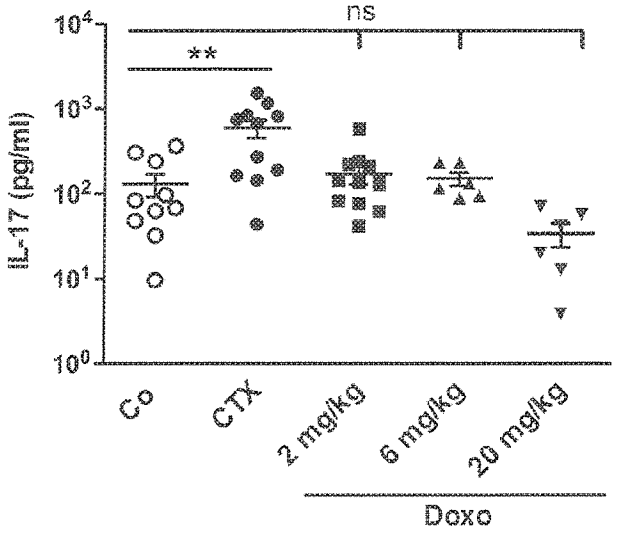

FIGURE 8A

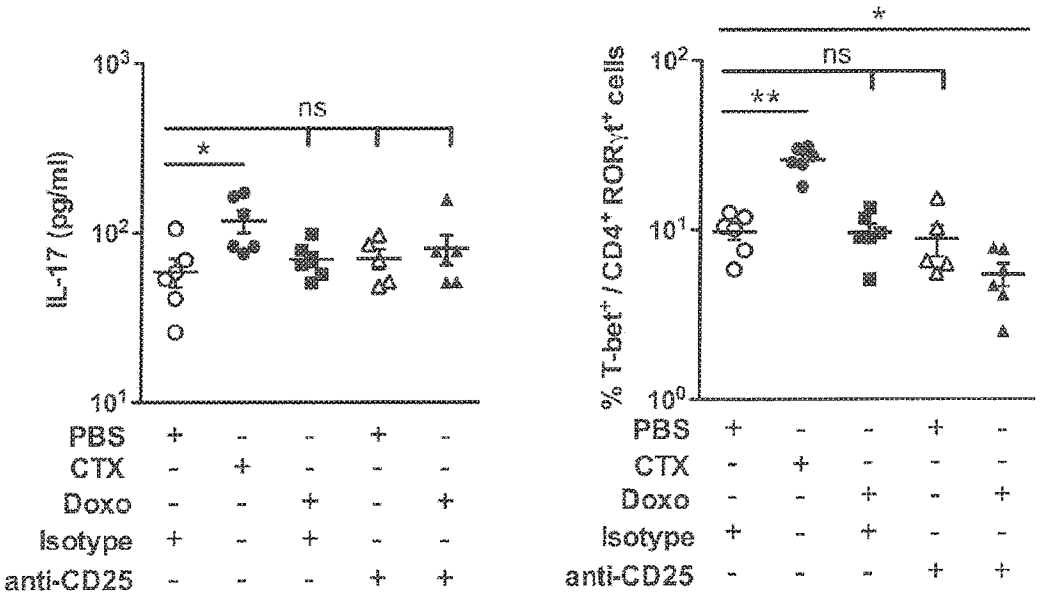

FIGURE 8B

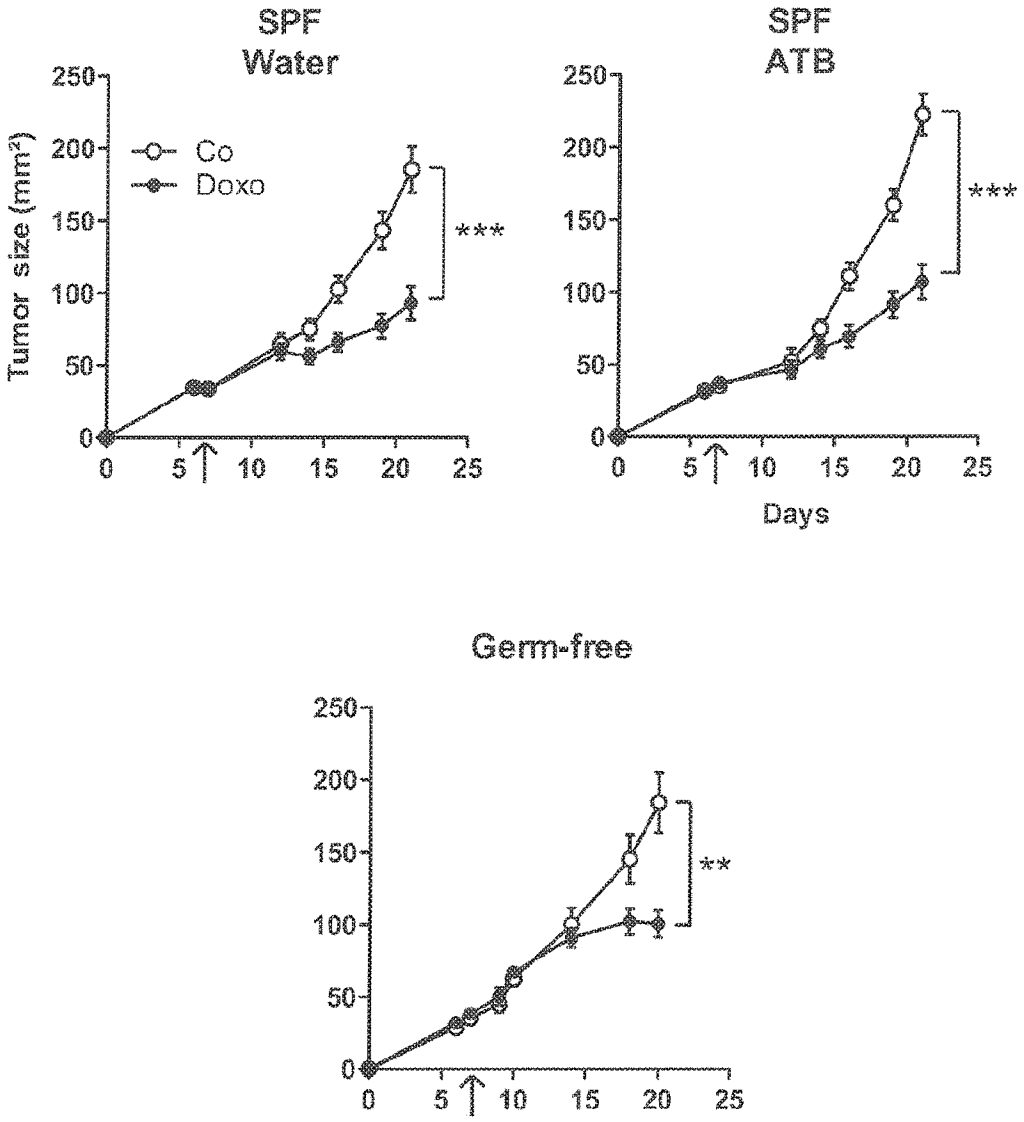

FIGURE 8C

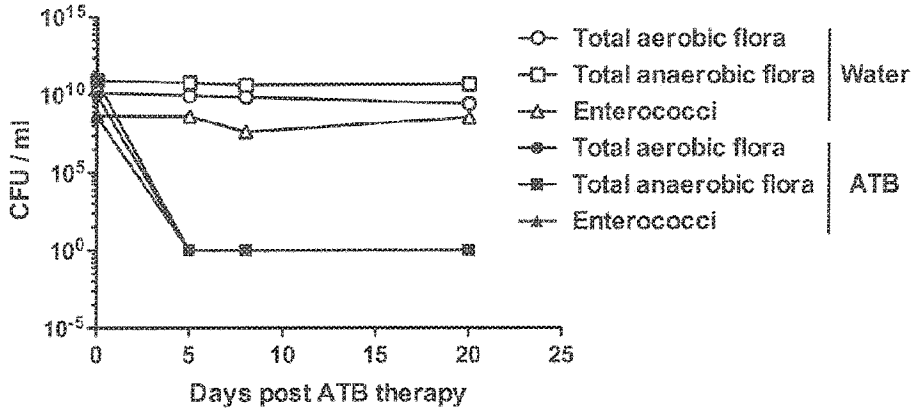

FIGURE 9

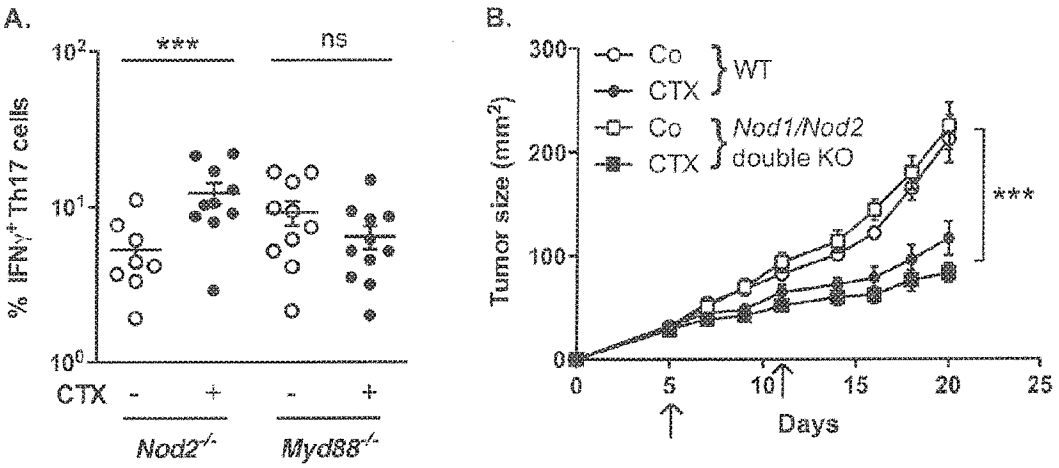

FIGURE 10

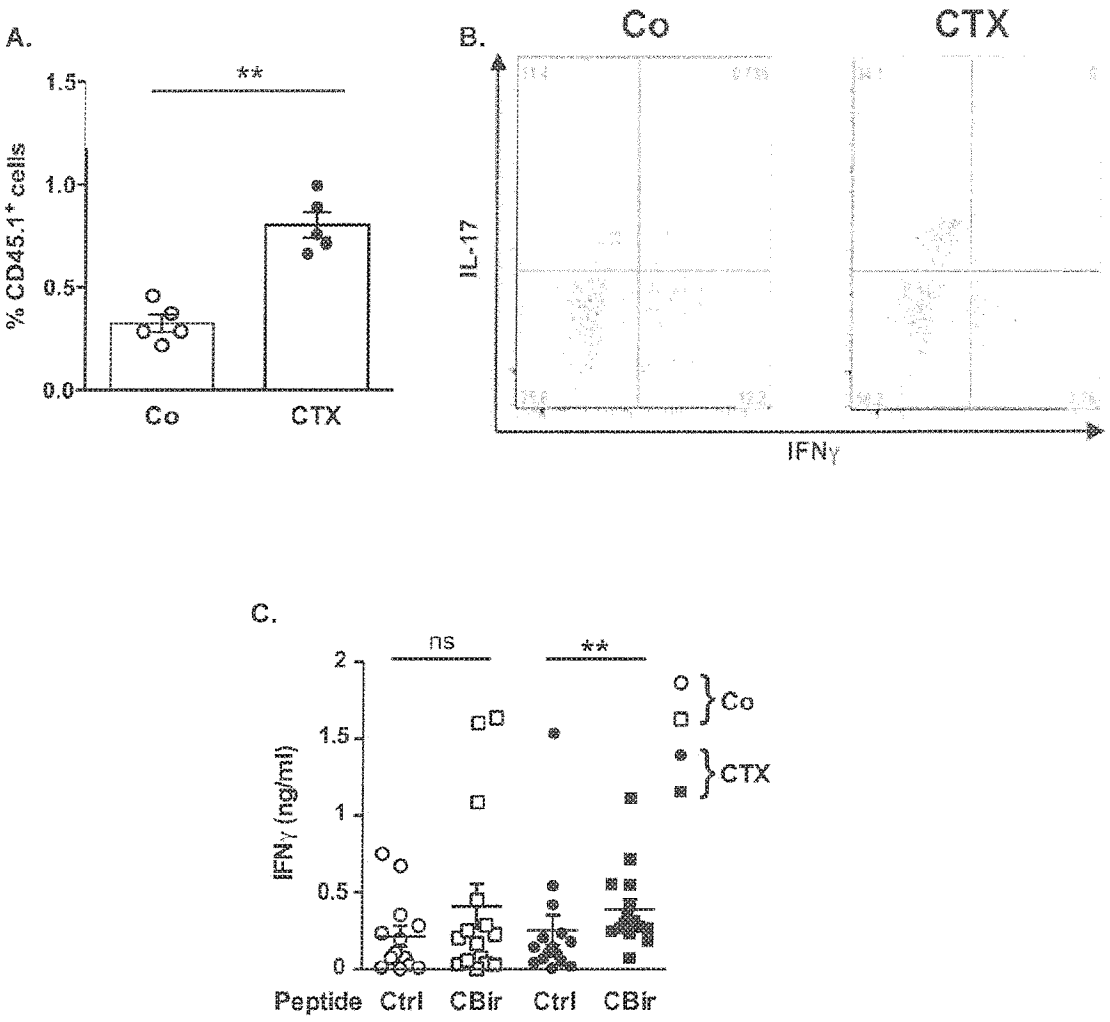

FIGURE 11

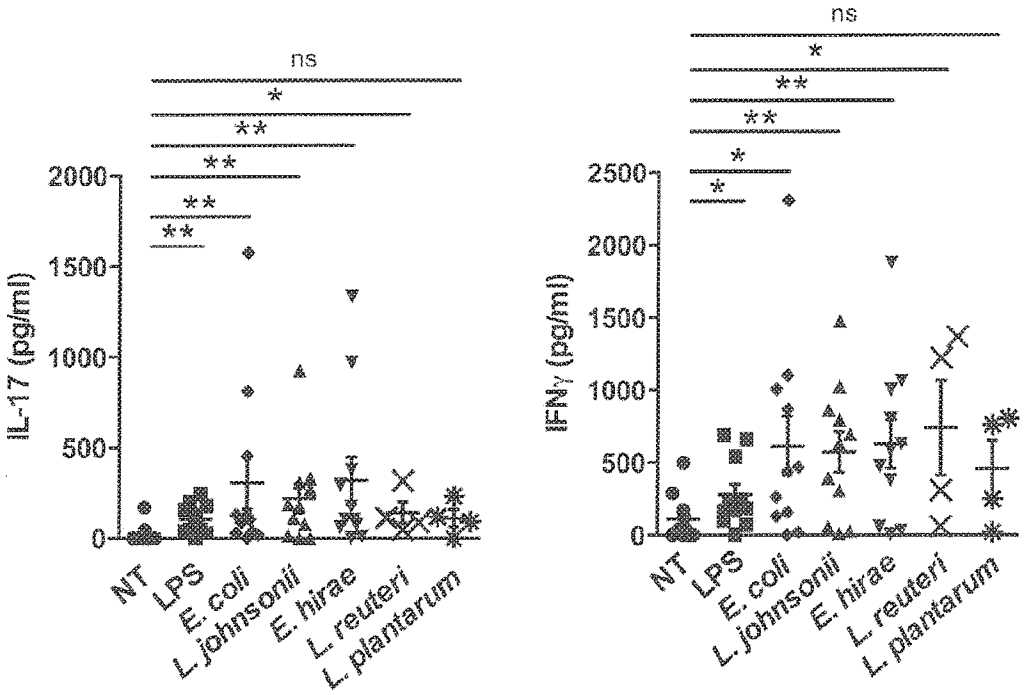

FIGURE 12

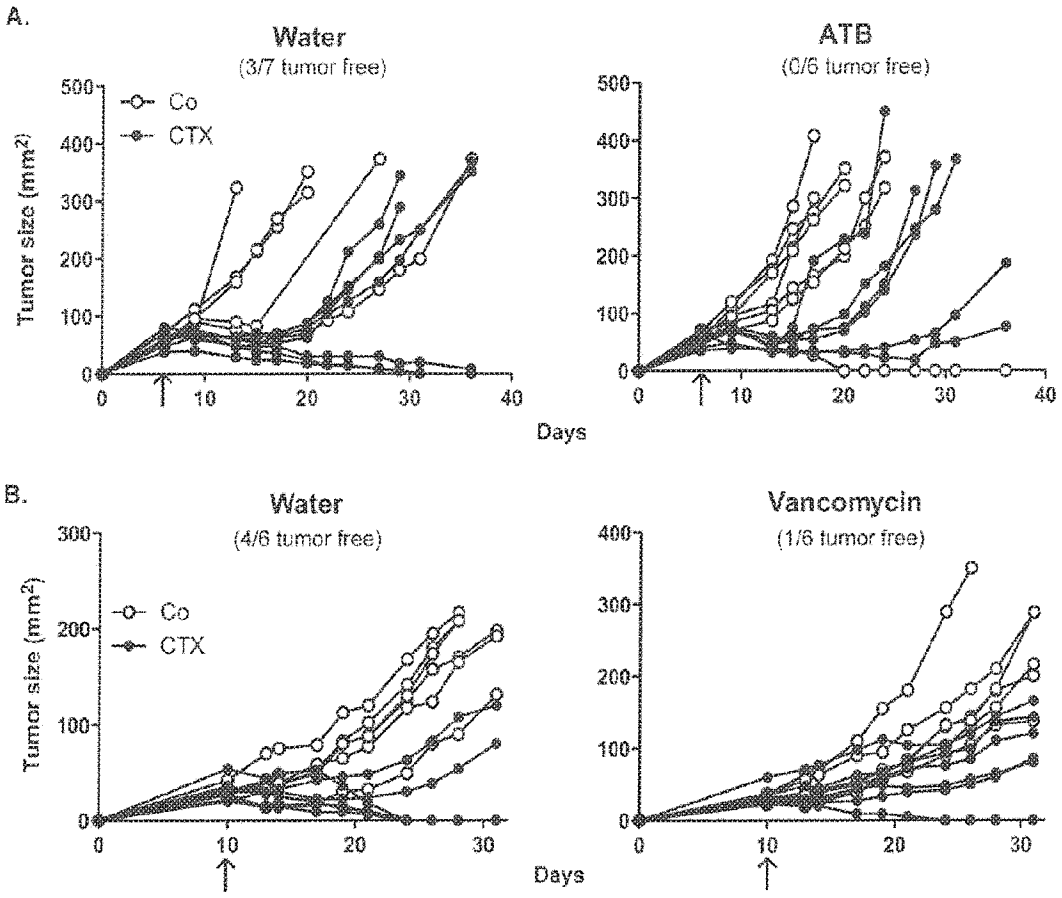

FIGURE 13

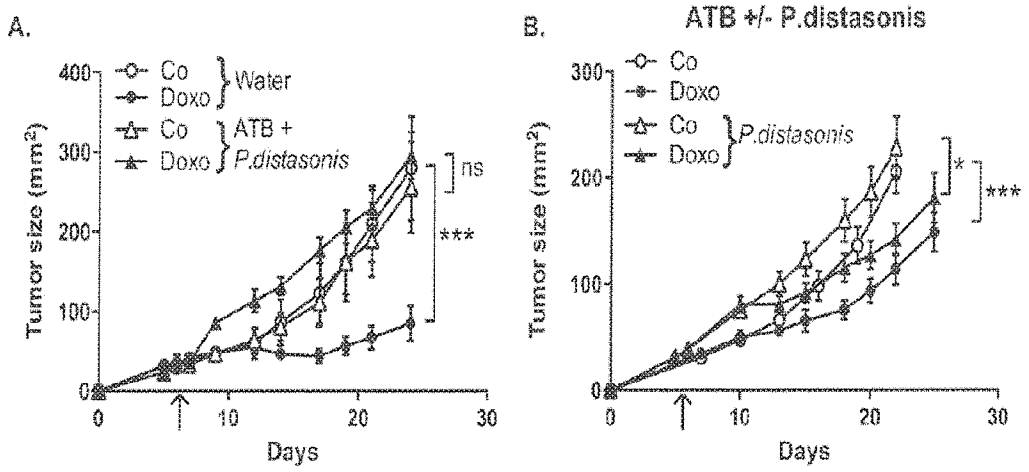

FIGURE 14

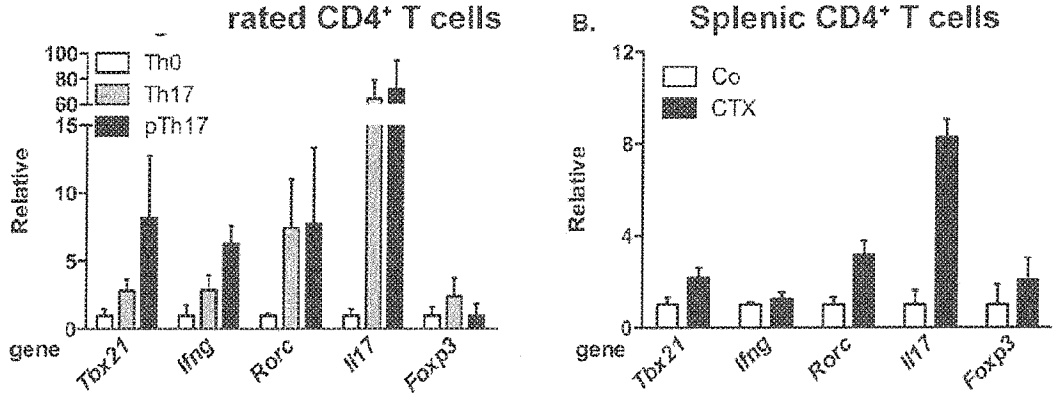

FIGURE 15

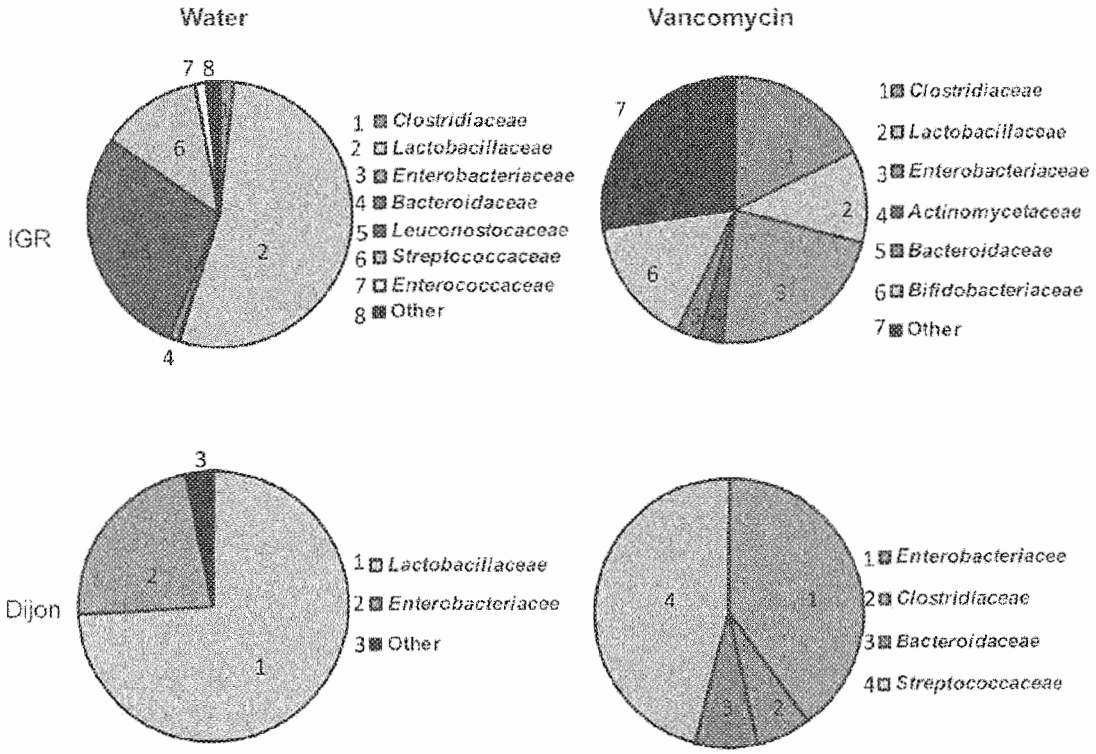

FIGURE 16

1

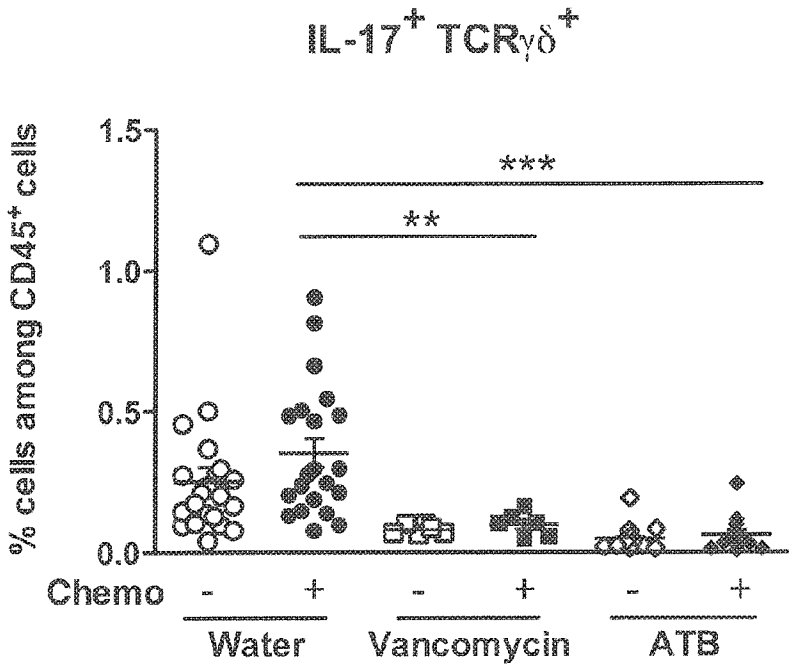

FIGURE 17

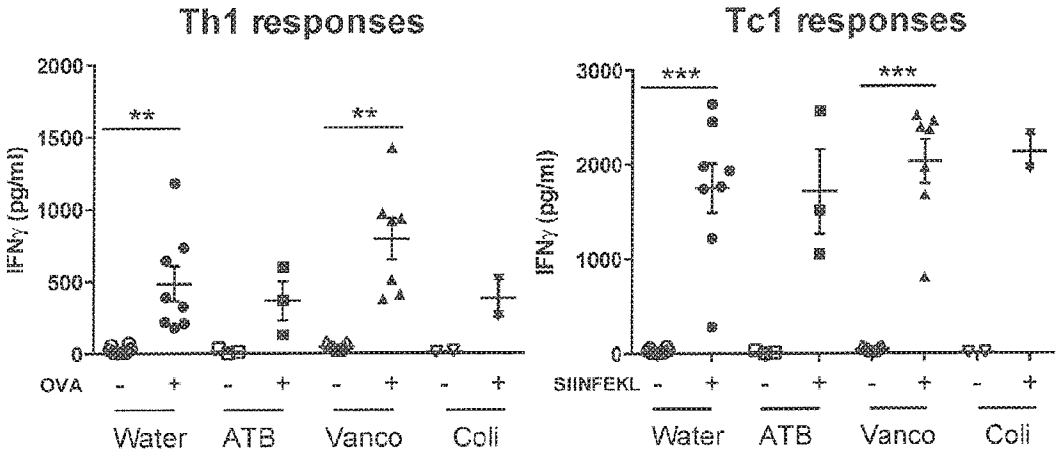

FIGURE 18

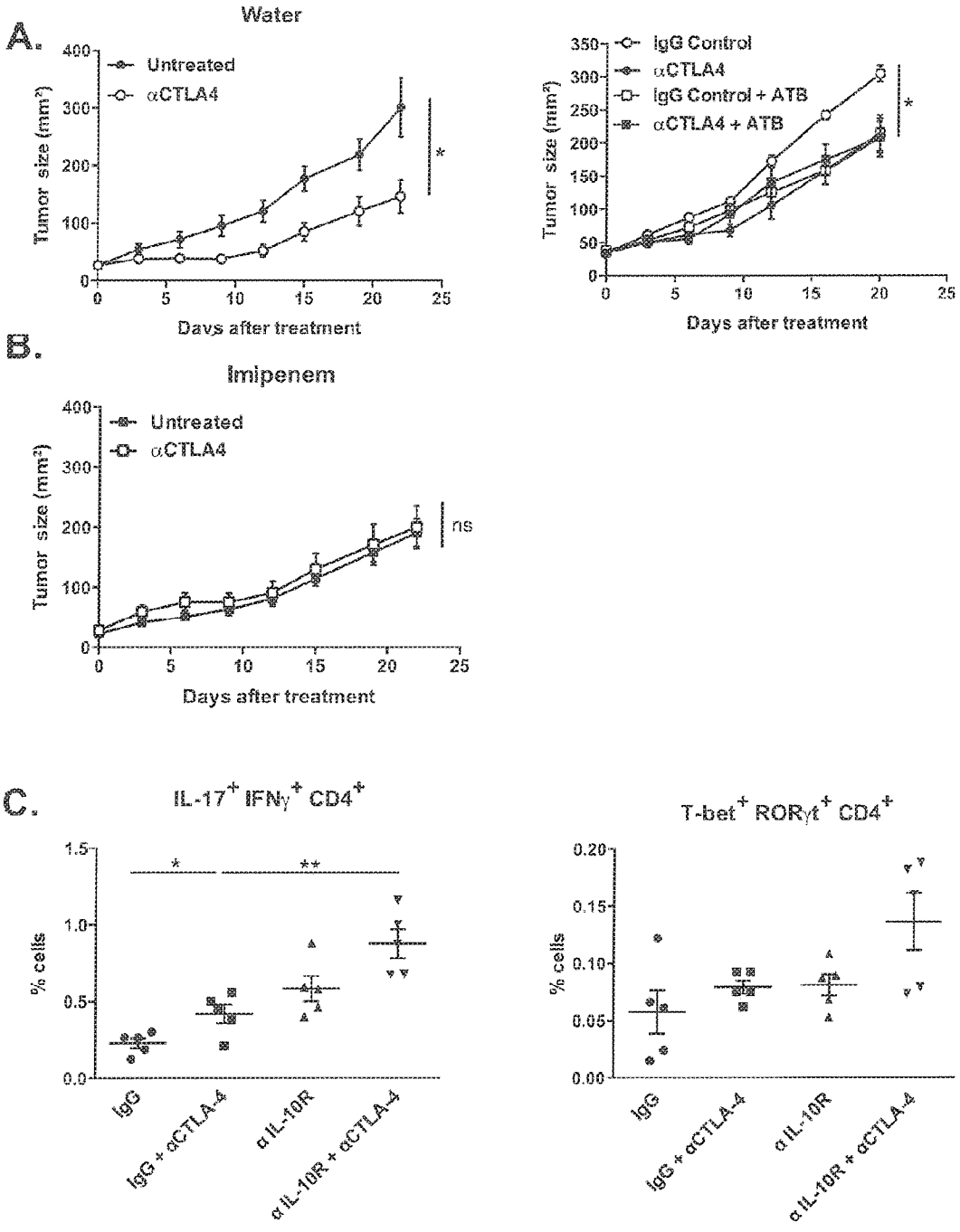

FIGURE 19

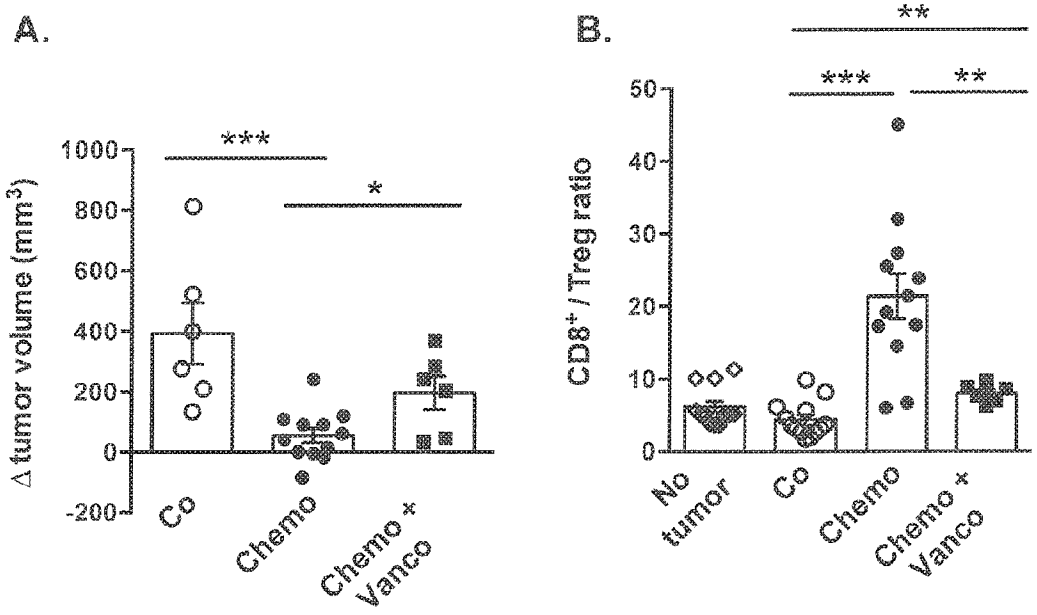

FIGURE 20

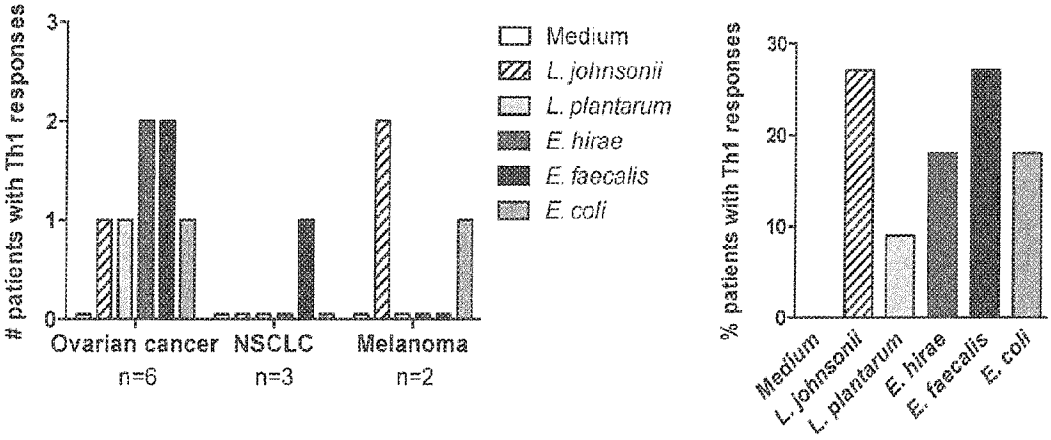

FIGURE 21A

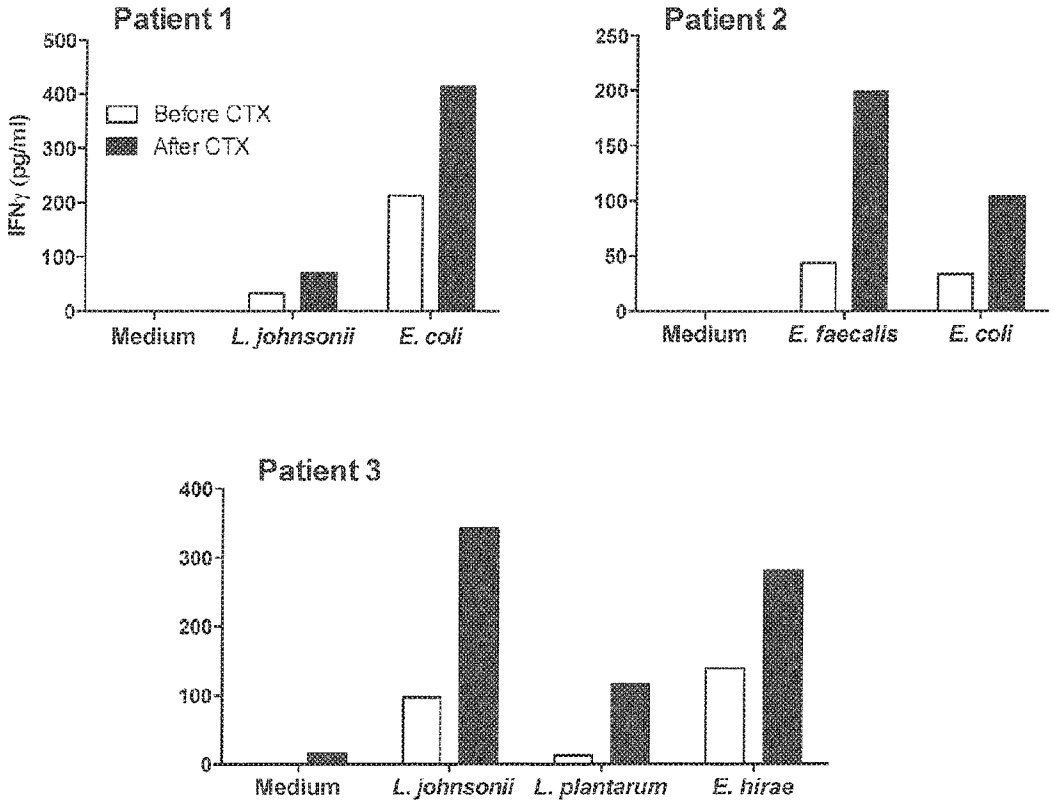

FIGURE 21B

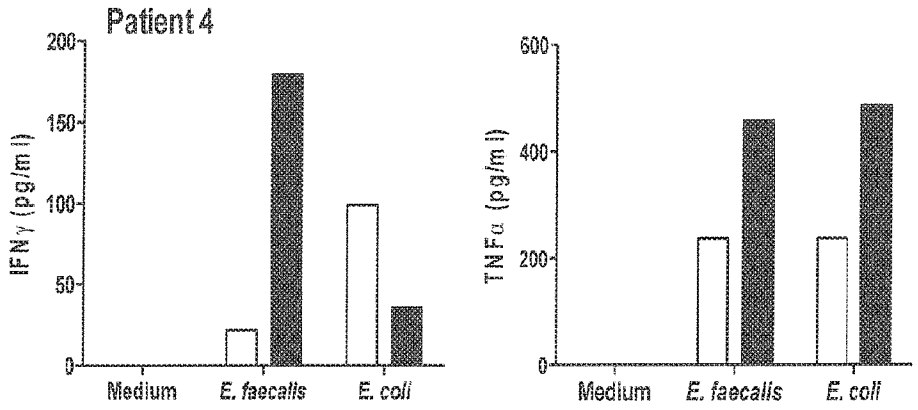

FIGURE 21C

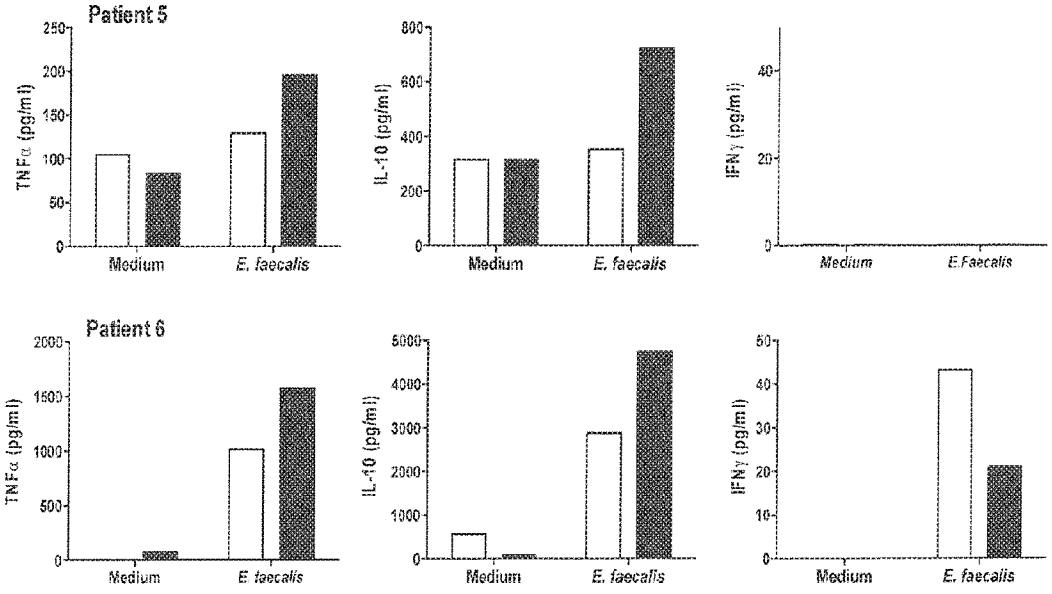

FIGURE 21D

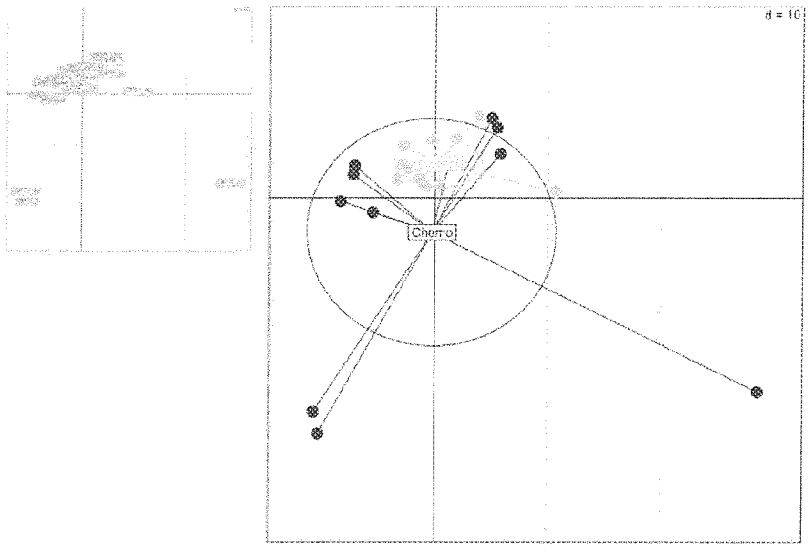

FIGURE 22

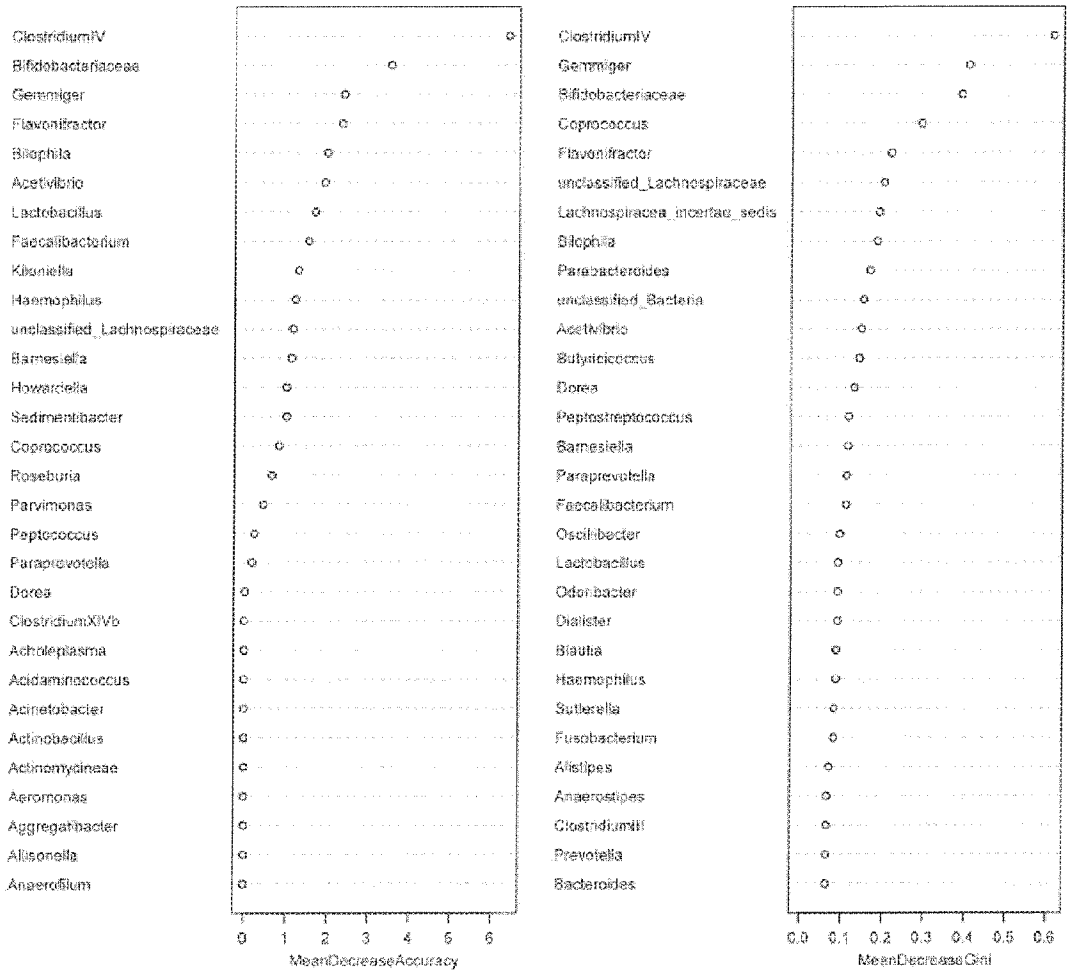

FIGURE 23

FIGURE 24

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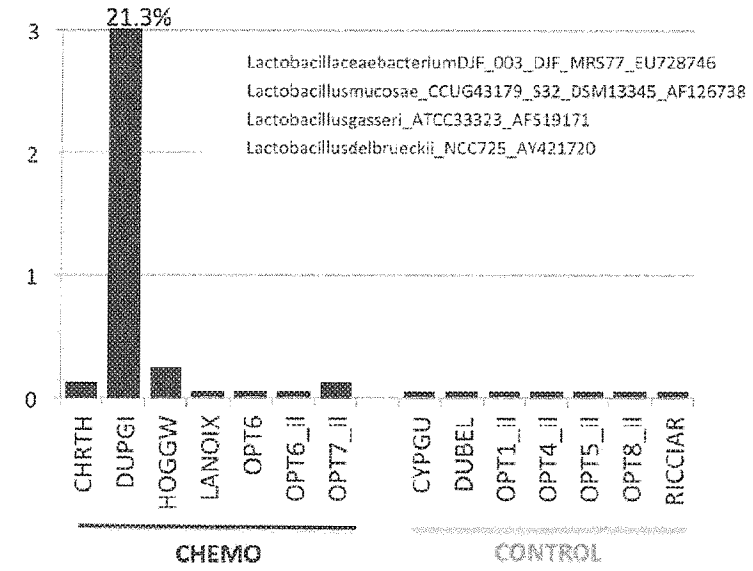

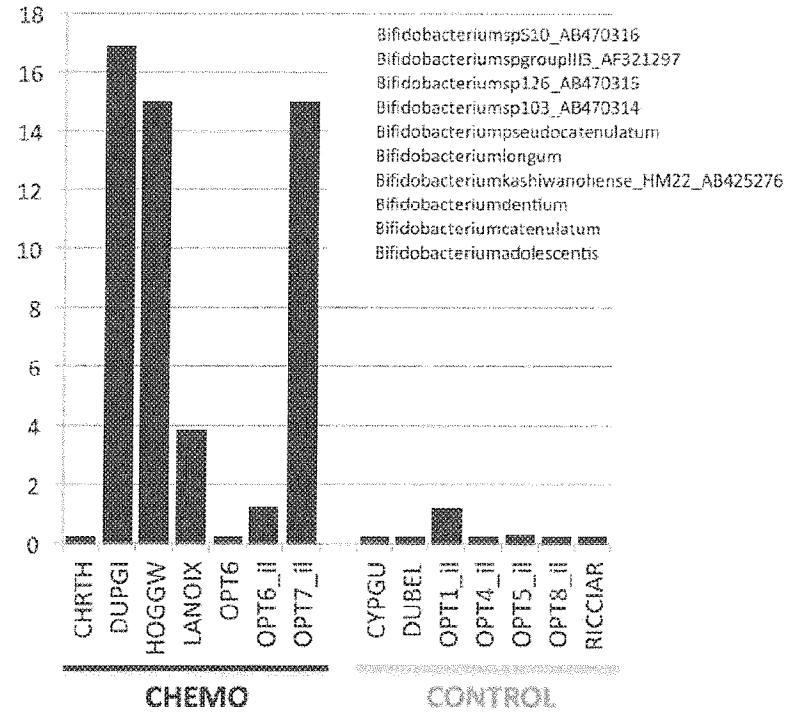

FIGURE 25

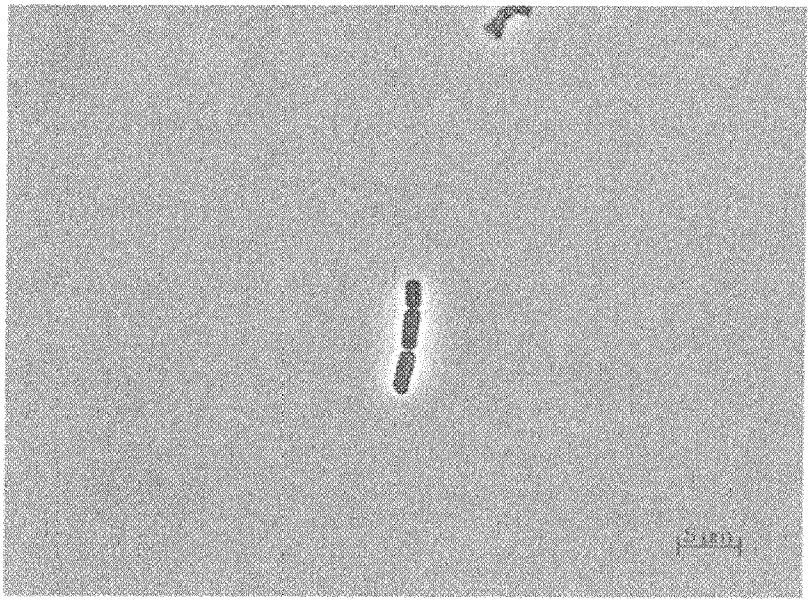

FIGURE 26 A

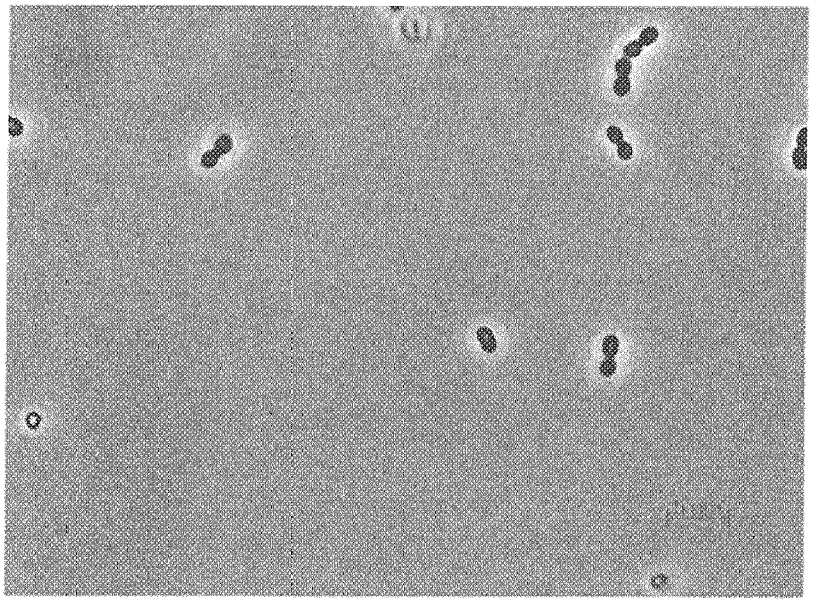

FIGURE 26 B

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EUROPEAN SEARCH REPORT

Application Number
EP 13 30 6597

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A	WO 2011/131472 A1 (ROUSSY INST GUSTAVE [FR]; ZITVOGEL LAURENCE [FR]; KROEMER GUIDO [FR];) 27 October 2011 (2011-10-27) * summary of invention *	1-17	INV. C12Q1/68
A	JUTTA ZWIELEHNER ET AL: "Changes in Human Fecal Microbiota Due to Chemotherapy Analyzed by TaqMan-PCR, 454 Sequencing and PCR-DGGE Fingerprinting", PLOS ONE, vol. 6, no. 12, 14 December 2011 (2011-12-14), page e28654, XP055110348, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0028654 * the whole document *	1-17	
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	27 March 2014	Cornelis, Karen

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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EUROPEAN SEARCH REPORT

Application Number
EP 13 30 6597

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
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Place of search	Date of completion of the search	Examiner
The Hague	27 March 2014	Cornelis, Karen

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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(54) **Title**: DELIVERY OF MRNA FOR THE AUGMENTATION OF PROTEINS AND ENZYMES IN HUMAN GENETIC DISEASES

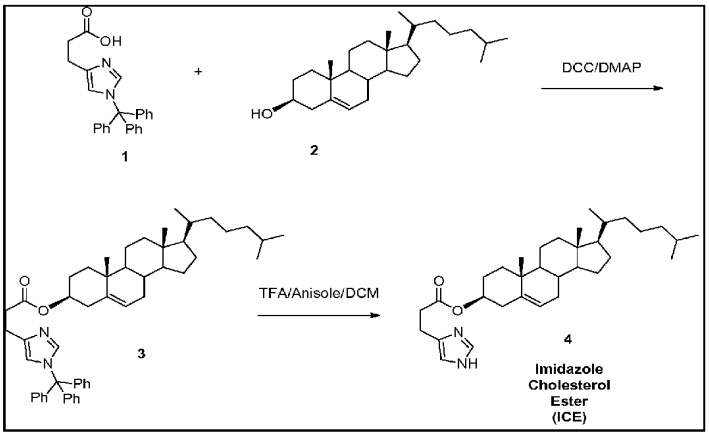

FIG. 1

(57) **Abstract**: Disclosed herein are compositions and methods of modulating the expression of gene or the production of a protein by transfecting target cells with nucleic acids. The compositions disclosed herein demonstrate a high transfection efficacy and are capable of ameliorating diseases associated with protein or enzyme deficiencies.

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DELIVERY OF MRNA FOR THE AUGMENTATION OF PROTEINS AND ENZYMES IN HUMAN GENETIC DISEASES

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 61/265,653, filed December 1, 2009 (Attorney Docket No. SHIR-004-001), the entire teachings of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Novel approaches and therapies are still needed for the treatment of protein and enzyme deficiencies, particularly strategies and therapies which overcome the challenges and limitations associated with the administration of nucleic acids and the transfection of target cells. Additional approaches which modulate or supplement the expression of a deficient protein or enzyme and thus ameliorate the underlying deficiency would be useful in the development of appropriate therapies for associated disorders.

For example, the urea cycle metabolic disorders represent protein and enzyme deficiencies for which there are no currently available cures. The urea cycle is a series of biochemical reactions which occurs in many animals that produce urea ($(NH_2)_2CO$) from ammonia (NH_3) and, in mammals, takes place only in the liver. Specifically, the urea cycle consists of a series of five biochemical reactions and serves two primary functions: the elimination of nitrogen as urea and the synthesis of arginine. Defects in the urea cycle result in the accumulation of ammonia and its precursor amino acids (glutamine, glutamic acid, aspartic acid, and glycine). The

resulting high levels of ammonia are neurotoxic, and the triad of hyperammonemia, encephalopathy, and respiratory alkalosis characterizes the urea cycle disorders.

Ornithine transcarbamylase (OTC) deficiency represents one such urea cycle genetic disorder. Typically, a subject with OTC deficiency has a reduced level of the enzyme OTC. In the classic severe form of OTC deficiency, within the first days of life patients present with lethargy, convulsions, coma and severe hyperammonemia that quickly lead to a deteriorating and fatal outcome absent appropriate medical intervention. If left untreated, complications from OTC deficiency may include developmental delay, mental retardation and/or death.

Treatment of OTC deficient patients primarily involves the regulation of serum ammonia and hemodialysis remains the only effective means to rapidly lower serum ammonia levels. Generally, the treatment goal of urea cycle metabolic disorders is to provide sufficient protein and arginine for growth, development, and energy while preventing the development of hyperammonemia and hyperglutaminemia. Therapeutic approaches that are currently available for the therapeutic management of urea cycle metabolic disorders such as OTC deficiency rely heavily upon dietary management. There are no currently available long-term treatments or cures for urea cycle metabolic disorders. Novel therapies that increase the level or production of an affected protein or enzyme in target cells, such as hepatocytes, or that modulate the expression of nucleic acids encoding the affected protein or enzyme could provide a treatment or even a cure for metabolic disorders, including metabolic disorders such as OTC deficiency.

SUMMARY OF THE INVENTION

Disclosed are methods of intracellular delivery of nucleic acids that are capable of correcting existing genetic defects and/or providing beneficial functions to one or more target cells. Following successful delivery to target tissues and cells, the compositions and nucleic acids of the present invention transfect that target cell and the nucleic acids (e.g., mRNA) can be translated into the gene product of interest (e.g., a functional protein or enzyme) or can otherwise modulate or regulate the presence or expression of the gene product of interest.

The compositions and methods provided herein are useful in the management and treatment of a large number of diseases, in particular diseases which result from protein and/or enzyme deficiencies. Individuals suffering from such diseases may have underlying genetic defects that lead to the compromised expression of a protein or enzyme, including, for example, the non-synthesis of the protein, the reduced synthesis of the protein, or synthesis of a protein lacking or having diminished biological activity. In particular, the methods and compositions provided herein are useful for the treatment of the urea cycle metabolic disorders that occur as a result of one or more defects in the biosynthesis of enzymes involved in the urea cycle. The methods and compositions provided herein are also useful in various *in vitro* and *in vivo* applications in which the delivery of a nucleic acid (e.g., mRNA) to a target cell and transfection of that target cell are desired.

In one embodiment, the compositions provided herein may comprise a nucleic acid, a transfer vehicle and an agent to facilitate contact with, and subsequent transfection of a target cell. The nucleic acid can encode a clinically useful gene product or protein. For example, the nucleic acid may encode a functional urea cycle enzyme. In preferred embodiments, the nucleic acid is RNA, or more preferably mRNA encoding a functional protein or enzyme.

In some embodiments, compositions and methods for increasing expression of a functional protein or enzyme in a target cell are provided. For example, the compositions and methods provided herein may be used to increase the expression of a urea cycle enzyme (e.g., OTC, CPS1, ASS1, ASL or ARG1). In some embodiments, the composition comprises an mRNA and a transfer vehicle. In some embodiments, the mRNA encodes a urea cycle enzyme. In some embodiments the mRNA can comprise one or more modifications that confer stability to the mRNA (e.g., compared to a wild-type or native version of the mRNA) and may also comprise one or more modifications relative to the wild-type which correct a defect implicated in the associated aberrant expression of the protein. For example, the nucleic acids of the present invention may comprise modifications to one or both the 5' and 3' untranslated regions. Such modifications may include, but are not limited to, the inclusion of a partial sequence of a cytomegalovirus (CMV) immediate-early 1 (IE1)

gene, a poly A tail, a Cap1 structure or a sequence encoding human growth hormone (hGH)).

Methods of treating a subject, wherein the subject has a protein or enzyme deficiency are also provided. The methods can comprise administering a composition provided herein. For example, methods of treating or preventing conditions in which production of a particular protein and/or utilization of a particular protein is inadequate or compromised are provided. In one embodiment, the methods provided herein can be used to treat a subject having a deficiency in one or more urea cycle enzymes. The method can comprise contacting and transfecting target cells or tissues (such as hepatocytes that are deficient in one or more urea cycle enzymes) with a composition provided herein, wherein the nucleic acid encodes the deficient urea cycle enzyme. In this manner, the expression of the deficient enzyme in the target cell is increased, which in turn is expected to ameliorate the effects of the underlying enzyme deficiency. The protein or enzyme expressed by the target cell from the translated mRNA may be retained within the cytosol of the target cell or alternatively may be secreted extracellularly. In some embodiments, the nucleic acid is an mRNA. In some embodiments, the mRNA comprises a modification that confers stability to the mRNA code (e.g., when compared to the wild-type or native version of the mRNA). For example, the mRNA encoding a functional enzyme may comprise one or more modifications to one or both the 5' and 3' untranslated regions.

In a preferred embodiment, the nucleic acids (e.g., mRNA) provided herein are formulated in a lipid or liposomal transfer vehicle to facilitate delivery to the target cells and/or to stabilize the nucleic acids contained therein. Contemplated transfer vehicles may comprise one or more cationic lipids, non-cationic lipids, and/or PEG-modified lipids. For example, the transfer vehicle may comprise a mixture of the lipids CHOL, DOPE, DLinDMA and DMG-PEG-2000. In another embodiment, the transfer vehicle may comprise the lipids ICE, DOPE and DMG-PEG-2000. In still another embodiment the transfer vehicle may comprise one or more lipids selected from the group consisting of ICE, DSPC, CHOL, DODAP, DOTAP and C8-PEG-2000 ceramide. In a preferred embodiment, the transfer vehicle is a liposome or a lipid nanoparticle which is capable of preferentially distributing to the target cells and tissues *in vivo*.

Methods of expressing a functional protein or enzyme (e.g., a urea cycle enzyme) in a target cell are also provided. In some embodiments, the target cell is deficient in a urea cycle enzyme. The methods comprise contacting the target cell with a composition comprising an mRNA and a transfer vehicle. Following expression of the protein or enzyme encoded by the mRNA, the expressed protein or enzyme may be retained within the cytosol of the target cell or alternatively may be secreted extracellularly. In some embodiments, the mRNA encodes a urea cycle enzyme. In some embodiments the mRNA can comprise one or more modifications that confer stability to the mRNA and may also comprise one or more modifications relative to the wild-type that correct a defect implicated in the associated aberrant expression of the protein. In some embodiments, the compositions and methods of the present invention rely on the target cells to express the functional protein or enzyme encoded by the exogenously administered nucleic acid (e.g., mRNA). Because the protein or enzyme encoded by the exogenous mRNA are translated by the target cell, the proteins and enzymes expressed may be characterized as being less immunogenic relative to their recombinantly prepared counterparts.

Also provided are compositions and methods useful for facilitating the transfection and delivery of one or more nucleic acids (e.g., mRNA) to target cells. For example, the compositions and methods of the present invention contemplate the use of targeting ligands capable of enhancing the affinity of the composition to one or more target cells. In one embodiment, the targeting ligand is apolipoprotein-B or apolipoprotein-E and corresponding target cells express low-density lipoprotein receptors, thereby facilitating recognition of the targeting ligand. The methods and compositions of the present invention may be used to preferentially target a vast number of target cells. For example, contemplated target cells include, but are not limited to, hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, lung cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

The above discussed and many other features and attendant advantages of the present invention will become better understood by reference to the following detailed description of the invention when taken in conjunction with the accompanying examples. The various embodiments described herein are complimentary and can be combined or used together in a manner understood by the skilled person in view of the teachings contained herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the synthesis of the imidazole cholesterol ester lipid ICE.

FIG. 2 illustrates the presence of firefly luciferase activity produced from the delivery of exogenous mRNA in the livers and spleens of treated and untreated CD-1 mice.

FIG. 3 illustrates codon-optimized firefly luciferase mRNA *in situ* hybridization in control and treated (B1 and B2) mouse livers observed on x-ray film under low (2X) magnification. (A) represents cresyl violet staining of control (Ct) and treated liver sections B1 and B2 mice; (B) represents X-ray film autoradiography detection by antisense probes of CO-FF luciferase mRNA in B1 and B2 mouse livers; and (C) represents control (sense) hybridization. The abbreviations "cv", "as" and "s" correspond to cresyl violet, antisense, and sense, respectively.

FIG. 4 illustrates codon-optimized firefly luciferase mRNA labeling in treated (B1) and control livers. (A) represents emulsion autoradiography detection of CO-FF luciferase mRNA in a B1 liver section seen as bright labeling under darkfield illumination; (B) represents the same region as (A) seen under brightfield illumination using cresyl violet as a counter-stain; (C) represents B1 liver section treated with the CO-FF luciferase control (sense) riboprobe establishing the level of non-specific labeling; (D) represents the same region as (C) seen under brightfield illumination; (E) represents untreated control liver section treated with CO-FF luciferase antisense probe, no signal was detected; (F) represents the same region as (E) seen under brightfield illumination; (G) represents control liver section treated with the CO-FF luciferase control (sense) riboprobe establishing the level of non-specific labeling; and (H) represents the same region as (G) seen under brightfield illumination. The abbreviations "BD", "HA", "H", "PV", "as" and "s" correspond to bile duct, hepatic

artery, hepatocyte, portal vein, antisense and sense respectively. Magnification: 100X.

FIG. 5 illustrates immunohistochemical staining of mouse livers for the detection of firefly luciferase protein. (A) represents negative luciferase staining for control liver of mouse treated with 1x PBS (20X); (B) represents positive luciferase protein detection via immunohistochemical fluorescence-based methods, demonstrating that firefly luciferase protein is observed in the hepatocytes (20X), as well as a small number of sinusoidal endothelial cells that were positive for luciferase protein as well; (C) represents a positive firefly luciferase protein staining shown at higher magnification (40X). Luciferase protein is observed throughout the cytoplasm of the hepatocytes. The abbreviations (S) and (H) correspond to sinusoidal cells and hepatocytes, respectively.

FIG. 6 shows the nucleotide sequence of CO-FF luciferase mRNA (SEQ ID NO: 1).

FIG. 7 shows the nucleotide sequences of a 5' CMV sequence (SEQ ID NO: 2) and a 3' hGH sequence (SEQ ID NO: 3) which may be used to flank an mRNA sequence of interest.

DETAILED DESCRIPTION OF THE INVENTION

Disclosed herein are compositions that facilitate the delivery of nucleic acids to, and the subsequent transfection of, target cells. In particular, the compositions provided herein are useful for the treatment of diseases which result from the deficient production of proteins and/or enzymes. For example, suitable diseases that may be treated are those in which a genetic mutation in a particular gene causes affected cells to not express, have reduced expression of, or to express a non-functional product of that gene. Contacting such target cells with the compositions of the present invention such that the target cells are transfected with a nucleic acid encoding a functional version of the gene product allows the production of a functional protein or enzyme product this is useful in the treatment of such deficiency.

Provided herein are compositions for modulating the expression of a protein in a target cell. In some embodiments, the composition comprises an RNA molecule and a transfer vehicle. Compositions for increasing expression of a urea cycle

enzyme in a target cell are also provided. The compositions comprise, for example, an mRNA and a transfer vehicle. The mRNA encodes, for example, a functional urea cycle enzyme. In some embodiments, the mRNA of the composition can be modified to impart enhanced stability (e.g., relative to the wild-type version of the mRNA and/or the version of the mRNA found endogenously in the target cell). For example, the mRNA of the composition can include a modification compared to a wild-type version of the mRNA, wherein the modification confers stability to the mRNA of the composition.

Methods of expressing a urea cycle enzyme in a target cell are provided. In some embodiments, the target cell is deficient in a urea cycle enzyme. The methods provided herein comprise contacting the target cell with a composition comprising an mRNA and a transfer vehicle, wherein the mRNA encodes one or more urea cycle enzymes. In some embodiments, the mRNA of the composition is more stable than the wild-type version of the mRNA and/or more stable than the version of the mRNA found endogenously in the target cell.

Methods of treating a subject with a urea cycle deficiency are provided. The methods comprise administering a composition comprising an mRNA and a transfer vehicle, wherein the mRNA encodes a urea cycle enzyme. In some embodiments, the mRNA of the composition is more stable than the wild-type version of the mRNA and/or more stable than the version of the mRNA found endogenously in the target.

Provided herein are methods of and compositions for modulating the level of mRNA and/or the expression of proteins. In some embodiments, the compositions provided herein are capable of modulating the expression of a particular protein by decreasing expression of mRNA encoding that protein in a target cell or tissue. For example, in one embodiment, the composition comprises a miRNA or a nucleic acid encoding miRNA where the miRNA is capable of reducing or eliminating expression of a particular mRNA in a target cell. In some embodiments, the nucleic acid of the composition is more stable (e.g., limited nuclease susceptibility) compared to a wild-type and/or endogenous version of the nucleic acid.

As used herein, the term "nucleic acid" refers to genetic material (e.g., oligonucleotides or polynucleotides comprising DNA or RNA). In some embodiments, the nucleic acid of the compositions is RNA. Suitable RNA includes

mRNA, siRNA, miRNA, snRNA and snoRNA. Contemplated nucleic acids also include large intergenic non-coding RNA (lincRNA), which generally do not encode proteins, but rather function, for example, in immune signaling, stem cell biology and the development of disease. (See, e.g., Guttman, et al., 458: 223-227 (2009); and Ng, et al., Nature Genetics 42: 1035-1036 (2010), the contents of which are incorporated herein by reference). In a preferred embodiment, the nucleic acids of the invention include RNA or stabilized RNA encoding a protein or enzyme. The present invention contemplates the use of such nucleic acids (and in particular RNA or stabilized RNA) as a therapeutic capable of facilitating the expression of a functional enzyme or protein, and preferably the expression of a functional enzyme of protein in which a subject is deficient (e.g., a urea cycle enzyme). The term "functional", as used herein to qualify a protein or enzyme, means that the protein or enzyme has biological activity, or alternatively is able to perform the same, or a similar function as the native or normally-functioning protein or enzyme. The subject nucleic acid compositions of the present invention are useful for the treatment of a various metabolic or genetic disorders, and in particular those genetic or metabolic disorders which involve the non-expression, misexpression or deficiency of a protein or enzyme.

In the context of the present invention the term "expression" is used in its broadest sense to refer to either the transcription of a specific gene or nucleic acid into at least one mRNA transcript, or the translation of at least one mRNA or nucleic acid into a protein or enzyme. For example, contemplated by the present invention are compositions which comprise one or more mRNA nucleic acids which encode functional proteins or enzymes, and in the context of such mRNA nucleic acids, the term expression refers to the translation of such mRNA to produce the protein or enzyme encoded thereby.

The nucleic acids provided herein can be introduced into cells or tissues of interest. In some embodiments, the nucleic acid is capable of being expressed (e.g., the transcription of mRNA from a gene), translated (e.g., the translation of the encoded protein or enzyme from a synthetic or exogenous mRNA transcript) or otherwise capable of conferring a beneficial property to the target cells or tissues (e.g., reducing the expression of a target nucleic acid or gene). The nucleic acid may encode, for example, a hormone, enzyme, receptor, polypeptide, peptide or other

protein of interest. A nucleic acid may also encode a small interfering RNA (siRNA) or antisense RNA for the purpose of decreasing or eliminating expression of an endogenous nucleic acid or gene. In one embodiment of the present invention, the nucleic acid (e.g., mRNA encoding a deficient protein or enzyme) may optionally have chemical or biological modifications which, for example, improve the stability and/or half-life of such nucleic acid or which improve or otherwise facilitate translation.

The nucleic acids of the present invention may be natural or recombinant in nature and may exert their therapeutic activity using either sense or antisense mechanisms of action.

Also contemplated by the present invention is the co-delivery of one or more unique nucleic acids to target cells, for example, by combining two unique nucleic acids into a single transfer vehicle. In one embodiment of the present invention, a therapeutic first nucleic acid, such as mRNA encoding galactose-1-phosphate uridyltransferase (GALT), and a therapeutic second nucleic acid, such as mRNA encoding galatokinase (GALK), may be formulated in a single transfer vehicle and administered (e.g., for the treatment of galactosemia). The present invention also contemplates co-delivery and/or co-administration of a therapeutic first nucleic acid and a second nucleic acid to facilitate and/or enhance the function or delivery of the therapeutic first nucleic acid. For example, such a second nucleic acid (e.g., exogenous or synthetic mRNA) may encode a membrane transporter protein that upon expression (e.g., translation of the exogenous or synthetic mRNA) facilitates the delivery or enhances the biological activity of the first nucleic acid. Alternatively, the therapeutic first nucleic acid may be administered with a second nucleic acid that functions as a "chaperone" for example, to direct the folding of either the therapeutic first nucleic acid or endogenous nucleic acids.

Also contemplated is the delivery of one or more therapeutic nucleic acids to treat a single disorder or deficiency, wherein each such therapeutic nucleic acid functions by a different mechanism of action. For example, the compositions of the present invention may comprise a therapeutic first nucleic acid which, for example, is administered to correct an endogenous protein or enzyme deficiency, and which is accompanied by a second nucleic acid, which is administered to deactivate or "knock-

down" a malfunctioning endogenous nucleic acid and its protein or enzyme product. Such nucleic acids may encode, for example mRNA and siRNA.

While *in vitro* transcribed nucleic acids (e.g., mRNA) may be transfected into target cells, such nucleic acids are readily and efficiently degraded by the cell *in vivo*, thus rendering such nucleic acids ineffective. Moreover, some nucleic acids are unstable in bodily fluids (particularly human serum) and can be degraded even before reaching a target cell. In addition, within a cell, a natural mRNA can decay with a half-life of between 30 minutes and several days.

The nucleic acids provided herein, and in particular the mRNA nucleic acids provided herein, preferably retain at least some ability to be translated, thereby producing a functional protein or enzyme within a target cell. Accordingly, the present invention relates to the administration of a stabilized nucleic acid (e.g., mRNA which has been stabilized against *in vivo* nuclease digestion or degradation) to modulate the expression of a gene or the translation of a functional enzyme or protein within a target cell. In a preferred embodiment of the present invention, the activity of the nucleic acid (e.g., mRNA encoding a functional protein or enzyme) is prolonged over an extended period of time. For example, the activity of the nucleic acids may be prolonged such that the compositions of the present invention are administered to a subject on a semi-weekly or bi-weekly basis, or more preferably on a monthly, bi-monthly, quarterly or an annual basis. The extended or prolonged activity of the compositions of the present invention, and in particular of the mRNA comprised therein, is directly related to the quantity of functional protein or enzyme translated from such mRNA. Similarly, the activity of the compositions of the present invention may be further extended or prolonged by modifications made to improve or enhance translation of the mRNA nucleic acids. For example, the Kozac consensus sequence plays a role in the initiation of protein translation, and the inclusion of such a Kozac consensus sequence in the mRNA nucleic acids of the present invention may further extend or prolong the activity of the mRNA nucleic acids. Furthermore, the quantity of functional protein or enzyme translated by the target cell is a function of the quantity of nucleic acid (e.g., mRNA) delivered to the target cells and the stability of such nucleic acid. To the extent that the stability of the nucleic acids of the present invention may be improved or enhanced, the half-life, the activity of the translated

protein or enzyme and the dosing frequency of the composition may be further extended.

Accordingly, in a preferred embodiment, the nucleic acids provided herein comprise at least one modification which confers increased or enhanced stability to the nucleic acid, including, for example, improved resistance to nuclease digestion *in vivo*. As used herein, the terms "modification" and "modified" as such terms relate to the nucleic acids provided herein, include at least one alteration which preferably enhances stability and renders the nucleic acid more stable (e.g., resistant to nuclease digestion) than the wild-type or naturally occurring version of the nucleic acid. As used herein, the terms "stable" and "stability" as such terms relate to the nucleic acids of the present invention, and particularly with respect to the mRNA, refer to increased or enhanced resistance to degradation by, for example nucleases (i.e., endonucleases or exonucleases) which are normally capable of degrading such RNA. Increased stability can include, for example, less sensitivity to hydrolysis or other destruction by endogenous enzymes (e.g., endonucleases or exonucleases) or conditions within the target cell or tissue, thereby increasing or enhancing the residence of such nucleic acids in the target cell, tissue, subject and/or cytoplasm. The stabilized nucleic acid molecules provided herein demonstrate longer half-lives relative to their naturally occurring, unmodified counterparts (e.g. the wild-type version of the nucleic acid). Also contemplated by the terms "modification" and "modified" as such terms related to the nucleic acids of the present invention are alterations which improve or enhance translation of mRNA nucleic acids, including for example, the inclusion of sequences which function in the initiation of protein translation (e.g., the Kozac consensus sequence). (Kozak, M., Nucleic Acids Res 15 (20): 8125-48 (1987)).

In some embodiments, the nucleic acids of the present invention have undergone a chemical or biological modification to render them more stable. Exemplary modifications to a nucleic acid include the depletion of a base (e.g., by deletion or by the substitution of one nucleotide for another) or modification of a base, for example, the chemical modification of a base. The phrase "chemical modifications" as used herein, includes modifications which introduce chemistries which differ from those seen in naturally occurring nucleic acids, for example, covalent modifications such as the introduction of modified nucleotides, (e.g.,

nucleotide analogs, or the inclusion of pendant groups which are not naturally found in such nucleic acid molecules).

In addition, suitable modifications include alterations in one or more nucleotides of a codon such that the codon encodes the same amino acid but is more stable than the codon found in the wild-type version of the nucleic acid. For example, an inverse relationship between the stability of RNA and a higher number cytidines (C's) and/or uridines (U's) residues has been demonstrated, and RNA devoid of C and U residues have been found to be stable to most RNases (Heidenreich, *et al.* J Biol Chem 269, 2131-8 (1994)). In some embodiments, the number of C and/or U residues in an mRNA sequence is reduced. In a another embodiment, the number of C and/or U residues is reduced by substitution of one codon encoding a particular amino acid for another codon encoding the same or a related amino acid. Contemplated modifications to the mRNA nucleic acids of the present invention also include the incorporatation of pseudouridines. The incorporation of pseudouridines into the mRNA nucleic acids of the present invention may enhance stability and translational capacity, as well as diminishing immunogenicity *in vivo*. (See, e.g., Karikó, K., et al., Molecular Therapy 16 (11): 1833–1840 (2008)). Substitutions and modifications to the nucleic acids of the present invention may be performed by methods readily known to one or ordinary skill in the art.

The constraints on reducing the number of C and U residues in a sequence will likely be greater within the coding region of an mRNA, compared to an untranslated region, (i.e., it will likely not be possible to eliminate all of the C and U residues present in the message while still retaining the ability of the message to encode the desired amino acid sequence). The degeneracy of the genetic code, however presents an opportunity to allow the number of C and/or U residues that are present in the sequence to be reduced, while maintaining the same coding capacity (i.e., depending on which amino acid is encoded by a codon, several different possibilities for modification of RNA sequences may be possible). For example, the codons for Gly can be altered to GGA or GGG instead of GGU or GGC.

The term modification also includes, for example, the incorporation of non-nucleotide linkages or modified nucleotides into the nucleic acid sequences of the present invention (e.g., modifications to one or both the 3' and 5' ends of an mRNA

molecule encoding a functional protein or enzyme). Such modifications include the addition of bases to a nucleic acid sequence (e.g., the inclusion of a poly A tail or a longer poly A tail), the alteration of the 3' UTR or the 5' UTR, complexing the nucleic acid with an agent (e.g., a protein or a complementary nucleic acid molecule), and inclusion of elements which change the structure of a nucleic acid molecule (e.g., which form secondary structures).

The poly A tail is thought to stabilize natural messengers and synthetic sense RNA. Therefore, in one embodiment a long poly A tail can be added to an mRNA molecule thus rendering the RNA more stable. Poly A tails can be added using a variety of art-recognized techniques. For example, long poly A tails can be added to synthetic or *in vitro* transcribed RNA using poly A polymerase (Yokoe, *et al.* Nature Biotechnology. 1996; 14: 1252-1256). A transcription vector can also encode long poly A tails. In addition, poly A tails can be added by transcription directly from PCR products. Poly A may also be ligated to the 3' end of a sense RNA with RNA ligase (see, e.g., Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1991 edition)). In one embodiment, the length of the poly A tail is at least about 90, 200, 300, 400 at least 500 nucleotides. In one embodiment, the length of the poly A tail is adjusted to control the stability of a modified sense mRNA molecule of the invention and, thus, the transcription of protein. For example, since the length of the poly A tail can influence the half-life of a sense mRNA molecule, the length of the poly A tail can be adjusted to modify the level of resistance of the mRNA to nucleases and thereby control the time course of protein expression in a cell. In one embodiment, the stabilized nucleic acid molecules are sufficiently resistant to *in vivo* degradation (e.g., by nucleases), such that they may be delivered to the target cell without a transfer vehicle.

In one embodiment, a nucleic acid encoding a protein can be modified by the incorporation 3' and/or 5' untranslated (UTR) sequences which are not naturally found in the wild-type nucleic acid. In one embodiment, 3' and/or 5' flanking sequence which naturally flanks an mRNA and encodes a second, unrelated protein can be incorporated into the nucleotide sequence of an mRNA molecule encoding a therapeutic or functional protein in order to modify it. For example, 3' or 5' sequences

from mRNA molecules which are stable (e.g., globin, actin, GAPDH, tubulin, histone, or citric acid cycle enzymes) can be incorporated into the 3' and/or 5' region of a sense mRNA nucleic acid molecule to increase the stability of the sense mRNA molecule.

Also contemplated by the present invention are modifications to the nucleic acid sequences made to one or both of the 3' and 5' ends of the nucleic acid. For example, the present invention contemplates modifications to the 5' end of the nucleic acids (e.g., mRNA) to include a partial sequence of a CMV immediate-early 1 (IE1) gene, or a fragment thereof (e.g., SEQ ID NO: 2) to improve the nuclease resistance and/or improve the half-life of the nucleic acid. In addition to increasing the stability of the mRNA nucleic acid sequence, it has been surprisingly discovered the inclusion of a partial sequence of a CMV immediate-early 1 (IE1) gene enhances the translation of the mRNA and the expression of the functional protein or enzyme. Also contemplated is the inclusion of a sequence encoding human growth hormone (hGH), or a fragment thereof (e.g., SEQ ID NO: 3) to one or both of the 3' and 5' ends of the nucleic acid (e.g., mRNA) to further stabilize the nucleic acid. Generally, preferred modifications improve the stability and/or pharmacokinetic properties (e.g., half-life) of the nucleic acid relative to their unmodified counterparts, and include, for example modifications made to improve such nucleic acid's resistance to *in vivo* nuclease digestion.

In some embodiments, the composition can comprise a stabilizing reagent. The compositions can include one or more formulation reagents that bind directly or indirectly to, and stabilize the nucleic acid, thereby enhancing residence time in the cytoplasm of a target cell. Such reagents preferably lead to an improved half-life of a nucleic acid in the target cells. For example, the stability of an mRNA and efficiency of translation may be increased by the incorporation of "stabilizing reagents" that form complexes with the nucleic acids (e.g., mRNA) that naturally occur within a cell (see e.g., U.S. Pat. No. 5,677,124). Incorporation of a stabilizing reagent can be accomplished for example, by combining the poly A and a protein with the mRNA to be stabilized *in vitro* before loading or encapsulating the mRNA within a transfer vehicle. Exemplary stabilizing reagents include one or more proteins, peptides, aptamers, translational accessory protein, mRNA binding proteins, and/or translation initiation factors.

Stabilization of the compositions may also be improved by the use of opsonization-inhibiting moieties, which are typically large hydrophilic polymers that are chemically or physically bound to the transfer vehicle (e.g., by the intercalation of a lipid-soluble anchor into the membrane itself, or by binding directly to active groups of membrane lipids). These opsonization-inhibiting hydrophilic polymers form a protective surface layer which significantly decreases the uptake of the liposomes by the macrophage-monocyte system and reticulo-endothelial system (e.g., as described in U.S. Pat. No. 4,920,016, the entire disclosure of which is herein incorporated by reference). Transfer vehicles modified with opsonization-inhibition moieties thus remain in the circulation much longer than their unmodified counterparts.

When RNA is hybridized to a complementary nucleic acid molecule (e.g., DNA or RNA) it may be protected from nucleases. (Krieg, *et al.* Melton. Methods in Enzymology. 1987; 155, 397-415). The stability of hybridized mRNA is likely due to the inherent single strand specificity of most RNases. In some embodiments, the stabilizing reagent selected to complex a nucleic acid is a eukaryotic protein, (e.g., a mammalian protein). In yet another embodiment, the nucleic acid molecule (e.g., mRNA) for use in sense therapy can be modified by hybridization to a second nucleic acid molecule. If an entire mRNA molecule were hybridized to a complementary nucleic acid molecule translation initiation may be reduced. In some embodiments the 5' untranslated region and the AUG start region of the mRNA molecule may optionally be left unhybridized. Following translation initiation, the unwinding activity of the ribosome complex can function even on high affinity duplexes so that translation can proceed. (Liebhaber. J. Mol. Biol. 1992; 226: 2-13; Monia, *et al.* J Biol Chem. 1993; 268: 14514-22.)

It will be understood that any of the above described methods for enhancing the stability of nucleic acids may be used either alone or in combination with one or more of any of the other above-described methods and/or compositions.

In one embodiment, the compositions of the present invention facilitate the delivery of nucleic acids to target cells. In some embodiments, facilitating delivery to target cells includes increasing the amount of nucleic acid that comes in contact with the target cells. In some embodiments, facilitating delivery to target cells includes reducing the amount of nucleic acid that comes into contact with non-target cells. In

some embodiments, facilitating delivery to target cells includes allowing the transfection of at least some target cells with the nucleic acid. In some embodiments, the level of expression of the product encoded by the delivered nucleic acid is increased in target cells.

The nucleic acids of the present invention may be optionally combined with a reporter gene (e.g., upstream or downstream of the coding region of the nucleic acid) which, for example, facilitates the determination of nucleic acid delivery to the target cells or tissues. Suitable reporter genes may include, for example, Green Fluorescent Protein mRNA (GFP mRNA), *Renilla* Luciferase mRNA (Luciferase mRNA), Firefly Luciferase mRNA, or any combinations thereof. For example, GFP mRNA may be fused with a nucleic acid encoding OTC mRNA to facilitate confirmation of mRNA localization in the target cells, tissues or organs.

As used herein, the terms "transfect" or "transfection" mean the intracellular introduction of a nucleic acid into a cell, or preferably into a target cell. The introduced nucleic acid may be stably or transiently maintained in the target cell. The term "transfection efficiency" refers to the relative amount of nucleic acid up-taken by the target cell which is subject to transfection. In practice, transfection efficiency is estimated by the amount of a reporter nucleic acid product expressed by the target cells following transfection. Preferred are compositions with high transfection efficacies and in particular those compositions that minimize adverse effects which are mediated by transfection of non-target cells and tissues. The compositions of the present invention that demonstrate high transfection efficacies improve the likelihood that appropriate dosages of the nucleic acid will be delivered to the site of pathology, while minimizing potential systemic adverse effects.

As provided herein, the compositions can include a transfer vehicle. As used herein, the term "transfer vehicle" includes any of the standard pharmaceutical carriers, diluents, excipients and the like which are generally intended for use in connection with the administration of biologically active agents, including nucleic acids. The compositions and in particular the transfer vehicles described herein are capable of delivering nucleic acids of varying sizes to their target cells or tissues. In one embodiment of the present invention, the transfer vehicles of the present invention are capable of delivering large nucleic acid sequences (e.g., nucleic acids of

at least 1kDa, 1.5kDa, 2 kDa, 2.5kDa, 5kDa, 10kDa, 12kDa, 15kDa, 20kDa, 25kDa, 30kDa, or more). The nucleic acids can be formulated with one or more acceptable reagents, which provide a vehicle for delivering such nucleic acids to target cells. Appropriate reagents are generally selected with regards to a number of factors, which include, among other things, the biological or chemical properties of the nucleic acids (e.g., charge), the intended route of administration, the anticipated biological environment to which such nucleic acids will be exposed and the specific properties of the intended target cells. In some embodiments, transfer vehicles, such as liposomes, encapsulate the nucleic acids without compromising biological activity. In some embodiments, the transfer vehicle demonstrates preferential and/or substantial binding to a target cell relative to non-target cells. In a preferred embodiment, the transfer vehicle delivers its contents to the target cell such that the nucleic acids are delivered to the appropriate subcellular compartment, such as the cytoplasm.

In some embodiments, the transfer vehicle is a liposomal vesicle, or other means to facilitate the transfer of a nucleic acid to target cells and tissues. Suitable transfer vehicles include, but are not limited to, liposomes, nanoliposomes, ceramide-containing nanoliposomes, proteoliposomes, nanoparticulates, calcium phosphor-silicate nanoparticulates, calcium phosphate nanoparticulates, silicon dioxide nanoparticulates, nanocrystalline particulates, semiconductor nanoparticulates, poly(D-arginine), nanodendrimers, starch-based delivery systems, micelles, emulsions, niosomes, plasmids, viruses, calcium phosphate nucleotides, aptamers, peptides and other vectorial tags. Also contemplated is the use of bionanocapsules and other viral capsid proteins assemblies as a suitable transfer vehicle. (Hum. Gene Ther. 2008 Sep;19(9):887-95). In a preferred embodiment of the present invention, the transfer vehicle is formulated as a lipid nanoparticle. As used herein, the phrase "lipid nanoparticle" refers to a transfer vehicle comprising one or more lipids (e.g., cationic and/or non-cationic lipids). Preferably, the lipid nanoparticles are formulated to deliver one or more nucleic acids (e.g., mRNA) to one or more target cells or tissues. The use of lipids, either alone or as a component of the transfer vehicle, and in particular lipid nanoparticles, is preferred. Examples of suitable lipids include, for example, the phosphatidyl compounds (e.g., phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids,

cerebrosides, and gangliosides). Also contemplated is the use of polymers as transfer vehicles, whether alone or in combination with other transfer vehicles. Suitable polymers may include, for example, polyacrylates, polyalkycyanoacrylates, polylactide, polylactide-polyglycolide copolymers, polycaprolactones, dextran, albumin, gelatin, alginate, collagen, chitosan, cyclodextrins and polyethylenimine. In one embodiment, the transfer vehicle is selected based upon its ability to facilitate the transfection of a nucleic acid to a target cell.

In one embodiment of the present invention, the transfer vehicle may be selected and/or prepared to optimize delivery of the nucleic acid to the target cell, tissue or organ. For example, if the target cell is a hepatocyte the properties of the transfer vehicle (e.g., size, charge and/or pH) may be optimized to effectively deliver such transfer vehicle to the target cell or organ, reduce immune clearance and/or promote retention in that target organ. Alternatively, if the target tissue is the central nervous system (e.g., mRNA administered for the treatment of neurodegenerative diseases may specifically target brain or spinal tissue) selection and preparation of the transfer vehicle must consider penetration of, and retention within the blood brain barrier and/or the use of alternate means of directly delivering such transfer vehicle to such target tissue. In one embodiment, the compositions of the present invention may be combined with agents that facilitate the transfer of exogenous nucleic acids (e.g., agents which disrupt or improve the permeability of the blood brain barrier and thereby enhance the transfer of exogenous mRNA to the target cells).

The use of liposomal transfer vehicles to facilitate the delivery of nucleic acids to target cells is contemplated by the present invention. Liposomes (e.g., liposomal lipid nanoparticles) are generally useful in a variety of applications in research, industry, and medicine, particularly for their use as transfer vehicles of diagnostic or therapeutic compounds *in vivo* (Lasic, Trends Biotechnol., 16: 307-321, 1998; Drummond *et al.*, Pharmacol. Rev., 51: 691-743, 1999) and are usually characterized as microscopic vesicles having an interior aqua space sequestered from an outer medium by a membrane of one or more bilayers. Bilayer membranes of liposomes are typically formed by amphiphilic molecules, such as lipids of synthetic or natural origin that comprise spatially separated hydrophilic and hydrophobic domains (Lasic, Trends Biotechnol., 16: 307-321, 1998). Bilayer membranes of the liposomes can also

be formed by amphiphilic polymers and surfactants (e.g., polymerosomes, niosomes, etc.).

In the context of the present invention, a liposomal transfer vehicle typically serves to transport the nucleic acid to the target cell. For the purposes of the present invention, the liposomal transfer vehicles are prepared to contain the desired nucleic acids. The process of incorporation of a desired entity (e.g., a nucleic acid) into a liposome is often referred to as "loading" (Lasic, *et al.*, FEBS Lett., 312: 255-258, 1992). The liposome-incorporated nucleic acids may be completely or partially located in the interior space of the liposome, within the bilayer membrane of the liposome, or associated with the exterior surface of the liposome membrane. The incorporation of a nucleic acid into liposomes is also referred to herein as "encapsulation" wherein the nucleic acid is entirely contained within the interior space of the liposome.

The purpose of incorporating a nucleic acid into a transfer vehicle, such as a liposome, is often to protect the nucleic acid from an environment which may contain enzymes or chemicals that degrade nucleic acids and/or systems or receptors that cause the rapid excretion of the nucleic acids. Accordingly, in a preferred embodiment of the present invention, the selected transfer vehicle is capable of enhancing the stability of the nucleic acid(s) (e.g., mRNA encoding a functional protein) contained therein. The liposome can allow the encapsulated nucleic acid to reach the target cell and/or may preferentially allow the encapsulated nucleic acid to reach the target cell, or alternatively limit the delivery of such nucleic acids to other sites or cells where the presence of the administered nucleic acid may be useless or undesirable. Furthermore, incorporating the nucleic acids into a transfer vehicle, such as for example, a cationic liposome, also facilitates the delivery of such nucleic acids into a target cell.

Ideally, liposomal transfer vehicles are prepared to encapsulate one or more desired nucleic acids (e.g., mRNA encoding a urea cycle enzyme) such that the compositions demonstrate a high transfection efficiency and enhanced stability. While liposomes can facilitate introduction of nucleic acids into target cells, the addition of polycations (e.g., poly L-lysine and protamine), as a copolymer can facilitate, and in some instances markedly enhance the transfection efficiency of

several types of cationic liposomes by 2–28 fold in a number of cell lines both *in vitro* and *in vivo*. (See N.J. Caplen, *et al.*, Gene Ther. 1995; 2: 603; S. Li, *et al.*, Gene Ther. 1997; 4, 891.)

The present invention contemplates the use of cationic lipids and liposomes to encapsulate and/or enhance the delivery of nucleic acids into their target cells and tissues. As used herein, the phrase "cationic lipid" refers to any of a number of lipid species that carry a net positive charge at a selected pH, such as physiological pH. The contemplated liposomal transfer vehicles and lipid nanoparticles may be prepared by including multi-component lipid mixtures of varying ratios employing one or more cationic lipids, non-cationic lipids and PEG-modified lipids. Several cationic lipids have been described in the literature, many of which are commercially available. In some embodiments, the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride or "DOTMA" is used. (Felgner *et al.* (Proc. Nat'l Acad. Sci. 84, 7413 (1987); U.S. Pat. No. 4,897,355). DOTMA can be formulated alone or can be combined with dioleoylphosphatidylethanolamine or "DOPE" or other cationic or non-cationic lipids into a liposomal transfer vehicle or a lipid nanoparticle, and such liposomes can be used to enhance the delivery of nucleic acids into target cells. Other suitable cationic lipids include, for example, 5-carboxyspermylglycinedioctadecylamide or "DOGS," 2,3-dioleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-1-propanaminium or "DOSPA" (Behr *et al.* Proc. Nat.'l Acad. Sci. 86, 6982 (1989); U.S. Pat. No. 5,171,678; U.S. Pat. No. 5,334,761), 1,2-Dioleoyl-3-Dimethylammonium-Propane or "DODAP", 1,2-Dioleoyl-3-Trimethylammonium-Propane or "DOTAP". Contemplated cationic lipids also include 1,2-distearyloxy-N,N-dimethyl-3-aminopropane or "DSDMA", 1,2-dioleyloxy-N,N-dimethyl-3-aminopropane or "DODMA", 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane or "DLinDMA", 1,2-dilinolenyloxy-N,N-dimethyl-3-aminopropane or "DLenDMA", N-dioleyl-N,N-dimethylammonium chloride or "DODAC", N,N-distearyl-N,N-dimethylammonium bromide or "DDAB", N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide or "DMRIE", 3-dimethylamino-2-(cholest-5-en-3-beta-oxybutan-4-oxy)-1-(ci s,cis-9,12-octadecadienoxy)propane or "CLinDMA", 2-[5′-(cholest-5-en-3-beta-oxy)-3′-oxapentoxy)-3-dimethy l-1-(cis,cis-9′, 1-2′-octadecadienoxy)propane or

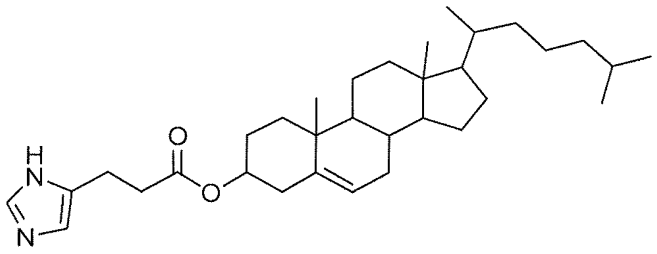
"CpLinDMA", N,N-dimethyl-3,4-dioleyloxybenzylamine or "DMOBA", 1,2-N,N'-dioleylcarbamyl-3-dimethylaminopropane or "DOcarbDAP", 2,3-Dilinoleoyloxy-N,N-dimethylpropylamine or "DLinDAP", 1,2-N,N'-Dilinoleylcarbamyl-3-dimethylaminopropane or "DLincarbDAP", 1,2-Dilinoleoylcarbamyl-3-dimethylaminopropane or "DLinCDAP", 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane or "DLin-K-DMA", 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane or "DLin-K-XTC2-DMA", or mixtures thereof. (Heyes, J., *et al.*, J Controlled Release 107: 276-287 (2005); Morrissey, DV., *et al.*, Nat. Biotechnol. 23(8): 1003-1007 (2005); PCT Publication WO2005/121348A1).

The use of cholesterol-based cationic lipids is also contemplated by the present invention. Such cholesterol-based cationic lipids can be used, either alone or in combination with other cationic or non-cationic lipids. Suitable cholesterol-based cationic lipids include, for example, DC-Chol (N,N-dimethyl-N-ethylcarboxamidocholesterol), 1,4-bis(3-N-oleylamino-propyl)piperazine (Gao, *et al.* Biochem. Biophys. Res. Comm. 179, 280 (1991); Wolf *et al.* BioTechniques 23, 139 (1997); U.S. Pat. No. 5,744,335).

In addition, several reagents are commercially available to enhance transfection efficacy. Suitable examples include LIPOFECTIN (DOTMA:DOPE) (Invitrogen, Carlsbad, Calif.), LIPOFECTAMINE (DOSPA:DOPE) (Invitrogen), LIPOFECTAMINE2000. (Invitrogen), FUGENE, TRANSFECTAM (DOGS), and EFFECTENE.

Also contemplated are cationic lipids such as the dialkylamino-based, imidazole-based, and guanidinium-based lipids. For example, certain embodiments are directed to a composition comprising one or more imidazole-based cationic lipids, for example, the imidazole cholesterol ester or "ICE" lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate, as represented by structure (I) below. In a preferred embodiment, a transfer vehicle (e.g., a lipid nanoparticle) for delivery of RNA (e.g., mRNA) or protein (e.g., an enzyme), for example a therapeutic amount of RNA or protein, may comprise one or more imidazole-based cationic lipids, for example, the imidazole cholesterol ester or "ICE" lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-

methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate, as represented by structure (I).



(I)

Without wishing to be bound by a particular theory, it is believed that the fusogenicity of the imidazole-based cationic lipid ICE is related to the endosomal disruption which is facilitated by the imidazole group, which has a lower pKa relative to traditional cationic lipids. The endosomal disruption in turn promotes osmotic swelling and the disruption of the liposomal membrane, followed by the transfection or intracellular release of the nucleic acid(s) contents loaded therein into the target cell.

The imidazole-based cationic lipids are also characterized by their reduced toxicity relative to other cationic lipids. The imidazole-based cationic lipids (e.g., ICE) may be used as the sole cationic lipid in the transfer vehicle or lipid nanoparticle, or alternatively may be combined with traditional cationic lipids (e.g., DOPE, DC-Chol), non-cationic lipids, PEG-modified lipids and/or helper lipids. The cationic lipid may comprise a molar ratio of about 1% to about 90%, about 2% to about 70%, about 5% to about 50%, about 10% to about 40% of the total lipid present in the transfer vehicle, or preferably about 20% to about 70% of the total lipid present in the transfer vehicle.

The use of polyethylene glycol (PEG)-modified phospholipids and derivatized lipids such as derivatized cerarmides (PEG-CER), including N-Octanoyl-Sphingosine-1-[Succinyl(Methoxy Polyethylene Glycol)-2000] (C8 PEG-2000 ceramide) is also contemplated by the present invention, either alone or preferably in combination with other lipid formulations together which comprise the transfer vehicle (e.g., a lipid nanoparticle). Contemplated PEG-modified lipids include, but is not limited to, a polyethylene glycol chain of up to 5 kDa in length covalently

attached to a lipid with alkyl chain(s) of C_6-C_{20} length. The addition of such components may prevent complex aggregation and may also provide a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid composition to the target tissues, (Klibanov *et al.* (1990) FEBS Letters, 268 (1): 235–237), or they may be selected to rapidly exchange out of the formulation *in vivo* (see U.S. Pat. No. 5,885,613). Particularly useful exchangeable lipids are PEG-ceramides having shorter acyl chains (e.g., C14 or C18). The PEG-modified phospholipid and derivitized lipids of the present invention may comprise a molar ratio from about 0% to about 20%, about 0.5% to about 20%, about 1% to about 15%, about 4% to about 10%, or about 2% of the total lipid present in the liposomal transfer vehicle.

The present invention also contemplates the use of non-cationic lipids. As used herein, the phrase "non-cationic lipid" refers to any neutral, zwitterionic or anionic lipid. As used herein, the phrase "anionic lipid" refers to any of a number of lipid species that carry a net negative charge at a selected pH, such as physiological pH. Non-cationic lipids include, but are not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. Such non-cationic lipids may be used alone, but are preferably used in combination with other excipients, for example, cationic lipids. When used in combination with a cationic lipid, the non-cationic lipid may comprise a molar ratio of 5% to about 90%, or preferably about 10 % to about 70% of the total lipid present in the transfer vehicle.

Preferably, the transfer vehicle (e.g., a lipid nanoparticle) is prepared by combining multiple lipid and/or polymer components. For example, a transfer vehicle may be prepared using DSPC/CHOL/DODAP/C8-PEG-5000 ceramide in a molar

ratio of about 1 to 50 : 5 to 65 : 5 to 90 : 1 to 25, respectively. A transfer vehicle may be comprised of additional lipid combinations in various ratios, including for example, DSPC/CHOL/DODAP/mPEG-5000 (e.g., combined at a molar ratio of about 33:40:25:2), DSPC/CHOL/DODAP/C8 PEG-2000-Cer (e.g., combined at a molar ratio of about 31:40:25:4), POPC/DODAP/C8-PEG-2000-Cer (e.g., combined at a molar ratio of about 75-87:3-14:10) or DSPC/CHOL/DOTAP/C8 PEG-2000-Cer (e.g., combined at a molar ratio of about 31:40:25:4). The selection of cationic lipids, non-cationic lipids and/or PEG-modified lipids which comprise the liposomal transfer vehicle or lipid nanoparticle, as well as the relative molar ratio of such lipids to each other, is based upon the characteristics of the selected lipid(s), the nature of the intended target cells or tissues and the characteristics of the nucleic acids to be delivered by the liposomal transfer vehicle. Additional considerations include, for example, the saturation of the alkyl chain, as well as the size, charge, pH, pKa, fusogenicity and toxicity of the selected lipid(s).

The liposomal transfer vehicles for use in the present invention can be prepared by various techniques which are presently known in the art. Multi-lamellar vesicles (MLV) may be prepared conventional techniques, for example, by depositing a selected lipid on the inside wall of a suitable container or vessel by dissolving the lipid in an appropriate solvent, and then evaporating the solvent to leave a thin film on the inside of the vessel or by spray drying. An aqueous phase may then added to the vessel with a vortexing motion which results in the formation of MLVs. Uni-lamellar vesicles (ULV) can then be formed by homogenization, sonication or extrusion of the multi-lamellar vesicles. In addition, unilamellar vesicles can be formed by detergent removal techniques.

In certain embodiments of this invention, the compositions of the present invention comprise a transfer vehicle wherein the therapeutic RNA (e.g., mRNA encoding OTC) is associated on both the surface of the transfer vehicle (e.g., a liposome) and encapsulated within the same transfer vehicle. For example, during preparation of the compositions of the present invention, cationic liposomal transfer vehicles may associate with the nucleic acids (e.g., mRNA) through electrostatic interactions with such therapeutic mRNA.

In certain embodiments, the compositions of the present invention may be loaded with diagnostic radionuclide, fluorescent materials or other materials that are detectable in both *in vitro* and *in vivo* applications. For example, suitable diagnostic materials for use in the present invention may include Rhodamine-dioleoylphosphatidylethanolamine (Rh-PE), Green Fluorescent Protein mRNA (GFP mRNA), *Renilla* Luciferase mRNA and Firefly Luciferase mRNA.

During the preparation of liposomal transfer vehicles, water soluble carrier agents may be encapsulated in the aqueous interior by including them in the hydrating solution, and lipophilic molecules may be incorporated into the lipid bilayer by inclusion in the lipid formulation. In the case of certain molecules (e.g., cationic or anionic lipophilic nucleic acids), loading of the nucleic acid into preformed liposomes may be accomplished, for example, by the methods described in U.S. Pat. No. 4,946,683, the disclosure of which is incorporated herein by reference. Following encapsulation of the nucleic acid, the liposomes may be processed to remove un-encapsulated mRNA through processes such as gel chromatography, diafiltration or ultrafiltration. For example, if it is desirous to remove externally bound nucleic acid from the surface of the liposomal transfer vehicle formulation, such liposomes may be subject to a Diethylaminoethyl SEPHACEL column.

In addition to the encapsulated nucleic acid, one or more therapeutic or diagnostic agents may be included in the transfer vehicle. For example, such additional therapeutic agents may be associated with the surface of the liposome, can be incorporated into the lipid bilayer of a liposome by inclusion in the lipid formulation or loading into preformed liposomes (see U.S. Pat. Nos. 5,194,654 and 5,223,263, which are incorporated by reference herein).

There are several methods for reducing the the size, or "sizing", of liposomal transfer vehicles, and any of these methods may generally be employed when sizing is used as part of the invention. The extrusion method is a preferred method of liposome sizing. (Hope, M J *et al.* Reduction of Liposome Size and Preparation of Unilamellar Vesicles by Extrusion Techniques. In: *Liposome Technology* (G. Gregoriadis, Ed.) Vol. 1. p 123 (1993). The method consists of extruding liposomes through a small-pore polycarbonate membrane or an asymmetric ceramic membrane to reduce liposome sizes to a relatively well-defined size distribution. Typically, the suspension

is cycled through the membrane one or more times until the desired liposome size distribution is achieved. The liposomes may be extruded through successively smaller pore membranes to achieve gradual reduction in liposome size.

A variety of alternative methods known in the art are available for sizing of a population of liposomal transfer vehicles. One such sizing method is described in U.S. Pat. No. 4,737,323, incorporated herein by reference. Sonicating a liposome suspension either by bath or probe sonication produces a progressive size reduction down to small ULV less than about 0.05 microns in diameter. Homogenization is another method that relies on shearing energy to fragment large liposomes into smaller ones. In a typical homogenization procedure, MLV are recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns, are observed. The size of the liposomal vesicles may be determined by quasi-electric light scattering (QELS) as described in Bloomfield, Ann. Rev. Biophys. Bioeng., 10:421–450 (1981), incorporated herein by reference. Average liposome diameter may be reduced by sonication of formed liposomes. Intermittent sonication cycles may be alternated with QELS assessment to guide efficient liposome synthesis.

Selection of the appropriate size of a liposomal transfer vehicle must take into consideration the site of the target cell or tissue and to some extent the application for which the liposome is being made. In some embodiments, it may be desirable to limit transfection of the nucleic acids to certain cells or tissues. For example, the liver represents an important target organ for the compositions of the present invention in part due to its central role in metabolism and production of proteins and accordingly diseases which are caused by defects in liver-specific gene products (e.g., the urea cycle disorders) may benefit from specific targeting of cells (e.g., hepatocytes). Accordingly, in one embodiment of the present invention, the structural characteristics of the target tissue may be exploited to direct the distribution of the liposomal transfer vehicle to such target tissues. For example, to target hepatocytes a liposomal transfer vehicle may be sized such that its dimensions are smaller than the fenestrations of the endothelial layer lining hepatic sinusoids in the liver; accordingly the liposomal transfer vehicle can readily penetrate such endothelial fenestrations to reach the target hepatocytes. Alternatively, a liposomal transfer vehicle may be sized

such that the dimensions of the liposome are of a sufficient diameter to limit or expressly avoid distribution into certain cells or tissues. For example, a liposomal transfer vehicle may be sized such that its dimensions are larger than the fenestrations of the endothelial layer lining hepatic sinusoids to thereby limit distribution of the liposomal transfer vehicle to hepatocytes. In such an embodiment, large liposomal transfer vehicles will not easily penetrate the endothelial fenestrations, and would instead be cleared by the macrophage Kupffer cells that line the liver sinusoids. Generally, the size of the transfer vehicle is within the range of about 25 to 250 nm, prefereably less than about 250nm, 175nm, 150nm, 125nm, 100nm, 75nm, 50nm, 25nm or 10nm.

Similarly, the compositions of the present invention may be prepared to preferentially distribute to other target tissues, cells or organs, such as the heart, lungs, kidneys, spleen. For example, the transfer vehicles of the present invention may be prepared to achieve enhanced delivery to the target cells and tissues. Accordingly, the compositions of the present invention may be enriched with additional cationic, non-cationic and PEG-modified lipids to further target tissues or cells.

In some embodiments, the compositions of the present invention distribute into the cells and tissues of the liver to facilitate the delivery and the subsequent expression of the nucleic acids (e.g., mRNA) comprised therein by the cells and tissues of the liver (e.g., hepatocytes). While such compositions may preferentially distribute into the cells and tissues of the liver, the therapeutic effects of the expressed nucleic acids need not be limited to the target cells and tissues. For example, the targeted hepatocytes may function as a "reservoir" or "depot" capable of expressing or producing, and systemically excreting a functional protein or enzyme. Accordingly, in one embodiment of the present invention the liposomal transfer vehicle may target hepatocyes and/or preferentially distribute to the cells and tissues of the liver and upon delivery. Following transfection of the target hepatocytes, the mRNA nucleic acids(s) loaded in the liposomal vehicle are translated and a functional protein product expressed, excreted and systemically distributed.

In some embodiments, the compositions of the present invention comprise one or more additional molecules (e.g., proteins, peptides, aptamers or oliogonucleotides) which facilitate the transfer of the nucleic acids (e.g., mRNA, miRNA, snRNA and

snoRNA) from the transfer vehicle into an intracellular compartment of the target cell. In one embodiment, the additional molecule facilitates the delivery of the nucleic acids into, for example, the cytosol, the lysosome, the mitochondrion, the nucleus, the nucleolae or the proteasome of a target cell. Also included are agents that facilitate the transport of the translated protein of interest from the cytoplasm to its normal intercellular location (e.g., in the mitochondrion) to treat deficiencies in that organelle. In some embodiments, the agent is selected from the group consisting of a protein, a peptide, an aptamer, and an oligonucleotide.

In one embodiment, the compositions of the present invention facilitate a subject's endogenous production of one or more functional proteins and/or enzymes, and in particular the production of proteins and/or enzymes which demonstrate less immunogenicity relative to their recombinantly-prepared counterparts. In a preferred embodiment of the present invention, the transfer vehicles comprise nucleic acids which encode mRNA of a deficient protein or enzyme. Upon distribution of such compositions to the target tissues and the subsequent transfection of such target cells, the exogenous mRNA loaded into the liposomal transfer vehicle (e.g., a lipid nanoparticle) may be translated *in vivo* to produce a functional protein or enzyme encoded by the exogenously administered mRNA (e.g., a protein or enzyme in which the subject is deficient). Accordingly, the compositions of the present invention exploit a subject's ability to translate exogenously- or recombinantly-prepared mRNA to produce an endogenously-translated protein or enzyme, and thereby produce (and where applicable excrete) a functional protein or enzyme. The expressed or translated proteins or enzymes may also be characterized by the *in vivo* inclusion of native post-translational modifications which may often be absent in recombinantly-prepared proteins or enzymes, thereby further reducing the immunogenicity of the translated protein or enzyme.

The administration of mRNA encoding a deficient protein or enzyme avoids the need to deliver the nucleic acids to specific organelles within a target cell (e.g., mitochondria). Rather, upon transfection of a target cell and delivery of the nucleic acids to the cytoplasm of the target cell, the mRNA contents of a transfer vehicle may be translated and a functional protein or enzyme expressed.

The present invention also contemplates the discriminatory targeting of target cells and tissues by both passive and active targeting means. The phenomenon of passive targeting exploits the natural distributions patterns of a transfer vehicle *in vivo* without relying upon the use of additional excipients or means to enhance recognition of the transfer vehicle by target cells. For example, transfer vehicles which are subject to phagocytosis by the cells of the reticulo-endothelial system are likely to accumulate in the liver or spleen, and accordingly may provide means to passively direct the delivery of the compositions to such target cells.

Alternatively, the present invention contemplates active targeting, which involves the use of additional excipients, referred to herein as "targeting ligands" that may be bound (either covalently or non-covalently) to the transfer vehicle to encourage localization of such transfer vehicle at certain target cells or target tissues. For example, targeting may be mediated by the inclusion of one or more endogenous targeting ligands (e.g., apolipoprotein E) in or on the transfer vehicle to encourage distribution to the target cells or tissues. Recognition of the targeting ligand by the target tissues actively facilitates tissue distribution and cellular uptake of the transfer vehicle and/or its contents in the target cells and tissues (e.g., the inclusion of an apolipoprotein-E targeting ligand in or on the transfer vehicle encourages recognition and binding of the transfer vehicle to endogenous low density lipoprotein receptors expressed by hepatocytes). As provided herein, the composition can comprise a ligand capable of enhancing affinity of the composition to the target cell. Targeting ligands may be linked to the outer bilayer of the lipid particle during formulation or post-formulation. These methods are well known in the art. In addition, some lipid particle formulations may employ fusogenic polymers such as PEAA, hemagluttinin, other lipopeptides (see U.S. Patent Application Ser. Nos. 08/835,281, and 60/083,294, which are incorporated herein by reference) and other features useful for *in vivo* and/or intracellular delivery. In other some embodiments, the compositions of the present invention demonstrate improved transfection efficacies, and/or demonstrate enhanced selectivity towards target cells or tissues of interest. Contemplated therefore are compositions which comprise one or more ligands (e.g., peptides, aptamers, oligonucleotides, a vitamin or other molecules) that are capable of enhancing the affinity of the compositions and their nucleic acid contents for the

target cells or tissues. Suitable ligands may optionally be bound or linked to the surface of the transfer vehicle. In some embodiments, the targeting ligand may span the surface of a transfer vehicle or be encapsulated within the transfer vehicle. Suitable ligands and are selected based upon their physical, chemical or biological properties (e.g., selective affinity and/or recognition of target cell surface markers or features.) Cell-specific target sites and their corresponding targeting ligand can vary widely. Suitable targeting ligands are selected such that the unique characteristics of a target cell are exploited, thus allowing the composition to discriminate between target and non-target cells. For example, compositions of the present invention may bear surface markers (e.g., apolipoprotein-B or apolipoprotein-E) that selectively enhance recognition of, or affinity to hepatocytes (e.g., by receptor-mediated recognition of and binding to such surface markers). Additionally, the use of galactose as a targeting ligand would be expected to direct the compositions of the present invention to parenchymal hepatocytes, or alternatively the use of mannose containing sugar residues as a targeting ligand would be expected to direct the compositions of the present invention to liver endothelial cells (e.g., mannose containing sugar residues that may bind preferentially to the asialoglycoprotein receptor present in hepatocytes). (See Hillery AM, *et al.* "Drug Delivery and Targeting: For Pharmacists and Pharmaceutical Scientists" (2002) Taylor & Francis, Inc.) The presentation of such targeting ligands that have been conjugated to moieties present in the transfer vehicle (e.g., a lipid nanoparticle) therefore facilitate recognition and uptake of the compositions of the present invention in target cells and tissues. Examples of suitable targeting ligands include one or more peptides, proteins, aptamers, vitamins and oligonucleotides.

As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, to which the compositions and methods of the present invention are administered. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

As used herein, the term "target cell" refers to a cell or tissue to which a composition of the invention is to be directed or targeted. In some embodiments, the target cells are deficient in a protein or enzyme of interest. For example, where it is

desired to deliver a nucleic acid to a hepatocyte, the hepatocyte represents the target cell. In some embodiments, the nucleic acids and compositions of the present invention transfect the target cells on a discriminatory basis (i.e., do not transfect non-target cells). The compositions and methods of the present invention may be prepared to preferentially target a variety of target cells, which include, but are not limited to, hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, lung cells, bone cells, stem cells, mesenchymal cells, neural cells (e.g., meninges, astrocytes, motor neurons, cells of the dorsal root ganglia and anterior horn motor neurons), photoreceptor cells (e.g., rods and cones), retinal pigmented epithelial cells, secretory cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

Following transfection of one or more target cells by the compositions and nucleic acids of the present invention, expression of the protein encoded by such nucleic acid may be preferably stimulated and the capability of such target cells to express the protein of interest is enhanced. For example, transfection of a target cell with an mRNA OTC will allow expression of the protein product OTC following translation of the nucleic acid.

The urea cycle metabolic disorders and protein or enzyme deficiencies generally may be amenable to treatment with the methods and compositions provided herein. The nucleic acids of the compositions and/or methods provided herein preferably encode a product (e.g., a protein, enzyme, polypeptide, peptide, functional RNA, and/or antisense molecule), and preferably encodes a product whose *in vivo* production is desired.

The urea cycle metabolic disorders represent examples of protein and enzyme deficiencies which may be treated using the methods and compositions provided herein. Such urea cycle metabolic disorders include OTC deficiency, arginosuccinate synthetase deficiency (ASD) and argininosuccinate lyase deficiency (ALD). Therefore, in some embodiments, the nucleic acid of the methods and compositions provided herein encode an enzyme involved in the urea cycle, including, for example, ornithine transcarbamylase (OTC), carbamyl phosphate synthetase (CPS),

argininosuccinate synthetase 1 (ASS1) argininosuccinate lyase (ASL), and arginase (ARG).

Five metabolic disorders which result from defects in the biosynthesis of the enzymes involved in the urea cycle have been described, and include ornithine transcarbamylase (OTC) deficiency, carbamyl phosphate synthetase (CPS) deficiency, argininosuccinate synthetase 1 (ASS1) deficiency (citrullinemia), argininosuccinate lyase (ASL) deficiency and arginase deficiency (ARG). Of these five metabolic disorders, OTC deficiency represents the most common, occurring in an estimated one out of every 80,000 births.

OTC is a homotrimeric mitochondrial enzyme which is expressed almost exclusively in the liver and which encodes a precursor OTC protein that is cleaved in two steps upon incorporation into the mitchondrial matrix. (Horwich AL., *et al.* Cell 1986; 44: 451-459). OTC deficiency is a genetic disorder which results in a mutated and biologically inactive form of the enzyme ornithine transcarbamylase. OTC deficiency often becomes evident in the first few days of life, typically after protein ingestion. In the classic severe form of OTC deficiency, within the first days of life patients present with lethargy, convulsions, coma and severe hyperammonemia, which quickly leads to a deteriorating and fatal outcome absent appropriate medical intervention. (Monish S., *et al.*, Genetics for Pediatricians; Remedica, Cold Spring Harbor Laboratory (2005)). If improperly treated or if left untreated, complications from OTC deficiency may include developmental delay and mental retardation. OTC deficient subjects may also present with progressive liver damage, skin lesions, and brittle hair. In some affected individuals, signs and symptoms of OTC deficiency may be less severe, and may not appear until later in life.

The *OTC* gene, which is located on the short arm of the X chromosome within band Xp21.1, spans more than 85 kb and is comprised of 10 exons encoding a protein of 1062 amino acids. (Lindgren V., *et al.* Science 1984; 226: 698-7700; Horwich, AL., *et al.* Science 224: 1068-1074, 1984; Horwich, AL. *et al.*, Cell 44: 451-459, 1986; Hata, A., *et al.*, J. Biochem. 100: 717-725, 1986, which are incorporated herein by reference). The OTC enzyme catalyzes the conversion or ornithine and carbamoyl phosphate to citrulline. Since *OTC* is on the X chromosome, females are primarily

carriers while males with nonconservative mutations rarely survive past 72 hours of birth.

In healthy subjects, *OTC* is expressed almost exclusively in hepatocellular mitochondria. Although not expressed in the brain of healthy subjects, OTC deficiency can lead to neurological disorders. For example, one of the usual symptoms of OTC deficiency, which is heterogeneous in its presentation, is hyperammonaemic coma (Gordon, N., Eur J Paediatr Neurol 2003;7:115-121.).

OTC deficiency is very heterogeneous, with over 200 unique mutations reported and large deletions that account for approximately 10-15% of all mutations, while the remainder generally comprises missense point mutations with smaller numbers of nonsense, splice-site and small deletion mutations. (Monish A., *et al.*) The phenotype of OTC deficiency is extremely heterogeneous, which can range from acute neonatal hyperammonemic coma to asymptomatic hemizygous adults. (Gordon N. Eur J Paediatr Neurol 2003; 7: 115-121). Those mutations that result in severe and life threatening neonatal disease are clustered in important structural and functional domains in the interior of the protein at sites of enzyme activity or at the interchain surface, while mutations associated with late-onset disease are located on the protein surface (Monish A., *et al.*) Patients with milder or partial forms of OTC deficiency may have onset of disease later in life, which may present as recurrent vomiting, neurobehavioral changes or seizures associated with hyperammonemia.

The compositions and methods of the present invention are broadly applicable to the delivery of nucleic acids, and in particular mRNA, to treat a number of disorders. In particular, the compositions and methods of the present invention are suitable for the treatment of diseases or disorders relating to the deficiency of proteins and/or enzymes. In one embodiment, the nucleic acids of the present invention encode functional proteins or enzymes that are excreted or secreted by the target cell into the surrounding extracellular fluid (e.g., mRNA encoding hormones and neurotransmitters). Alternatively, in another embodiment, the nucleic acids of the present invention encode functional proteins or enzymes that remain in the cytosol of the target cell (e.g., mRNA encoding urea cycle metabolic disorders). Other disorders for which the present invention are useful include disorders such as SMN1-related spinal muscular atrophy (SMA); amyotrophic lateral sclerosis (ALS); GALT-related

galactosemia; Cystic Fibrosis (CF); SLC3A1-related disorders including cystinuria; COL4A5-related disorders including Alport syndrome; galactocerebrosidase deficiencies; X-linked adrenoleukodystrophy and adrenomyeloneuropathy; Friedreich's ataxia; Pelizaeus-Merzbacher disease; TSC1 and TSC2-related tuberous sclerosis; Sanfilippo B syndrome (MPS IIIB); CTNS-related cystinosis; the FMR1-related disorders which include Fragile X syndrome, Fragile X-Associated Tremor/Ataxia Syndrome and Fragile X Premature Ovarian Failure Syndrome; Prader-Willi syndrome; hereditary hemorrhagic telangiectasia (AT); Niemann-Pick disease Type C1; the neuronal ceroid lipofuscinoses-related diseases including Juvenile Neuronal Ceroid Lipofuscinosis (JNCL), Juvenile Batten disease, Santavuori-Haltia disease, Jansky-Bielschowsky disease, and PTT-1 and TPP1 deficiencies; EIF2B1, EIF2B2, EIF2B3, EIF2B4 and EIF2B5-related childhood ataxia with central nervous system hypomyelination/vanishing white matter; CACNA1A and CACNB4-related Episodic Ataxia Type 2; the MECP2-related disorders including Classic Rett Syndrome, MECP2-related Severe Neonatal Encephalopathy and PPM-X Syndrome; CDKL5-related Atypical Rett Syndrome; Kennedy's disease (SBMA); Notch-3 related cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL); SCN1A and SCN1B-related seizure disorders; the Polymerase G-related disorders which include Alpers-Huttenlocher syndrome, POLG-related sensory ataxic neuropathy, dysarthria, and ophthalmoparesis, and autosomal dominant and recessive progressive external ophthalmoplegia with mitochondrial DNA deletions; X-Linked adrenal hypoplasia; X-linked agammaglobulinemia; and Wilson's disease. In one embodiment, the nucleic acids, and in particular mRNA, of the present invention may encode functional proteins or enzymes. For example, the compositions of the present invention may include mRNA encoding erythropoietin, α1-antitrypsin, carboxypeptidase N or human growth hormone.

Alternatively the nucleic acids may encode full length antibodies or smaller antibodies (e.g., both heavy and light chains) to confer immunity to a subject. While one embodiment of the present invention relates to methods and compositions useful for conferring immunity to a subject (e.g., via the translation of mRNA nucleic acids encoding functional antibodies), the inventions disclosed herein and contemplated

hereby are broadly applicable. In an alternative embodiment the compositions of the present invention encode antibodies that may be used to transiently or chronically effect a functional response in subjects. For example, the mRNA nucleic acids of the present invention may encode a functional monoclonal or polyclonal antibody, which upon translation (and as applicable, systemic excretion from the target cells) may be useful for targeting and/or inactivating a biological target (e.g., a stimulatory cytokine such as tumor necrosis factor). Similarly, the mRNA nucleic acids of the present invention may encode, for example, functional anti-nephritic factor antibodies useful for the treatment of membranoproliferative glomerulonephritis type II or acute hemolytic uremic syndrome, or alternatively may encode anti-vascular endothelial growth factor (VEGF) antibodies useful for the treatment of VEGF-mediated diseases, such as cancer.

The compositions of the present invention can be administered to a subject. In some embodiments, the composition is formulated in combination with one or more additional nucleic acids, carriers, targeting ligands or stabilizing reagents, or in pharmacological compositions where it is mixed with suitable excipients. For example, in one embodiment, the compositions of the present invention may be prepared to deliver nucleic acids (e.g., mRNA) encoding two or more distinct proteins or enzymes. Alternatively, the compositions of the present invention may be prepared to deliver a single nucleic acid and two or more populations or such compositions may be combined in a single dosage form or co-administered to a subject. Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, Pa., latest edition.

A wide range of molecules that can exert pharmaceutical or therapeutic effects can be delivered into target cells using compositions and methods of the present invention. The molecules can be organic or inorganic. Organic molecules can be peptides, proteins, carbohydrates, lipids, sterols, nucleic acids (including peptide nucleic acids), or any combination thereof. A formulation for delivery into target cells can comprise more than one type of molecule, for example, two different nucleotide sequences, or a protein, an enzyme or a steroid.

The compositions of the present invention may be administered and dosed in accordance with current medical practice, taking into account the clinical condition of the subject, the site and method of administration, the scheduling of administration, the subject's age, sex, body weight and other factors relevant to clinicians of ordinary skill in the art. The "effective amount" for the purposes herein may be determined by such relevant considerations as are known to those of ordinary skill in experimental clinical research, pharmacological, clinical and medical arts. In some embodiments, the amount administered is effective to achieve at least some stabilization, improvement or elimination of symptoms and other indicators as are selected as appropriate measures of disease progress, regression or improvement by those of skill in the art. For example, a suitable amount and dosing regimen is one that causes at least transient expression of the nucleic acid in the target cell.

Suitable routes of administration include, for example, oral, rectal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, the compositions of the present invention may be administered in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a targeted tissue, preferably in a depot or sustained release formulation. Local delivery can be affected in various ways, depending on the tissue to be targeted. For example, aerosols containing compositions of the present invention can be inhaled (for nasal, tracheal, or bronchial delivery); compositions of the present invention can be injected into the site of injury, disease manifestation, or pain, for example; compositions can be provided in lozenges for oral, tracheal, or esophageal application; can be supplied in liquid, tablet or capsule form for administration to the stomach or intestines, can be supplied in suppository form for rectal or vaginal application; or can even be delivered to the eye by use of creams, drops, or even injection. Formulations containing compositions of the present invention complexed with therapeutic molecules or ligands can even be surgically administered, for example in association with a polymer or other structure or substance that can allow the compositions to diffuse from the site of implantation to

surrounding cells. Alternatively, they can be applied surgically without the use of polymers or supports.

In one embodiment, the compositions of the present invention are formulated such that they are suitable for extended-release of the nucleic acids contained therein. Such extended-release compositions may be conveniently administered to a subject at extended dosing intervals. For example, in one embodiment, the compositions of the present invention are administered to a subject twice day, daily or every other day. In a preferred embodiment, the compositions of the present invention are administered to a subject twice a week, once a week, every ten days, every two weeks, every three weeks, or more preferably every four weeks, once a month, every six weeks, every eight weeks, every other month, every three months, every four months, every six months, every eight months, every nine months or annually. Also contemplated are compositions and liposomal vehicles which are formulated for depot administration (e.g., intramuscularly, subcutaneously, intravitreally) to either deliver or release a nucleic acids (e.g., mRNA) over extended periods of time. Preferably, the extended-release means employed are combined with modifications made to the nucleic acid to enhance stability.

While certain compounds, compositions and methods of the present invention have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds of the invention and are not intended to limit the same. Each of the publications, reference materials, accession numbers and the like referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference in their entirety.

The articles "a" and "an" as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also

includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. The publications and other reference materials referenced herein to describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

EXAMPLES

Example 1

General Preparation of Transfer Vehicles by Solvent Dilution Technique

This example generally illustrates a process for the manufacture of small (< 100 nm) liposomal formulations containing mRNA and the means to evaluate the amount of mRNA encapsulated. Parameters which may be modified to further optimize transfection efficiency include, but are not limited to, the selection of lipid, the ratio of lipids, the molar ratio of the PEG-containing lipid, the length of the lipid anchor of the PEG-containing lipid and the sizing of the liposomal transfer vehicles.

Appropriate quantities of lipids (e.g., DSPC/CHOL/DODAP/C8-PEG2000-Cer) to construct a transfer vehicle of a desired lipid ratio (e.g., a molar ratio of

31:40:25:4) were weighed and dissolved in absolute ethanol at 70°C to obtain the desired lipid ratios and concentrations. In order to monitor the lipid, a small amount (typically 0.05 mole%) of rhodamine-dioleoylphosphatidylethanolamine (Rh-PE) was routinely added to the lipid solution.

mRNA, for example, encoding for GFP, OTC or Luciferase was denatured by heating for 10 minutes at 70°C, followed by cooling on ice. This solution was analyzed to confirm the mRNA concentration prior to formulation. An aliquot of mRNA was diluted with water, and then combined with an equal volume of 10 mM citrate pH 5.0 buffer such that the final citrate content following lipid addition (from solvent) was 4 mM.

The mRNA/citrate buffer solutions were then heated to 90°C for 5 minutes to completely denature the mRNA. While stirring or vortexing the denatured mRNA, the ethanolic lipid solution (at 70°C) was added to the mRNA to generate multi-lamellar vesicles (MLVs). The MLVs were then cooled to 70°C prior to extrusion. For samples prepared at high solvent concentrations (> 20%), the MLVs were diluted with 5 mM pH 5.0 citrate buffer (at 70°C) to produce a solvent concentration of 20% before extrusion to generate large unilamellar vesicles (LUVs).

MLVs were extruded at 70°C through 3 stacked 80 nm polycarbonate filters, using a thermo-jacketed extruder. Five passes were routinely used to generate large unilamellar vesicles (LUVs) of the desired size range. Following extrusion, the formulations were filtered through a 0.2μm syringe filter to remove small amounts of particulate material that tended to interfere with the determination of vesicle size.

mRNA that was not associated with the liposomes or was associated with the exterior surface of DODAP-containing liposomes was removed by anion exchange, such that all remaining associated mRNA was encapsulated in the liposomes. Two suitable methods include the use of anion exchange using Acrodisc units with MUSTANG Q membranes (Pall Life Sciences), or anion exchange using DEAE-SEPHACEL (Sigma-Aldrich, suspension in 20% ethanol). These techniques allowed for efficient removal of unencapsulated mRNA without significant dilution of the formulations.

Following removal of external mRNA, buffer could be exchanged by use of PD-10 gel filtration columns (SEPHADEX G-25, GE Healthcare) using a spin

protocol, which permits small molecular weight constituents (such as solvent and borate) in the liposome formulation to be retained in the gel and replaced by the equilibration buffer, without significant dilution of the sample. Alternatively, in some experiments, solvent may be removed and buffer exchanged using a Spectrum 500,000 MWCO diafiltration cartridge. Samples were ultrafiltered to 2-10 mL, then diafiltered against 10 wash volumes of the desired final buffer to remove solvent and exchange the buffer. The sample was sometimes further concentrated by ultrafiltration after the diafiltration process.

To quantify mRNA in samples with low lipid:mRNA ratios, a standard curve of mRNA was prepared by diluting the stock solution with water to obtain standards in the range of 0-200μg/mL. Samples were diluted (based on expected mRNA concentrations) with the appropriate buffer to produce mRNA concentrations within the standard range. 180μL aliquots of the standards or samples were combined with 300μL of 5% SDS and 120μL of ethanol. The samples were incubated for 10 min. at 50°C to dissolve the lipid. After cooling, the samples were transferred in duplicate (250μL aliquots) into the wells of a UV-transparent microplate. The absorbance at 260nm was measured and the mRNA concentration in the samples calculated from the standard curve. In samples where the lipid:mRNA (weight: weight) ratio was 10:1 (target ratio) or less, interference from the lipids with the absorbance at 260 nm was relatively low and could be ignored.

In samples where the lipid:mRNA (weight: weight) ratio was greater than 10:1, lipid interference became more significant as the amount of lipid increased, and therefore the lipid had to be removed in order to accurately quantify the mRNA content. A standard curve of mRNA was prepared by diluting the stock solution with water to obtain standards in the range of 0-250μg/mL. The samples to be assessed were diluted (based on expected mRNA concentrations) with the appropriate buffer to produce mRNA concentrations within the standard range. 180 μL of the standards or samples were combined with 20 μL 0.1 M sodium borate (to increase the pH, thus neutralizing the charge on the DODAP in the liposome samples, and causing the mRNA to dissociate from the DODAP). 600 μL of chloroform: methanol (1:2, v:v) was added to each standard or sample and the samples were vortexed. 200 μL of chloroform was added with vortexing followed by the addition of 200 μL of water.

The samples were vigorously vortexed and then centrifuged for 2 min. at 1000xg to separate the phases. 250 µL aliquots of the upper (aqueous) phase were transferred (in duplicate) into the wells of a UV-transparent microplate and the absorbance at 260 nm was measured. The mRNA concentration in samples was calculated from the standard curve. Note that for liposome samples containing DOTAP (or any other cationic lipid that cannot be neutralized by incubation at high pH), this assay is unsuitable for determining mRNA concentration as the mRNA cannot be disassociated from the DOTAP and a proportion of the mRNA tends to be extracted into the solvent ($CHCl_3$) phase in conjunction with the lipid.

mRNA encapsulation was determined by separation of samples on DEAE-SEPHACEL (anion exchange gel) columns as follows. Using 2 mL glass Pasteur pipettes plugged with glass wool, columns of DEAE-SEPHACEL were poured and equilibrated with 5 volumes (~10 mL) of 145 mM sodium chloride-10 mM borate buffer pH 8.0. 0.5 mL of sample was loaded onto a column and the eluate collected. The columns were washed with 7x0.5 mL aliquots of 145 mM sodium chloride-10 mM borate buffer pH 8.0, collecting each eluted fraction separately. The initial sample and each aliquot was assayed for mRNA and lipid as described above. The % encapsulation was calculated by 100 x (mRNA/lipid) of material eluted from the column / (mRNA/lipid) of initial sample). Based on the calculated mRNA concentration from extraction analyses described above liposomal mRNA samples were diluted to a desired mRNA concentration (1 µg) in a total volume of 5 µL (i.e. 0.2 mg/mL).

Example 2

Preparation of DSPC/CHOL/DODAP/C8-PEG-2000 ceramide (molar ratio of 31:40:25:4)/Renilla Luciferase mRNA (Formulation 1)

Formulation 1 was prepared by dissolving the appropriate masses of DSPC, CHOL, DODAP and C8-PEG-2000 ceramide in absolute ethanol, then adding this to a solution of *Renilla* Luciferase mRNA in buffer to produce MLVs at 10.8 mg/mL lipid, 250 µg/mL mRNA, 20% solvent. The MLVs were extruded to produce LUVs, and then passed through a 0.2 µm filter. The pH was increased by combining with an equal volume of 100 mM NaCl-50 mM borate pH 8.0 and the external mRNA

removed by anion exchange using the DEAE-Sephacel centrifugation method, as described in Example 1. The solvent was removed, the external buffer exchanged and the sample concentrated by diafiltration/ultrafiltration. The concentrated sample was then passed through a 0.2 μm filter and aliquots were transferred to vials and stored at 2-8°C.

Example 3

Preparation of DSPC/CHOL/DOTAP/C8-PEG-2000 ceramide (molar ratio of 31:40:25:4)/Renilla Luciferase mRNA (Formulation 2)

Formulation 2 was prepared using a similar methodology as Formulation 1 with minor changes. In brief, the appropriate masses of DSPC, CHOL, DOTAP and C8-PEG-2000 ceramide were dissolved in absolute ethanol and then added to a solution of *Renilla* Luciferase mRNA in buffer to produce MLVs at 10.8 mg/mL lipid, 250 μg/mL mRNA, 20% solvent. The MLVs were extruded to produce LUVs. As DOTAP was used in this formulation, the external mRNA could not be effectively removed by anion exchange and therefore this step was omitted. The solvent was removed, the external buffer exchanged and the sample concentrated by diafiltration/ultrafiltration. The concentrated sample was then passed through a 0.2 μm filter and aliquots were transferred to vials and stored at 2-8°C.

Example 4

Preparation of DSPC/CHOL/DODAP/C8-PEG-2000 ceramide (molar ratio of 31:40:25:4)/Firefly Luciferase mRNA (Formulation 3)

To prepare Formulation 3 the appropriate masses of DSPC, CHOL, DODAP and C8-PEG-2000 ceramide were dissolved in absolute ethanol, then added to a solution of Firefly Luciferase mRNA in buffer to produce MLVs at 10.8 mg/mL lipid, 250 μg/mL mRNA, 20% solvent. The MLVs were extruded to produce LUVs, and then passed through a 0.2 μm filter. The pH was increased by combining with 0.1 volumes of 0.1 M sodium borate and the external mRNA removed by anion exchange using the DEAE-Sephacel column method described in Example 1. The solvent was removed, the external buffer exchanged and the sample concentrated by

diafiltration/ultrafiltration. The concentrated sample was then passed through a 0.2 μm filter and aliquots were transferred to vials and stored at 2-8°C.

Example 5

Preparation of DSPC/CHOL/DODAP/C8-PEG-2000 ceramide (molar ratio of 31:40:2:4)/Murine OTC mRNA (Formulation 4)

Formulation 4 was prepared by dissolving the appropriate mass of DSPC, CHOL, DODAP and C8-PEG-2000 ceramide in absolute ethanol, then adding this to a solution of murine OTC mRNA in buffer to produce MLVs at 10.8 mg/mL lipid, 250 μg/mL mRNA, 20% solvent. The MLVs were extruded to produce LUVs, and then passed through a 0.2 μm filter. The pH was increased by combining with 0.1 volumes of 0.1 M sodium borate and the external mRNA removed by anion exchange using the DEAE-Sephacel column method as described in Example 1. The solvent was removed, the external buffer exchanged and the sample concentrated by diafiltration/ultrafiltration. The concentrated sample was then passed through a 0.2 μm filter and aliquots were transferred to vials and stored at 2-8°C.

Example 6

Preparation and Characterization of the imidiazole cholesterol ester lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17-((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate; Imidazole Cholesterol Ester (ICE)

FIG. 1 depicts the reaction scheme for the synthesis of ICE. A mixture of trityl-deamino-histidine **(1)**, (1.97g, 5.15mmol), cholesterol **(2)**, (1.97 g, 5.1 mmol), dicyclohexylcarbodiimide **(DCC)**, (2.12g, 5.2mmol) and dimethylaminopyridine **(DMAP)**, (0.13g, 1.0mmol) in anhydrous benzene (100ml) was stirred at ambient temperature for two days. The resulting suspension was filtered through Celite and the filtrate was removed under reduced pressure. The resulting foam was dried under high vacuum overnight to provide crude ester **(3)** which was used on the following step without purification.

The crude ester **(3)** was dissolved in anhydrous dichloromethane **(DCM)**, (200ml) and trifluoroacetic acid **(TFA)**, (50 ml) was added at room temperature. The

resulting solution was stirred at ambient temperature for 4 hours. Aqueous saturated $NaHCO_3$ (250ml) was added carefully, followed by solid Na_2CO_3 until slightly basic.

The phases were separated and the aqueous layer was extracted with DCM (200 ml). The organic phases were washed with brine (200ml), dried (Na_2SO_4) and filtered. The resulting filtrate was evaporated and the residue was dried under high vacuum overnight. Flash chromatography purification (silica gel, 0-10% methanol in chloroform) afforded the desired pure product **(4)** (1.07g, 37% yield for two steps) as a white solid (mp: 192-194°C).

^{1}H NMR ($CDCl_3$): δ 0.66 (s, 3H), 0.84-1.64 (m, 33H), 1.76-2.05 (m, 5H), 2.29 (d, 2H), 2.63 (t, 2H), 2.90 (t, 2H), 4.61 (m, 1H), 5.36 (d, 1H), 6.80 (s, 1H), 7.53 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 11.9, 18.8, 19.4, 21.1, 21.6, 22.6, 22.9, 23.9, 24.4, 27.8, 28.1, 28.3, 31.9, 34.5, 35.9, 36.3, 36.7, 37.0, 38.2, 39.6, 39.8, 42.4, 50.1, 56.2, 56.8, 74.1, 122.8, 134.7, 139.6, 173.4. APCI(+)-MS (*m/z*): Calcd. 509, Found 509. Elem. Anal. (C,H,N): Calcd. 77.90, 10.30, 5.51; Found 77.65, 10.37, 5.55.

Example 7

Formulation Protocol

A codon-optimized firefly luciferase messenger RNA represented by SEQ ID NO: 1 (FFL mRNA) was synthesized by *in vitro* transcription from a plasmid DNA template encoding the gene, which was followed by the addition of a 5' cap structure (Cap1) and a 3' poly(A) tail of approximately 200 nucleotides in length as determined by gel electrophoresis. (See, *e.g.*, Fechter, P. *et al.*, J. Gen. Virology, 86, 1239-1249 (2005), the contents of which are incorporated herein by reference in its entirety.) The 5' and 3' untranslated regions present in the FFL mRNA product are underlined (SEQ ID NO: 1).

Nanoparticulate transfer vehicles were formed via standard ethanol injection methods. (See, *e.g.*, Ponsa, M., *et al.*, Int. J. Pharm. 95, 51-56 (1993), the contents of which are incorporated herein by reference.) Ethanolic stock solutions of the lipids were prepared ahead of time at a concentration of 50mg/mL and stored at –20°C. FFL mRNA was stored in water at a final concentration of 1mg/mL at –80°C until the time of use.

All mRNA concentrations were determined via the Ribogreen assay (Invitrogen). Encapsulation of mRNA was calculated by performing the Ribogreen assay both with and without the presence of 0.1% Triton-X 100. Particle sizes (dynamic light scattering (DLS)) and zeta potentials were determined using a Malvern Zetasizer instrument in 1x PBS and 1mM KCl solutions, respectively.

Aliquots of 50mg/mL ethanolic solutions of an imidazole cholesterol ester lipid (ICE), DOPE and DMG-PEG-2000 were mixed and diluted with ethanol to a final volume of 3mL. The molar ratio of the prepared ICE:DOPE:DMG-PEG-2000 transfer vehicle was 70:25:5. Separately, an aqueous buffered solution (10mM citrate/150mM NaCl, pH 4.5) of FFL mRNA was prepared from a 1mg/mL stock. The lipid solution was injected rapidly into the aqueous mRNA solution and shaken to yield a final suspension in 20% ethanol. The resulting nanoparticulate suspension was filtered, diafiltrated with 1x PBS (pH 7.4), concentrated and stored at 2-8°C. The final concentration was equal to 1.73mg/mL CO-FF mRNA (encapsulated), the Z_{ave} was equal to 68.0nm (with a $Dv_{(50)}$ of 41.8nm, and a $Dv_{(90)}$ of 78.0nm) and the Zeta potential was equal to +25.7 mV.

Biodistribution Analysis

All studies were performed using female CD-1 mice of approximately 3-weeks age at the beginning of each experiment. Samples were introduced by a single bolus tail-vein injection of an equivalent total dose of 200μg of encapsulated FFL mRNA. Four hours post-injection the mice were sacrificed and perfused with saline.

The liver and spleen of each mouse was harvested, apportioned into three parts, and stored in either, (i) 10% neutral buffered formalin, (ii) snap-frozen and stored at –80°C for bioluminescence analysis (see below), or for *in situ* hybridization studies, or (iii) liver sections were isolated in isopentane (2-methylbutane) bath, maintained at –35°C, rinsed with 1x PBS, lightly patted with a kimwipe to remove any excess fluid, placed in the bath for approximately 5-7 minutes, after which the liver was removed, wrapped in foil and stored in a small sterile plastic bag at –80°C until ready for assay.

The bioluminescence assay was conducted using a Promega Luciferase Assay System (Item # E1500 Promega). Tissue preparation was performed as follows:

Portions of the desired tissue sample (snap-frozen) were thawed, washed with RODI water and placed in a ceramic bead homogenization tube. The tissue was treated with lysis buffer and homogenized. Upon subjection to five freeze/thaw cycles followed by centrifugation at 4°C, the supernatant was transferred to new microcentrifuge tubes. Repeat and store tissue extracts at –80°C.

The Luciferase Assay Reagent was prepared by adding 10mL of Luciferase Assay Buffer to Luciferase Assay Substrate and mix via vortex. 20µL of homogenate samples was loaded onto a 96-well plate followed by 20µL of plate control to each sample. Separately, 120µL of Luciferase Assay Reagent (prepared as described above) was loaded onto each well of a 96-well flat bottomed plate. Each plate was inserted into the appropriate chambers using a Molecular Device Flex Station instrument and measure the luminescence (measured in relative light units (RLU)).

In Situ Hybridization

Tissue Slide Preparation

Slide preparation and analysis was performed as follows: Each liver was frozen at –35°C according to the procedure described above. The frozen livers were cut into 6 micrometer sections and mounted onto glass microscope slides. Prior to *in situ* hybridization, the sections were fixed in 4% formaldehyde in phosphate buffered saline (PBS), treated with trienthanolamine/acetic anhydride and washed and dehydrated through a series of ethanol solutions.

cRNA Probe Preparation

DNA templates were designed consisting of pBSKII+ vector containing EcoRI and XbaI restriction sites for generation of the antisense and sense strands, respectively. cRNA transcripts were synthesized from these DNA templates (antisense and sense strands, each 700bp) with T3 and T7 RNA polymerase, respectively. Templates were validated by cold RNA probe synthesis prior to making riboprobes with ^{35}S-UTP. Both antisense and sense radiolabeled riboprobes were synthesized *in vitro* according to the manufacturer's protocol (Ambion) and labeled with 35S-UTP (>1,000 Ci/mmol).

Hybridization and Washing Procedures

Sections were hybridized overnight at 55°C in deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl (pH 7.4), 5 mM EDTA, 10 mM Na_2HPO_4, 10% dextran sulfate, 1X Denhardt's reagent, 50µg/mL total yeast RNA and 50-80,000 cpm/µL 35S labeled cRNA probe. The tissues were subjected to stringent washing at 65°C in 50% formamide, 2X SSC, 10 mM DTT and washed in PBS before treatment with 20µg/ml RNAse A at 37°C for 30 minutes. Following washes in 2X SSC and 0.1X SSC for 10 minutes at 37°C, the slides were dehydrated and exposed to Kodak BioMaxMR x-ray film for 90 minutes then submitted to emulsion autoradiography for 11 and 24 hours exposure times.

Imaging of Liver Sections

Photographic development was carried out in Kodak D-19. Sections were counterstained lightly with cresyl violet and analyzed using brightfield and darkfield microscopy. Sense (control) riboprobes established the level of background signal.

In Vivo Bioluminescence Results

Animals were injected intravenously with a single 200µg dose of encapsulated mRNA and sacrificed after four hours. Activity of expressed firefly luciferase protein in livers and spleens was determined in a bioluminescence assay. As demonstrated in FIG. 2, detectable signal over baseline was observed in every animal tested. The presence of a luminescent signal over background infers the expression of firefly luciferase protein from the exogenous mRNA. Luminescence observed in the liver was enhanced over similar signals observed in the spleen.

In Situ Hybridization Results

In situ hybridization studies were performed on liver taken from two different animals from the group of mice treated using an ICE:DOPE:DMG-PEG-2000 transfer vehicle (prepared as previously described) and one control liver from the untreated group of mice. X-Ray film autoradiography was employed for the detection of codon-optimized firefly luciferase mRNA via ^{35}S-U labeled riboprobes. (See, Wilcox, J.N. J. Histochem. Cytochem. 41, 1725-1733 (1993)). FIG. 3 demonstrates both

brightfield illumination (cresyl violet counterstain) and darkfield illumination of control and treated livers under low (2X) magnification. CO-FF luciferase mRNA was detected in both treated livers (B1 and B2, thin arrows) but not the control liver (Ct) when using the antisense riboprobe (FIG 3B). High-level mRNA labeling was observed in the liver marginal tissue band (large arrow). No signal was detected in any liver when applying the control (sense) riboprobe (FIG. 3C).

Under a dark field illumination labeled FFL mRNA was detected as bright spots (100X magnification) in the livers of injected animals by hybridization of an antisense probe of FFL mRNA (FIG. 4A), while the same liver showed few bright spots when a sense strand probe of FFL mRNA was used for hybridization (FIG. 4C). A control liver taken from an animal that did not receive any nanoparticles by injection did not produce any significant signal under dark field illumination when either the antisense (FIG. 4E) or sense probes (FIG. 4G) were used for hybridization.

Example 8

Immunohistochemical Analysis Results

The FFL mRNA was packaged and delivered via a lipid transfer vehicle formulation consisting of cholesterol, DOPE, DLinDMA, and DMG-PEG2000 in a manner similar to that described *supra*.

The translation of the FFL mRNA into its respective protein has been successfully identified via immunohistochemical analysis (FIG. 5). Using an anti-firefly antibody, the detection of expressed firefly protein can be observed in the hepatocytes of treated mice (FIG 5B and 5C). The analysis of control mice treated with 1x PBS demonstrated no detectable firefly protein (FIG. 5A).

Discussion

A synthetic messenger RNA encapsulted in lipid-based materials can be used for the delivery and expression of genes *in vivo* in liver including heptocytes. Mixtures of cationic, non-cationic and PEG-modified lipids were used to express a reporter protein molecule. The imidazole-based cationic lipid ICE resulted in enriched delivery of mRNA to liver versus spleen *in vivo*. The observation of a bioluminescent signal demonstrates that a protein reporter molecule was translated

from the exogenous mRNA that was delivered in a lipid nanoparticle in vivo. *In situ* hybridization studies demonstrated the direct detection of the exogenous mRNA through ^{35}S-U riboprobe labeling. Emulsion autoradiography produced a signal that can be used to localize the mRNA to liver tissue and more specifically to hepatocytes present in the livers of treated animals (See, FIGS. 3 and 4). FFL mRNA was not detected in the livers of untreated control mice.

The successful delivery of such mRNA to the liver and in particular to hepatocytes supports the conclusion that the methods, formulations and compositions of the present invention can be used for the treatment and the correction of in-born errors of metabolism that are localized to the liver. For example, diseases such as ASD, ARG, CPS, ASS1 and OTC deficiencies, as well as other disorders may be treated through mRNA replacement therapy of a missing or malfunctioning gene. Metabolic zonation of the urea cycle to hepatocytes means that replacement of the missing enzyme activity in these cells should greatly improve normal biochemical processing in subjects afflicted by an enzyme deficiency, and in particular subjects afflicted with a urea cycle disorder.

CLAIMS

What is claimed is:

1. A composition for modulating the expression of a protein in a target cell, wherein said composition comprises at least one RNA molecule and a transfer vehicle.

2. The composition of claim 1, wherein the RNA molecule is selected from the group consisting of mRNA, miRNA, snRNA, and snoRNA.

3. The composition of claim 2, wherein the RNA molecule comprises at least one modification which confers stability to the RNA molecule.

4. The composition of claim 3, wherein the RNA molecule comprises a modification of the 5' untranslated region of said RNA molecule.

5. The composition of claim 4, wherein said modification comprises a partial sequence of a CMV immediate-early 1 (IE1) gene.

6. The composition of claim 5, wherein said partial sequence of the CMV immediate-early 1 (IE1) gene comprises SEQ ID NO: 2.

7. The composition of claim 4, wherein said modification comprises the inclusion of a poly A tail.

8. The composition of claim 4, wherein said modification comprises the inclusion of a Cap1 structure.

9. The composition of claim 3, wherein the RNA molecule comprises a modification of the 3' untranslated of said RNA molecule.

10. The composition of claim 9, wherein said modification comprises the inclusion of a sequence encoding human growth hormone (hGH).

11. The composition of claim 10, wherein said sequence encoding human growth hormone (hGH) comprises SEQ ID NO: 3.

12. The composition of claim 9, wherein said modification comprises the inclusion of a poly A tail.

13. The composition of claim 1, wherein the transfer vehicle is a liposome.

14. The composition of claim 1, wherein the transfer vehicle is a lipid nanoparticle.

15. The composition of claim 1, comprising an agent for facilitating transfer of the RNA molecule to an intracellular compartment of the target cell.

16. The composition of claim 15, wherein the agent is selected from the group consisting of a protein, a peptide, an aptamer, and an oligonucleotide.

17. The composition of claim 1, comprising a ligand capable of enhancing affinity of the composition for the target cell.

18. The composition of claim 17, wherein the ligand is selected from the group consisting of a peptide, a protein, an aptamer, a vitamin, and an oligonucleotide.

19. The composition of claim 17, wherein said ligand is selected from the group consisting of apolipoprotein-B and apolipoprotein-E.

20. The composition of claim 1, comprising a stabilizing reagent.

21. The composition of claim 20, wherein the stabilizing reagent is selected from the group consisting of a protein, a peptide, and an aptamer.

22. The composition of claim 21, wherein the stabilizing reagent binds to the RNA molecule.

23. The composition of claim 1, wherein said transfer vehicle comprises one or more cationic lipids.

24. The composition of claim 1, wherein said transfer vehicle comprises one or more non-cationic lipids.

25. The composition of claim 1, wherein said transfer vehicle comprises one or more PEG-modified lipids.

26. The composition of claim 1, wherein said transfer vehicle comprises CHOL, DOPE, DLinDMA and DMG-PEG-2000.

27. The composition of claim 1, wherein said transfer vehicle comprises ICE, DOPE and DMG-PEG-2000.

28. The composition of claim 1, wherein the transfer vehicle comprises one or more lipids selected from the group consisting of ICE, DSPC, CHOL, DODAP, DOTAP and C8-PEG-2000 ceramide.

29. The composition of claim 14, wherein the transfer vehicle comprises DSPC, CHOL, DODAP and C8-PEG-2000 ceramide.

30. The composition of claim 1, wherein said target cell is selected from the group consisting of hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, lung cells, bone cells, stem cells,

mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

31. The composition of claim 1, wherein said RNA molecule is mRNA and wherein said mRNA is greater than 1 kDa.

32. A composition for increasing expression of a urea cycle enzyme a target cell, the composition comprising an mRNA and a transfer vehicle, wherein the mRNA encodes a urea cycle enzyme and wherein the mRNA comprises a modification, wherein the modification confers stability to the mRNA.

33. The composition of claim 32, wherein the modification comprises an alteration of a 5' untranslated of said mRNA.

34. The composition of claim 33, wherein said modification comprises a partial sequence of a CMV immediate-early 1 (IE1) gene.

35. The composition of claim 34, wherein said partial sequence of the CMV immediate-early 1 (IE1) gene comprises SEQ ID NO: 2.

36. The composition of claim 33, wherein said modification comprises the inclusion of a poly A tail.

37. The composition of claim 33, wherein said modification comprises the inclusion of a Cap1 structure.

38. The composition of claim 32, wherein the modification comprises an alteration of a 3' untranslated region of said mRNA.

39. The composition of claim 38, wherein said modification comprises the inclusion of a sequence encoding human growth hormone (hGH).

40. The composition of claim 39, wherein said sequence encoding human growth hormone (hGH) comprises SEQ ID NO: 3.

41. The composition of claim 38, wherein said modification comprises the inclusion of a poly A tail.

42. The composition of claim 32, wherein the urea cycle enzyme is selected from the group consisting of ornithine transcarbamylase (OTC), carbamoyl-phosphate synthetase 1 (CPS1), argininosuccinate synthetase (ASS1), argininosuccinate lyase (ASL), and arginase 1 (ARG1).

43. The composition of claim 32, wherein following expression of said urea cycle enzyme by said target cell, said urea cycle enzyme is secreted from said target cell.

44. The composition of claim 32, wherein said transfer vehicle comprises one or more cationic lipids.

45. The composition of claim 32, wherein said transfer vehicle comprises one or more non-cationic lipids.

46. The composition of claim 32, wherein said transfer vehicle comprises one or more PEG-modified lipids.

47. The composition of claim 32, wherein said transfer vehicle comprises CHOL, DOPE, DLinDMA and DMG-PEG-2000.

48. The composition of claim 32, wherein said transfer vehicle comprises ICE, DOPE and DMG-PEG-2000.

49. The composition of claim 32, wherein the transfer vehicle comprises one or more lipids selected from the group consisting of ICE, DSPC, CHOL, DODAP, DOTAP and C8-PEG-2000 ceramide.

50. The composition of claim 49, wherein the transfer vehicle comprises DSPC, CHOL, DODAP and C8-PEG-2000 ceramide.

51. The composition of claim 32, wherein the transfer vehicle is a liposome.

52. The composition of claim 32, wherein said transfer vehicle is a lipid nanoparticle.

53. The composition of claim 32, further comprising an agent for facilitating transfer of the mRNA to an intracellular compartment of the target cell.

54. The composition of claim 53, wherein the agent is selected from the group consisting of a protein, a peptide, an aptamer, and an oligonucleotide.

55. The composition of claim 32, further comprising a ligand capable of enhancing affinity of the composition for the target cell.

56. The composition of claim 55, wherein the ligand is selected from the group consisting of a peptide, a protein, an aptamer, a vitamin, and an oligonucleotide.

57. The composition of claim 55, wherein said ligand is selected from the group consisting of apolipoprotein-B and apolipoprotein-E.

58. The composition of claim 32, further comprising a stabilizing reagent.

59. The composition of claim 58, wherein the stabilizing reagent is selected from the group consisting of a protein, a peptide, and an aptamer.

60. The composition of claim 58, wherein the stabilizing reagent binds to the mRNA.

61. The composition of claim 32, wherein said target cell is a hepatocyte.

62. The composition of claim 32, wherein said mRNA is greater than 1 kDa.

63. A method of treating a subject, wherein the subject has a protein deficiency, comprising, administering a composition comprising an mRNA and a transfer vehicle, wherein the mRNA encodes a functional protein, and wherein the mRNA comprises a modification, wherein the modification confers stability to the administered mRNA.

64. The method claim 63, wherein following expression of said mRNA by a target cell a functional protein is produced.

65. The method of claim 64, wherein said functional protein is secreted from said target cell.

66. The method of claim 63, wherein the mRNA encodes a functional urea cycle enzyme..

67. The method of claim 66, wherein the urea cycle enzyme is selected from the group consisting of OTC, CPS1, ASS1, ASL, and ARG1.

68. The method of claim 63, wherein the modification comprises an alteration of a 5' untranslated region of said mRNA.

69. The method of claim 68, wherein said modification comprises a partial sequence of a CMV immediate-early 1 (IE1) gene.

70. The method of claim 69, wherein said partial sequence of a CMV immediate-early 1 (IE1) gene comprises SEQ ID NO: 2.

71. The method of claim 68, wherein said modification comprises the inclusion of a poly A tail.

72. The method of claim 68, wherein said modification comprises the inclusion of a Cap1 structure.

73. The method of claim 63, wherein the modification comprises an alteration of a 3' untranslated region of said mRNA.

74. The method of claim 73, wherein said modification comprises the inclusion of a sequence encoding human growth hormone (hGH).

75. The method of claim 74, wherein said sequence encoding human growth hormone (hGH) comprises SEQ ID NO: 3.

76. The method of claim 73, wherein said modification comprises the inclusion of a poly A tail.

77. The method of claim 63, wherein said transfer vehicle comprises one or more cationic lipids.

78. The method of claim 63, wherein said transfer vehicle comprises one or more non-cationic lipids.

79. The method of claim 63, wherein said transfer vehicle comprises one or more PEG-modified lipids.

80. The method of claim 63, wherein said transfer vehicle comprises CHOL, DOPE, DLinDMA and DMG-PEG-2000.

81. The method of claim 63, wherein said transfer vehicle comprises ICE, DOPE and DMG-PEG-2000.

82. The method of claim 63, wherein the transfer vehicle is a liposome.

83. The method of claim 63, wherein said transfer vehicle is a lipid nanoparticle.

84. The method of claim 63, wherein the composition comprises an agent for facilitating transfer of the mRNA to an intracellular compartment of a target cell of the subject.

85. The method of claim 84, wherein the agent is selected from the group consisting of a protein, a peptide, an aptamer, and an oligonucleotide.

86. The method of claim 63, wherein the composition comprises a ligand capable of enhancing affinity of the composition for a target cell of the subject.

87. The method of claim 86, wherein the ligand is selected from the group consisting of a peptide, a protein, an aptamer, a vitamin, and an oligonucleotide.

88. The method of claim 87, wherein said ligand is selected from the group consisting of apolipoprotein-B and apolipoprotein-E.

89. The method of claim 63, wherein the composition comprises a stabilizing reagent.

90. The method of claim 89, wherein the stabilizing reagent is selected from the group consisting of a protein, a peptide, and an aptamer.

91. The method of claim 89, wherein the stabilizing reagent binds to the mRNA.

92. The method of claim 63, wherein said mRNA is greater than 1 kDa.

93. A method of expressing a functional protein in a target cell wherein the target cell is deficient in said functional protein, comprising contacting the target cell with a composition comprising an mRNA and a transfer vehicle, wherein the mRNA encodes said functional protein and wherein the mRNA comprises a modification, wherein the modification confers stability to the mRNA.

94. The method claim 93, wherein following expression of said mRNA by a target cell a functional protein is produced.

95. The method of claim 94, wherein said functional protein is secreted from said target cell.

96. The method of claim 93, wherein said deficient functional protein is a urea cycle enzyme.

97. The method of claim 96, wherein the urea cycle enzyme is selected from the group consisting of OTC, CPS1, ASS1, ASL, and ARG1.

98. The method of claim 93, wherein the modification comprises an alteration of a 5' untranslated region of said mRNA.

99. The method of claim 98, wherein said modification comprises a partial sequence of a CMV immediate-early 1 (IE1) gene.

100. The method of claim 99, wherein said partial sequence of the CMV immediate-early 1 (IE1) gene comprises SEQ ID NO: 2.

101. The method of claim 98, wherein said modification comprises the inclusion of a poly A tail.

102. The method of claim 98, wherein said modification comprises the inclusion of a Cap1 structure.

103. The method of claim 93, wherein the modification comprises an alteration of a 3' untranslated region of said mRNA.

104. The method of claim 103, wherein said modification comprises the inclusion of a sequence encoding human growth hormone (hGH).

105. The method of claim 104, wherein said sequence encoding human growth hormone (hGH) comprises SEQ ID NO: 3.

106. The method of claim 103, wherein said modification comprises the inclusion of a poly A tail.

107. The method of claim 93, wherein said transfer vehicle comprises one or more cationic lipids.

108. The method of claim 93, wherein said transfer vehicle comprises one or more non-cationic lipids.

109. The method of claim 93, wherein said transfer vehicle comprises one or more PEG-modified lipids.

110. The method of claim 93, wherein said transfer vehicle comprises CHOL, DOPE, DLinDMA and DMG-PEG-2000.

111. The method of claim 93, wherein said transfer vehicle comprises ICE, DOPE and DMG-PEG-2000.

112. The method of claim 93, wherein the transfer vehicle is a liposome.

113. The method of claim 93, wherein said transfer vehicle is a lipid nanoparticle.

114. The method of claim 93, wherein the composition comprises an agent for facilitating transfer of the mRNA to an intracellular compartment of the target cell.

115. The method of claim 114, wherein the agent is selected from the group consisting of a protein, a peptide, an aptamer, and an oligonucleotide.

116. The method of claim 93, wherein the composition comprises a ligand capable of enhancing affinity of the composition for the target cell.

117. The method of claim 116, wherein said ligand is selected from the group consisting of apolipoprotein-B and apolipoprotein-E.

118. The method of claim 117, wherein said target cell expresses one or more low density lipoprotein receptors.

119. The method of claim 116, wherein the ligand is selected from the group consisting of a peptide, a protein, an aptamer, a vitamin, and an oligonucleotide.

120. The method of claim 93, wherein the composition comprises a stabilizing reagent.

121. The method of claim 120, wherein the stabilizing reagent is selected from the group consisting of a protein, a peptide, and an aptamer.

122. The method of claim 120, wherein the stabilizing reagent binds to the mRNA.

123. The method of claim 93, wherein the transfer vehicle comprises one or more lipids selected from the group consisting of ICE, DSPC, CHOL, DODAP, DOTAP and C8-PEG-2000 ceramide.

124. The method of claim 93 wherein the transfer vehicle comprises DSPC, CHOL, DODAP and C8-PEG-2000 ceramide.

125. The method of claim 93, wherein said target cell is selected from the group consisting of hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, lung cells, bone cells, stem cells, mesenchymal cells, neural cells, cardiac cells, adipocytes, vascular smooth muscle cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testicular cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes and tumor cells.

126. The method of claim 93, wherein said mRNA is greater than 1 kDa.

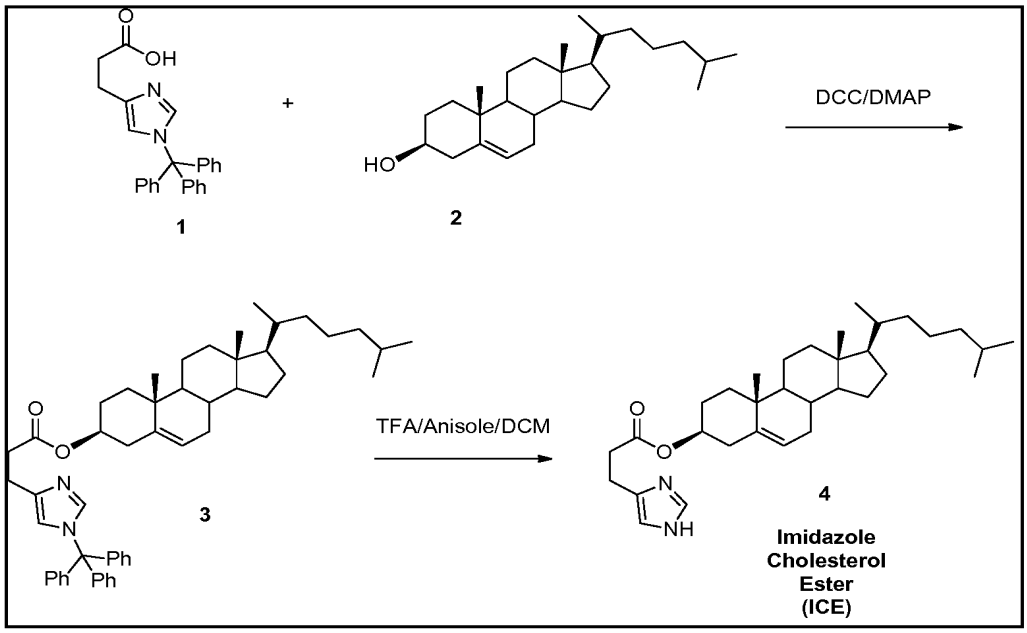
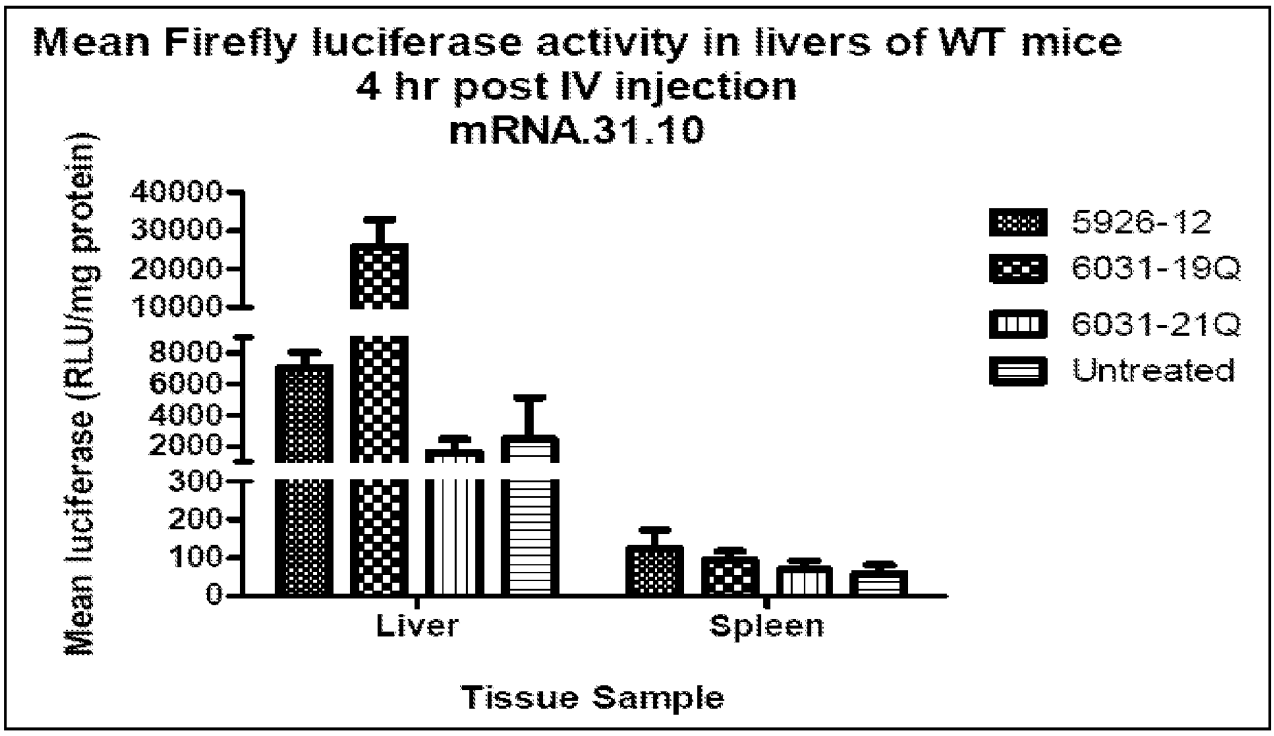

OH
N
N
Ph
Ph
Ph
1
+
HO
2
DCC/DMAP
TFA/Anisole/DCM
3
4
NH
Imidazole
Cholesterol
Ester
(ICE)

FIG. 1


Mean Firefly luciferase activity in livers of WT mice
4 hr post IV injection
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Mean luciferase (RLU/mg protein)
40000
30000
20000
10000
8000
6000
4000
2000
300
200
100
0
Liver
Spleen
Tissue Sample
5926-12
6031-19Q
6031-21Q
Untreated

FIG. 2

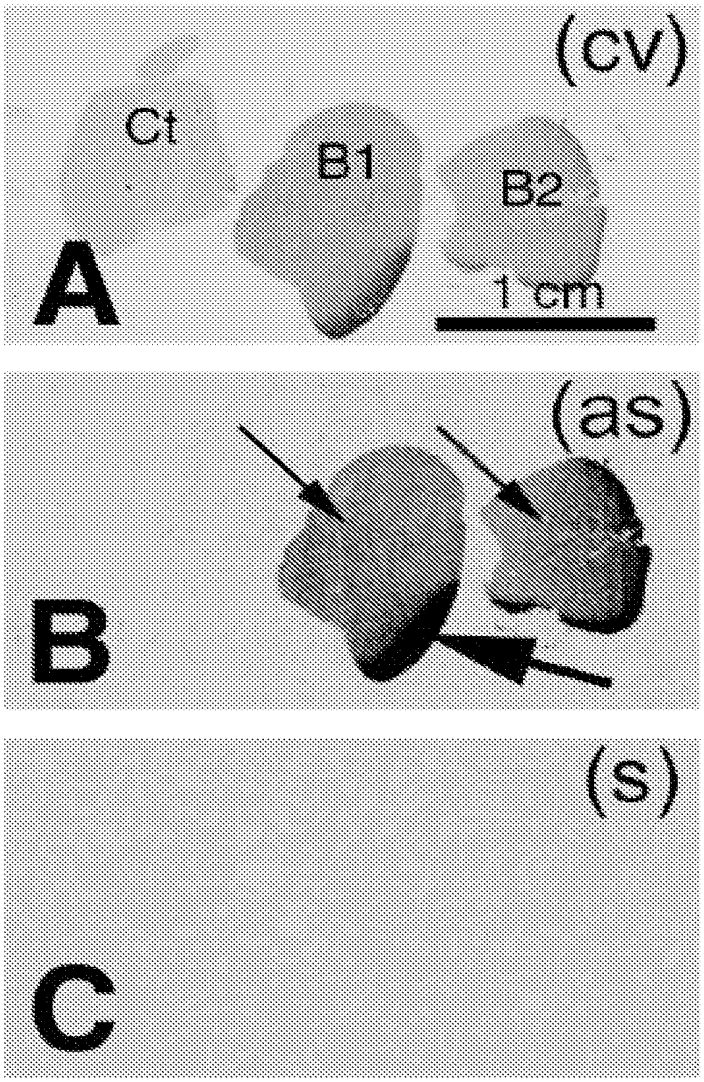
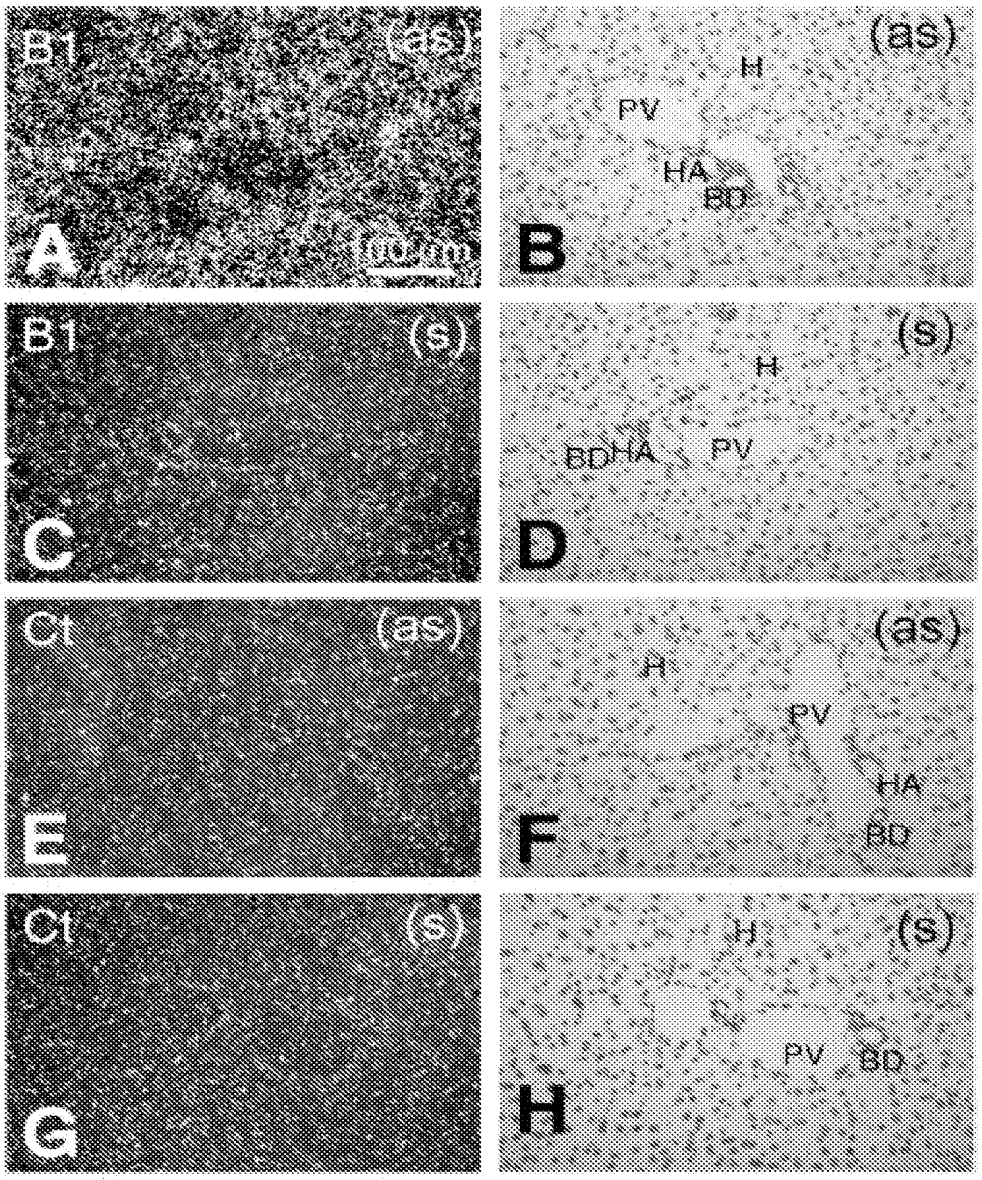


FIG. 3


B1
(as)
A
100 µm
H
PV
HA
BD
B
(as)
B1
(s)
C
H
BD
HA
PV
D
(s)
Ct
(as)
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(as)
Ct
(s)
G
H
PV
BD
H
(s)

FIG. 4

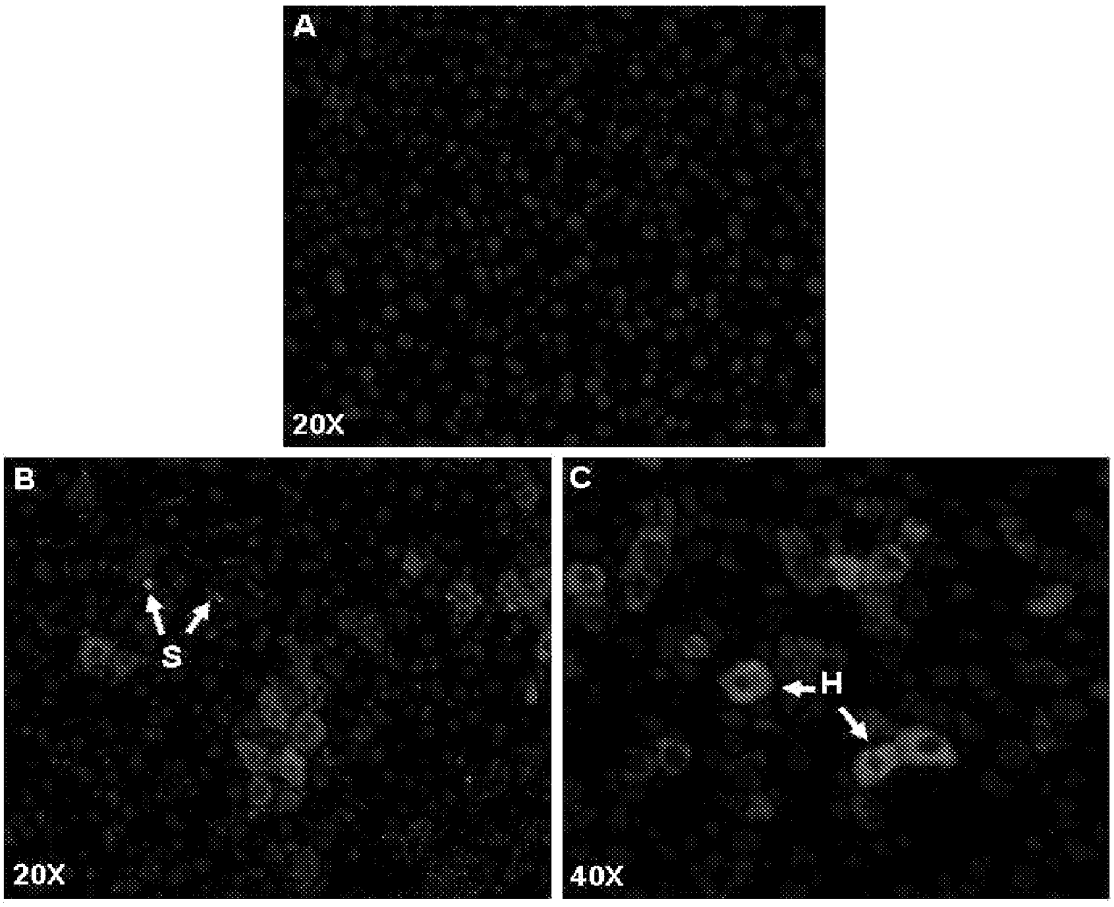

FIG. 5

CO-FF Luciferase mRNA:

GGGAUCCUACCAUGGAAGAUGCCAAAAACAUUAAGAAGGGCCCAGCGCCAUUCUACC
CACUCGAAGACGGGACCGCCGGCGAGCAGCUGCACAAAGCCAUGAAGCGCUACGCCC
UGGUGCCCGGCACCAUCGCCUUUACCGACGCACAUAUCGAGGUGGACAUUACCUACG
CCGAGUACUUCGAGAUGAGCGUUCGGCUGGCAGAAGCUAUGAAGCGCUAUGGGCUGA
AUACAAACCAUCGGAUCGUGGUGUGCAGCGAGAAUAGCUUGCAGUUCUUCAUGCCCG
UGUUGGGUGCCCUGUUCAUCGGUGUGGCUGUGGCCCCAGCUAACGACAUCUACAACG
AGCGCGAGCUGCUGAACAGCAUGGGCAUCAGCCAGCCCACCGUCGUAUUCGUGAGCA
AGAAAGGGCUGCAAAAGAUCCUCAACGUGCAAAAGAAGCUACCGAUCAUACAAAAGA
UCAUCAUCAUGGAUAGCAAGACCGACUACCAGGGCUUCCAAAGCAUGUACACCUUCG
UGACUUCCCAUUUGCCACCCGGCUUCAACGAGUACGACUUCGUGCCCGAGAGCUUCG
ACCGGGACAAAACCAUCGCCCUGAUCAUGAACAGUAGUGGCAGUACCGGAUUGCCCA
AGGGCGUAGCCCUACCGCACCGCACCGCUUGUGUCCGAUUCAGUCAUGCCCGCGACC
CCAUCUUCGGCAACCAGAUCAUCCCCGACACCGCUAUCCUCAGCGUGGUGCCAUUUC
ACCACGGCUUCGGCAUGUUCACCACGCUGGGCUACUUGAUCUGCGGCUUUCGGGUCG
UGCUCAUGUACCGCUUCGAGGAGGAGCUAUUCUUGCGCAGCUUGCAAGACUAUAAGA
UUCAAUCUGCCCUGCUGGUGCCCACACUAUUUAGCUUCUUCGCUAAGAGCACUCUCA
UCGACAAGUACGACCUAAGCAACUUGCACGAGAUCGCCAGCGGCGGGGCGCCGCUCA
GCAAGGAGGUAGGUGAGGCCGUGGCCAAACGCUUCCACCUACCAGGCAUCCGCCAGG
GCUACGGCCUGACAGAAACAACCAGCGCCAUUCUGAUCACCCCCGAAGGGGACGACA
AGCCUGGCGCAGUAGGCAAGGUGGUGCCCUUCUUCGAGGCUAAGGUGGUGGACUUGG
ACACCGGUAAGACACUGGGUGUGAACCAGCGCGGCGAGCUGUGCGUCCGUGGCCCCA
UGAUCAUGAGCGGCUACGUUAACAACCCCGAGGCUACAAACGCUCUCAUCGACAAGG
ACGGCUGGCUGCACAGCGGCGACAUCGCCUACUGGGACGAGGACGAGCACUUCUUCA
UCGUGGACCGGCUGAAGAGCCUGAUCAAAUACAAGGGCUACCAGGUAGCCCCAGCCG
AACUGGAGAGCAUCCUGCUGCAACACCCCAACAUCUUCGACGCCGGGGUCGCCGGCC
UGCCCGACGACGAUGCCGGCGAGCUGCCCGCCGCAGUCGUCGUGCUGGAACACGGUA
AAACCAUGACCGAGAAGGAGAUCGUGGACUAUGUGGCCAGCCAGGUUACAACCGCCA
AGAAGCUGCGCGGUGGUGUUGUGUUCGUGGACGAGGUGCCUAAAGGACUGACCGGCA
AGUUGGACGCCCGCAAGAUCCGCGAGAUUCUCAUUAAGGCCAAGAAGGGCGGCAAGA
UCGCCGUGUAAUUUGAAUU (SEQ ID NO: 1)

FIG. 6

5' CMV Sequence:

UAAUACGACUCACUAUAGGACAGAUCGCCUGGAGACGCCAUCCACGCUG
UUUUGACCUCCAUAGAAGACACCGGGACCGAUCCAGCCUCCGCGGCCGG
GAACGGUGCAUUGGAACGCGGAUUCCCCGUGCCAAGAGUGACUCACCGU
CCUUGACACG (SEQ ID NO: 2)

3' hGH Sequence:

CGGGUGGCAUCCCUGUGACCCCUCCCCAGUGCCUCUCCUGGCCCUGGAA
GUUGCCACUCCAGUGCCCACCAGCCUUGUCCUAAUAAAAUUAAGUUGCA
UC (SEQ ID NO: 3)

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/58457

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/7105, C07H 21/02 (2011.01)
USPC - 514/44R; 536/23.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8)-A61K 31/7105, C07H 21/02 (2011.01)
USPC-514/44R; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC 435/91.1, 424/450

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google Patents; Google Scholar
liposome, RNA, mRNA, delivery, gene therapy, urea cycle, ornithine transcarbamylase or carbamoyl-phosphate synthetase 1 OR argininosuccinate synthetase or argininosuccinate lyase or arginase, lipofectin, peg OR pegylated or polyethylene glycol, ceramide, dod

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---------- Y	US 2008/0260706 A1 (RABINOVICH et al.) 23 October 2008 (23.10.2008) para [0016], [0017], [0019], [0020], [0021], [0035], [0052]-[0054], [0066], [0089], Figs. 4, 12A	1-4, 7-9, 12-14, 23, 24, 30, 31 ---------- 5, 10, 15-22, 25-29, 32-34, 36-39, 41-62
Y	US 6,743,823 B1 (SUMMAR et al.) 01 June 2004 (01.06.2004) col 3 ln 65-col 4 ln 10	32-34, 36-39, 41-62
Y	US 6,147,055 A (HOBART et al.) 14 November 2000 (14.11.2000) col 1 ln 60-67, Fig.1.	5, 34
Y	US 6,670,178 B1 (SELDEN et al.) 30 December 2003 (30.12.2003) col 23 ln 5-60	10, 39
Y	US 2006/0172003 A1 (MEERS et al.) 03 August 2006 (03.08.2006) para [0002]-[0006], [0143]-[0146], [0153]	15-22, 53-61
Y	US 2006/0204566 A1 (SMYTH-TEMPLETON et al.) 14 September 2006 (14.09.2006) para [0058]	19, 57, 61
Y	US 2006/0083780 A1 (HEYES et al.) 20 April 2006 (20.04.2006) para [0006], [0010], [0015], [0043], [0058], [0059], [0063], [0138], [0172], [0228], [0248], [0252]	25-29, 46-50
Y	US 2005/0054026 A1 (ATSUSHI et al.) 10 March 2005 (10.03.2005) para [0004]-[0010], [0014], [0081], [0184]	26, 27, 47, 48

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 2011 (24.04.2011)	06 MAY 2011
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2007/0252295 A1 (PANZNER et al.) 01 November 2007 (01.11.2007) para [0011], [0014], [0063], [0072]	27, 48

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/58457

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 6, 11, 35, 40, 70, 75, 100, 105
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 6, 11, 35, 40, 70, 75, 100, 105 are directed toward sequences. The applicant failed to submit a valid CRF to the ISA/225 of 17 December 2010. Accordingly, the USPTO cannot supply a search for the sequences listed in this application and claims 6, 11, 35, 40, 70, 75, 100, 105 are unsearchable.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Group I: Claims 1-5, 7-10, 12-34, 36-39, and 41-62, drawn to compositions for modulating the expression of a protein in a target cell
Group II: Claims 63-69, 71-74, 76-99, 101-104, and 106-126, drawn to a methods for treating a subject who has a protein deficiency and for expressing a functional protein in a target cell.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The special technical feature of the inventions listed as Groups I and II is an mRNA and a transfer vehicle, wherein the mRNA encodes a functional protein, and wherein the mRNA comprises a modification, wherein the modification confers stability to the administered mRNA. This special technical feature fails to provide a contribution over the prior art, as evidenced by US 2009/0093433 A1 to Woolf et al. (published April 9, 2009) which teaches an mRNA and a transfer vehicle (para [0021]), wherein the mRNA encodes a functional protein (para [0037]), and wherein the mRNA comprises a modification (para [0043]), wherein the modification confers stability to the administered mRNA (abstract, para [0037], [0043]). In the absence of a contribution over the prior art, the shared technical feature is not a shared special technical feature. Without a shared special technical feature, the inventions lack unity with one another.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 7-10, 12-31, 32-34, 36-39, and 41-62

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

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(19) World Intellectual Property Organization
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18 September 2014 (18.09.2014)

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(10) International Publication Number
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61/798,666 15 March 2013 (15.03.2013) US

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(74) Agents: **SHUSTER, Michael, J.** et al.; FENWICK & WEST LLP, Silicon Valley Center, 801 California Street, Mountain View, CA 94041 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

[Continued on next page]

(54) Title: NETWORK-BASED MICROBIAL COMPOSITIONS AND METHODS

(57) Abstract: Provided are therapeutic compositions containing combinations of bacteria, for the maintenance or restoration of a healthy microbiota in the gastrointestinal tract of a mammalian subject, and methods for use thereof.

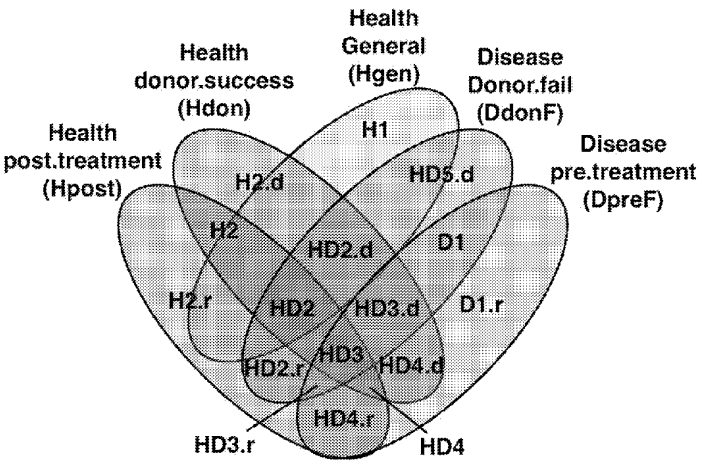


Figure 19

WO 2014/145958 A2

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

TITLE

[001] Network-Based Microbial Compositions and Methods

RELATED APPLICATIONS

[002] This application claims the benefit of U.S. Provisional Application No. 61/798,666, filed March 15, 2013, which is incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING

[003] This application includes a Sequence Listing submitted electronically as a text file named 26184PCT_sequencelisting.txt, created on March 10, 2014, with a size of 4,196,066 bytes. The sequence listing is incorporated by reference.

BACKGROUND

[004] Mammals are colonized by microbes in the gastrointestinal (GI) tract, on the skin, and in other epithelial and tissue niches such as the oral cavity, eye surface and vagina. The gastrointestinal tract harbors an abundant and diverse microbial community. It is a complex system, providing an environment or niche for a community of many different species or organisms, including diverse strains of bacteria. Hundreds of different species can form a commensal community in the GI tract in a healthy person, and this complement of organisms evolves from birth to ultimately form a functionally mature microbial population by about 3 years of age. Interactions between microbial strains in these populations and between microbes and the host (*e.g.* the host immune system) shape the community structure, with availability of and competition for resources affecting the distribution of microbes. Such resources may be food, location and the availability of space to grow or a physical structure to which the microbe may attach. For example, the host's diet is involved in shaping the GI tract flora.

[005] A healthy microbiota provides the host with multiple benefits, including colonization resistance to a broad spectrum of pathogens, essential nutrient biosynthesis and absorption, and immune stimulation that maintains a healthy gut epithelium and an appropriately controlled systemic immunity. In settings of 'dysbiosis' or disrupted symbiosis, microbiota functions can be lost or deranged, resulting in increased susceptibility to pathogens, altered metabolic profiles, or induction of proinflammatory signals that can result in local or systemic inflammation or autoimmunity. Thus, the intestinal microbiota plays a significant role in the pathogenesis of many diseases and disorders. Many of these diseases and disorders are chronic conditions that significantly decrease a subject's quality of life and can be ultimately fatal.

[006] Manufacturers of probiotics have asserted that their preparations of bacteria promote mammalian health by preserving the natural microflora in the GI tract and reinforcing the normal controls on aberrant immune responses. *See, e.g.,* U.S. Patent No. 8,034,601. Probiotics, however, have been limited to a very narrow group of genera and a correspondingly limited number of species.

As such, they do not adequately replace the missing natural microflora nor correct dysbioses of the GI tract in many situations.

[007] Therefore, in response to the need for durable, efficient, and effective compositions and methods for prevention, diagnosis and/or treatment of prediabetes and diabetes by way of restoring or enhancing microbiota functions, we address these and other shortcomings of the prior art by providing compositions and methods for treating subjects.

SUMMARY OF THE INVENTION

[008] Disclosed herein are methods for treating, preventing, or reducing the severity of a disorder selected from the group consisting of Clostridium difficile Associated Diarrhea (CDAD), Type 2 Diabetes, Obesity, Irritable Bowel Disease (IBD), colonization with a pathogen or pathobiont, and infection with a drug-resistant pathogen or pathobiont, comprising: administering to a mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria or the purified bacterial preparation capable of forming a network ecology selected from the group consisting of N262.S, N290.S, N284.S, N271.S, N282.S, N288.S, N302.S, N279.S, N310.S, N323.S, N331.S, N332.S, N301.S, N312.S, N339.S, N325.S, N340.S, N341.S, N346.S, N338.S, N336.S, N345.S, N355.S, N356.S, N343.S, N329.S, N361.S, N353.S, N381.S, N344.S, N352.S, N357.S, N358.S, N369.S, N372.S, N375.S, N380.S, N374.S, N377.S, N368.S, N370.S, N373.S, N376.S, N389.S, N394.S, N431.S, N434.S, N390.S, N397.S, N387.S, N440.S, N396.S, N399.S, N403.S, N414.S, N430.S, N432.S, N436.S, N437.S, N457.S, N545, N386.S, N402.S, N405.S, N415.S, N421.S, N422.S, N423.S, N458.S, N459.S, N493.S, N416.S, N439.S, N447.S, N490.S, N526, N429.S, N433.S, N448.S, N488.S, N508.S, N509.S, N510.S, N511.S, N408.S, N446.S, N451.S, N474.S, N520.S, N521.S, N535.S, N516.S, N463.S, N518.S, N586, N450.S, N465.S, N519.S, N537.S, N419.S, N468.S, N477.S, N514.S, N382.S, N460.S, N462.S, N512.S, N517.S, N523.S, N547.S, N548.S, N577.S, N581.S, N585.S, N616.S, N466.S, N469.S, N480.S, N482.S, N484.S, N515.S, N533.S, N709, N730, N478.S, N572.S, N400.S, N543.S, N582.S, N621.S, N689, N769, N481.S, N525.S, N528.S, N534.S, N574.S, N580.S, N590.S, N591.S, N597.S, N664, N693, N530.S, N687, N470.S, N529.S, N539.S, N546.S, N570.S, N579.S, N602.S, N614.S, N648.S, N652.S, N655.S, N672.S, N681.S, N690.S, N692.S, N698.S, N737.S, N738.S, N785, N841, N878, N880, N881, N987, N988, N996, N1061, N479.S, N538.S, N542.S, N578.S, N609.S, N611.S, N617.S, N666.S, N675.S, N682.S, N844, N845, N846, N852, N876, N982, N1008, N649.S, N657.S, N678.S, N686.S, N710.S, N522.S, N651.S, N653.S, N654.S, N680.S, N712.S, N792, N802, N804, N807, N849, N858, N859, N875, N885, N942, N961, N972, N1051, N587.S, N589.S, N612.S, N625.S, N656.S, N714.S, N779, N781, N828, N829, N860, N894, N925, N927, N935, N947, N983, N1023, N441.S, N584.S, N794, N788, N524.S, N604.S, N610.S, N623.S, N663.S, N669.S, N676.S, N703.S, N775.S, N777.S, N780.S, N817.S, N827.S, N836.S, N871.S, N874.S, N898.S, N907.S, N998.S, N1088, N1089, N660.S, N665.S, N667.S, N733.S, N734.S,

N739.S, N741.S, N782.S, N789.S, N796.S, N798.S, N800.S, N809.S, N816.S, N842.S, N843.S, N869.S, N986.S, N995.S, N1002.S, N1004.S, N1019.S, N1093, N668.S, N685.S, N835.S, N851.S, N464.S, N695.S, N776.S, N793.S, N815.S, N833.S, N891.S, N1070.S, N1092, N795.S, N797.S, N808.S, N811.S, N826.S, N830.S, N832.S, N840.S, N945.S, N960.S, N968.S, N1091, N805.S, N822.S, N928.S, N936.S, N1078.S, and N913.S.

[009] In some embodiments, the therapeutic bacterial composition comprises at least one bacterial entity, wherein said bacterial entity is capable of forming the network ecology in combination with one more bacterial entities present in the gastrointestinal tract of the mammalian subject at the time of the administering or thereafter. In certain embodiments, the network ecology is selected from the group consisting of N1008, N1023, N1051, N1061, N1070.S, N1088, N1089, N1092, N381.S, N382.S, N387.S, N399.S, N400.S, N402.S, N403.S, N414.S, N429.S, N430.S, N432.S, N433.S, N436.S, N437.S, N439.S, N441.S, N447.S, N448.S, N457.S, N460.S, N462.S, N463.S, N464.S, N470.S, N474.S, N488.S, N490.S, N493.S, N508.S, N509.S, N510.S, N511.S, N512.S, N514.S, N515.S, N517.S, N518.S, N519.S, N520.S, N523.S, N524.S, N529.S, N539.S, N543.S, N546.S, N547.S, N548.S, N570.S, N574.S, N577.S, N579.S, N580.S, N582.S, N584.S, N585.S, N589.S, N591.S, N597.S, N602.S, N604.S, N609.S, N610.S, N611.S, N612.S, N614.S, N616.S, N621.S, N623.S, N625.S, N648.S, N651.S, N652.S, N653.S, N654.S, N655.S, N660.S, N663.S, N664, N665.S, N666.S, N669.S, N672.S, N676.S, N681.S, N687, N689, N690.S, N692.S, N693, N695.S, N698.S, N703.S, N709, N712.S, N714.S, N730, N734.S, N737.S, N738.S, N769, N775.S, N777.S, N779, N780.S, N781, N785, N788, N792, N793.S, N794, N797.S, N798.S, N802, N804, N807, N817.S, N827.S, N828, N830.S, N832.S, N833.S, N836.S, N840.S, N841, N844, N845, N849, N852, N858, N859, N860, N869.S, N871.S, N874.S, N875, N878, N880, N881, N885, N894, N898.S, N907.S, N913.S, N925, N927, N942, N947, N961, N968.S, N972, N982, N983, N986.S, N987, N988, N996, and N998.S.

[010] In one embodiment, the network ecology consists essentially of N1008, N1023, N1051, N1061, N1070.S, N1088, N1089, N1092, N381.S, N382.S, N387.S, N399.S, N400.S, N402.S, N403.S, N414.S, N429.S, N430.S, N432.S, N433.S, N436.S, N437.S, N439.S, N441.S, N447.S, N448.S, N457.S, N460.S, N462.S, N463.S, N464.S, N470.S, N474.S, N488.S, N490.S, N493.S, N508.S, N509.S, N510.S, N511.S, N512.S, N514.S, N515.S, N517.S, N518.S, N519.S, N520.S, N523.S, N524.S, N529.S, N539.S, N543.S, N546.S, N547.S, N548.S, N570.S, N574.S, N577.S, N579.S, N580.S, N582.S, N584.S, N585.S, N589.S, N591.S, N597.S, N602.S, N604.S, N609.S, N610.S, N611.S, N612.S, N614.S, N616.S, N621.S, N623.S, N625.S, N648.S, N651.S, N652.S, N653.S, N654.S, N655.S, N660.S, N663.S, N664, N665.S, N666.S, N669.S, N672.S, N676.S, N681.S, N687, N689, N690.S, N692.S, N693, N695.S, N698.S, N703.S, N709, N712.S, N714.S, N730, N734.S, N737.S, N738.S, N769, N775.S, N777.S, N779, N780.S, N781, N785, N788, N792, N793.S, N794, N797.S, N798.S, N802, N804, N807, N817.S, N827.S, N828, N830.S, N832.S, N833.S, N836.S, N840.S, N841, N844, N845, N849, N852, N858, N859, N860, N869.S, N871.S,

N874.S, N875, N878, N880, N881, N885, N894, N898.S, N907.S, N913.S, N925, N927, N942, N947, N961, N968.S, N972, N982, N983, N986.S, N987, N988, N996, or N998.S.

[011] In another embodiment, the network ecology is selected from the group consisting of N387.S, N399.S, N512.S, N462.S, N651.S, N982, and N845. In one embodiment, network ecology comprises N387.S and the therapeutic bacterial composition comprises at least one bacterium selected from each of clade_262, clade_396, clade_444, clade_478, clade_500, and clade_553. In another embodiment, the network ecology comprises N387.S and the therapeutic bacterial composition consists essentially of at least one bacterium selected from each of clade_262, clade_396, clade_444, clade_478, clade_500, and clade_553. In certain embodiments, clade_262 comprises one or more bacteria selected from the group consisting Clostridium glycyrrhizinilyticum, Clostridium nexile, Coprococcus comes, Lachnospiraceae bacterium 1_1_57FAA, Lachnospiraceae bacterium 1_4_56FAA, Lachnospiraceae bacterium 8_1_57FAA, Ruminococcus lactaris, and Ruminococcus torques, wherein clade_396 comprises one or more bacteria selected from the group consisting Acetivibrio ethanolgignens, Anaerosporobacter mobilis, Bacteroides pectinophilus, Clostridium aminovalericum, Clostridium phytofermentans, Eubacterium hallii, and Eubacterium xylanophilum, wherein clade_444 comprises one or more bacteria selected from the group consisting Butyrivibrio fibrisolvens, Eubacterium rectale, Eubacterium sp. oral clone GI038, Lachnobacterium bovis, Roseburia cecicola, Roseburia faecalis, Roseburia faecis, Roseburia hominis, Roseburia intestinalis, Roseburia inulinivorans, Roseburia sp. 11SE37, Roseburia sp. 11SE38, Shuttleworthia satelles, Shuttleworthia sp. MSX8B, and Shuttleworthia sp. oral taxon G69, wherein clade_478 comprises one or more bacteria selected from the group consisting Faecalibacterium prausnitzii, Gemmiger formicilis, and Subdoligranulum variabile, wherein clade_500 comprises one or more bacteria selected from the group consisting Alistipes finegoldii, Alistipes onderdonkii, Alistipes putredinis, Alistipes shahii, Alistipes sp. HGB5, Alistipes sp. JC50, and Alistipes sp. RMA 9912, and wherein clade_553 comprises one or more bacteria selected from the group consisting Collinsella aerofaciens, Collinsella intestinalis, Collinsella stercoris, and Collinsella tanakaei.

[012] In one embodiment, clade_262 comprises one or more bacteria of Ruminococcus torques, wherein clade_396 comprises one or more bacteria of Eubacterium hallii, wherein clade_444 comprises one or more bacteria selected from the group consisting of Eubacterium rectale and Roseburia inulinivorans, wherein clade_478 comprises one or more bacteria of Faecalibacterium prausnitzii, wherein clade_500 comprises one or more bacteria of Alistipes putredinis, and wherein clade_553 comprises one or more bacteria of Collinsella aerofaciens.

[013] In another embodiment, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_396 comprises one more bacteria selected from the

group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 161, Seq. ID No.: 288, Seq. ID No.: 551, Seq. ID No.: 6, Seq. ID No.: 613, Seq. ID No.: 848, and Seq. ID No.: 875, wherein clade_444 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865, wherein clade_478 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1896, Seq. ID No.: 880, and Seq. ID No.: 932, wherein clade_500 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 129, Seq. ID No.: 131, Seq. ID No.: 132, Seq. ID No.: 133, Seq. ID No.: 134, Seq. ID No.: 135, and Seq. ID No.: 136, and wherein clade_553 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 659, Seq. ID No.: 660, Seq. ID No.: 661, and Seq. ID No.: 662.

[014] In other embodiments, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_396 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 161, Seq. ID No.: 288, Seq. ID No.: 551, Seq. ID No.: 6, Seq. ID No.: 613, Seq. ID No.: 848, and Seq. ID No.: 875, wherein clade_444 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865, wherein clade_478 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1896, Seq. ID No.: 880, and Seq. ID No.: 932, wherein clade_500 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 129, Seq. ID No.: 131, Seq. ID No.: 132, Seq. ID No.: 133, Seq. ID No.: 134, Seq. ID No.: 135, and Seq. ID No.: 136, and wherein clade_553 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 659, Seq. ID No.: 660, Seq. ID No.: 661, and Seq. ID No.: 662.

[015] In one embodiment, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1670, wherein clade_396 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 848, wherein clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1639 and Seq. ID No.: 856, wherein clade_478 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences

having 97% or greater identity to Seq. ID No.: 880, wherein clade_500 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 132, and wherein clade_553 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 659.

[016] In other embodiments, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1670, wherein clade_396 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 848, wherein clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1639 and Seq. ID No.: 856, wherein clade_478 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 880, wherein clade_500 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 132, and wherein clade_553 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 659.

[017] In another embodiment, network ecology comprises N399.S and the therapeutic bacterial composition comprises at least one bacterium selected from each of clade_262, clade_360, clade_396, clade_444, clade_478, and clade_494. In yet another embodiment, the network ecology comprises N399.S and the therapeutic bacterial composition consists essentially of at least one bacterium selected from each of clade_262, clade_360, clade_396, clade_444, clade_478, and clade_494.

[018] In some embodiments, clade_262 comprises one or more bacteria selected from the group consisting of Clostridium glycyrrhizinilyticum, Clostridium nexile, Coprococcus comes, Lachnospiraceae bacterium 1_1_57FAA, Lachnospiraceae bacterium 1_4_56FAA, Lachnospiraceae bacterium 8_1_57FAA, Ruminococcus lactaris, and Ruminococcus torques, wherein clade_360 comprises one or more bacteria selected from the group consisting of Dorea formicigenerans, Dorea longicatena, Lachnospiraceae bacterium 2_1_46FAA, Lachnospiraceae bacterium 2_1_58FAA, Lachnospiraceae bacterium 4_1_37FAA, Lachnospiraceae bacterium 9_1_43BFAA, Ruminococcus gnavus, and Ruminococcus sp. ID8, wherein clade_396 comprises one or more bacteria selected from the group consisting of Acetivibrio ethanolgignens, Anaerosporobacter mobilis, Bacteroides pectinophilus, Clostridium aminovalericum, Clostridium phytofermentans, Eubacterium hallii, and Eubacterium xylanophilum, wherein clade_444 comprises one or more bacteria selected from the group consisting of Butyrivibrio fibrisolvens, Eubacterium rectale, Eubacterium sp. oral clone GI038, Lachnobacterium bovis, Roseburia cecicola, Roseburia faecalis, Roseburia faecis, Roseburia hominis, Roseburia intestinalis, Roseburia inulinivorans, Roseburia sp. 11SE37, Roseburia sp. 11SE38, Shuttleworthia satelles, Shuttleworthia sp. MSX8B, and Shuttleworthia sp. oral taxon G69, wherein clade_478 comprises one or more bacteria selected from the group consisting of Faecalibacterium prausnitzii, Gemmiger formicilis, and Subdoligranulum variabile, and wherein clade_494 comprises one or more bacteria selected from the group consisting of Clostridium orbiscindens, Clostridium sp.

NML 04A032, Flavonifractor plautii, Pseudoflavonifractor capillosus, and Ruminococcaceae bacterium D16.

[019] In another embodiment, clade_262 comprises one or more bacteria of Ruminococcus torques, wherein clade_360 comprises one or more bacteria of Dorea longicatena, wherein clade_396 comprises one or more bacteria of Eubacterium hallii, wherein clade_444 comprises one or more bacteria of Eubacterium rectale, wherein clade_478 comprises one or more bacteria of Faecalibacterium prausnitzii, and wherein clade_494 comprises one or more bacteria of Pseudoflavonifractor capillosus.

[020] In one embodiment, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, wherein clade_396 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 161, Seq. ID No.: 288, Seq. ID No.: 551, Seq. ID No.: 6, Seq. ID No.: 613, Seq. ID No.: 848, and Seq. ID No.: 875, wherein clade_444 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865, wherein clade_478 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1896, Seq. ID No.: 880, and Seq. ID No.: 932, and wherein clade_494 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1591, Seq. ID No.: 1655, Seq. ID No.: 609, Seq. ID No.: 637, and Seq. ID No.: 886.

[021] In some embodiments, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, wherein clade_396 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 161, Seq. ID No.: 288, Seq. ID No.: 551, Seq. ID No.: 6, Seq. ID No.: 613, Seq. ID No.: 848, and Seq. ID No.: 875, wherein clade_444 comprises one more bacteria selected from the group

consisting of bacteria having 16S sequences Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865, wherein clade_478 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1896, Seq. ID No.: 880, and Seq. ID No.: 932, and wherein clade_494 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1591, Seq. ID No.: 1655, Seq. ID No.: 609, Seq. ID No.: 637, and Seq. ID No.: 886.

[022] In other embodiments, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1670, wherein clade_360 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 774, wherein clade_396 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 848, wherein clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 856, wherein clade_478 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 880, and wherein clade_494 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1591.

[023] In one aspect, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1670, wherein clade_360 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 774, wherein clade_396 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 848, wherein clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 856, wherein clade_478 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 880, and wherein clade_494 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1591.

[024] In another aspect, the network ecology comprises N462.S and the therapeutic bacterial composition comprises at least one bacterium selected from each of clade_262, clade_360, and clade_478. In yet another aspect, the network ecology comprises N462.S and the therapeutic bacterial composition consists essentially of at least one bacterium selected from each of clade_262, clade_360, and clade_478.

[025] In other aspects, clade_262 comprises one or more bacteria selected from the group consisting of Clostridium glycyrrhizinilyticum, Clostridium nexile, Coprococcus comes, Lachnospiraceae bacterium 1_1_57FAA, Lachnospiraceae bacterium 1_4_56FAA, Lachnospiraceae

bacterium 8_1_57FAA, Ruminococcus lactaris, and Ruminococcus torques, wherein clade_360 comprises one or more bacteria selected from the group consisting of Dorea formicigenerans, Dorea longicatena, Lachnospiraceae bacterium 2_1_46FAA, Lachnospiraceae bacterium 2_1_58FAA, Lachnospiraceae bacterium 4_1_37FAA, Lachnospiraceae bacterium 9_1_43BFAA, Ruminococcus gnavus, and Ruminococcus sp. ID8, and wherein clade_478 comprises one or more bacteria selected from the group consisting of Faecalibacterium prausnitzii, Gemmiger formicilis, and Subdoligranulum variabile.

[026] In another aspect, clade_262 comprises one or more bacteria of Coprococcus comes, wherein clade_360 comprises one or more bacteria of Dorea longicatena, and wherein clade_478 comprises one or more bacteria selected from the group consisting Faecalibacterium prausnitzii and Subdoligranulum variabile.

[027] In yet another aspect, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, and wherein clade_478 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1896, Seq. ID No.: 880, and Seq. ID No.: 932.

[028] In certain aspects, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, and wherein clade_478 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1896, Seq. ID No.: 880, and Seq. ID No.: 932.

[029] In another aspect, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 674, wherein clade_360 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 774, and wherein clade_478 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1896 and Seq. ID No.: 880.

[030] In other aspects, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 674, wherein clade_360 comprises one or

more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 774, and wherein clade_478 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1896 and Seq. ID No.: 880.

[031] In another embodiment, network ecology comprises N512.S and the therapeutic bacterial composition comprises at least one bacterium selected from each of clade_262, clade_360, and clade_444. In one embodiment, the network ecology comprises N512.S and the therapeutic bacterial composition consists essentially of at least one bacterium selected from each of clade_262, clade_360, and clade_444.

[032] In other embodiments, clade_262 comprises one or more bacteria selected from the group consisting of Clostridium glycyrrhizinilyticum, Clostridium nexile, Coprococcus comes, Lachnospiraceae bacterium 1_1_57FAA, Lachnospiraceae bacterium 1_4_56FAA, Lachnospiraceae bacterium 8_1_57FAA, Ruminococcus lactaris, and Ruminococcus torques, wherein clade_360 comprises one or more bacteria selected from the group consisting of Dorea formicigenerans, Dorea longicatena, Lachnospiraceae bacterium 2_1_46FAA, Lachnospiraceae bacterium 2_1_58FAA, Lachnospiraceae bacterium 4_1_37FAA, Lachnospiraceae bacterium 9_1_43BFAA, Ruminococcus gnavus, and Ruminococcus sp. ID8, and wherein clade_444 comprises one or more bacteria selected from the group consisting of Butyrivibrio fibrisolvens, Eubacterium rectale, Eubacterium sp. oral clone GI038, Lachnobacterium bovis, Roseburia cecicola, Roseburia faecalis, Roseburia faecis, Roseburia hominis, Roseburia intestinalis, Roseburia inulinivorans, Roseburia sp. 11SE37, Roseburia sp. 11SE38, Shuttleworthia satelles, Shuttleworthia sp. MSX8B, and Shuttleworthia sp. oral taxon G69.

[033] In certain embodiments, clade_262 comprises one or more bacteria selected from the group consisting of Coprococcus comes and Ruminococcus torques, wherein clade_360 comprises one or more bacteria of Dorea longicatena, and wherein clade_444 comprises one or more bacteria of Eubacterium rectale.

[034] In one embodiment, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, and wherein clade_444 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865.

[035] In another embodiment, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, and wherein clade_444 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865.

[036] In certain embodiments, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1670 and Seq. ID No.: 674, wherein clade_360 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 774, and wherein clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 856.

[037] In one aspect, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1670 and Seq. ID No.: 674, wherein clade_360 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 774, and wherein clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 856.

[038] In another aspect, the network ecology comprises N845 and the therapeutic bacterial composition comprises at least one bacterium selected from each of clade_262, clade_360, and clade_378. In certain aspects, the network ecology comprises N845 and the therapeutic bacterial composition consists essentially of at least one bacterium selected from each of clade_262, clade_360, and clade_378.

[039] In other aspects, clade_262 comprises one or more bacteria selected from the group consisting of Clostridium glycyrrhizinilyticum, Clostridium nexile, Coprococcus comes, Lachnospiraceae bacterium 1_1_57FAA, Lachnospiraceae bacterium 1_4_56FAA, Lachnospiraceae bacterium 8_1_57FAA, Ruminococcus lactaris, and Ruminococcus torques, wherein clade_360 comprises one or more bacteria selected from the group consisting of Dorea formicigenerans, Dorea longicatena, Lachnospiraceae bacterium 2_1_46FAA, Lachnospiraceae bacterium 2_1_58FAA, Lachnospiraceae bacterium 4_1_37FAA, Lachnospiraceae bacterium 9_1_43BFAA, Ruminococcus gnavus, and Ruminococcus sp. ID8, and wherein clade_378 comprises one or more bacteria selected from the group consisting of Bacteroides barnesiae, Bacteroides coprocola, Bacteroides coprophilus,

Bacteroides dorei, Bacteroides massiliensis, Bacteroides plebeius, Bacteroides sp. 3_1_33FAA, Bacteroides sp. 3_1_40A, Bacteroides sp. 4_3_47FAA, Bacteroides sp. 9_1_42FAA, Bacteroides sp. NB_8, and Bacteroides vulgatus.

[040] In certain aspects, clade_262 comprises one or more bacteria of Coprococcus comes, wherein clade_360 comprises one or more bacteria of Dorea longicatena, and wherein clade_378 comprises one or more bacteria of Bacteroides dorei.

[041] In another aspect, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, and wherein clade_378 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 267, Seq. ID No.: 272, Seq. ID No.: 273, Seq. ID No.: 274, Seq. ID No.: 284, Seq. ID No.: 289, Seq. ID No.: 309, Seq. ID No.: 310, Seq. ID No.: 313, Seq. ID No.: 314, Seq. ID No.: 323, and Seq. ID No.: 331.

[042] In certain aspects, clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, wherein clade_360 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1050, Seq. ID No.: 1051, Seq. ID No.: 1053, Seq. ID No.: 1058, Seq. ID No.: 1661, Seq. ID No.: 1668, Seq. ID No.: 773, and Seq. ID No.: 774, and wherein clade_378 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 267, Seq. ID No.: 272, Seq. ID No.: 273, Seq. ID No.: 274, Seq. ID No.: 284, Seq. ID No.: 289, Seq. ID No.: 309, Seq. ID No.: 310, Seq. ID No.: 313, Seq. ID No.: 314, Seq. ID No.: 323, and Seq. ID No.: 331.

[043] In one embodiment, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 674, wherein clade_360 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 774, and wherein clade_378 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 274.

[044] In another embodiment, clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 674, wherein clade_360 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 774,

and wherein clade_378 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 274.

[045] In some embodiments, the network ecology comprises N982 and the therapeutic bacterial composition comprises at least one bacterium selected from each of clade_172, clade_262, and clade_396. In another embodiment, the network ecology comprises N982 and the therapeutic bacterial composition consists essentially of at least one bacterium selected from each of clade_172, clade_262, and clade_396.

[046] In certain aspects, clade_172 comprises one or more bacteria selected from the group consisting of Bifidobacteriaceae genomosp. C1, Bifidobacterium adolescentis, Bifidobacterium angulatum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium catenulatum, Bifidobacterium dentium, Bifidobacterium gallicum, Bifidobacterium infantis, Bifidobacterium kashiwanohense, Bifidobacterium longum, Bifidobacterium pseudocatenulatum, Bifidobacterium pseudolongum, Bifidobacterium scardovii, Bifidobacterium sp. HM2, Bifidobacterium sp. HMLN12, Bifidobacterium sp. M45, Bifidobacterium sp. MSX5B, Bifidobacterium sp. TM_7, and Bifidobacterium thermophilum, wherein clade_262 comprises one or more bacteria selected from the group consisting of Clostridium glycyrrhizinilyticum, Clostridium nexile, Coprococcus comes, Lachnospiraceae bacterium 1_1_57FAA, Lachnospiraceae bacterium 1_4_56FAA, Lachnospiraceae bacterium 8_1_57FAA, Ruminococcus lactaris, and Ruminococcus torques, and wherein clade_396 comprises one or more bacteria selected from the group consisting of Acetivibrio ethanolgignens, Anaerosporobacter mobilis, Bacteroides pectinophilus, Clostridium aminovalericum, Clostridium phytofermentans, Eubacterium hallii, and Eubacterium xylanophilum.

[047] In another aspect, clade_172 comprises one or more bacteria of Bifidobacterium longum, wherein clade_262 comprises one or more bacteria of Coprococcus comes, and wherein clade_396 comprises one or more bacteria of Eubacterium hallii.

[048] In one aspect, clade_172 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 345, Seq. ID No.: 346, Seq. ID No.: 347, Seq. ID No.: 348, Seq. ID No.: 350, Seq. ID No.: 351, Seq. ID No.: 352, Seq. ID No.: 353, Seq. ID No.: 354, Seq. ID No.: 355, Seq. ID No.: 356, Seq. ID No.: 357, Seq. ID No.: 358, Seq. ID No.: 359, Seq. ID No.: 360, Seq. ID No.: 361, Seq. ID No.: 362, Seq. ID No.: 363, Seq. ID No.: 364, and Seq. ID No.: 365, wherein clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, and wherein clade_396 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 161, Seq. ID No.: 288, Seq. ID No.: 551, Seq. ID No.: 6, Seq. ID No.: 613, Seq. ID No.: 848, and Seq. ID No.: 875.

[049] In another aspect, clade_172 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 345, Seq. ID No.: 346, Seq. ID No.: 347, Seq. ID No.: 348, Seq. ID No.: 350, Seq. ID No.: 351, Seq. ID No.: 352, Seq. ID No.: 353, Seq. ID No.: 354, Seq. ID No.: 355, Seq. ID No.: 356, Seq. ID No.: 357, Seq. ID No.: 358, Seq. ID No.: 359, Seq. ID No.: 360, Seq. ID No.: 361, Seq. ID No.: 362, Seq. ID No.: 363, Seq. ID No.: 364, and Seq. ID No.: 365, wherein clade_262 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1048, Seq. ID No.: 1049, Seq. ID No.: 1057, Seq. ID No.: 1663, Seq. ID No.: 1670, Seq. ID No.: 588, Seq. ID No.: 607, and Seq. ID No.: 674, and wherein clade_396 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 161, Seq. ID No.: 288, Seq. ID No.: 551, Seq. ID No.: 6, Seq. ID No.: 613, Seq. ID No.: 848, and Seq. ID No.: 875.

[050] In another aspect, clade_172 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 356, wherein clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 674, and wherein clade_396 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 848.

[051] In certain aspects, clade_172 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 356, wherein clade_262 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 674, and wherein clade_396 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 848.

[052] In another embodiment, the network ecology comprises N651.S and the therapeutic bacterial composition comprises at least one bacterium selected from each of clade_444, clade_516, and clade_522. In yet another embodiment, the network ecology comprises N651.S and the therapeutic bacterial composition consists essentially of at least one bacterium selected from each of clade_444, clade_516, and clade_522.

[053] In one embodiment, clade_444 comprises one or more bacteria selected from the group consisting of Butyrivibrio fibrisolvens, Eubacterium rectale, Eubacterium sp. oral clone GI038, Lachnobacterium bovis, Roseburia cecicola, Roseburia faecalis, Roseburia faecis, Roseburia hominis, Roseburia intestinalis, Roseburia inulinivorans, Roseburia sp. 11SE37, Roseburia sp. 11SE38, Shuttleworthia satelles, Shuttleworthia sp. MSX8B, and Shuttleworthia sp. oral taxon G69, wherein clade_516 comprises one or more bacteria selected from the group consisting of Anaerotruncus colihominis, Clostridium methylpentosum, Clostridium sp. YIT 12070, Hydrogenoanaerobacterium saccharovorans, Ruminococcus albus, and Ruminococcus flavefaciens, and wherein clade_522 comprises one or more bacteria selected from the group consisting of Bacteroides galacturonicus,

Eubacterium eligens, Lachnospira multipara, Lachnospira pectinoschiza, and Lactobacillus rogosae. In another embodiment, clade_444 comprises one or more bacteria of Roseburia inulinivorans, wherein clade_516 comprises one or more bacteria of Anaerotruncus colihominis, and wherein clade_522 comprises one or more bacteria of Eubacterium eligens. In some embodiments, clade_444 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865, wherein clade_516 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1005, Seq. ID No.: 164, Seq. ID No.: 1656, Seq. ID No.: 1660, Seq. ID No.: 606, and Seq. ID No.: 642, and wherein clade_522 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1046, Seq. ID No.: 1047, Seq. ID No.: 1114, Seq. ID No.: 280, and Seq. ID No.: 845.

[054] In other embodiments, clade_444 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1045, Seq. ID No.: 1634, Seq. ID No.: 1635, Seq. ID No.: 1636, Seq. ID No.: 1637, Seq. ID No.: 1638, Seq. ID No.: 1639, Seq. ID No.: 1640, Seq. ID No.: 1641, Seq. ID No.: 1728, Seq. ID No.: 1729, Seq. ID No.: 1730, Seq. ID No.: 456, Seq. ID No.: 856, and Seq. ID No.: 865, wherein clade_516 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1005, Seq. ID No.: 164, Seq. ID No.: 1656, Seq. ID No.: 1660, Seq. ID No.: 606, and Seq. ID No.: 642, and wherein clade_522 comprises one more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1046, Seq. ID No.: 1047, Seq. ID No.: 1114, Seq. ID No.: 280, and Seq. ID No.: 845.

[055] In one embodiment, clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 1639, wherein clade_516 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 164, and wherein clade_522 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences having 97% or greater identity to Seq. ID No.: 845.

[056] In one aspect, clade_444 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 1639, wherein clade_516 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 164, and wherein clade_522 comprises one or more bacteria selected from the group consisting of bacteria having 16S sequences Seq. ID No.: 845.

[057] In another aspect, the composition further comprises a pharmaceutically-acceptable excipient. In one aspect, the therapeutic bacterial composition is substantially depleted of a residual

habitat product of a fecal material. In certain aspects, the composition is formulated for oral administration. In other embodiemtns, the composition is capable of inducing the formation of IgA, RegIII-gamma, IL-10, regulatory T cells, TGF-beta, alpha-defensin, beta-defensin, or an antimicrobial peptide in the mammalian subject. In another embodiment, the composition is comestible.

[058] The invention provides a composition, comprising any of the compositions administered according to the methods described above. The invention also includes a dosage unit comprising predetermined ratios of the isolated bacteria present in the network ecology as described above.

[059] The invention provides a method for producing short chain fatty acids (SCFA) within a mammalian subject, comprising:administering to said mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria of the purified bacterial preparation capable of forming one or a plurality of bacterial functional pathways, the one or plurality of bacterial functional pathways capable of forming a functional network ecology selected from the group consisting of N262.S, N290.S, N284.S, N271.S, N282.S, N288.S, N302.S, N279.S, N310.S, N323.S, N331.S, N332.S, N301.S, N312.S, N339.S, N325.S, N340.S, N341.S, N346.S, N338.S, N336.S, N345.S, N355.S, N356.S, N343.S, N329.S, N361.S, N353.S, N381.S, N344.S, N352.S, N357.S, N358.S, N369.S, N372.S, N375.S, N380.S, N374.S, N377.S, N368.S, N370.S, N373.S, N376.S, N389.S, N394.S, N431.S, N434.S, N390.S, N397.S, N387.S, N440.S, N396.S, N399.S, N403.S, N414.S, N430.S, N432.S, N436.S, N437.S, N457.S, N545, N386.S, N402.S, N405.S, N415.S, N421.S, N422.S, N423.S, N458.S, N459.S, N493.S, N416.S, N439.S, N447.S, N490.S, N526, N429.S, N433.S, N448.S, N488.S, N508.S, N509.S, N510.S, N511.S, N408.S, N446.S, N451.S, N474.S, N520.S, N521.S, N535.S, N516.S, N463.S, N518.S, N586, N450.S, N465.S, N519.S, N537.S, N419.S, N468.S, N477.S, N514.S, N382.S, N460.S, N462.S, N512.S, N517.S, N523.S, N547.S, N548.S, N577.S, N581.S, N585.S, N616.S, N466.S, N469.S, N480.S, N482.S, N484.S, N515.S, N533.S, N709, N730, N478.S, N572.S, N400.S, N543.S, N582.S, N621.S, N689, N769, N481.S, N525.S, N528.S, N534.S, N574.S, N580.S, N590.S, N591.S, N597.S, N664, N693, N530.S, N687, N470.S, N529.S, N539.S, N546.S, N570.S, N579.S, N602.S, N614.S, N648.S, N652.S, N655.S, N672.S, N681.S, N690.S, N692.S, N698.S, N737.S, N738.S, N785, N841, N878, N880, N881, N987, N988, N996, N1061, N479.S, N538.S, N542.S, N578.S, N609.S, N611.S, N617.S, N666.S, N675.S, N682.S, N844, N845, N846, N852, N876, N982, N1008, N649.S, N657.S, N678.S, N686.S, N710.S, N522.S, N651.S, N653.S, N654.S, N680.S, N712.S, N792, N802, N804, N807, N849, N858, N859, N875, N885, N942, N961, N972, N1051, N587.S, N589.S, N612.S, N625.S, N656.S, N714.S, N779, N781, N828, N829, N860, N894, N925, N927, N935, N947, N983, N1023, N441.S, N584.S, N794, N788, N524.S, N604.S, N610.S, N623.S, N663.S, N669.S, N676.S, N703.S, N775.S, N777.S, N780.S, N817.S, N827.S, N836.S, N871.S, N874.S, N898.S, N907.S, N998.S, N1088, N1089, N660.S, N665.S, N667.S, N733.S, N734.S, N739.S, N741.S, N782.S, N789.S, N796.S, N798.S, N800.S, N809.S, N816.S, N842.S, N843.S, N869.S, N986.S, N995.S,

N1002.S, N1004.S, N1019.S, N1093, N668.S, N685.S, N835.S, N851.S, N464.S, N695.S, N776.S, N793.S, N815.S, N833.S, N891.S, N1070.S, N1092, N795.S, N797.S, N808.S, N811.S, N826.S, N830.S, N832.S, N840.S, N945.S, N960.S, N968.S, N1091, N805.S, N822.S, N928.S, N936.S, N1078.S, and N913.S.

[060] In one embodiment, the functional network ecology is selected from the group consisting of N1008, N1023, N1051, N1061, N1070.S, N1088, N1089, N1092, N381.S, N382.S, N399.S, N400.S, N402.S, N403.S, N414.S, N429.S, N430.S, N432.S, N433.S, N436.S, N437.S, N439.S, N441.S, N447.S, N448.S, N457.S, N460.S, N462.S, N463.S, N464.S, N470.S, N474.S, N488.S, N490.S, N493.S, N508.S, N509.S, N510.S, N511.S, N512.S, N514.S, N515.S, N517.S, N518.S, N519.S, N520.S, N523.S, N524.S, N528.S, N529.S, N539.S, N543.S, N546.S, N547.S, N548.S, N570.S, N574.S, N577.S, N579.S, N580.S, N582.S, N584.S, N585.S, N589.S, N591.S, N597.S, N602.S, N604.S, N609.S, N610.S, N611.S, N612.S, N614.S, N616.S, N621.S, N623.S, N625.S, N648.S, N651.S, N652.S, N653.S, N654.S, N655.S, N660.S, N663.S, N664, N665.S, N666.S, N669.S, N672.S, N676.S, N681.S, N687, N689, N690.S, N692.S, N693, N695.S, N698.S, N703.S, N709, N712.S, N714.S, N730, N734.S, N737.S, N738.S, N769, N775.S, N777.S, N779, N780.S, N781, N785, N788, N792, N793.S, N794, N797.S, N798.S, N802, N804, N807, N817.S, N827.S, N828, N830.S, N832.S, N833.S, N836.S, N840.S, N841, N844, N845, N849, N852, N858, N859, N860, N869.S, N871.S, N874.S, N875, N878, N880, N881, N885, N894, N898.S, N907.S, N913.S, N925, N927, N942, N947, N961, N968.S, N972, N982, N983, N986.S, N987, N988, N996, and N998.S. In another embodiment, the functional network ecology is N528.S, and the plurality of bacterial functional pathways comprises the functional pathways of of KO:K00656, KO:K01069, KO:K01734, KO:K03417, KO:K03778, KO:K07246.

[061] The invention includes a method for catalyzing secondary metabolism of bile acids within a mammalian subject, comprising: administering to said mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria of the purified bacterial preparation capable of forming one or a plurality of bacterial functional pathways, the one or plurality of bacterial functional pathways capable of forming a functional network ecology selected from the group consisting of N262.S, N290.S, N284.S, N271.S, N282.S, N288.S, N302.S, N279.S, N310.S, N323.S, N331.S, N332.S, N301.S, N312.S, N339.S, N325.S, N340.S, N341.S, N346.S, N338.S, N336.S, N345.S, N355.S, N356.S, N343.S, N329.S, N361.S, N353.S, N381.S, N344.S, N352.S, N357.S, N358.S, N369.S, N372.S, N375.S, N380.S, N374.S, N377.S, N368.S, N370.S, N373.S, N376.S, N389.S, N394.S, N431.S, N434.S, N390.S, N397.S, N387.S, N440.S, N396.S, N399.S, N403.S, N414.S, N430.S, N432.S, N436.S, N437.S, N457.S, N545, N386.S, N402.S, N405.S, N415.S, N421.S, N422.S, N423.S, N458.S, N459.S, N493.S, N416.S, N439.S, N447.S, N490.S, N526, N429.S, N433.S, N448.S, N488.S, N508.S, N509.S, N510.S, N511.S, N408.S, N446.S, N451.S, N474.S, N520.S, N521.S, N535.S, N516.S, N463.S, N518.S, N586,

N450.S, N465.S, N519.S, N537.S, N419.S, N468.S, N477.S, N514.S, N382.S, N460.S, N462.S, N512.S, N517.S, N523.S, N547.S, N548.S, N577.S, N581.S, N585.S, N616.S, N466.S, N469.S, N480.S, N482.S, N484.S, N515.S, N533.S, N709, N730, N478.S, N572.S, N400.S, N543.S, N582.S, N621.S, N689, N769, N481.S, N525.S, N528.S, N534.S, N574.S, N580.S, N590.S, N591.S, N597.S, N664, N693, N530.S, N687, N470.S, N529.S, N539.S, N546.S, N570.S, N579.S, N602.S, N614.S, N648.S, N652.S, N655.S, N672.S, N681.S, N690.S, N692.S, N698.S, N737.S, N738.S, N785, N841, N878, N880, N881, N987, N988, N996, N1061, N479.S, N538.S, N542.S, N578.S, N609.S, N611.S, N617.S, N666.S, N675.S, N682.S, N844, N845, N846, N852, N876, N982, N1008, N649.S, N657.S, N678.S, N686.S, N710.S, N522.S, N651.S, N653.S, N654.S, N680.S, N712.S, N792, N802, N804, N807, N849, N858, N859, N875, N885, N942, N961, N972, N1051, N587.S, N589.S, N612.S, N625.S, N656.S, N714.S, N779, N781, N828, N829, N860, N894, N925, N927, N935, N947, N983, N1023, N441.S, N584.S, N794, N788, N524.S, N604.S, N610.S, N623.S, N663.S, N669.S, N676.S, N703.S, N775.S, N777.S, N780.S, N817.S, N827.S, N836.S, N871.S, N874.S, N898.S, N907.S, N998.S, N1088, N1089, N660.S, N665.S, N667.S, N733.S, N734.S, N739.S, N741.S, N782.S, N789.S, N796.S, N798.S, N800.S, N809.S, N816.S, N842.S, N843.S, N869.S, N986.S, N995.S, N1002.S, N1004.S, N1019.S, N1093, N668.S, N685.S, N835.S, N851.S, N464.S, N695.S, N776.S, N793.S, N815.S, N833.S, N891.S, N1070.S, N1092, N795.S, N797.S, N808.S, N811.S, N826.S, N830.S, N832.S, N840.S, N945.S, N960.S, N968.S, N1091, N805.S, N822.S, N928.S, N936.S, N1078.S, and N913.S.

[062] In one embodiment, the functional network ecology is selected from the group consisting of N1008, N1023, N1051, N1061, N1070.S, N1088, N1089, N1092, N381.S, N382.S, N399.S, N400.S, N402.S, N403.S, N414.S, N429.S, N430.S, N432.S, N433.S, N436.S, N437.S, N439.S, N441.S, N447.S, N448.S, N457.S, N460.S, N462.S, N463.S, N464.S, N470.S, N474.S, N488.S, N490.S, N493.S, N508.S, N509.S, N510.S, N511.S, N512.S, N514.S, N515.S, N517.S, N518.S, N519.S, N520.S, N523.S, N524.S, N529.S, N539.S, N543.S, N546.S, N547.S, N548.S, N570.S, N574.S, N577.S, N579.S, N580.S, N582.S, N584.S, N585.S, N589.S, N591.S, N597.S, N602.S, N604.S, N609.S, N610.S, N611.S, N612.S, N614.S, N616.S, N621.S, N623.S, N625.S, N648.S, N651.S, N652.S, N653.S, N654.S, N655.S, N660.S, N663.S, N664, N665.S, N666.S, N669.S, N672.S, N676.S, N681.S, N687, N689, N690.S, N692.S, N693, N695.S, N698.S, N703.S, N709, N712.S, N714.S, N730, N734.S, N737.S, N738.S, N769, N775.S, N777.S, N779, N780.S, N781, N785, N788, N792, N793.S, N794, N797.S, N798.S, N802, N804, N807, N817.S, N827.S, N828, N830.S, N832.S, N833.S, N836.S, N840.S, N841, N844, N845, N849, N852, N858, N859, N860, N869.S, N871.S, N874.S, N875, N878, N880, N881, N885, N894, N898.S, N907.S, N913.S, N925, N927, N942, N947, N961, N968.S, N972, N982, N983, N986.S, N987, N988, N996, and N998.S. In another embodiment, the functional network ecology is N660.S and the plurality of bacterial functional pathways comprises the functional pathways of of KO:K00656, and KO:K01442.

[063] In some embodiment, the invention includes a composition further comprising a pharmaceutically-acceptable excipient. In one embodiment, the composition is formulated for oral administration. In another embodiment, the composition is capable of inducing the formation of butyrate, propionate, acetate, 7-deoxybile acids, deoxycholate acide (DCA) and lithocholic acid (LCA)in the mammalian subject. In other embodiments, the composition is capable of inducing the depletion of glucose, pyruvate, lactate, cellulose, fructans, starch, xylans, pectins, taurocholate, glycocholate, ursocholate, cholate, glycochenodeoxycholate, taurochenodeoxycholate, ursodeoxycholate, or chenodeoxycholate; or the formation and depletion of intermediary metabolites acetyl-CoA, butyryl-CoA, propanoyl-CoA, chenodeoxycholoyl-CoA, or ursodeoxycholoyl-CoA in the mammalian subject. In another embodiment, the composition is formulated with one or more prebiotic compounds. In some embodiment,s the composition is comestible.

[064] The invention includes a composition, comprising any of the compositions administered according to the methods described above.

[065] The invention also includes a dosage unit comprising predetermined ratios of the isolated bacteria present in the network ecology described above.

[066] The invention comprises a pharmaceutical formulation comprising a purified bacterial population consisting essentially of a bacterial network capable of forming germinable bacterial spores, wherein the bacterial network is present in an amount effective to populate the gastrointestinal tract in a mammalian subject in need thereof to whom the formulation is administered, under conditions such that at least one type of bacteria not detectably present in the bacterial network or in the gastrointestinal tract prior to administration is augmented.

[067] The invention also includes a pharmaceutical formulation comprising a purified bacterial population comprising a plurality of bacterial entities, wherein the bacterial entities are present in an amount effective to induce the formation of a functional bacterial network in the gastrointestinal tract in a mammalian subject in need thereof to whom the formulation is administered. In some embodiments, the functional bacterial network comprises bacterial entities present in the formulation. In other embodiments, the functional bacterial network comprises bacterial entities present in the gastrointestinal tract at the time of administration. In another embodiment, the functional bacterial network comprises bacterial entities not present in the formulation or the gastrointestinal tract at the time of administration. In one embodiment, the formulation can be provided as an oral finished pharmaceutical dosage form including at least one pharmaceutically acceptable carrier In some embodiments, the formulation of claim 83 or claim 84, wherein the mammalian subject suffers from a dysbiosis comprising a gastrointestinal disease, disorder or condition selected from the group consisting of *Clostridium difficile* Associated Diarrhea (CDAD), Type 2 Diabetes, Type 1 Diabetes, Obesity, Irritable Bowel Syndrome (IBS), Irritable Bowel Disease (IBD), Ulcerative Colitis, Crohn's

Disease, colitis, colonization with a pathogen or pathobiont, and infection with a drug-resistant pathogen or pathobiont.

[068] In another embodiment, the bacterial network is purified from a fecal material subjected to a treatment step that comprises depleting or inactivating a pathogenic material. In one embodiment, the bacterial network is substantially depleted of a detectable level of a first pathogenic material. In some embodiments, the bacterial network is substantially depleted of a residual habitat product of the fecal material.

[069] In one embodiment, the invention provides a method of treating or preventing a dysbiosis in a human subject, comprising administering to the human subject the formulation of claim 83 or claim 84 in an amount effective to treat or prevent a dysbiosis or to reduce the severity of at least one symptom of the dysbiosis in the human subject to whom the formulation is administered.

[070] In another embodiment, the formulation is provided as an oral finished pharmaceutical dosage form including at least one pharmaceutically acceptable carrier, the dosage form comprising at least about $1x10^4$ colony forming units of bacterial spores per dose of the composition, wherein the bacterial spores comprise at least two bacterial entities comprising 16S rRNA sequences at least 97% identical to the nucleic acid sequences selected from the group consisting of Seq. ID No.: 674, Seq. ID No.: 1670, Seq. ID No.: 774, Seq. ID No.: 848, Seq. ID No.: 856, Seq. ID No.: 1639, Seq. ID No.: 880, Seq. ID No.: 1896, Seq. ID No.: 1591, Seq. ID No.: 164, Seq. ID No.: 845, and Seq. ID No.: 659.

[071] In yet another embodiment, the administration of the formulation results in a reduction or an elimination of at least one pathogen and/or pathobiont present in the gastrointestinal tract when the therapeutic composition is administered. In one embodiment, the administration of the formulation results in engraftment of at least one type of spore-forming bacteria present in the therapeutic composition.

[072] In one aspect, the administration of the formulation results in augmentation in the gastrointestinal tract of the subject to whom the formulation is administered of at least one type of bacteria not present in the formulation. In another aspect, the at least one type of spore-forming bacteria are not detectably present in the gastrointestinal tract of the subject to whom the formulation is administered when the formulation is administered. In yet another aspect, the administration of the formulation results in at least two of: i) reduction or elimination of at least one pathogen and/or pathobiont present in the gastrointestinal tract when the formulation is administered; ii) engraftment of at least one type of spore-forming bacteria present in the therapeutic composition; and iii) augmentation of at least one type of spore-forming or non-spore forming bacteria not present in the therapeutic composition.

[073] In some aspects, the administration of the therapeutic composition results in at reduction or elimination of at least one pathogen and/or pathobiont present in the gastrointestinal tract when the therapeutic composition is administered and at least one of: i) engraftment of at least one type of

spore-forming bacteria present in the therapeutic composition; and ii) augmentation of at least one type of bacteria not present in the therapeutic composition.

[074] In another aspect, the method of inducing engraftment of a bacterial population in the gastrointestinal tract of a human subject, comprising the step of administering to the human subject an orally acceptable pharmaceutical formulation comprising a purified bacterial network, under conditions such that at least i) a subset of the spore-forming bacteria sustainably engraft within the gastrointestinal tract, or ii) at least one type of bacteria not present in the therapeutic composition is augmented within the gastrointestinal tract.

[075] The invention provides a pharmaceutical formulation comprising a purified first bacterial entity and a purified second bacterial entity, wherein the first bacterial entity comprises a first nucleic acid sequence encoding a first polypeptide capable of catalyzing a first chemical reaction, wherein the second bacterial entity comprises a second nucleic acid sequence encoding a second polypeptide capable of catalyzing a second chemical reaction, wherein the pharmaceutical formulation is formulated for oral administration to a mammalian subject in need thereof, wherein the first chemical reaction and the second chemical reaction are capable of occurring in the gastrointestinal tract of the mammalian subject under conditions such that a first product of the first chemical reaction, a substance present within said mammalian subject, or a combination of said first product with the substance is used as a substrate in the second chemical reaction to form a second product, wherein the second product induces a host cell response. In one embodiment, the substance is a mammalian subject protein or a food-derived protein. In another embodiment, the host cell response comprises production by the host cell of a biological material. In certain embodiments, the biological material comprises a cytokine, growth factor or signaling polypeptide.

[076] In one embodiment, the host cell response comprises an immune response. In another embodiment, the host cell response comprises decreased gastric motility. In yet another embodiment, the host cell response comprises change in host gene expression, increased host metabolism, reduced gut permeability, enhanced epithelial cell junction integrity, reduced lipolysis by the action of Lipoprotein Lipase in adipose tissue, decreased hepatic gluconeogenesis, increased insulin sensitivity, increased production of FGF-19, or change in energy harvesting and/or storage.

[077] The invention includes a pharmaceutical formulation comprising a purified first bacterial entity and a purified second bacterial entity, wherein the first bacterial entity and the second bacterial entity form a functional bacterial network in the gastrointestinal tract of a mammalian subject to whom the pharmaceutical formulation is administered, wherein the functional network modulates the level and/or activity of a biological material capable of inducing a host cell response.

[078] The invention also includes a pharmaceutical formulation comprising a purified first bacterial entity and a purified second bacterial entity, wherein the first bacterial entity and the second bacterial entity form a functional bacterial network in the gastrointestinal tract of a mammalian

subject to whom the pharmaceutical formulation is administered, wherein the functional network induces the production of a biological material capable of inducing a host cell response.

[079] The invention comprises a therapeutic composition, comprising a network of at least two bacterial entities, wherein the network comprises at least one keystone bacterial entity and at least one non-keystone bacterial entity, wherein the at least two bacterial entities are each provided in amounts effective for the treatment or prevention of a gastrointestinal disease, disorder or condition in a mammalian subject. In one aspect, the network comprises at least three bacterial entities. In another aspect, the network comprises at least three bacterial entities including at least two keystone bacterial entities.

[080] The invention comprises a therapeutic composition, comprising a network of at least two keystone bacterial entities capable of forming germination-competent spores, wherein the at least two keystone bacterial entities are each provided in amounts effective for the treatment or prevention of a gastrointestinal disease, disorder or condition in a mammalian subject. In one aspect, the comprision comprises a network of at least two keystone bacterial entities capable of forming germination-competent spores.

[081] In one embodiment, the invention comprises a therapeutic composition, comprising: a first network of at least two bacterial entities, wherein the first network comprises a keystone bacterial entity and a non-keystone bacterial entity; and a second network of at least two bacterial entities, wherein the second network comprises at least one keystone bacterial entity and at least one non-keystone bacterial entity, wherein the networks are each provided in amounts effective for the treatment or prevention of a gastrointestinal disease, disorder or condition in a mammalian subject.

[082] The invention includes a therapeutic composition, comprising a network of at least two bacterial entities, wherein the network comprises a first keystone bacterial entity and a second keystone bacterial entity, wherein the two bacterial entities are each provided in amounts effective for the treatment or prevention of a gastrointestinal disease, disorder or condition in a mammalian subject. In one aspect, the first and second keystone bacterial entities are present in the same network. In another aspect, the first and second keystone bacterial entities are present in different networks.

[083] In some aspects, the invention comprises a diagnostic composition for the detection of a dysbiosis, comprising a first detection moiety capable of detecting a first keystone bacterial entity and a second detection moiety capable of detecting a first non-keystone bacterial entity, wherein the keystone bacterial entity and the non-keystone bacterial entity comprise a network, wherein the absence of at least one of the keystone bacterial entity and the non-keystone bacterial entity in a mammalian subject is indicative of a dysbiosis.

[084] The invention comprises a method of altering a microbiome population present in a mammalian subject, comprising the steps of determining the presence of an incomplete network of bacterial entities in the gastrointestinal tract of the mammalian subject, and introducing to the

gastrointestinal tract of the mammalian subject an effective amount of one or more supplemental bacterial entities not detectable in the gastrointestinal tract of the mammalian subject prior to such administration, under conditions such that the incomplete network is completed, thereby altering the microbiome population.

[085] In one embodiment, the one or more supplemental bacterial entities become part of the incomplete network, thereby forming a complete network. In another embodiment, the one or more supplemental bacterial entities alter the microbiota of the mammalian subject such that one or more additional bacterial entities complete the incomplete network. In yet another embodiment, the one or more supplemental bacterial entities comprise a network.

[086] The invention includes a method for detection and correction of a dysbiosis in a mammalian subject in need thereof, comprising the steps of: providing a fecal sample from the mammalian subject comprising a plurality of bacterial entities; contacting the fecal sample with a first detection moiety capable of detecting a first bacterial entity present in an network; detecting the absence of the first bacterial entity in the fecal sample, thereby detecting a dysbiosis in the mammalian subject; and administering to the mammalian subject a composition comprising an effective amount of the first bacterial entity. In one embodiment, the method includes confirming that the dysbiosis in the mammalian subject has been corrected.

[087] The invention comprises a system for predicting a dysbiosis in a subject, the system comprising: a storage memory for storing a dataset associated with a sample obtained from the subject, wherein the dataset comprises content data for at least one network of bacterial entities; and a processor communicatively coupled to the storage memory for determining a score with an interpretation function wherein the score is predictive of dysbiosis in the subject.

[088] The invention also comprises a kit for diagnosis of a state of dysbiosis in a mammalian subject in need thereof, comprising a plurality of detection means suitable for use in detecting (1) a first bacterial entity comprising a keystone bacterial entity and (2) a second bacterial entity, wherein the first and second bacterial entities comprise a functional network ecology.

BRIEF DESCRIPTION OF THE DRAWINGS

[089] Figure 1 provides a schematic of 16S rRNA gene and denotes the coordinates of hypervariable regions 1-9 (V1-V9), according to an embodiment of the invention. Coordinates of V1-V9 are 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294, and 1435-1465 respectively, based on numbering using E. coli system of nomenclature defined by Brosius et al., Complete nucleotide sequence of a 16S ribosomal RNA gene (16S rRNA) from Escherichia coli, PNAS 75(10):4801-4805 (1978).

[090] Figure 2 highlights in bold the nucleotide sequences for each hypervariable region in the exemplary reference E. coli 16S sequence described by Brosius et al.

[091] Figure 3 provides the OTU and clade composition of networks tested in experiment SP-376, according to an embodiment of the invention.

[092] Figure 4 illustrates the results of a nutrient utilization assay with Clostridium difficile and potential competitors of the pathogen. A plus sign (+) indicates that it is a nutrient for the isolate tested. A minus sign (-) indicates that it is not a nutrient for the isolate tested.

[093] Figure 5 demonstrates the microbial diversity measured in the ethanol-treated spore treatment sample and patient pre- and post-treatment samples, according to an embodiment of the invention. Total microbial diversity is defined using the Chao1 Alpha-Diversity Index and is measured at the same genomic sampling depths to confirm adequate and comparable sequence coverage of the target samples. The patient pretreatment (purple) harbored a microbiome that was significantly reduced in total diversity as compared to the ethanol-treated spore treatment (red) and patient post treatment at days 5 (blue), 14 (orange), and 25 (green).

[094] Figure 6 demonstrates how patient microbial ecology is shifted by treatment with an ethanol-treated spore treatment from a dysbiotic state to a state of health. Principal coordinates analysis based on the total diversity and structure of the microbiome (Bray Curtis Beta Diversity) of the patient pre- and post-treatment delineates that the combination of engraftment of the OTUs from the spore treatment and the augmentation of the patient microbial ecology leads to a microbial ecology that is distinct from both the pretreatment microbiome and the ecology of the ethanol-treated spore treatment.

[095] Figure 7 demonstrates the augmentation of Bacteroides species in patients treated with the spore population, according to an embodiment of the invention.

[096] Figure 8 shows species engrafting versus species augmenting in patients microbiomes after treatment with a bacterial composition such as but not limited to an ethanol-treated spore population, according to an embodiment of the invention. Relative abundance of species that engrafted or augmented as described were determined based on the number of 16S sequence reads. Each plot is from a different patient treated with the bacterial composition such as but not limited to an ethanol-treated spore population for recurrent C. difficile.

[097] Figure 9 shows a set of survival curves demonstrating efficacy of the ethanol enriched spore population in a mouse prophylaxis model of C. difficile, according to an embodiment of the invention.

[098] Figure 10 illustrates an in vivo hamster Clostridium difficile relapse prevention model to validate efficacy of ethanol-treated spores and ethanol treated, gradient purified spores, according to an embodiment of the invention.

[099] Figure 11 shows an in vivo hamster Clostridium difficile relapse prevention model to validate efficacy of network ecology bacterial composition, according to an embodiment of the invention.

[0100] Figure 12 shows secondary bile acid metabolism KEGG Orthology Pathway and associated enzymatic gene products defined by EC numbers.

[0101] Figure 13 shows Butyrate (a.k.a butanoate) production KEGG Orthology Pathway and associated enzymatic gene products defined by EC numbers.

[0102] Figure 14 shows Propionate (a.k.a. propanoate) production KEGG Orthology Pathway and associated enzymatic gene products defined by EC numbers.

[0103] Figure 15 shows Acetate production KEGG Orthology Pathway and associated enzymatic gene products defined by EC numbers.

[0104] Figure 16 is an overview of a method to computationally derive network ecologies, according to an embodiment of the invention.

[0105] Figure 17 is a schematic representation of how Keystone OTUs (nodes 2 and 4, shaded circles) are central members of many network ecologies that contain non-Keystone OTUs (nodes 1, 3, and 5-9). Distinct network ecologies include [node 2--node 7], [node--3--node 2--node--4], [node 2--node 4--node 5--node 6--node 7], [node 1--node 2--node 8--node 9], and [node --node 3].

[0106] Figure 18 exemplifies a Derivation of Network Ecology Classes, according to an embodiment of the invention. Subsets of networks are selected for use in defining Network Classes based on key biological criteria. Hierarchical Network clusters are defined by the presence (white) and absence (blue) of OTUs and/or Functional Metabolic Pathways and Classes are defined as branches of the hierarchical clustering tree based on the topological overlap measure.

[0107] Figure 19 shows phenotypes assigned to samples for the computational derivation of Network Ecologies that typify microbiome states of health (Hpost, Hdon, & Hgen) and states of disease (DdonF & DpreF). The composition of the microbiome of samples in different phenotypes can overlap with the intersections, defined by H, HD, D designations, having different biological meanings.

[0108] Figure 20 shows an exemplary phylogenetic tree and the relationship of OTUs and Clades. A, B, C, D, and E represent OTUs, also known as leaves in the tree. Clade 1 comprises OTUs A and B, Clade 2 comprises OTUs C, D and E, and Clade 3 is a subset of Clade 2 comprising OTUs D and E. Nodes in a tree that define clades in the tree can be either statistically supported or not statistically supported. OTUs within a clade are more similar to each other than to OTUs in another clade and the robustness the clade assignment is denoted by the degree of statistical support for a node upstream of the OTUs in the clade.

[0109] Figure 21 is a high-level block diagram illustrating an example of a computer for use as a server or a user device, in accordance with one embodiment.

[0110] The figures depict various embodiments of the present invention for purposes of illustration only. One skilled in the art will readily recognize from the following discussion that alternative

embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the invention described herein.

DETAILED DESCRIPTION

OVERVIEW

[0111] Disclosed herein are therapeutic compositions containing combinations of bacteria for the prevention, control, and treatment of gastrointestinal diseases, and other disorders and conditions that result in or are caused by a dysbiotic microbiome in a niche of a host. Such indications include, but are not limited to Clostridium difficile associated diarrhea (CDAD), Type 2 Diabetes, Ulcerative colitis, as well as infection by antibiotic resistant bacteria such as Carbapenem resistant Klebsiella pneomoniae (CRKp) and Vancomycin Resistant Enterococcus (VRE). These compositions are advantageous in being suitable for safe administration to humans and other mammalian subjects and are efficacious in numerous gastrointestinal diseases, disorders and conditions and in general nutritional health. While bacterial compositions are known, these are generally single bacterial strains or combinations of bacteria that are combined without understanding the ecology formed by a consortium of bacterial organisms, resulting in poor efficacy, instability, substantial variability and lack of adequate safety.

[0112] The human body is an ecosystem in which the microbiota and the microbiome play a significant role in the basic healthy function of human systems (*e.g.* metabolic, immunological, and neurological). The microbiota and resulting microbiome comprise an ecology of microorganisms that co-exist within single subjects interacting with one another and their host (*i.e.*, the mammalian subject) to form a dynamic unit with inherent biodiversity and functional characteristics. Within these networks of interacting microbes (*i.e.* ecologies), particular members can contribute more significantly than others; as such these members are also found in many different ecologies, and the loss of these microbes from the ecology can have a significant impact on the functional capabilities of the specific ecology. Robert Paine coined the concept "Keystone Species" in 1969 (see Paine RT. 1969. A note on trophic complexity and community stability. The American Naturalist 103: 91–93.) to describe the existence of such lynchpin species that are integral to a given ecosystem regardless of their abundance in the ecological community. Paine originally describe the role of the starfish Pisaster ochraceus in marine systems and since the concept has been experimentally validated in numerous ecosystems.

[0113] The present invention provides methods to define important network ecologies and functional network ecologies that occur in healthy and diseased subjects, and provides the compositional constituents of these network ecologies. The method enables the derivation of ecological modules (*i.e.* groups or networks of organisms and metabolic functions) within a broader ecology that can catalyze a change from a dysbiotic microbiome to one that represents a state of health. In another embodiment the methods enable the de novo construction of a network ecology

based on desired biological characteristics, including functional characteristics, e.g. a functional network ecology. The methods further provide keystone species (*i.e.* operational taxonomic units, or OTUs) and keystone metabolic functions that are members of these microbial communities based on their ubiquitous appearance in many different networks. Importantly, this method is distinguished from previous computational approaches by being the first method to define actual network ecologies that are found in many healthy subjects. Network ecologies comprise consortia of bacterial OTUs (*i.e.* genera, species, or strains) that form coherent intact biological communities with defined phylogenetic and/or functional properties. In other words, the structure-function relationships contained within any Network Ecology possess an inherent biodiversity profile and resulting biological functional capabilities. The specific bacterial combinations and functional capabilities of the resulting microbiome are efficacious for the treatment or prevention of diseases, disorders and conditions of the gastrointestinal tract or for the treatment or prevention of systemic diseases, disorders and conditions that are influenced by the microbiota of the gastrointestinal tract. Further the network ecologies have a modularity to their structure and function with specific nodes (as example OTUs, phylogenetic clades, functional pathways) comprising a backbone of the network onto which different r-groups (as example OTUs, phylogenetic clades, functional pathways) can be incorporated to achieve specific biological properties of the network ecology. Network Ecologies defined in terms of functional modalities are referred to as Functional Network Ecologies.

[0114] The network ecologies provided herein are useful in settings where a microbial dysbiosis is occurring, given their capacities to achieve one or more of the following actions: i) disrupting the existing microbiota and/or microbiome; ii) establishing a new microbiota and/or microbiome; and (iii) stabilizing a functional microbiota and/or microbiome that supports health. Such network ecologies may be sustainably present upon introduction into a mammalian subject, or may be transiently present until such time as the functional microbiota and/or microbiome are re-established. In therapeutic settings, treatment with a consortium of microbial OTUs will change the microbiome of the treated host from a state of disease to a state of health. This change in the total diversity and composition can be mediated by both: (i) engraftment of OTUs that comprise the therapeutic composition into the host's ecology (Engrafted Ecology), and (ii) the establishment of OTUs that are not derived from the therapeutic composition, but for which the treatment with the therapeutic composition changes the environmental conditions such that these OTUs can establish. This Augmented Ecology is comprised of OTUs that were present at lower levels in the host pre-treatment or that were exogenously introduced from a source other than the therapeutic composition itself.

[0115] Provided herein are computational methods based at least in part on network theory (Proulx SR, Promislow DEL, Phillips PC. 2005. Network thinking in ecology and evolution. Trends in Ecology & Evolution 20: 345–353.), that delineate ecological and functional structures of a group of microorganisms based on the presence or absence of the specific OTUs (*i.e.* microbial orders, families, genera, species or strains) or functions inherent to those OTUs in a population of sampled

mammalian subjects. Notably, these network ecologies and functional network ecologies are not simply inferred based on the clustering of OTUs according to binary co-occurrences computed from average relative abundances across a set of subject samples (*See e.g.* Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, and Huttenhower C. 2012. Microbial co-occurrence relationships in the human microbiome. PLoS Computational Bioliology 8: e1002606. Lozupone C, Faust K, Raes J, Faith JJ, Frank DN, Zaneveld J, Gordon JI, and Knight R. 2012. Identifying genomic and metabolic features that can underlie early successional and opportunistic lifestyles of human gut symbionts. Genome Research 22: 1974–1984), but instead the ecologies represent actual communities of bacterial OTUs that are computationally derived and explicitly exist as an ecological network within one or more subjects. Further, we provide methods by which to characterize the biological significance of a given ecological network in terms of its phylogenetic diversity, functional properties, and association with health or disease. The present invention delineates ecologies suitable for the treatment or prevention of diseases, disorders, and conditions of the gastrointestinal tract or which are distal to the gastrointestinal tract but caused or perpetuated by a dysbiosis of the gut microbiota.

DEFINITIONS

[001] As used herein, the term "purified bacterial preparation" refers to a preparation that includes bacteria that have been separated from at least one associated substance found in a source material or any material associated with the bacteria in any process used to produce the preparation.

[002] A "bacterial entity" includes one or more bacteria. Generally, a first bacterial entity is distinguishable from a second bacterial entity

[003] As used herein, the term "formation" refers to synthesis or production.

[004] As used herein, the term "inducing" means increasing the amount or activity of a given material as dictated by context.

[005] As used herein, the term "depletion" refers to reduction in amount of.

[006] As used herein, a "prebiotic" is a comestible food or beverage or ingredient thereof that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health. Prebiotics may include complex carbohydrates, amino acids, peptides, or other essential nutritional components for the survival of the bacterial composition. Prebiotics include, but are not limited to, amino acids, biotin, fructooligosaccharide, galactooligosaccharides, inulin, lactulose, mannan oligosaccharides, oligofructose-enriched inulin, oligofructose, oligodextrose, tagatose, trans-galactooligosaccharide, and xylooligosaccharides.

[007] As used herein, "predetermined ratios" refer to ratios determined or selected in advance.

[008] As used herein, "germinable bacterial spores" are spores capable of forming vegetative cells under certain environmental conditions.

[009] As used herein, "detectably present" refers to present in an amount that can be detected using assays provided herein or otherwise known in the art that exist as of the filing date.

[010] As used herein, "augmented" refers to an increase in amount and/or localization within to a point where it becomes detectably present.

[011] As used herein, a "fecal material" refers to a solid waste product of digested food and includes feces or bowel washes.

[012] As used herein, a "host cell response" is a response produced by a cell comprising a host organism.

[013] As used herein, a "mammalian subject protein" refers to a protein produced by a mammalian subject and encoded by the mammalian subject genome.

[014] As used herein, the term "food-derived" refers to a protein found in a consumed food.

[015] As used herein, the term "biological material" refers to a material produced by a biological organism.

[016] As used herein, the term "detection moiety" refers to an assay component that functions to detect an analyte.

[017] As used herein, the term "incomplete network" refers to a partial network that lacks the entire set of components needed to carry out one or more network functions.

[018] As used herein, the term "supplemental" refers to something that is additional and non-identical.

[019] As used herein, the term "Antioxidant" refers to, without limitation, any one or more of various substances such as beta-carotene (a vitamin A precursor), vitamin C, vitamin E, and selenium that inhibit oxidation or reactions promoted by Reactive Oxygen Species ("ROS") and other radical and non-radical species. Additionally, antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. Non-limiting examples of antioxidants include astaxanthin, carotenoids, coenzyme Q10 ("CoQ10"), flavonoids, glutathione, Goji (wolfberry), hesperidin, lactowolfberry, lignan, lutein, lycopene, polyphenols, selenium, vitamin A, vitamin C, vitamin E, zeaxanthin, or combinations thereof.

[020] "Backbone Network Ecology" or simply "Backbone Network" or "Backbone" are compositions of microbes that form a foundational composition that can be built upon or subtracted from to optimize a Network Ecology or Functional Network Ecology to have specific biological characteristics or to comprise desired functional properties, respectively. Microbiome therapeutics can be comprised of these "Backbone Networks Ecologies" in their entirety, or the "Backbone Networks" can be modified by the addition or subtraction of "R-Groups" to give the network ecology desired characteristics and properties. "R-Groups" can be defined in multiple terms including, but not limited to: individual OTUs, individual or multiple OTUs derived from a specific phylogenetic clade or a desired phenotype such as the ability to form spores, or functional bacterial compositions that comprise. "Backbone Networks" can comprise a computationally derived Network Ecology in its entirety or can be subsets of the computed network that represent key nodes in the network that

contributed to efficacy such as but not limited to a composition of Keystone OTUs. The number of organisms in the human gastrointestinal tract, as well as the diversity between healthy individuals, is indicative of the functional redundancy of a healthy gut microbiome ecology. *See* The Human Microbiome Consortia. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486: 207-214. This redundancy makes it highly likely that non-obvious subsets of OTUs or functional pathways (*i.e.* "Backbone Networks") are critical to maintaining states of health and or catalyzing a shift from a dysbiotic state to one of health. One way of exploiting this redundancy is through the substitution of OTUs that share a given clade (see below) or of adding members of a clade not found in the Backbone Network.

[021] "Bacterial Composition" refers to a consortium of microbes comprising two or more OTUs. Backbone Network Ecologies, Functional Network Ecologies, Network Classes, and Core Ecologies are all types of bacterial compositions. A "Bacterial Composition" can also refer to a composition of enzymes that are derived from a microbe or multiple microbes. As used herein, Bacterial Composition includes a therapeutic microbial composition, a prophylactic microbial composition, a Spore Population, a Purified Spore Population, or ethanol treated spore population.

[022] "Clade" refers to the OTUs or members of a phylogenetic tree that are downstream of a statistically valid node in a phylogenetic tree (Figure 20). The clade comprises a set of terminal leaves in the phylogenetic tree (i.e. tips of the tree) that are a distinct monophyletic evolutionary unit and that share some extent of sequence similarity. Clades are hierarchical. In one embodiment, the node in a phylogenetic tree that is selected to define a clade is dependent on the level of resolution suitable for the underlying data used to compute the tree topology.

[023] The "Colonization" of a host organism includes the non-transitory residence of a bacterium or other microscopic organism. As used herein, "reducing colonization" of a host subject's gastrointestinal tract (or any other microbiotal niche) by a pathogenic or non-pathogenic bacterium includes a reduction in the residence time of the bacterium the gastrointestinal tract as well as a reduction in the number (or concentration) of the bacterium in the gastrointestinal tract or adhered to the luminal surface of the gastrointestinal tract. The reduction in colonization can be permanent or occur during a transient period of time. Reductions of adherent pathogens can be demonstrated directly, *e.g.*, by determining pathogenic burden in a biopsy sample, or reductions may be measured indirectly, *e.g.*, by measuring the pathogenic burden in the stool of a mammalian host.

[024] A "Combination" of two or more bacteria includes the physical co-existence of the two bacteria, either in the same material or product or in physically connected products, as well as the temporal co-administration or co-localization of the two bacteria.

[025] "Cytotoxic" activity of bacterium includes the ability to kill a bacterial cell, such as a pathogenic bacterial cell. A "cytostatic" activity or bacterium includes the ability to inhibit, partially

or fully, growth, metabolism, and/or proliferation of a bacterial cell, such as a pathogenic bacterial cell. Cytotoxic activity may also apply to other cell types such as but not limited to Eukaryotic cells.

[026] "Dysbiosis" refers to a state of the microbiota or microbiome of the gut or other body area, including mucosal or skin surfaces in which the normal diversity and/or function of the ecological network is disrupted. Any disruption from the preferred (*e.g.*, ideal) state of the microbiota can be considered a dysbiosis, even if such dysbiosis does not result in a detectable decrease in health. This state of dysbiosis may be unhealthy, it may be unhealthy under only certain conditions, or it may prevent a subject from becoming healthier. Dysbiosis may be due to a decrease in diversity, the overgrowth of one or more pathogens or pathobionts, symbiotic organisms able to cause disease only when certain genetic and/or environmental conditions are present in a patient, or the shift to an ecological network that no longer provides a beneficial function to the host and therefore no longer promotes health.

[027] "Ecological Niche" or simply "Niche" refers to the ecological space in which an organism or group of organisms occupies. Niche describes how an organism or population or organisms responds to the distribution of resources, physical parameters (*e.g.*, host tissue space) and competitors (*e.g.*, by growing when resources are abundant, and when predators, parasites and pathogens are scarce) and how it in turn alters those same factors (*e.g.*, limiting access to resources by other organisms, acting as a food source for predators and a consumer of prey).

[028] "Germinant" is a material or composition or physical-chemical process capable of inducing vegetative growth of a bacterium that is in a dormant spore form, or group of bacteria in the spore form, either directly or indirectly in a host organism and/or in vitro.

[029] "Inhibition" of a pathogen or non-pathogn encompasses the inhibition of any desired function or activity y the bacterial compositions of the present invention. Demonstrations of inhibition, such as decrease in the growth of a pathogenic bacterium or reduction in the level of colonization of a pathogenic bacterium are provided herein and otherwise recognized by one of ordinary skill in the art. Inhibition of a pathogenic or non-pathogenic bacterium's "growth" may include inhibiting the increase in size of the pathogenic or non-pathogenic bacterium and/or inhibiting the proliferation (or multiplication) of the pathogenic or non-pathogenic bacterium. Inhibition of colonization of a pathogenic or non-pathogenic bacterium may be demonstrated by measuring the amount or burden of a pathogen before and after a treatment. An "inhibition" or the act of "inhibiting" includes the total cessation and partial reduction of one or more activities of a pathogen, such as growth, proliferation, colonization, and function. Inhibition of function includes, for example, the inhibition of expression of pathogenic gene products such as a toxin or invasive pilus induced by the bacterial composition.

[030] "Isolated" encompasses a bacterium or other entity or substance that has been (1) separated from at least some of the components with which it was associated when initially produced (whether

in nature or in an experimental setting), and/or (2) produced, prepared, purified, and/or manufactured by the hand of man. Isolated bacteria include those bacteria that are cultured, even if such cultures are not monocultures. Isolated bacteria may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated bacteria are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. The terms "purify," "purifying" and "purified" refer to a bacterium or other material that has been separated from at least some of the components with which it was associated either when initially produced or generated (*e.g.*, whether in nature or in an experimental setting), or during any time after its initial production. A bacterium or a bacterial population may be considered purified if it is isolated at or after production, such as from a material or environment containing the bacterium or bacterial population, or by passage through culture, and a purified bacterium or bacterial population may contain other materials up to about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or above about 90% and still be considered "isolated." In some embodiments, purified bacteria and bacterial populations are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. In the instance of bacterial compositions provided herein, the one or more bacterial types present in the composition can be independently purified from one or more other bacteria produced and/or present in the material or environment containing the bacterial type. Bacterial compositions and the bacterial components thereof are generally purified from residual habitat products.

[031] "Keystone OTU" or "Keystone Function" refers to one or more OTUs or Functional Pathways (*e.g.* KEGG or COG pathways) that are common to many network ecologies or functional network ecologies and are members of networks that occur in many subjects (*i.e.* are pervasive). Due to the ubiquitous nature of Keystone OTUs and their associated Functions Pathways, they are central to the function of network ecologies in healthy subjects and are often missing or at reduced levels in subjects with disease. Keystone OTUs and their associated functions may exist in low, moderate, or high abundance in subjects. "Non-Keystone OTU" or "non-Keystone Function" refers to an OTU or Function that is observed in a Network Ecology or a Functional Network Ecology and is not a keystone OTU or Function.

[032] "Microbiota" refers to the community of microorganisms that occur (sustainably or transiently) in and on an animal subject, typically a mammal such as a human, including eukaryotes, archaea, bacteria, and viruses (including bacterial viruses *i.e.*, phage).

[033] "Microbiome" refers to the genetic content of the communities of microbes that live in and on the human body, both sustainably and transiently, including eukaryotes, archaea, bacteria, and

viruses (including bacterial viruses (*i.e.*, phage)), wherein "genetic content" includes genomic DNA, RNA such as ribosomal RNA, the epigenome, plasmids, and all other types of genetic information.

[034] "Microbial Carriage" or simply "Carriage" refers to the population of microbes inhabiting a niche within or on humans. Carriage is often defined in terms of relative abundance. For example, OTU1 comprises 60% of the total microbial carriage, meaning that OTU1 has a relative abundance of 60% compared to the other OTUs in the sample from which the measurement was made. Carriage is most often based on genomic sequencing data where the relative abundance or carriage of a single OTU or group of OTUs is defined by the number of sequencing reads that are assigned to that OTU/s relative to the total number of sequencing reads for the sample. Alternatively, Carriage may be measured using microbiological assays.

[035] "Microbial Augmentation" or simply "augmentation" refers to the establishment or significant increase of a population of microbes that are (i) absent or undetectable (as determined by the use of standard genomic and microbiological techniques) from the administered therapeutic microbial composition, (ii) absent, undetectable, or present at low frequencies in the host niche (for example: gastrointestinal tract, skin, anterior-nares, or vagina) before the delivery of the microbial composition, and (iii) are found after the administration of the microbial composition or significantly increased, for instance 2-fold, 5-fold, $1x10^2$, $1x10^3$, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, or greater than $1x10^8$, in cases where they were present at low frequencies. The microbes that comprise an augmented ecology can be derived from exogenous sources such as food and the environment, or grow out from micro-niches within the host where they reside at low frequency. The administration of a bacterial microbial composition induces an environmental shift in the target niche that promotes favorable conditions for the growth of these commensal microbes. In the absence of treatment with a bacterial composition, the host can be constantly exposed to these microbes; however, sustained growth and the positive health effects associated with the stable population of increased levels of the microbes comprising the augmented ecology are not observed.

[036] "Microbial Engraftment" or simply "engraftment" refers to the establishment of OTUs present in the bacterial composition in a target niche that are absent in the treated host prior to treatment. The microbes that comprise the engrafted ecology are found in the therapeutic microbial composition and establish as constituents of the host microbial ecology upon treatment. Engrafted OTUs can establish for a transient period of time, or demonstrate long-term stability in the microbial ecology that populates the host post treatment with a bacterial composition. The engrafted ecology can induce an environmental shift in the target niche that promotes favorable conditions for the growth of commensal microbes capable of catalyzing a shift from a dysbiotic ecology to one representative of a health state.

[037] As used herein, the term "Minerals" is understood to include boron, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, silicon, tin, vanadium, zinc, or combinations thereof.

[038] "Network Ecology" refers to a consortium of clades or OTUs that co-occur in some number of subjects. As used herein, a "network" is defined mathematically by a graph delineating how specific nodes (*i.e.* clades or OTUs) and edges (connections between specific clades or OTUs) relate to one another to define the structural ecology of a consortium of clades or OTUs. Any given Network Ecology will possess inherent phylogenetic diversity and functional properties. A Network Ecology can also be defined in terms of its functional capabilities where for example the nodes would be comprised of elements such as, but not limited to, enzymes, clusters of orthologous groups (COGS; http://www.ncbi.nlm.nih.gov/books/NBK21090/), or KEGG Orthology Pathways (www.genome.jp/kegg/); these networks are referred to as a "Functional Network Ecology". Functional Network Ecologies can be reduced to practice by defining the group of OTUs that together comprise the functions defined by the Functional Network Ecology.

[039] "Network Class" and "Network Class Ecology" refer to a group of network ecologies that in general are computationally determined to comprise ecologies with similar phylogenetic and/or functional characteristics. A Network Class therefore contains important biological features, defined either phylogenetically or functionally, of a group (*i.e.*, a cluster) of related network ecologies. One representation of a Network Class Ecology is a designed consortium of microbes, typically non-pathogenic bacteria, that represents core features of a set of phylogenetically or functionally related network ecologies seen in many different subjects. In many occurrences, a Network Class, while designed as described herein, exists as a Network Ecology observed in one or more subjects. Network Class ecologies are useful for reversing or reducing a dysbiosis in subjects where the underlying, related Network Ecology has been disrupted. Exemplary Network Classes are provided in Table 12 and examples of phylogenetic signature by family of Network Classes are given in Table 13.

[040] To be free of "non-comestible products" means that a bacterial composition or other material provided herein does not have a substantial amount of a non-comestible product, *e.g.*, a product or material that is inedible, harmful or otherwise undesired in a product suitable for administration, *e.g.*, oral administration, to a human subject. Non-comestible products are often found in preparations of bacteria from the prior art.

[041] "Operational taxonomic units" and "OTU" (or plural, "OTUs") refer to a terminal leaf in a phylogenetic tree and is defined by a nucleic acid sequence, *e.g.*, the entire genome, or a specific genetic sequence, and all sequences that share sequence identity to this nucleic acid sequence at the level of species. In some embodiments the specific genetic sequence may be the 16S sequence or a portion of the 16S sequence. In other embodiments, the entire genomes of two entities are sequenced and compared. In another embodiment, select regions such as multilocus sequence tags (MLST),

specific genes, or sets of genes may be genetically compared. In 16S embodiments, OTUs that share ≥ 97% average nucleotide identity across the entire 16S or some variable region of the 16S are considered the same OTU. *See e.g.* Claesson MJ, Wang Q, O'Sullivan O, Greene-Diniz R, Cole JR, Ross RP, and O'Toole PW. 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res 38: e200. Konstantinidis KT, Ramette A, and Tiedje JM. 2006. The bacterial species definition in the genomic era. Philos Trans R Soc Lond B Biol Sci 361: 1929–1940. In embodiments involving the complete genome, MLSTs, specific genes, other than 16S, or sets of genes OTUs that share ≥ 95% average nucleotide identity are considered the same OTU. *See e.g.*, Achtman M, and Wagner M. 2008. Microbial diversity and the genetic nature of microbial species. Nat. Rev. Microbiol. 6: 431–440. Konstantinidis KT, Ramette A, and Tiedje JM. 2006. The bacterial species definition in the genomic era. Philos Trans R Soc Lond B Biol Sci 361: 1929–1940. OTUs are frequently defined by comparing sequences between organisms. Generally, sequences with less than 95% sequence identity are not considered to form part of the same OTU. OTUs may also be characterized by any combination of nucleotide markers or genes, in particular highly conserved genes (*e.g.*, "house-keeping" genes), or a combination thereof. Such characterization employs, *e.g.*, WGS data or a whole genome sequence.

[042] Table 1 below shows a List of Operational Taxonomic Units (OTU) with taxonomic assignments made to Genus, Species, and Phylogenetic Clade. Clade membership of bacterial OTUs is based on 16S sequence data. Clades are defined based on the topology of a phylogenetic tree that is constructed from full-length 16S sequences using maximum likelihood methods familiar to individuals with ordinary skill in the art of phylogenetics. Clades are constructed to ensure that all OTUs in a given clade are: (i) within a specified number of bootstrap supported nodes from one another, and (ii) within 5% genetic similarity. OTUs that are within the same clade can be distinguished as genetically and phylogenetically distinct from OTUs in a different clade based on 16S-V4 sequence data, while OTUs falling within the same clade are closely related. OTUs falling within the same clade are evolutionarily closely related and may or may not be distinguishable from one another using 16S-V4 sequence data. Members of the same clade, due to their evolutionary relatedness, play similar functional roles in a microbial ecology such as that found in the human gut. Compositions substituting one species with another from the same clade are likely to have conserved ecological function and therefore are useful in the present invention. All OTUs are denoted as to their putative capacity to form spores and whether they are a Pathogen or Pathobiont (see Definitions for description of "Pathobiont"). NIAID Priority Pathogens are denoted as 'Category-A', 'Category-B', or 'Category-C', and Opportunistic Pathogens are denoted as 'OP'. OTUs that are not pathogenic or for which their ability to exist as a pathogen is unknown are denoted as 'N'. The 'SEQ ID Number' denotes the identifier of the OTU in the Sequence Listing File and 'Public DB Accession' denotes the identifier of the OTU in a public sequence repository.

[043] "Pathobiont" refer to specific bacterial species found in healthy hosts that may trigger immune-mediated pathology and/or disease in response to certain genetic or environmental factors (Chow et al. 2011. Curr Op Immunol. Pathobionts of the intestinal microbiota and inflammatory disease. 23: 473-80). Thus, a pathobiont is an opportunistic microbe that is mechanistically distinct from an acquired infectious organism. Thus, the term "pathogen" includes both acquired infectious organisms and pathobionts.

[044] "Pathogen", "pathobiont" and "pathogenic" in reference to a bacterium or any other organism or entity that includes any such organism or entity that is capable of causing or affecting a disease, disorder or condition of a host organism containing the organism or entity, including but not limited to pre-diabetes, type 1 diabetes or type 2 diabetes.

[045] "Phenotype" refers to a set of observable characteristics of an individual entity. As example an individual subject may have a phenotype of "health" or "disease". Phenotypes describe the state of an entity and all entities within a phenotype share the same set of characteristics that describe the phenotype. The phenotype of an individual results in part, or in whole, from the interaction of the entity's genome and/or microbiome with the environment, especially including diet.

[046] "Phylogenetic Diversity" is a biological characteristic that refers to the biodiversity present in a given Network Ecology or Network Class Ecology based on the OTUs that comprise the network. Phylogenetic diversity is a relative term, meaning that a Network Ecology or Network Class that is comparatively more phylogenetically diverse than another network contains a greater number of unique species, genera, and taxonomic families. Uniqueness of a species, genera, or taxonomic family is generally defined using a phylogenetic tree that represents the genetic diversity all species, genera, or taxonomic families relative to one another. In another embodiment phylogenetic diversity may be measured using the total branch length or average branch length of a phylogenetic tree. Phylogenetic Diversity may be optimized in a bacterial composition by including a wide range of biodiversity.

[047] "Phylogenetic tree" refers to a graphical representation of the evolutionary relationships of one genetic sequence to another that is generated using a defined set of phylogenetic reconstruction algorithms (*e.g.* parsimony, maximum likelihood, or Bayesian). Nodes in the tree represent distinct ancestral sequences and the confidence of any node is provided by a bootstrap or Bayesian posterior probability, which measures branch uncertainty.

[048] "Prediabetes" refers a condition in which blood glucose levels are higher than normal, but not high enough to be classified as diabetes. Individuals with pre-diabetes are at increased risk of developing type 2 diabetes within a decade. According to CDC, prediabetes can be diagnosed by fasting glucose levels between 100-125 mg/dL, 2 hour post-glucose load plasma glucose in oral glucose tolerance test (OGTT) between 140 and 199 mg/dL, or hemoglobin A1c test between 5.7%-6.4%.

[049] "rDNA", "rRNA", "16S-rDNA", "16S-rRNA", "16S", "16S sequencing", "16S-NGS", "18S", "18S-rRNA", "18S-rDNA", "18S sequencing", and "18S-NGS" refer to the nucleic acids that encode for the RNA subunits of the ribosome. rDNA refers to the gene that encodes the rRNA that comprises the RNA subunits. There are two RNA subunits in the ribosome termed the small subunit (SSU) and large subunit (LSU); the RNA genetic sequences (rRNA) of these subunits is related to the gene that encodes them (rDNA) by the genetic code. rDNA genes and their complementary RNA sequences are widely used for determination of the evolutionary relationships amount organisms as they are variable, yet sufficiently conserved to allow cross organism molecular comparisons. Typically 16S rDNA sequence (approximately 1542 nucleotides in length) of the 30S SSU is used for molecular-based taxonomic assignments of Prokaryotes and the 18S rDNA sequence (approximately 1869 nucleotides in length) of 40S SSU is used for Eukaryotes. 16S sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most bacteria.

[050] "Residual habitat products" refers to material derived from the habitat for microbiota within or on a human or animal. For example, microbiota live in feces in the gastrointestinal tract, on the skin itself, in saliva, mucus of the respiratory tract, or secretions of the genitourinary tract (*i.e.*, biological matter associated with the microbial community). Substantially free of residual habitat products means that the bacterial composition no longer contains the biological matter associated with the microbial environment on or in the human or animal subject and is 100% free, 99% free, 98% free, 97% free, 96% free, or 95% free of any contaminating biological matter associated with the microbial community. Residual habitat products can include abiotic materials (including undigested food) or it can include unwanted microorganisms. Substantially free of residual habitat products may also mean that the bacterial composition contains no detectable cells from a human or animal and that only microbial cells are detectable. In one embodiment, substantially free of residual habitat products may also mean that the bacterial composition contains no detectable viral (including bacterial viruses (*i.e.*, phage)), fungal, mycoplasmal contaminants. In another embodiment, it means that fewer than 1x10-2%, 1x10-3%, 1x10-4%, 1x10-5%, 1x10-6%, 1x10-7%, 1x10-8 of the viable cells in the bacterial composition are human or animal, as compared to microbial cells. There are multiple ways to accomplish this degree of purity, none of which are limiting. Thus, contamination may be reduced by isolating desired constituents through multiple steps of streaking to single colonies on solid media until replicate (such as, but not limited to, two) streaks from serial single colonies have shown only a single colony morphology. Alternatively, reduction of contamination can be accomplished by multiple rounds of serial dilutions to single desired cells (*e.g.*, a dilution of 10-8 or 10-9), such as through multiple 10-fold serial dilutions. This can further be confirmed by showing that multiple isolated colonies have similar cell shapes and Gram staining behavior. Other methods for confirming adequate purity include genetic analysis (*e.g.* PCR, DNA sequencing), serology and antigen analysis, enzymatic

and metabolic analysis, and methods using instrumentation such as flow cytometry with reagents that distinguish desired constituents from contaminants.

[051] "Spore" or a population of "spores" includes bacteria (or other single-celled organisms) that are generally viable, more resistant to environmental influences such as heat and bacteriocidal agents than vegetative forms of the same bacteria, and typically capable of germination and out-growth. Spores are characterized by the absence of active metabolism until they respond to specific environmental signals, causing them to germinate. "Spore-formers" or bacteria "capable of forming spores" are those bacteria containing the genes and other necessary abilities to produce spores under suitable environmental conditions. A Table of preferred spore-forming bacterial compositions is provided in Table 11.

[052] "Spore population" refers to a plurality of spores present in a composition. Synonymous terms used herein include spore composition, spore preparation, ethanol-treated spore fraction and spore ecology. A spore population may be purified from a fecal donation, *e.g.* via ethanol or heat treatment, or a density gradient separation or any combination of methods described herein to increase the purity, potency and/or concentration of spores in a sample. Alternatively, a spore population may be derived through culture methods starting from isolated spore former species or spore former OTUs or from a mixture of such species, either in vegetative or spore form.

[053] In one embodiment, the spore preparation comprises spore forming species wherein residual non-spore forming species have been inactivated by chemical or physical treatments including ethanol, detergent, heat, sonication, and the like; or wherein the non-spore forming species have been removed from the spore preparation by various separations steps including density gradients, centrifugation, filtration and/or chromatography; or wherein inactivation and separation methods are combined to make the spore preparation. In yet another embodiment, the spore preparation comprises spore forming species that are enriched over viable non-spore formers or vegetative forms of spore formers. In this embodiment, spores are enriched by 2-fold, 5-fold, 10-fold, 50-fold, 100-fold, 1000-fold, 10,000-fold or greater than 10,000-fold compared to all vegetative forms of bacteria. In yet another embodiment, the spores in the spore preparation undergo partial germination during processing and formulation such that the final composition comprises spores and vegetative bacteria derived from spore forming species.

[054] "Sporulation induction agent" is a material or physical-chemical process that is capable of inducing sporulation in a bacterium, either directly or indirectly, in a host organism and/or in vitro.

[055] To "increase production of bacterial spores" includes an activity or a sporulation induction agent. "Production" includes conversion of vegetative bacterial cells into spores and augmentation of the rate of such conversion, as well as decreasing the germination of bacteria in spore form, decreasing the rate of spore decay in vivo, or ex vivo, or to increasing the total output of spores (*e.g.* via an increase in volumetric output of fecal material).

[056] "Subject" refers to any animal subject including humans, laboratory animals (*e.g.*, primates, rats, mice), livestock (*e.g.*, cows, sheep, goats, pigs, turkeys, and chickens), and household pets (*e.g.*, dogs, cats, and rodents). The subject may be suffering from a dysbiosis, that contributes to or causes a condition classified as diabetes or pre-diabetes, including but not limited to mechanisms such as metabolic endotoxemia, altered metabolism of primary bile acids, immune system activation, or an imbalance or reduced production of short chain fatty acids including butyrate, propionate, acetate, and branched chain fatty acids.

[057] As used herein the term "Vitamin" is understood to include any of various fat-soluble or water-soluble organic substances (non-limiting examples include Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin or niacinamide), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (folic acid), and Vitamin B12 (various cobalamins; commonly cyanocobalamin in vitamin supplements), Vitamin C, Vitamin D, Vitamin E, Vitamin K, K1 and K2 (*i.e.*, MK-4, MK-7), folic acid and biotin) essential in minute amounts for normal growth and activity of the body and obtained naturally from plant and animal foods or synthetically made, pro-vitamins, derivatives, analogs.

[058] "V1-V9 regions" or "16S V1-V9 regions" refers to the 16S rRNA refers to the first through ninth hypervariable regions of the 16S rRNA gene that are used for genetic typing of bacterial samples. These regions in bacteria are defined by nucleotides 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294 and 1435-1465 respectively using numbering based on the E. coli system of nomenclature. Brosius et al., Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli, PNAS 75(10):4801-4805 (1978). In some embodiments, at least one of the V1, V2, V3, V4, V5, V6, V7, V8, and V9 regions are used to characterize an OTU. In one embodiment, the V1, V2, and V3 regions are used to characterize an OTU. In another embodiment, the V3, V4, and V5 regions are used to characterize an OTU. In another embodiment, the V4 region is used to characterize an OTU. A person of ordinary skill in the art can identify the specific hypervariable regions of a candidate 16S rRNA by comparing the candidate sequence in question to a reference sequence and identifying the hypervariable regions based on similarity to the reference hypervariable regions, or alternatively, one can employ Whole Genome Shotgun (WGS) sequence characterization of microbes or a microbial community.

Interactions Between Microbiome and Host

[059] Interactions between the human microbiome and the host shape host health and disease via multiple mechanisms, including the provision of essential functions by the microbiota. . Examples of these mechanisms include but are not limited to the function of the microbiota in ensuring a healthy level of bile acid metabolism, energy harvesting and storage, and regulation of immune responses, and reducing deleterious and unhealthy levels of gut permeability or metabolic endotoxemia.

Importance of Bile Acids to Human Health and Role of Microbiota

[060] Primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized from cholesterol in the liver in humans. The synthesized primary bile acids are conjugated to glycine, taurine, or sulfate before secretion into the bile by specific transporters located in the basolateral membrane of the hepatocyte. The ingestion of a meal triggers the release of bile from the gallbladder into the intestinal lumen, where bile acids form micelles with dietary lipids and lipid-soluble vitamins, facilitating their absorption. ~95% of bile acids are reabsorbed via specific transporters expressed in the distal ileum, and the remaining 5% escapes the enterohepatic cycle and travels towards the large intestine to be excreted in the feces. In the colon, the bile acids may undergo deconjugation and dehydroxylation by the gut microflora. The resulting secondary bile acids are mainly deoxycholic acid (DCA) and lithocholic acid (LCA). The bile acid pool undergoes this enterohepatic cycle around 12x/day in humans. Although the bile acid pool size is constant, the flux of bile acids varies during the day; bile acid flux and plasma bile acid concentrations are highest postprandially (See reviews Prawitt, J et al. 2011 Curr Diab Rep Bile acid metabolism and the pathogenesis of type 2 diabetes 11: 160-166; Nieuwdorp et al. 2014 Gastroenterology. Role of the Microbiome in Energy Regulation and Metabolism. pii: S0016-5085(14)00219-4. doi: 10.1053/j.gastro.2014.02.008).

[061] Commensal bacteria are involved in processing primary bile acids to secondary bile acids in the colon. Known biotransformations of bile acids by commensal GI bacteria include deconjugation of the conjugated bile salts to liberate free bile acids and amino acid moieties, removal of hydroxyl groups principally the C-7 hydroxyl group of the cholic acid moiety, oxidative and reductive reactions of the existing hydroxyl groups, and epimerization of bile acids.

[062] The canonical first step in bile acid metabolism is deconjugation of the taurine or glycine group through enzymes termed bile salt hydrolases, to yield CA and CDCA. These bile acids are then substrates for a series of enzymatic steps that remove the 7-alphahydroxy group to form deoxycholic acid (DCA) and lithocholic acid (LCA). LCA has particularly low solubility due to the loss of hydrophilic side chains compared to any of the other bile acids. It is also feasible for microbes to dehydroxylate the conjugated primary bile acids, giving rise to gluco-DCA; gluco-LCA; tauro-DCA; and tauro-LCA. Further modifications are possible, including the microbial conversion of CDCA to a 7-betahydroxy epimer, ursodeoxycholic acid (UDCA). Many other secondary bile acids are made in smaller amounts by the gut microbiota, for example, alpha-, beta-, gamma-, and omega-muricholic acids and many others (see Swann JR et al., 2011 PNAS Systemic gut microbial modulation of bile acid metabolism in host tissue compartments 108: 4523-30).

[063] Intestinal microbiota play a key role in bile acid metabolism. Germ-free mice have altered metabolism of bile acids, including increased levels of conjugated bile acids throughout the intestine, with no deconjugation, and strongly decreased fecal excretion. Provision of ampicillin to mice increases biliary bile acid output 3-fold and decreases fecal output by 70%.

[064] Dysbiosis of the gut microbiome affecting bile acid metabolism may affect adiposity, glucose regulation, and inflammation, among other effects. Bile acids are essential solubilizers of lipids, fats, and lipid soluble vitamins to enhance absorption of nutrients in the small intestine, and are also signaling molecules that regulate metabolism, including glucose homeostasis and basal metabolic rate. *See* Houten, SM *et al.* 2006 EMBO J. Endocrine function of bile acids. 25: 1419-25; Prawitt, J et al. 2011 Curr Diab Rep. Bile acid metabolism and the pathogenesis of type 2 diabetes. 11: 160-166. For example, bile acid sequestrants (non-absorbable polymers that complex bile acids in the intestinal lumen and divert them from the enterohepatic cycle) can improve glycemic control in Type 2 diabetes patients. Prawitt, J *et al.* 2011 Curr Diab Rep Bile acid metabolism and the pathogenesis of type 2 diabetes 11: 160-166.

[065] The most prominent targets of action by bile acids and their metabolites include FXR (farnesoid X receptor), an orphan nuclear receptor within the liver and intestine, and TGR5, a G-protein coupled receptor found on gallbladder, ileum, colon, brown and white adipose tissue. FXR is preferentially activated by CDCA, and in turn upregulates the expression of gene products including FGF-19 (fibroblast growth factor 19) in humans. FGF-15 (the murine analogue of FGF-19) increases basal metabolic rate and reverses weight gain in mice given a high fat diet. FXR activation also down-regulates hepatic gluconeogenesis. Although both conjugated and unconjugated bile acids can bind to FXR, the conjugated forms must be actively transported into the cell to initiate signaling whereas the unconjugated bile acids can diffuse through the membrane owing to their lower molecular weight, higher pKa and tendency to exist in the protonated form.

[066] TGR5 is preferentially activated by the secondary bile acid LCA and tauro-LCA with downstream effects, among others, on expression of incretin hormones such as GLP-1 that modulate insulin production and help maintain glucose homeostasis.

[067] Bile acids are therefore important metabolic regulators. Additional insight into the importance of the interplay between the gut microbiome, bile acid metabolism, and glucose homeostasis is provided by the observation that treating obese male patients with a 7-day course of vancomycin decreases total microbiota diversity, specifically depleting species in the diverse Clostridium IV and XIVa clusters. Among the Clostridia are various organisms that metabolize bile acids as well as others that produce short chain fatty acids, including butyrate and propionate. In contrast, treatment with a 7-day course of amoxicillin produces a trend toward increased microbiota diversity. Moreover, fecal bile acid composition is markedly changed following vancomycin treatment; secondary bile acids DCA, LCA and iso-LCA decrease whereas primary bile acids CA and CDCA increase. Amoxicillin treatment does not alter the ratio of bile acids in fecal samples. FGF-19 levels in serum are also decreased following vancomycin treatment, but not amoxicillin treatment. Peripheral insulin sensitivity changes following vancomycin but not amoxicillin treatment. Vrieze, A

et al., 2013 J Hepatol Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensistivity dx.doi.org/10.1016/j.jhep.2013.11.034.

[068] While the study by Vrieze et al. points out the potential for microbes to improve insulin homeostasis through enhanced secondary bile acid metabolism, the authors point out several limitations of their work. Most importantly, while the HIT-Chip analysis used to generate fecal microbial profiles provides valuable information regarding classes of organisms, it does not provide mechanistic information or identify specific species or functional enzymatic pathways responsible for the observed effects. Moreover, the HIT-Chip is a hybridization based assay and the similarity of sequences among the organisms in the Clostridial clusters may lead to mis-assignments. As a result, others have failed to define specific compositions that can be used to modulate insulin sensitivity via bile acid metabolism.

[069] In addition to the role for bile acids as metabolic regulators, bile acids are also linked to inflammatory disease. Crohn's Disease patients in the Metagenomics of the Human Intestinal Tract (MetaHIT) cohort show reduced bile salt hydrolase (BSH) gene abundance compared to patients without disease, and increased primary bile acids in inflammatory bowel syndrome patients is correlated with stool frequency and consistency (Duboc et al. 2012 Neurogastroenterol Motil. Increase in fecal primary bile acids and dysbiosis in patients with diarrhea-predominant irritable bowel syndrome. doi: 10.1111/j.1365-2982.2012.01893.x). Furthermore, TGR5 is expressed on immune monocytes and macrophages in addition to GI and liver tissues, and FXR and TGR5 are known to be involved in regulation of inflammation in enterohepatic tissues (Jones et al., 2014 Expert Opin Biol Ther The human microbiome and bile acid metabolism: dysbiosis, dysmetabolism, disease and intervention doi:10.1517/14712598.2014.880420)

[070] Multiple methods are available for determination of bile acids in serum, bile and faces of individuals. As reviewed by Sharma (Sharma, KR, Review on bile acid analysis, Int J Pharm Biomed Sci 2012, 3(2), 28-34), a variety of methods can be used, such as chemical (Carey JB, et al, 1958, The bile acids in normal human serum with comparative observations in patients with jaundice. J Lab Clin Med 1958, 46, 860-865), thin layer chromatography (Fausa O, and Shalhegg BA. 1976 Quantitative determination of bile acids and their conjugates using thin-layer chromatography and a purified 3-α hydroxysteroid dehydrogenase. Scand J Gastroenterol 9, 249-254.), high performance liquid chromatography (Islam S, et al Fasting serum bile acid level in cirrhosis "A semi quantitative index of hepatic function". J Hepatol 1985, 1, 609-617; Paauw JD, at al. Assay for taurine conjugates of bile acids in serum by reversed phase high performance liquid chromatography. J Chromatograph B Biomed Appl 1996, 685, 171-175), radioimmunoassay (Wildgrube J, Stockhausan H, Peter M, Mauritz G, Mahdawi R. Radioimmunoassay of bile acids in tissues, bile and urine. Clin Chem 1983, 29, 494-498), enzyme linked colorimetric and radioimmunoassay (Guo W, et al. A study on detection of serum fasting total bile acid and cholog lycin in neonates for cholestasis. Chin Med Sci J 1996, 11,

244-247.), mass spectrometry (Sjovell J, et al. Mass spectrometry of bile acids. Method in enzymology. Vol. III, Academic Press, New York 1985.), tandem mass spectrometry (Griffiths WJ. Tandem mass spectrometry in study of fatty acids, bile acids and steroids. Mass Spectrum Rev 2003, 22, 81-152.), gas chromatography using high resolution glass capillary columns and mass spectrometry (Setchell KDR, Matsui A. Serum bile acid analysis. Clin Chim Acta 1983, 127, 1-17.), gas chromatography (Fischer S, et al. Hepatic levels of bile acids in end stage chronic cholestatic liver disease. Clin Chim Acta 1996, 251, 173-186.), gas liquid chromatography (Van Berge Hengouwen GP et al., Quantitative anaylsis of bile acids in serum and bile, using gas liquid chromatography. Clin Chim Acta 1974, 54, 249-261; Batta AK, et al. Characterization of serum and urinary bile acids in patients with primary biliary cirrhosis by gas-liquid chromatography-mass spectrometry: effect of ursodeoxycholic acid treatment. J Lipid Res 1989, 30, 1953- 1962), luminometric method (Styrellius I, Thore A, Bjorkhem I. Luminometric method. In: Methods of enzymatic analysis. (Ed. III). Bergmeyer, Hans Ulirch [Hrsg]. 8: 274-281, 1985.), UV method for bile assay (Staver E, et al. Fluorimetric method for serum. In: Methods of enzymatic analysis. (Ed.III). Bergmeyer, Hans Ulrich; [Hrsg]. 8, 288-290, 1985; Staver E, et al. UV method for bile, gastric juice and duodenai aspirates. In: Methods of enzymatic analysis, (e.d.III). Bergmeyer, Hans Ulrich [Hrsg]. 8: 285-287, 1985), enzymatic colorimetric method (Collins BJ, et al. Measurement of total bile acids in gastric juice. J Clin Pathol 1984, 37, 313-316) and enzymatic fluorimetric method can be used (Murphy GM, et al. A fluorometric and enzymatic method for the estimation of serum total bile acids. J Clin Path 1970, 23, 594-598; Hanson NQ, Freier E F. Enzymic measurement of total bile acid adapted to the Cobas Fara Centrifugal analyzer. Clin Chem. 1985, 35, 1538-1539).

Importance of Short Chain Fatty Acids (SCFA) to Human Health and Role of Microbiota

[071] Short chain fatty acids (SCFAs) are a principal product of bacterial fermentation in the colon. SCFAs, particularly acetate, propionate and butyrate, are thought to have many potential benefits to the mammalian host. SCFAs are organic acids with fewer than 6 carbons and include acetate, propionate, butyrate, valerate, isovalerate, and 2-methyl butyrate. While longer chain fatty acids are derived primarily from dietary sources, SCFAs are derived from the breakdown of non-digestible plant fiber. Butyrate is a primary energy source for colonocytes, whereas propionate is thought to be metabolized mostly by the liver via portal vein circulation from the colon. Acetate is derived from the microbiota is thought to be more generally available to tissues.

[072] In addition to acting as metabolic substrates, SCFAs have multiple benefits, including that SCFAs produced by the microbiota are essential for immune homeostasis and particularly for immune modulation by regulatory T cells. Direct ingestion of acetate, propionate or butyrate, or a mixture of all three by mice, stimulates the proliferation and maturation of regulatory T cells (Tregs) that reside in the colon. Mice given SCFAs in drinking water have significantly higher levels of colonic CD4+ FoxP3+ T cells (Tregs) than germ free and SPFA controls, and these Treg cells are functionally more

potent as measured by the expression of IL-10 mRNA and protein, and by their ability to inhibit CD8+ effector T cells in vitro (Smith PM et al. 2013 Science The microbial metabolites, short-chain fatty acid regulate colonic Treg cell homeostasis 341: 569-73). This effect of SCFAs is mediated via signaling through GPR43 (FFAR2), a G protein coupled receptor expressed on a variety of cells but with high frequency on colonic Treg cells. GPR43 signaling is upstream of modification of histone deacetylase activity (particularly HDAC9 and HDCA3), which is known to alter gene expression via reconfiguration of chromatin. Furthermore, the effects of experimental colitis induced by adoptive T cell transfer are reduced by SCFAs including propionate alone and a mixture of acetate, propionate or butyrate in a GPR43 dependent fashion.

[073] Beyond the direct effects of SCFA administered orally to animals, microbes can produce SCFA in situ in the colon and improve outcomes in several disease models. Daily administration of 10^9 cfu of Butyricicoccus pullicaecorum, a butyrate forming organism first isolated from chickens, for 1 week ameliorates TNBS-induced colitis in a rat model (Eeckhaut V et al., 2013 Gut Progress towards butyrate-producing pharmabiotics: Butyricicoccus pullicaecorum capsule and efficacy in TNBS models in comparison with therapeutics doi:10.1136/gutjnl-2013-305293). In humans, topical administration of butyrate or sodium butyrate via a rectal enema may be beneficial to ulcerative colitis patients (Scheppach W et al.1992 Gastroenterol Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis 103: 51–56; Vernia P et al. 2003 Eur. J. Clin. Investig Topical butyrate improves efficacy of 5-ASA in refractory distal ulcerative colitis: results of a multicentre trial. 33: 244–48). Butyrate has effects at multiple levels including signaling via GPR109A, which is expressed on the apical surface of intestinal epithelial cells (IECs). GPR109A lowers NFKB-mediated gene expression, including reduced expression of the inflammatory cytokines TNF-alpha, IL-6 and IL-1beta.

[074] Oral administration of SCFAs in mice also has direct effects on metabolism. SCFAs are a significant energy source and thus fermentation by the microbiota can contribute up 5-10% of the basal energy requirements of a human. SCFAs upregulate production of glucagon-like peptide 1 (GLP-1), peptide (P)YY and insulin. GLP-1 and PYY are noted to play a role in enhancing satiety and reducing food intake. Furthermore, fecal transplantation from lean human donors to obese recipients with metabolic syndrome results in a significant increase in insulin sensitivity after 6 weeks. This change is most correlated with the transfer of Eubacterium hallii, a gram-positive, butyrate-fermenting microbe (Vrieze, A., et al., 2012 Gastroenterol Transfer of intestinal microbiota from lean donors increases insulin sensitivity 143: 913-6).

[075] A common factor underlying both diabetes and obesity is the phenomenon of low-level inflammation termed metabolic endotoxemia (see below). Metabolic endotoxemia refers to increased permeability of the gut ("leaky gut syndrome") coupled with increased translocation of lipopolysaccharide (LPS), mediating an inflammatory response that triggers insulin resistance,

changes in lipid metabolism, and liver inflammation responsible for non-alcoholic fatty liver disease (NAFLD). Low level bacteremia may also lead to the translocation of viable gram-negative organisms into distal tissues, such as adipocytes, and further drive inflammation. SCFAs provide a benefit here as well, both by providing an energy source to enhance colonic epithelial cell integrity and by stimulating the expression of tight junction proteins to reduce translocation of gram-negative LPS, bacterial cells and their fragments (Wang HB et al, 2012 Dig Dis Sci Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription 57: 3126-35).

[076] For all of these reasons, it would be useful to have microbial communities with an enhanced ability to produce SCFAs for the treatment of diseases such as diabetes, obesity, inflammation, ulcerative colitis and NAFLD.

[077] Acetate, propionate and butyrate are formed as end-products in anaerobic fermentation. SCFA producing bacteria in the gut gain energy by substrate-level phosphorylation during oxidative breakdown of carbon precursors. However, the resulting reducing equivalents, captured in the form of NADH, must be removed to maintain redox balance, and hence the energetic driving force to produce large amounts of reduced end-products such as butyrate and propionate, in order to regenerates NAD+. Acetate, propionate and butyrate are not the only end products of fermentation: microbes in the gut also produce lactate, formate, hydrogen and carbon dioxide depending on the conditions. As discussed below, lactate and acetate can also drive the formation of butyrate and propionate through cross-feeding by one microorganism to another.

[078] The rate of SCFA production in the colon is highly dependent on many factors including the availability of polysaccharide carbon sources (such as, but not limited to, fructans, starches, cellulose, galactomannans, xylans, arabinoxylans, pectins, inulin, fructooligosaccharides, and the like), the presence of alternative electron sinks such as sulfate and nitrate, the redox potential, hydrogen (H_2) partial pressure and pH. As described above, cross-feeding among organisms can also play a role, for instance when a lactate forming organism provides lactate as a substrate for a butyrate or propionate producer, or when a saccharolytic organism breaks down a complex carbohydrate to provide a mono- or disaccharide for fermentation. Acetate, which can be as high as 30 mM in the gut, is also a key building block of butyrate through the action of the enzyme butyryl-CoA:acetate CoA transferase, the final step in the production of butyrate. Importantly, this enzyme can also function as a propionyl-CoA: acetate CoA transferase, resulting in the production of propionate.

[079] Since diet is a principal determinant of the variety of carbon sources and other nutrients available in the colon, it is clear that a functional ecology for SCFA production will comprise multiple organisms capable of adapting to diet-driven changes in in the gut environment. Thus, there exists a need for a bacterial composition that can ferment sufficient quantities of SCFA products in spite of

the varying environmental conditions imposed by a changing diet. Such bacterial compositions will comprise organisms capable of fermenting a variety of carbon sources into SCFA.

Role of Microbiota in Metabolic Endotoxemia/Bacteremia

[080] Chronic, low-grade inflammation is characteristic of obesity and is recognized to play an underlying pathogenic role in the metabolic complications and negative health outcomes of the disease. Notably, obesity is associated with elevated plasma levels of bacterial lipopolysaccharide (LPS). Energy intake, in particular a high fat diet (HFD), increases gut permeability and increases plasma LPS levels 2- to 3-fold. LPS in the circulatory system reflects passage of bacterial fragments across the gut into systemic circulation (termed "metabolic endotoxemia"), either through increases in diffusion due to intestinal paracellular permeability or through absorption by enterocytes during chylomicron secretion. Subcutaneous infusion of LPS into wild type mice maintained on a normal chow diet for 4 weeks leads to increased whole body, liver and adipose tissue weights, adipose and liver inflammation as well as fasted hyperglycemia and insulinemia, effects that are comparable to those induced by HFD (Cani et al., 2007 Diabetes. Metabolic endotoxemia initiates obesity and insulin resistance doi:10.2337/db06-1491). In addition to bacterial fragments, the translocation of live bacteria to host tissues may also be a feature of obesity (termed "metabolic bacteremia") (Shen et al., 2013 Mol Aspects Med. The gut microbiota, obesity and insulin resistance doi: 10.1016/j.mam.2012.11.001).

[081] Host–microbiota interactions at the gut mucosal interface are involved in intestinal barrier functionality and bacterial surveillance/detection. Dysbiosis can promote bacterial translocation and obesity-associated inflammation. In one instance, metabolic endotoxemia of HFD-induced obesity in mice is associated with reductions in Bifidobacterium, and both may be ameliorated through treatment with inulin (oligofructose) (Cani et al. Diabetologia Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia doi: 10.1007/s00125-007-0791-0). The beneficial effects of inulin and Bifidobacterium are associated with enhanced production of intestinotrophic proglucagon-derived peptide 2 (GLP-2), a peptide produced by L cells of the intestine that promotes intestinal growth (Cani et al. Gut. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. doi:10.1136/gut.2008.165886). Alternative pathways involving host-microbiota interactions and intestinal barrier integrity and metabolic endotoxemia/bacteremia include but are not limited to those involving intestinotrophic proglucagon-derived peptide (GLP)-2, endocannabinoid (eCB) signaling, and pattern recognition receptors including nucleotide-binding oligomerization domain (NOD)-like receptors (NLR) such as NOD1/NLRC1 and NOD2/NLRC2 as well as Toll like receptors (TLR) such as TLR-2, TLR-4, TLR-5 and TLR adapter protein myeloid differentiation primary-response protein 88 (MyD88) (see review Shen et al., 2013 Mol Aspects Med. The gut microbiota, obesity and insulin resistance doi: 10.1016/j.mam.2012.11.001).

Role of Microbiota in Energy Harvesting and Storage

[082] The gut microbiota is involved in host energy harvesting. Germ free (GF) mice consume more energy but are significantly leaner than wild type counterparts. Conventionalization of GF mice given a low-fat, polysaccharide-rich diet with the microbiota of conventionally-raised mice leads to 60% more adiposity and insulin resistance despite reduced food intake (Backhed et al., 2004 PNAS The gut microbiota as an environmental factor that regulates fat storage doi: 10.1073/pnas.0407076101). GF mice conventionalized with microbiota from obese mice show significantly greater increase in total body fat than GF mice conventionalized with microbiota from lean mice. Obese (ob/ob) mice have significantly less energy remaining in their feces relative to their lean littermates as measured by bomb calorimetry (Turnbaugh et al. 2006 Nature. An obesity-associated gut microbiome with increased capacity for energy harvest doi:10.1038/nature05414). In humans, "overnutrition" (defined as energy consumption as a percentage of weight-maintaining energy needs) is associated with proportionally more Firmicutes and fewer Bacteroidetes and energy loss (stool calories as a percentage of ingested calories) in lean subjects is negatively associated with the proportional change in Firmicutes and positively associated with the proportional change in Bacteroidetes, suggesting impact of the gut microbiota on host energy harvest (Jumpertz et al., 2011 Am J Clin Nutr. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. doi: 10.3945/ajcn.110.010132).

[083] In addition to affecting host energy harvesting, gut microbiota is also implicated in energy storage. The increase in body fat observed upon conventionalization of GF mice is associated with a decrease in Fasting-induced adipose factor (Fiaf) expression in the ileum and a 122% increase in Lipoprotein Lipase (LPL) activity in epididymal adipose tissue (Backhed et al., 2004 PNAS The gut microbiota as an environmental factor that regulates fat storage doi/10.1073/pnas.0407076101). Fiaf (also known as angiopoietin-like 4) is a protein secreted by adipose tissues, liver and intestine that inhibits the activity of LPL, a key enzyme in the hydrolysis of lipoprotein-associated triglycerides and the release of fatty acids for transport into cells. In adipocytes, fatty acids released by LPL are re-esterified into triglyceride and stored as fat (Shen et al., 2013 Mol Aspects Med. The gut microbiota, obesity and insulin resistance doi: 10.1016/j.mam.2012.11.001).

Other Functional Pathways

[084] The pathways and mechanisms discussed above on the functional pathways and mechanisms by which the microbiota shape host health and disease is not meant to be exhaustive. Alternative functional pathways and mechanisms exist, including but not limited to pathways involving AMP-activated protein kinase (AMPK), TLR-5, and SREBP-1c and ChREBP.

Emergence of Antibiotic Resistance in Bacteria

[085] Antibiotic resistance is an emerging public health issue (Carlet J, Collignon P, Goldmann D, Goossens H, Gyssens IC, Harbarth S, Jarlier V, Levy SB, N'Doye B, Pittet D, et al. 2011. Society's failure to protect a precious resource: antibiotics. Lancet 378: 369–371.). Numerous genera

of bacteria harbor species that are developing resistance to antibiotics. These include but are not limited to Vancomycin Resistant Enterococcus (VRE) and Carbapenem resistant Klebsiella (CRKp). Klebsiella pneumoniae and Escherichia coli strains are becoming resistant to carbapenems and require the use of old antibiotics characterized by high toxicity, such as colistin (Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, et al. 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect 18: 413–431.). Further multiply drug resistant strains of multiple species, including Pseudomonas aeruginosa, Enterobacter spp, and Acinetobacter spp are observed clinically including isolates that are highly resistant to ceftazidime, carbapenems, and quinolones (European Centre for Disease Prevention and Control: EARSS net database. http://ecdc.europa.eu.). The Centers for Disease Control and Prevention in 2013 released a Threat Report (http://www.cdc.gov/drugresistance/threat-report-2013/) citing numerous bacterial infection threats that included Clostridium difficile, Carbapenem-resistant Enterobacteriaceae (CRE), Multidrug-resistant Acinetobacter, Drug-resistant Campylobacter, Extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs), Vancomycin-resistant Enterococcus (VRE), Multidrug-resistant Pseudomonas aeruginosa, Drug-resistant Non-typhoidal Salmonella, Drug-resistant Salmonella Typhi, Drug-resistant Shigella, Methicillin-resistant Staphylococcus aureus (MRSA), Drug-resistant Streptococcus pneumoniae, Vancomycin-resistant Staphylococcus aureus (VRSA), Erythromycin-resistant Group A Streptococcus, and Clindamycin-resistant Group B Streptococcus. The increasing failure of antibiotics due the rise of resistant microbes demands new therapeutics to treat bacterial infections. Administration of a microbiome therapeutic bacterial composition offers potential for such therapies. Applicants have discovered that patients suffering from recurrent *C. difficile* associated diarrhea (CDAD) often harbor antibiotic resistant Gram-negative bacteria, in particular Enterobacteriaceae and that treatment with a bacterial composition effectively treats CDAD and reduces the antibiotic resistant Gram-negative bacteria. The gastrointestinal tract is implicated as a reservoir for many of these organisms including VRE, MRSA, Pseudomonas aeruginosa, Acinetobacter and the yeast Candida (Donskey, Clinical Infectious Diseases 2004 39:214,The Role of the Intestinal Tract as a Reservoir and Source for Transmission of Nosocomial Pathogens), and thus as a source of nosocomial infections. Antibiotic treatment and other decontamination procedures are among the tools in use to reduce colonization of these organisms in susceptible patients including those who are immunosuppressed. Bacterial-based therapeutics would provide a new tool for decolonization, with a key benefit of not promoting antibiotic resistance as antibiotic therapies do.

COMPOSITIONS OF THE INVENTION

Network Ecologies

[086] As described above, the Network Ecology and Functional Network Ecology refer to a consortium of OTUs or Functional modalities respectively that co-occur in a group of subjects. The network is defined mathematically by a graph delineating how specific nodes (*i.e.*, OTUs or

functional modalities) and edges (connections between specific OTUs or functional modalities) relate to one another to define the structural ecology of a consortium of OTUs or functions. Any given Network Ecology or Functional Network Ecology will possess inherent phylogenetic diversity and functional properties.

[087] A Network Class or Core Network refers to a group of Network Ecologies or Functional Network ecologies that are computationally determined to comprise ecologies with similar phylogenetic and/or functional characteristics. A Network Class or Core Network therefore contains important biological features, defined either phylogenetically or functionally, of a group (*i.e.*, a cluster) of related network ecologies.

[088] Keystone OTUs or Functions are one or more OTUs or Functions that are common to many network Ecologies or Functional Netwok Ecologies and are members of Networks Ecologies or Functional Network Ecologies that occur in many subjects (*i.e.*, are pervasive). Due to the ubiquitous nature of Keystone OTUs and Functions, they are central to the function of network ecologies in healthy subjects and are often missing or at reduced levels in subjects with disease. Keystone OTUs and Functions may exist in low, moderate, or high abundance in subjects.

[089] Bacteria that are members of the keystone OTUs, core network or network ecology are provided herein.

Bacterial Compositions

[090] Provided are bacteria and combinations of bacteria that comprise network ecologies and functional network ecologies of the human gut microbiota. The bacteria and combinations of bacteria that comprise network ecologies have a capacity to meaningfully provide functions of a healthy microbiota when administered to mammalian hosts. Without being limited to a specific mechanism, it is believed that the members of network ecologies can inhibit the growth, proliferation, germination and/or colonization of one or a plurality of pathogenic bacteria in the dysbiotic microbiotal niche, and may also augment the growth, proliferation, germination and/or colonization of desired bacteria so that a healthy, diverse and protective microbiota colonizes and populates the intestinal lumen to establish or reestablish ecological control over pathogens or potential pathogens (*e.g.*, some bacteria are pathogenic bacteria only when present in a dysbiotic environment). The term pathogens refers to a bacterium or a group of bacteria or any other organism or entity that is capable of causing or affecting a disease, disorder or condition of a host containing the bacterium, organism or entity, including but not limited to metabolic diseases such as prediabetes, type 1 diabetes, and type 2 diabetes.

[091] As used herein, a "type" or more than one "types" of bacteria may be differentiated at the genus level, the species, level, the sub-species level, the strain level or by any other taxonomic method, as described herein and otherwise known in the art.

[092] Bacterial compositions can comprise two types of bacteria (termed "binary combinations" or "binary pairs"), and typically a large number of bacteria types. For instance, a bacterial composition can comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, or at least 21, 22, 23, 24, 25, 26, 27, 28, 29 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or at least 40, at least 50 or greater than 50 types of bacteria, as defined by species or operational taxonomic unit (OTU), or otherwise as provided herein. In some embodiments, the bacterial composition includes at least 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or greater numbers of bacteria types.

[093] In another embodiment, the number of types of bacteria present in a bacterial composition is at or below a known value. For example, in such embodiments the network ecology comprises 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50 or fewer types of bacteria, such as 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 or fewer, or 9 or fewer types of bacteria, 8 or fewer types of bacteria, 7 or fewer types of bacteria, 6 or fewer types of bacteria, 5 or fewer types of bacteria, 4 or fewer types of bacteria, or 3 or fewer types of bacteria.

Bacterial Compositions Described by Species

[094] Bacterial compositions that comprise network ecologies may be prepared comprising at least two types of isolated bacteria, chosen from the species in Table 1.

[095] In one embodiment, the bacterial composition that comprises at least one and preferably more than one of the following: *Enterococcus faecalis* (previously known as *Streptococcus faecalis*), *Clostridium innocuum*, *Clostridium ramosum*, *Bacteroides ovatus*, *Bacteroides vulgatus*, *Bacteroides thetaoiotaomicron*, *Escherichia coli* (1109 and 1108-1), *Clostridum bifermentans*, and *Blautia producta* (previously known as *Peptostreptococcus productus*). In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[096] In one embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Enterococcus faecalis* (previously known as *Streptococcus faecalis*), *Clostridium innocuum*, *Clostridium ramosum*, *Bacteroides ovatus*, *Bacteroides vulgatus*, *Bacteroides thetaoiotaomicron*, *Escherichia coli* (1109 and 1108-1), *Clostridum bifermentans*, and *Blautia producta* (previously known as *Peptostreptococcus productus*). In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[097] In another embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Acidaminococcus intestinalis*, *Bacteroides ovatus*, two strains of *Bifidobacterium adolescentis*, two strains of *Bifidobacterium longum*, *Blautia producta*, *Clostridium cocleatum*, *Collinsella aerofaciens*, two strains of *Dorea longicatena*, *Escherichia coli*, *Eubacterium desmolans*, *Eubacterium eligens*, *Eubacterium limosum*, four strains of *Eubacterium rectale*,

Eubacterium ventriosumi, Faecalibacterium prausnitzii, Lachnospira pectinoshiza, Lactobacillus casei, Lactobacillus casei/paracasei, Paracateroides distasonis, Raoultella sp., one strain of *Roseburia* (chosen from *Roseburia faecalis* or *Roseburia faecis*), *Roseburia intestinalis,* two strains of *Ruminococcus torques, two* strains of *Ruminococcus obeum,* and *Streptococcus mitis*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[098] In yet another embodiment, the bacterial composition comprises at least one and preferably more than one of the following: *Barnesiella intestinihominis; Lactobacillus reuteri;* a species characterized as one of *Enterococcus hirae, Enterococus faecium*, or *Enterococcus durans;* a species characterized as one of *Anaerostipes caccae* or *Clostridium indolis;* a species characterized as one of *Staphylococcus warneri* or *Staphylococcus pasteuri*; and *Adlercreutzia equolifaciens.* In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[099] In other embodiments, the bacterial composition comprises at least one and preferably more than one of the following: *Clostridium absonum, Clostridium argentinense, Clostridium baratii, Clostridium bartlettii, Clostridium bifermentans, Clostridium botulinum, Clostridium butyricum, Clostridium cadaveris, Clostridium camis, Clostridium celatum, Clostridium chauvoei, Clostridium clostridioforme, Clostridium cochlearium, Clostridium difficile, Clostridium fallax, Clostridium felsineum, Clostridium ghonii, Clostridium glycolicum, Clostridium haemolyticum, Clostridium hastiforme, Clostridium histolyticum, Clostridium indolis, Clostridium innocuum, Clostridium irregulare, Clostridium limosum, Clostridium malenominatum, Clostridium novyi, Clostridium oroticum, Clostridium paraputrificum, Clostridium perfringens, Clostridium piliforme, Clostridium putrefaciens, Clostridium putrificum, Clostridium ramosum, Clostridium sardiniense, Clostridium sartagoforme, Clostridium scindens, Clostridium septicum, Clostridium sordellii, Clostridium sphenoides, Clostridium spiroforme, Clostridium sporogenes, Clostridium subterminale, Clostridium symbiosum, Clostridium tertium, Clostridium tetani, Clostridium welchii,* and *Clostridium villosum*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[0100] In one embodiment, the bacterial composition that comprises a network ecology comprises at least one and preferably more than one of the following: *Clostridium innocuum, Clostridium bifermentans, Clostridium butyricum, Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides uniformis*, three strains of *Escherichia coli*, and *Lactobacillus sp.* In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[0101] In one embodiment, the bacterial composition that comprises a network ecology comprises at least one and preferably more than one of the following: *Clostridium bifermentans, Clostridium innocuum, Clostridium butyricum*, three strains of *Escherichia coli*, three strains of *Bacteroides,* and

Blautia producta. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[0102] In one embodiment, the bacterial composition that comprises a network ecology comprises at least one and preferably more than one of the following: *Bacteroides sp.*, *Escherichia coli*, and non pathogenic *Clostridia*, including *Clostridium innocuum*, *Clostridium bifermentans* and *Clostridium ramosum*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[0103] In one embodiment, the bacterial composition that comprises a network ecology comprises at least one and preferably more than one of the following: *Bacteroides* species, *Escherichia coli* and non-pathogenic *Clostridia*, such as *Clostridium butyricum, Clostridium bifermentans* and *Clostridium innocuum*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[0104] In one embodiment, the bacterial composition that comprises a network ecology comprises at least one and preferably more than one of the following: *Bacteroides caccae, Bacteroides capillosus, Bacteroides coagulans, Bacteroides distasonis, Bacteroides eggerthii, Bacteroides forsythus, Bacteroides fragilis, Bacteroides fragilis-ryhm, Bacteroides gracilis, Bacteroides levii, Bacteroides macacae, Bacteroides merdae, Bacteroides ovatus, Bacteroides pneumosintes, Bacteroides putredinis, Bacteroides pyogenes, Bacteroides splanchnicus, Bacteroides stercoris, Bacteroides tectum, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides ureolyticus,* and *Bacteroides vulgatus*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[0105] In one embodiment, the bacterial composition that comprises a network ecology comprises at least one and preferably more than one of the following: *Bacteroides, Eubacteria, Fusobacteria, Propionibacteria,* Lactobacilli, *anaerobic cocci, Ruminococcus, Escherichia coli, Gemmiger, Desulfomonas,* and *Peptostreptococcus*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

[0106] In one embodiment, the bacterial composition that comprises a network ecology comprises at least one and preferably more than one of the following: *Bacteroides fragilis ss. Vulgatus, Eubacterium aerofaciens, Bacteroides fragilis ss. Thetaiotaomicron, Blautia producta* (previously known as *Peptostreptococcus productus II*), *Bacteroides fragilis ss. Distasonis, Fusobacterium prausnitzii, Coprococcus eutactus, Eubacterium aerofaciens III, Blautia producta* (previously known as *Peptostreptococcus productus I*), *Ruminococcus bronii, Bifidobacterium adolescentis, Gemmiger formicilis, Bifidobacterium longum, Eubacterium siraeum, Ruminococcus torques, Eubacterium rectale III-H, Eubacterium rectale IV, Eubacterium eligens, Bacteroides eggerthii, Clostridium leptum, Bacteroides fragilis ss. A, Eubacterium biforme, Bifidobacterium infantis, Eubacterium rectale III-F, Coprococcus comes, Bacteroides capillosus, Ruminococcus albus, Eubacterium*

formicigenerans, Eubacterium hallii, Eubacterium ventriosum I, Fusobacterium russii, Ruminococcus obeum, Eubacterium rectale II, Clostridium ramosum I, Lactobacillus leichmanii, Ruminococcus cailidus, Butyrivibrio crossotus, Acidaminococcus fermentans, Eubacterium ventriosum, Bacteroides fragilis ss. fragilis, Bacteroides AR, Coprococcus catus, Eubacterium hadrum, Eubacterium cylindroides, Eubacterium ruminantium, Eubacterium CH-1, Staphylococcus epidermidis, Peptostreptococcus BL, Eubacterium limosum, Bacteroides praeacutus, Bacteroides L, Fusobacterium mortiferum I, Fusobacterium naviforme, Clostridium innocuum, Clostridium ramosum, Propionibacterium acnes, Ruminococcus flavefaciens, Ruminococcus AT, Peptococcus AU-1, Eubacterium AG, -AK, -AL, -AL-1, -AN; Bacteroides fragilis ss. ovatus, -ss. d, -ss. f; Bacteroides L-1, L-5; Fusobacterium nucleatum, Fusobacterium mortiferum, Escherichia coli, Streptococcus morbiliorum, Peptococcus magnus, Peptococcus G, AU-2; Streptococcus intermedius, Ruminococcus lactaris, Ruminococcus CO Gemmiger X, Coprococcus BH, -CC; Eubacterium tenue, Eubacterium ramulus, Eubacterium AE, -AG-H, -AG-M, -AJ, -BN-1; Bacteroides clostridiiformis ss. clostridliformis, Bacteroides coagulans, Bacteroides orails, Bacteroides ruminicola ss. brevis, -ss. ruminicola, Bacteroides splanchnicus, Desuifomonas pigra, Bacteroides L-4, -N-i; Fusobacterium H, Lactobacillus G, and *Succinivibrio A*. In an alternative embodiment, at least one of the preceding species is not substantially present in the bacterial composition.

Bacterial Compositions Described by Operational Taxonomic Unit (OTUs)

[0107] Bacterial compositions may be prepared comprising at least two types of isolated bacteria, chosen from the SEQ ID Numbers (OTUs) in Table 1.

[0108] OTUs can be defined either by full 16S sequencing of the rRNA gene (Table 1), by sequencing of a specific hypervariable region of this gene (*i.e.* V1, V2, V3, V4, V5, V6, V7, V8, or V9), or by sequencing of any combination of hypervariable regions from this gene (*e.g.* V1-3 or V3-5). The bacterial 16S rDNA is approximately 1500 nucleotides in length and is used in reconstructing the evolutionary relationships and sequence similarity of one bacterial isolate to another using phylogenetic approaches. 16S sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most microbes.

[0109] Using well known techniques, in order to determine the full 16S sequence or the sequence of any hypervariable region of the 16S sequence, genomic DNA is extracted from a bacterial sample, the 16S rDNA (full region or specific hypervariable regions) amplified using polymerase chain reaction (PCR), the PCR products cleaned, and nucleotide sequences delineated to determine the genetic composition of 16S gene or subdomain of the gene. If full 16S sequencing is performed, the sequencing method used may be, but is not limited to, Sanger sequencing. If one or more hypervariable regions are used, such as the V4 region, the sequencing can be, but is not limited to

being, performed using the Sanger method or using a next-generation sequencing method, such as an Illumina (sequencing by synthesis) method using barcoded primers allowing for multiplex reactions.

[0110] OTUs can be defined by a combination of nucleotide markers or genes, in particular highly conserved genes (*e.g.*, "house-keeping" genes), or a combination thereof, full-genome sequence, or partial genome sequence generated using amplified genetic products, or whole genome sequence (WGS). Using well defined methods familiar to one with ordinary skill in the art, DNA extracted from a bacterial sample will have specific genomic regions amplified using PCR and sequenced to determine the nucleotide sequence of the amplified products. In the whole genome shotgun (WGS) method, extracted DNA will be directly sequenced without amplification. Sequence data can be generated using any sequencing technology including, but not limited to Sanger, Illumina, 454 Life Sciences, Ion Torrent, ABI, Pacific Biosciences, and/or Oxford Nanopore.

[0111] In one embodiment, the OTUs can be characterized by one or more of the variable regions of the 16S sequence (V1-V9). These regions in bacteria are defined by nucleotides 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294 and 1435-1465 respectively using numbering based on the *E. coli* system of nomenclature. (*See, e.g.*, Brosius *et al.*, Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli, PNAS 75(10):4801-4805 (1978)). In some embodiments, at least one of the V1, V2, V3, V4, V5, V6, V7, V8, and V9 regions are used to characterize an OTU. In one embodiment, the V1, V2, and V3 regions are used to characterize an OTU. In another embodiment, the V3, V4, and V5 regions are used to characterize an OTU. In another embodiment, the V4 region is used to characterize an OTU.

Bacterial Compositions Exclusive of Certain Bacterial Species Or Strains

[0112] In one embodiment, the bacterial composition does not comprise at least one of *Enterococcus faecalis* (previously known as *Streptococcus faecalis*), *Clostridium innocuum*, *Clostridium ramosum*, *Bacteroides ovatus*, *Bacteroides vulgatus*, *Bacteroides thetaoiotaomicron*, *Escherichia coli* (1109 and 1108-1), *Clostridum bifermentans*, and *Blautia producta* (previously known as *Peptostreptococcus productus*).

[0113] In another embodiment, the bacterial composition does not comprise at least one of *Acidaminococcus intestinalis*, *Bacteroides ovatus*, two species of *Bifidobacterium adolescentis*, two species of *Bifidobacterium longum*, *Collinsella aerofaciens*, two species of *Dorea longicatena*, *Escherichia coli*, *Eubacterium eligens*, *Eubacterium limosum*, four species of *Eubacterium rectale*, *Eubacterium ventriosumi*, *Faecalibacterium prausnitzii*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Paracateroides distasonis*, *Raoultella sp.*, one species of *Roseburia* (chosen from *Roseburia faecalis* or *Roseburia faecis*), *Roseburia intestinalis*, two species of *Ruminococcus torques*, and *Streptococcus mitis*.

[0114] In yet another embodiment, the bacterial composition does not comprise at least one of *Barnesiella intestinihominis; Lactobacillus reuteri;* a species characterized as one of *Enterococcus*

hirae, *Enterococus faecium*, or *Enterococcus durans;* a species characterized as one of *Anaerostipes caccae* or *Clostridium indolis;* a species characterized as one of *Staphylococcus warneri* or *Staphylococcus pasteuri*; and *Adlercreutzia equolifaciens.*

[0115] In other embodiments, the bacterial composition does not comprise at least one of *Clostridium absonum, Clostridium argentinense, Clostridium baratii, Clostridium bifermentans, Clostridium botulinum, Clostridium butyricum, Clostridium cadaveris, Clostridium camis, Clostridium celatum, Clostridium chauvoei, Clostridium clostridioforme, Clostridium cochlearium, Clostridium difficile, Clostridium fallax, Clostridium felsineum, Clostridium ghonii, Clostridium glycolicum, Clostridium haemolyticum, Clostridium hastiforme, Clostridium histolyticum, Clostridium indolis, Clostridium innocuum, Clostridium irregulare, Clostridium limosum, Clostridium malenominatum, Clostridium novyi, Clostridium oroticum, Clostridium paraputrificum, Clostridium perfringens, Clostridium piliforme, Clostridium putrefaciens, Clostridium putrificum, Clostridium ramosum, Clostridium sardiniense, Clostridium sartagoforme, Clostridium scindens, Clostridium septicum, Clostridium sordellii, Clostridium sphenoides, Clostridium spiroforme, Clostridium sporogenes, Clostridium subterminale, Clostridium symbiosum, Clostridium tertium, Clostridium tetani, Clostridium welchii,* and *Clostridium villosum.*

[0116] In another embodiment, the bacterial composition does not comprise at least one of *Clostridium innocuum, Clostridum bifermentans, Clostridium butyricum, Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides uniformis*, three strains of *Escherichia coli*, and *Lactobacillus sp.*

[0117] In another embodiment, the bacterial composition does not comprise at least one of *Clostridium bifermentans, Clostridium innocuum, Clostridium butyricum*, three strains of *Escherichia coli*, three strains of *Bacteroides,* and *Blautia producta* (previously known as *Peptostreptococcus productus).*

[0118] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides sp.*, *Escherichia coli*, and non pathogenic *Clostridia*, including *Clostridium innocuum, Clostridium bifermentans* and *Clostridium ramosum.*

[0119] In another embodiment, the bacterial composition does not comprise at least one of more than one *Bacteroides* species, *Escherichia coli* and non-pathogenic *Clostridia*, such as *Clostridium butyricum, Clostridium bifermentans* and *Clostridium innocuum.*

[0120] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides caccae, Bacteroides capillosus, Bacteroides coagulans, Bacteroides distasonis, Bacteroides eggerthii, Bacteroides forsythus, Bacteroides fragilis, Bacteroides fragilis-ryhm, Bacteroides gracilis, Bacteroides levii, Bacteroides macacae, Bacteroides merdae, Bacteroides ovatus, Bacteroides pneumosintes, Bacteroides putredinis, Bacteroides pyogenes, Bacteroides*

splanchnicus, Bacteroides stercoris, Bacteroides tectum, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides ureolyticus, and *Bacteroides vulgatus.*

[0121] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides, Eubacteria, Fusobacteria, Propionibacteria, Lactobacilli, anaerobic cocci, Ruminococcus, Escherichia coli, Gemmiger, Desulfomonas,* and *Peptostreptococcus.*

[0122] In another embodiment, the bacterial composition does not comprise at least one of *Bacteroides fragilis ss. Vulgatus, Eubacterium aerofaciens, Bacteroides fragilis ss. Thetaiotaomicron, Blautia producta* (previously known as *Peptostreptococcus productus II*), *Bacteroides fragilis ss. Distasonis, Fusobacterium prausnitzii, Coprococcus eutactus, Eubacterium aerofaciens III, Blautia producta* (previously known as *Peptostreptococcus productus I*), *Ruminococcus bromii, Bifidobacterium adolescentis, Gemmiger formicilis, Bifidobacterium longum, Eubacterium siraeum, Ruminococcus torques, Eubacterium rectale III-H, Eubacterium rectale IV, Eubacterium eligens, Bacteroides eggerthii, Clostridium leptum, Bacteroides fragilis ss. A, Eubacterium biforme, Bifidobacterium infantis, Eubacterium rectale III-F, Coprococcus comes, Bacteroides capillosus, Ruminococcus albus, Eubacterium formicigenerans, Eubacterium hallii, Eubacterium ventriosum I, Fusobacterium russii, Ruminococcus obeum, Eubacterium rectale II, Clostridium ramosum I, Lactobacillus leichmanii, Ruminococcus cailidus, Butyrivibrio crossotus, Acidaminococcus fermentans, Eubacterium ventriosum, Bacteroides fragilis ss. fragilis, Bacteroides AR, Coprococcus catus, Eubacterium hadrum, Eubacterium cylindroides, Eubacterium ruminantium, Eubacterium CH-1, Staphylococcus epidermidis, Peptostreptococcus BL, Eubacterium limosum, Bacteroides praeacutus, Bacteroides L, Fusobacterium mortiferum I, Fusobacterium naviforme, Clostridium innocuum, Clostridium ramosum, Propionibacterium acnes, Ruminococcus flavefaciens, Ruminococcus AT, Peptococcus AU-1, Eubacterium AG, -AK, -AL, -AL-1, -AN; Bacteroides fragilis ss. ovatus, -ss. d, -ss. f; Bacteroides L-1, L-5; Fusobacterium nucleatum, Fusobacterium mortiferum, Escherichia coli, Streptococcus morbiliorum, Peptococcus magnus, Peptococcus G, AU-2; Streptococcus intermedius, Ruminococcus lactaris, Ruminococcus CO Gemmiger X, Coprococcus BH, -CC; Eubacterium tenue, Eubacterium ramulus, Eubacterium AE, -AG-H, -AG-M, -AJ, -BN-1; Bacteroides clostridiiformis ss. clostridliformis, Bacteroides coagulans, Bacteroides orails, Bacteroides ruminicola ss. brevis, -ss. ruminicola, Bacteroides splanchnicus, Desuifomonas pigra, Bacteroides L-4, -N-i; Fusobacterium H, Lactobacillus G,* and *Succinivibrio A.*

Inhibition of Bacterial Pathogens

[0123] In some embodiments, the bacterial composition provides a protective or therapeutic effect against infection by one or more GI pathogens of interest. Table 1 provides a list of OTUs that are either pathogens, pathobionts, or opportunistic pathogens.

[0124] In some embodiments, the pathogenic bacterium is selected from the group consisting of Yersinia, Vibrio, Treponema, Streptococcus, Staphylococcus, Shigella, Salmonella, Rickettsia,

Orientia, Pseudomonas, Neisseria, Mycoplasma, Mycobacterium, Listeria, Leptospira, Legionella, Klebsiella, Helicobacter, Haemophilus, Francisella, Escherichia, Ehrlichia, Enterococcus, Coxiella, Corynebacterium, Clostridium, Chlamydia, Chlamydophila, Campylobacter, Burkholderia, Brucella, Borrelia, Bordetella, Bifidobacterium , Bacillus, multi-drug resistant bacteria, extended spectrum beta-lactam resistant Enterococci (ESBL), Carbapenem-resistent Enterobacteriaceae (CRE), and vancomycin-resistant Enterococci (VRE).

[0125] In some embodiments, these pathogens include, but are not limited to, Aeromonas hydrophila, Campylobacter fetus, Plesiomonas shigelloides, Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, enteroaggregative Escherichia coli, enterohemorrhagic Escherichia coli, enteroinvasive Escherichia coli, enterotoxigenic Escherichia coli (such as, but not limited to, LT and/or ST), Escherichia coli 0157:H7, Helicobacter pylori, Klebsiellia pneumonia, Lysteria monocytogenes, Plesiomonas shigelloides, Salmonella spp., Salmonella typhi, Salmonella paratyphi, Shigella spp., Staphylococcus spp., Staphylococcus aureus, vancomycin-resistant enterococcus spp., Vibrio spp., Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, and Yersinia enterocolitica.

[0126] In one embodiment, the pathogen of interest is at least one pathogen chosen from Clostridium difficile, Salmonella spp., pathogenic Escherichia coli, vancomycin-resistant Enterococcus spp., and extended spectrum beta-lactam resistant Enterococci (ESBL).

Purified Spore Populations

[0127] In some embodiments, the bacterial compositions comprise purified spore populations or a combination of a purified spore population with a non-spore population. Purified spore populations contain combinations of commensal bacteria of the human gut microbiota with the capacity to meaningfully provide functions of a healthy microbiota when administered to a mammalian subject. Without being limited to a specific mechanism, it is thought that such compositions inhibit the growth of a pathogen such as *C. difficile*, *Salmonella* spp., enteropathogenic *E. coli*, and vancomycin-resistant *Enterococcus* spp., so that a healthy, diverse and protective microbiota can be maintained or, in the case of pathogenic bacterial infections such as *C. difficile* infection, repopulate the intestinal lumen to reestablish ecological control over potential pathogens. In some embodiments, yeast spores and other fungal spores are also purified and selected for therapeutic use.

[0128] Disclosed herein are therapeutic and prophylactic compositions containing non-pathogenic, germination-competent bacterial spores, spore forming organisms and non-spore forming organisms, for the prevention, control, and treatment of gastrointestinal diseases, disorders and conditions and for general nutritional health. These compositions are advantageous in being suitable for safe administration to humans and other mammalian subjects and are efficacious in numerous gastrointestinal diseases, disorders and conditions and in general nutritional health. While spore-based compositions are known, these are generally prepared according to various techniques such as

lyophilization or spray-drying of liquid bacterial cultures, resulting in poor efficacy, instability, substantial variability and lack of adequate safety.

[0129] It has now been found that populations of bacterial spores can be obtained from biological materials obtained from mammalian subjects, including humans. These populations are formulated into compositions as provided herein, and administered to mammalian subjects using the methods as provided herein.

[0130] Provided herein are therapeutic bacterial compositions containing a purified population of bacterial spores, spore forming organisms and non-spore forming organisms.

[0131] As used herein, the terms "purify", "purified" and "purifying" refer to the state of a population (*e.g.*, a plurality of known or unknown amount and/or concentration) of desired bacterial spores or bacteria, that have undergone one or more processes of purification, *e.g.*, a selection or an enrichment of the desired bacterial spore, or alternatively a removal or reduction of residual habitat products as described herein. In some embodiments, a purified population has no detectable undesired activity or, alternatively, the level or amount of the undesired activity is at or below an acceptable level or amount. In other embodiments, a purified population has an amount and/or concentration of desired bacterial spores or bacteria at or above an acceptable amount and/or concentration. In other embodiments, the purified population of bacterial spores or bacteria is enriched as compared to the starting material (*e.g.*, a fecal material liquid culture) from which the population is obtained. This enrichment may be by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999% as compared to the starting material.

[0132] In certain embodiments, the purified populations of bacterial spores have reduced or undetectable levels of one or more pathogenic activities, such as toxicity, an infection of the mammalian recipient subject, an immunomodulatory activity, an autoimmune response, a metabolic response, or an inflammatory response or a neurological response. Such a reduction in a pathogenic activity may be by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999% as compared to the starting material. In other embodiments, the purified populations of bacterial spores have reduced sensory components as compared to fecal material, such as reduced odor, taste, appearance, and umami.

[0133] Provided are purified populations of bacterial spores or bacteria that are substantially free of residual habitat products. In certain embodiments, this means that the bacterial spore or bacterial composition no longer contains a substantial amount of the biological matter associated with the microbial community while living on or in the human or animal subject, and the purified population of spores may be 100% free, 99% free, 98% free, 97% free, 96% free, or 95% free of any contamination of the biological matter associated with the microbial community. Substantially free of residual habitat products may also mean that the bacterial spore composition contains no detectable

cells from a human or animal, and that only microbial cells are detectable, in particular, only desired microbial cells are detectable. In another embodiment, it means that fewer than $1x10^{-2}$%, $1x10^{-3}$%, $1x10^{-4}$%, $1x10^{-5}$%, $1x10^{-6}$%, $1x10^{-7}$%, $1x10^{-8}$% of the cells in the bacterial composition are human or animal, as compared to microbial cells. In another embodiment, the residual habitat product present in the purified population is reduced at least a certain level from the fecal material obtained from the mammalian donor subject, *e.g.*, reduced by at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, 99.99%, 99.999%, 99.9999%, or greater than 99.9999%.

[0134] In one embodiment, substantially free of residual habitat products or substantially free of a detectable level of a pathogenic material means that the bacterial composition contains no detectable viral (including bacterial viruses (*i.e.*, phage)), fungal, or mycoplasmal or toxoplasmal contaminants, or a eukaryotic parasite such as a helminth. Alternatively, the purified spore populations are substantially free of an acellular material, *e.g.*, DNA, viral coat material, or non-viable bacterial material.

[0135] As described herein, purified spore populations can be demonstrated by genetic analysis (*e.g.*, PCR, DNA sequencing), serology and antigen analysis, and methods using instrumentation such as flow cytometry with reagents that distinguish desired bacterial spores from non-desired, contaminating materials.

[0136] Exemplary biological materials include fecal materials such as feces or materials isolated from the various segments of the small and large intestines. Fecal materials are obtained from a mammalian donor subject, or can be obtained from more than one donor subject, *e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 200, 300, 400, 500, 750, 1000 or from greater than 1000 donors, where such materials are then pooled prior to purification of the desired bacterial spores.

[0137] In alternative embodiments, the desired bacterial spores or bacteria are purified from a single fecal material sample obtained from a single donor, and after such purification are combined with purified spore populations or bacteria from other purifications, either from the same donor at a different time, or from one or more different donors, or both.

[0138] Preferred bacterial genera include Acetonema, Alkaliphilus, Alicyclobacillus, Amphibacillus, Ammonifex, Anaerobacter, Anaerofustis, Anaerostipes, Anaerotruncus, Anoxybacillus, Bacillus, Blautia, Brevibacillus, Bryantella, Caldicellulosiruptor, Caloramator, Candidatus, Carboxydibrachium, Carboxydothermus, Clostridium, Cohnella, Coprococcus, Dendrosporobacter Desulfitobacterium, Desulfosporosinus, Desulfotomaculum, Dorea, Eubacterium, Faecalibacterium, Filifactor, Geobacillus, Halobacteroides, Heliobacillus, Heliobacterium, Heliophilum, Heliorestis, Lachnoanaerobaculum, Lysinibacillus, Moorella, Oceanobacillus, Orenia (S.), Oxalophagus, Oxobacter, Paenibacillus, Pelospora, Pelotomaculum, Propionispora, Roseburia, Ruminococcus, Sarcina, Sporobacterium, Sporohalobacter, Sporolactobacillus, Sporomusa,

Sporosarcina, Sporotomaculum, Subdoligranulum, Symbiobacterium, Syntrophobotulus, Syntrophospora, Terribacillus, Thermoanaerobacter, and Thermosinus.

[0139] In some embodiments, spore-forming bacteria are identified by the presence of nucleic acid sequences that modulate sporulation. In particular, signature sporulation genes are highly conserved across members of distantly related genera including Clostridium and Bacillus. Traditional approaches of forward genetics have identified many, if not all, genes that are essential for sporulation (spo). The developmental program of sporulation is governed in part by the successive action of four compartment-specific sigma factors (appearing in the order σF, σE, σG and σK), whose activities are confined to the forespore (σF and σG) or the mother cell (σE and σK).

[0140] Provided are spore populations containing more than one type of bacterium. As used herein, a "type" or more than one "types" of bacteria may be differentiated at the genus level, the species, level, the sub-species level, the strain level or by any other taxonomic method, as described herein and otherwise known in the art.

[0141] In some embodiments, all or essentially all of the bacterial spores or bacterial species present in a purified population are originally isolated obtained from a fecal material treated as described herein or otherwise known in the art. In alternative embodiments, one or more than one bacterial spores, bacteria, or types of bacterial spores are generated in culture and combined to form a purified bacterial composition, including a purified spore population. In other alternative embodiments, one or more of these culture-generated populations are combined with a fecal material-derived populations to generate a hybrid population. Bacterial compositions may contain at least two types of these preferred bacteria, including strains of the same species. For instance, a bacterial composition may comprise at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 or more than 20 types of bacteria, as defined by species or operational taxonomic unit (OTU) encompassing such species.

[0142] Thus, provided herein are methods for production of a bacterial composition containing a population of bacterial spores suitable and/or non-sporulating bacteria for therapeutic administration to a mammalian subject in need thereof. And the composition is produced by generally following the steps of: (a) providing a fecal material obtained from a mammalian donor subject; and (b) subjecting the fecal material to at least one purification treatment or step under conditions such that a population of bacterial spores is produced from the fecal material. The composition is formulated such that a single oral dose contains at least about $1x10^4$ colony forming units of the bacterial spores, and a single oral dose will typically contain about $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, or greater than $1x10^{15}$ CFUs of the bacterial spores. The presence and/or concentration of a given type of bacteria or bacterial spore may be known or unknown in a given purified spore population. If known, for example the concentration of bacteria or spores of a

given strain, or the aggregate of all strains, is *e.g.*, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, or greater than $1x10^{15}$ viable bacteria or bacterial spores per gram of composition or per administered dose.

[0143] In some formulations, the composition contains at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater than 90% spores on a mass basis. In some formulations, the administered dose does not exceed 200, 300, 400, 500, 600, 700, 800, 900 milligrams or 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, or 1.9 grams in mass.

[0144] The bacterial compositions are generally formulated for oral or gastric administration, typically to a mammalian subject. In particular embodiments, the composition is formulated for oral administration as a solid, semi-solid, gel, or liquid form, such as in the form of a pill, tablet, capsule, or lozenge. In some embodiments, such formulations contain or are coated by an enteric coating to protect the bacteria through the stomach and small intestine, although spores are generally resistant to the stomach and small intestines.

[0145] The bacterial compositions may be formulated to be effective in a given mammalian subject in a single administration or over multiple administrations. For example, a single administration is substantially effective to reduce *Cl. difficile* and/or *Cl. difficile* toxin content in a mammalian subject to whom the composition is administered. Substantially effective means that *Cl. difficile* and/or *Cl. difficile* toxin content in the subject is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99% or greater than 99% following administration of the composition.

Kits For Diagnosis of a State Of Dysbiosis in a Subject

[0146] In some embodiments, the invention includes kits for carrying out methods of the invention described herein and in the claims. In some embodiments, the invention includes a kit for diagnosis of a state of dysbiosis in a mammalian subject in need thereof. In one embodiment, the kit includes a plurality of detection means suitable for use in detecting (1) a first bacterial entity comprising a keystone bacterial entity and (2) a second bacterial entity, wherein the first and second bacterial entities comprise a Network Ecology, as described herein. The kit can include instructions for use of the kit.

[0147] In other embodiments, the kit provides detection means, reagents, and instructions for detecting a first bacterial entity and a second bacterial entity that comprise a Network Ecology by: obtaining a fecal sample from a mammalian subject comprising a plurality of bacterial entities, contacting the fecal sample with a first detection moiety (and in some cases, a second detection moiety) capable of detecting the first bacterial entity and the second bacterial entity present in the network, detecting the absence of the first and/or second bacterial entities in the fecal sample, and thereby detecting a dysbiosis in the mammailian subject. In some embodiments, the kit provides reagents and steps for administering to the mammailian subject a composition comprising an effect amount of the first and/or second bacterial species.

[0148] In some embodiments, the kit includes detection means and instructions for obtaining a fecal sample from the mammalian subject comprising a plurality of bacterial entities; contacting the fecal sample with a first detection moiety capable of detecting a first bacterial entity present in an network; detecting the absence of the first bacterial entity in the fecal sample, thereby detecting a dysbiosis in the mammalian subject; and administering to the mammalian subject a composition comprising an effective amount of the first bacterial entity.

[0149] In other embodiments, the kit includes reagents and instructions for a method for treating, preventing, or reducing the severity of a disorder selected from the group consisting of Clostridium difficile Associated Diarrhea (CDAD), Type 2 Diabetes, Obesity, Irritable Bowel Disease (IBD), colonization with a pathogen or pathobiont, and infection with a drug-resistant pathogen or pathobiont, comprising: administering to a mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria or the purified bacterial preparation capable of forming a network ecology selected from the group consisting of those in claims 1-61 below and described throughout the specification.

[0150] In another embodiment, the kit includes reagents and instructions for a method for producing short chain fatty acids (SCFA) within a mammalian subject, comprising: administering to said mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria of the purified bacterial preparation capable of forming one or a plurality of bacterial functional pathways, the one or plurality of bacterial functional pathways capable of forming a functional network ecology selected from the group consisting of those in claims 69-71 and described throughout in the specification.

[0151] In another embodiment, the kit includes reagents and instructions for a method for catalyzing secondary metabolism of bile acids within a mammalian subject, comprising: administering to said mammalian subject in need thereof an effective amount of a therapeutic bacterial composition, said therapeutic bacterial composition comprising a plurality of isolated bacteria or a purified bacterial preparation, the plurality of isolated bacteria of the purified bacterial preparation capable of forming one or a plurality of bacterial functional pathways, the one or plurality of bacterial functional pathways capable of forming a functional network ecology selected from the group consisting of claims 72-74 and throughout the specification.

Systems for Predicting a Dysbiosis in a Subject

[0152] The invention provides systems for predicting a dysbiosis in a subject, the system comprising: a storage memory for storing a dataset associated with a sample obtained from the subject, wherein the dataset comprises content data for at least one network of bacterial entities

described herein, and a processor communicatively coupled to the storage memory for determining a score with an interpretation function wherein the score is predictive of dysbiosis in the subject.

[0153] In some embodiments, the invention provides systems for detecting a dysbiosis in a subject comprising: a storage memory for storing a dataset associated with a sample obtained from the subject, wherein the dataset comprises content data for at least one network of bacterial entities described herein, and a processor communicatively coupled to the storage memory for determining a score with an interpretation function, wherein the score is predictive of dysbiosis in the subject.

[0154] An example of a computer system and its components that can be used to perform methods of the invention are described below in Figure 21.

Computer Overview

[0155] Figure 21 is a high-level block diagram illustrating an example of a computer 2100 for use as a server or a user device, in accordance with one embodiment. Illustrated are at least one processor 2102 coupled to a chipset 2104. The chipset 2104 includes a memory controller hub 2120 and an input/output (I/O) controller hub 2122. A memory 2106 and a graphics adapter 2112 are coupled to the memory controller hub 2120, and a display device 2118 is coupled to the graphics adapter 2112. A storage device 2108, keyboard 2110, pointing device 2114, and network adapter 2116 are coupled to the I/O controller hub 2122. Other embodiments of the computer 2100 have different architectures. For example, the memory 2106 is directly coupled to the processor 2102 in some embodiments.

[0156] The storage device 2108 is a non-transitory computer-readable storage medium such as a hard drive, compact disk read-only memory (CD-ROM), DVD, or a solid-state memory device. The memory 2106 holds instructions and data used by the processor 2102. The pointing device 2114 is used in combination with the keyboard 2110 to input data into the computer system 200. The graphics adapter 2112 displays images and other information on the display device 2118. In some embodiments, the display device 2118 includes a touch screen capability for receiving user input and selections. The network adapter 2116 couples the computer system 2100 to the network. Some embodiments of the computer 2100 have different and/or other components than those shown in Figure 21. For example, the server can be formed of multiple blade servers and lack a display device, keyboard, and other components.

[0157] The computer 2100 is adapted to execute computer program modules for providing functionality described herein. As used herein, the term "module" refers to computer program instructions and other logic used to provide the specified functionality. Thus, a module can be implemented in hardware, firmware, and/or software. In one embodiment, program modules formed of executable computer program instructions are stored on the storage device 2108, loaded into the memory 2106, and executed by the processor 2102.

METHODS OF THE INVENTION

Method of Determining Network Ecologies

[0158] Methods are provided for a computational approach based in part on network theory to construct the ecology of a group of microorganisms based on the presence or absence of specific OTUs (*i.e.*, microbial genera, species or strains) in a given set of sampled subjects. *See* Figure 16. *See e.g.*, Cormen TH, Leiserson CE, Rivest RL, and Stein C. 2009. Introduction to Algorithms. Third edition. The MIT Press. Garey MR, and Johnson DS. 1979. Computers and Intractability: A Guide to the Theory of NP-Completeness. First Edition. W. H. Freeman. The approach includes the following: (i) identifying the microbial network ecologies that are present in both healthy and diseased subjects, (ii) identifying the keystone OTUs and/or functions (Figure 17), and phylogenetic clades that characterize a given ecology, and (iii) providing specific metrics with which to prioritize the various network ecologies with respect to their capacity to be useful in restoring the microbiome from a state of dysbiosis to a state of health. In general the method first defines all low and high order networks within given sets of subjects, and then utilizes a comparative approach to define biologically significant networks.

[0159] This method comprises computing a co-occurrence matrix of OTUs (*i.e.*, presence or absence) for each subject across a defined subject population (populations are defined by a specific phenotype such as but not limited to "subjects who are healthy", or "subjects with disease"). The method comprises computing all nodes (OTUs, or species, or strains) and edges (co-occurrence between OTUs, or species, or strains) that define the Network Ecology in a given subject's sample. Each co-occurrence is scored using a discrete binary variable denoting presence or absence. While the algorithm allows co-occurrences to be weighted based on the relative abundance of OTUs in the samples, in general, this is undesirable since low abundance OTUs may be important ecologically. Furthermore, a discretized measure of presence or absence of nodes eliminates bias and errors in the computed network ecologies that will arise from bias in methods used to generate relative abundance measures. A discreet method measuring presence or absence enables the detection of low frequency OTUs and the elucidation of networks that are often missed by methods based on relative abundance measures. Following derivation of all low and high order networks in a given subject, one can define all the network ecologies in a given phenotype (*i.e.*, collections of data sets from subjects with a unifying characteristic, for example, all data sets from healthy subjects) by defining the node and edge combinations that are maximally observed across all subjects. Without being bound by theory, it is understood that such network ecologies are present in a mammalian subject. The algorithm iterates the construction of network ecologies to rank all ecologies (*i.e.* nodes and edges) within each sample based on co-occurrence, [maximum co-occurrence; maximum co-occurrence less 1; maximum co-occurrence less 2; *etc.*] until the networks with minimum co-occurrence are defined (*i.e.*, a minimum edge score is achieved). This method can be computationally intensive for data sets containing a large number of subjects. For data sets containing a large number of subjects the algorithm uses a strategy

whereby first seed network ecologies are constructed using the method defined above in a subset of subjects and then combinations of these seed networks are used to search for higher order networks across the entire data set.

[0160] Biological significance can be assigned to the observed network ecologies and members of a given Network Ecology based on multiple computed metrics including, but not limited to: (i) the frequency that a given OTU or Network Ecology is observed; (ii) the number of OTUs in the network; the frequency of occurrence of the network across subjects (*i.e.*, pervasiveness); (iii) the phylogenetic breadth of the network, (iv) specific functional properties, and (v) whether the network occurs preferentially in individuals that are healthy versus those harboring disease *(i.e.*, the various phenotypes). All network ecologies or OTUs that occur in one phenotype (*e.g.*, health) are compared to those that occur in other phenotypes (*e.g.*, one or more specific disease states) to core Network ecologies or OTUs that are found in one, two, or any multiple of phenotypes. Network ecologies are considered to be related if at least 70%, 80%, or 90% of their OTUs are in common. All network ecologies or OTUs are assigned a score for their biological significance based on but not limited to: (i) the intersections of phenotypes in which they occur or do not occur (e.g. present in health but not disease), and (ii) the various metrics above defined. The final output of all of these steps defines a set of Network Ecologies that are of high biological significance and a set of Keystone OTUs and/or metabolic functions that are integral components of these derived ecologies.

[0161] From these Network Ecologies, the method includes defining "Network Classes" that represent network groups or clusters with specific, related compositional characteristics with respect to OTU content, phylogenetic diversity, and metabolic functional capacity (Figure 18). Network Classes can be first defined by setting an inclusion threshold for networks to include in the analysis that is based on biological characteristics of the networks such as but not limited to the size (number of OTUs) and pervasiveness (*i.e.*, how frequently a given network is observed in a population of individuals). Selected network ecologies are then clustered using two vectors: one vector is phylogenetic relatedness of individual OTUs as defined by a computed phylogenetic tree, and the second vector is network relatedness based on the OTUs content in the individual networks. In another embodiment, clustering vectors are related based on metabolic functional pathways harbored by individual OTUs, and network relatedness is based on the functional pathways present in each individual network. Network Classes are defined by specific nodes in the dendrogram representing the computed network relatedness, and each class is characterized by a specific combination of OTUs. In one embodiment, these nodes are defined as branches of the hierarchical clustering tree based on the topological overlap measure; this measure is a highly robust measure of network interconnectedness. *See* Langfelder P, Zhang B, Horvath S. 2008. Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. Bioinformatics 24: 719–720.

[0162] From these Networks Classes, a target microbial composition's usefulness, *e.g.*, as a therapeutic, is selected using desired phylogenetic and functional properties for subsequent testing in in vitro and in vivo models. Exemplary Network Classes are delineated in Table 12, and Table 13 defines taxonomic families that are characteristic of Network Classes.

[0163] As described herein, provided are compositions (Table 8) containing keystone OTUs for states of health, including one or more of the OTUs provided below in Table 9.

[0164] As described herein, provided are compositions containing keystone OTUs, keystone metabolic functions and, optionally, non-keystone OTUs, including one or more of the OTUs provided below in Table 10.

[0165] In some therapeutic compositions, keystone OTUs are provided from members of a genera or family selected from Table 9.

[0166] Exemplary network ecologies are provided in Table 8, Table 11, Table 12, Table 14a 14b, and 14c, and Table 17.

[0167] Exemlary functional network ecologies are provided in Table 18 and Table 21.

[0168] Thus, provided herein are methods for production of a composition containing a population of bacteria as either vegetative cells or spores or both, suitable for therapeutic administration to a mammalian subject in need thereof. The composition is produced by generally following the steps of: (a) defining a target composition by selecting a Network Ecology, Functional Network Ecology, a Network Class, or a set of Keystone OTUs or Keystone Metabolic Functions that comprise the Network Ecology or Functional Network Ecology of interest (a) providing bacterial OTUs obtained from one or more bacterial cultures, biological or environmental sources, or a mammalian donor subject; and (b) combining the bacterial OTUs in a ratio and an amount sufficient to form a Network Ecology or Functional Network Ecology. The composition is formulated such that a single oral dose contains at least about $1x10^4$ colony forming units of the bacteria, and a single oral dose will typically contain about $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, or greater than $1x10^{15}$ CFUs of the bacteria. The concentration of bacterial of a given species or strain, or the aggregate of all species or strains, is *e.g.*, $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$, $1x10^{12}$, $1x10^{13}$, $1x10^{14}$, $1x10^{15}$, or greater than $1x10^{15}$ viable bacteria per gram of composition or per administered dose.

[0169] The bacterial compositions are generally formulated for oral or gastric administration, typically to a mammalian subject. In particular embodiments, the composition is formulated for oral administration as a solid, semi-solid, gel, or liquid form, such as in the form of a pill, tablet, capsule, or lozenge. In some embodiments, such formulations contain or are coated by an enteric coating to protect the bacteria through the stomach and small intestine, although compositions containing spores are generally resistant to the environment of the stomach and small intestine. Alternatively, the bacterial composition may be formulated for naso-gastric or rectal administration.

[0170] The bacterial compositions may be formulated to be effective in a given mammalian subject in a single administration or over multiple administrations. For example, a single administration is substantially effective to reduce Clostridium difficile (*i.e.*, C. difficile) and/or C. difficile toxin content and/or toxin activity, in a mammalian subject to whom the composition is administered. Substantially effective means that Cl. difficile and/or C. difficile toxin content in the subject is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99% or greater than 99% following administration of the composition.

Methods for Determining 16S Sequences

[0171] OTUs can be defined either by full 16S sequencing of the rRNA gene, by sequencing of a specific hypervariable region of this gene (*i.e.* V1, V2, V3, V4, V5, V6, V7, V8, or V9), or by sequencing of any combination of hypervariable regions from this gene (*e.g.* V1-3 or V3-5). The bacterial 16S rDNA is approximately 1500 nucleotides in length and is used in reconstructing the evolutionary relationships and sequence similarity of one bacterial isolate to another using phylogenetic approaches. 16S sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most microbes.

[0172] Using well known techniques, in order to determine the full 16S sequence or the sequence of any hypervariable region of the 16S sequence, genomic DNA is extracted from a bacterial sample, the 16S rDNA (full region or specific hypervariable regions) amplified using polymerase chain reaction (PCR), the PCR products cleaned, and nucleotide sequences delineated to determine the genetic composition of 16S gene or subdomain of the gene. If full 16S sequencing is performed, the sequencing method used may be, but is not limited to, Sanger sequencing. If one or more hypervariable regions are used, such as the V4 region, the sequencing can be, but is not limited to being, performed using the Sanger method or using a next-generation sequencing method, such as an Illumina (sequencing by synthesis) method using barcoded primers allowing for multiplex reactions.

[0173] OTUs can be defined by a combination of nucleotide markers or genes, in particular highly conserved genes (*e.g.*, "house-keeping" genes), or a combination thereof, full-genome sequence, or partial genome sequence generated using amplified genetic products, or whole genome sequence (WGS). Using well defined methods DNA extracted from a bacterial sample will have specific genomic regions amplified using PCR and sequenced to determine the nucleotide sequence of the amplified products. In the whole genome shotgun (WGS) method, extracted DNA will be directly sequenced without amplification. Sequence data can be generated using any sequencing technology including, but not limited to Sanger, Illumina, 454 Life Sciences, Ion Torrent, ABI, Pacific Biosciences, and/or Oxford Nanopore.

Methods for Preparing a Bacterial Composition for Administration to a Subject

[0174] Methods for producing bacterial compositions can include three main processing steps, combined with one or more mixing steps. The steps include organism banking, organism production, and preservation.

[0175] For banking, the strains included in the bacterial composition may be (1) isolated directly from a specimen or taken from a banked stock, (2) optionally cultured on a nutrient agar or broth that supports growth to generate viable biomass, and (3) the biomass optionally preserved in multiple aliquots in long-term storage.

[0176] In embodiments that use a culturing step, the agar or broth can contain nutrients that provide essential elements and specific factors that enable growth. An example would be a medium composed of 20 g/L glucose, 10 g/L yeast extract, 10 g/L soy peptone, 2 g/L citric acid, 1.5 g/L sodium phosphate monobasic, 100 mg/L ferric ammonium citrate, 80 mg/L magnesium sulfate, 10 mg/L hemin chloride, 2 mg/L calcium chloride, 1 mg/L menadione. A variety of microbiological media and variations are well known in the art (*e.g.* R.M. Atlas, *Handbook of Microbiological Media* (2010) CRC Press). Medium can be added to the culture at the start, may be added during the culture, or may be intermittently/continuously flowed through the culture. The strains in the bacterial composition may be cultivated alone, as a subset of the bacterial composition, or as an entire collection comprising the bacterial composition. As an example, a first strain may be cultivated together with a second strain in a mixed continuous culture, at a dilution rate lower than the maximum growth rate of either cell to prevent the culture from washing out of the cultivation.

[0177] The inoculated culture is incubated under favorable conditions for a time sufficient to build biomass. For bacterial compositions for human use, this is often at 37°C temperature, pH, and other parameter with values similar to the normal human niche. The environment can be actively controlled, passively controlled (*e.g.*, via buffers), or allowed to drift. For example, for anaerobic bacterial compositions (*e.g.*, gut microbiota), an anoxic/reducing environment can be employed. This can be accomplished by addition of reducing agents such as cysteine to the broth, and/or stripping it of oxygen. As an example, a culture of a bacterial composition can be grown at 37°C, pH 7, in the medium above, pre-reduced with 1 g/L cysteine·HCl.

[0178] When the culture has generated sufficient biomass, it can be preserved for banking. The organisms can be placed into a chemical milieu that protects from freezing (adding 'cryoprotectants'), drying ('lyoprotectants'), and/or osmotic shock ('osmoprotectants'), dispensing into multiple (optionally identical) containers to create a uniform bank, and then treating the culture for preservation. Containers are generally impermeable and have closures that assure isolation from the environment. Cryopreservation treatment is accomplished by freezing a liquid at ultra-low temperatures (*e.g.*, at or below -80°C). Dried preservation removes water from the culture by evaporation (in the case of spray drying or 'cool drying') or by sublimation (*e.g.*, for freeze drying, spray freeze drying). Removal of water improves long-term bacterial composition storage stability at

temperatures elevated above cryogenic. If the bacterial composition comprises spore forming species and results in the production of spores, the final composition can be purified by additional means, such as density gradient centrifugation preserved using the techniques described above. Bacterial composition banking can be done by culturing and preserving the strains individually, or by mixing the strains together to create a combined bank. As an example of cryopreservation, a bacterial composition culture can be harvested by centrifugation to pellet the cells from the culture medium, the supernate decanted and replaced with fresh culture broth containing 15% glycerol. The culture can then be aliquoted into 1 mL cryotubes, sealed, and placed at -80°C for long-term viability retention. This procedure achieves acceptable viability upon recovery from frozen storage.

[0179] Organism production can be conducted using similar culture steps to banking, including medium composition and culture conditions. It can be conducted at larger scales of operation, especially for clinical development or commercial production. At larger scales, there can be several subcultivations of the bacterial composition prior to the final cultivation. At the end of cultivation, the culture is harvested to enable further formulation into a dosage form for administration. This can involve concentration, removal of undesirable medium components, and/or introduction into a chemical milieu that preserves the bacterial composition and renders it acceptable for administration via the chosen route. For example, a bacterial composition can be cultivated to a concentration of 10^{10} CFU/mL, then concentrated 20-fold by tangential flow microfiltration; the spent medium can be exchanged by diafiltering with a preservative medium consisting of 2% gelatin, 100 mM trehalose, and 10 mM sodium phosphate buffer. The suspension can then be freeze-dried to a powder and titrated.

[0180] After drying, the powder can be blended to an appropriate potency, and mixed with other cultures and/or a filler such as microcrystalline cellulose for consistency and ease of handling, and the bacterial composition formulated as provided herein.

Methods of Treating a Subject

[0181] In some embodiments, the compositions disclosed herein are administered to a patient or a user (sometimes collectively referred to as a "subject"). As used herein "administer" and "administration" encompasses embodiments in which one person directs another to consume a bacterial composition in a certain manner and/or for a certain purpose, and also situations in which a user uses a bacteria composition in a certain manner and/or for a certain purpose independently of or in variance to any instructions received from a second person. Non-limiting examples of embodiments in which one person directs another to consume a bacterial composition in a certain manner and/or for a certain purpose include when a physician prescribes a course of conduct and/or treatment to a patient, when a parent commands a minor user (such as a child) to consume a bacterial composition, when a trainer advises a user (such as an athlete) to follow a particular course of conduct and/or treatment, and when a manufacturer, distributer, or marketer recommends conditions of use to

an end user, for example through advertisements or labeling on packaging or on other materials provided in association with the sale or marketing of a product.

[0182] The bacterial compositions offer a protective and/or therapeutic effect against diseases, disorders or conditions associated with dysbiosis of the gut microbiota, including but not limited to metabolic disorders such as pre-diabetes, type 1 diabetes, type 2 diabetes, obesity and non-alcoholic fatty liver disease (NAFLD), gastrointestinal disorders such as inflammatory bowel disease (IBD, such as ulcerative colitis and Crohns' disease), pouchitis and irritable bowel syndrome (IBS), and infectious diseases as described herein.

[0183] In some embodiments, the bacterial compositions offer a protective and/or therapeutic effect against diseases, disorders or conditions associated with dysbiosis of the gut microbiota, including but not limited to, metabolic diseases (e.g., Type 1 diabetes, Type 2 diabetes, Gestational diabetes, Diabetes complications, Prediabetes, NAFLD/NASH, Obesity, Weight Loss), GI diseases (Inflammatory bowel disease (IBD), Irritable bowel syndrome (IBS), Ulcerative Colitis, Crohn's Disease). Infectious diseases (Clostridium difficile Associated Diarrhea (CDAD), Carbapenem-resistant Enterobacteriaceae (CRE), Multidrug-resistant Acinetobacter, Drug-resistant Campylobacter, Extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs), Vancomycin-resistant Enterococcus (VRE), Multidrug-resistant Pseudomonas aeruginosa, Drug-resistant Non-typhoidal Salmonella, Drug-resistant Salmonella Typhi, Drug-resistant Shigella, Methicillin-resistant Staphylococcus aureus (MRSA), Drug-resistant Streptococcus pneumonia, Vancomycin-resistant Staphylococcus aureus (VRSA), Erythromycin-resistant Group A Streptococcus, Clindamycin-resistant Group B Streptococcus, Pathogenic fungus, or Candida infection).

[0184] The present bacterial compositions can be administered to animals, including humans, laboratory animals (*e.g.*, primates, rats, mice), livestock (*e.g.*, cows, sheep, goats, pigs, turkeys, chickens), and household pets (*e.g.*, dogs, cats, rodents).

[0185] In the present method, the bacterial composition can be administered enterically, in other words, by a route of access to the gastrointestinal tract. This includes oral administration, rectal administration (including enema, suppository, or colonoscopy), by an oral or nasal tube (nasogastric, nasojejunal, oral gastric, or oral jejunal), as detailed more fully herein.

Pretreatment Protocols

[0186] Prior to administration of the bacterial composition, the patient can optionally have a pretreatment protocol to prepare the gastrointestinal tract to receive the bacterial composition.

[0187] As one way of preparing the patient for administration of the microbial ecosystem, at least one antibiotic can be administered to alter the bacteria in the patient. As another way of preparing the patient for administration of the microbial ecosystem, a standard colon-cleansing preparation can be administered to the patient to substantially empty the contents of the colon, such as used to prepare a patient for a colonscopy. By "substantially emptying the contents of the colon," this application

means removing at least 75%, at least 80%, at least 90%, at least 95%, or about 100% of the contents of the ordinary volume of colon contents. Antibiotic treatment can precede the colon-cleansing protocol.

[0188] If a patient has received an antibiotic for treatment of an infection, or if a patient has received an antibiotic as part of a specific pretreatment protocol, in one embodiment, the antibiotic can be stopped in sufficient time to allow the antibiotic to be substantially reduced in concentration in the gut before the bacterial composition is administered. In one embodiment, the antibiotic can be discontinued 1, 2, or 3 days before the administration of the bacterial composition. In another embodiment, the antibiotic can be discontinued 3, 4, 5, 6, or 7 antibiotic half-lives before administration of the bacterial composition. In another embodiment, the antibiotic can be chosen so the constituents in the bacterial composition have an MIC50 that is higher than the concentration of the antibiotic in the gut.

[0189] MIC50 of a bacterial composition or the elements in the composition can be determined by methods well known in the art. Reller et al., Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices, Clinical Infectious Diseases 49(11):1749-1755 (2009). In such an embodiment, the additional time between antibiotic administration and administration of the bacterial composition is not necessary. If the pretreatment protocol is part of treatment of an acute infection, the antibiotic can be chosen so that the infection is sensitive to the antibiotic, but the constituents in the bacterial composition are not sensitive to the antibiotic.

Administration of Bacterial Compositions

[0190] The bacterial compositions of the invention are suitable for administration to mammals and non-mammalian animals in need thereof. In certain embodiments, the mammalian subject is a human subject who has one or more symptoms of a dysbiosis.

[0191] When the mammalian subject is suffering from a disease, disorder or condition characterized by an aberrant microbiota, the bacterial compositions described herein are suitable for treatment thereof. In some embodiments, the mammalian subject has not received antibiotics in advance of treatment with the bacterial compositions. For example, the mammalian subject has not been administered at least two doses of vancomycin, metronidazole and/or or similar antibiotic compound within one week prior to administration of the therapeutic composition. In other embodiments, the mammalian subject has not previously received an antibiotic compound in the one month prior to administration of the therapeutic composition. In other embodiments, the mammalian subject has received one or more treatments with one or more different antibiotic compounds and such treatment(s) resulted in no improvement or a worsening of symptoms.

[0192] In some embodiments, the gastrointestinal disease, disorder or condition is diarrhea caused by C. difficile including recurrent C. difficile infection, ulcerative colitis, colitis, Crohn's disease, or irritable bowel disease. Beneficially, the therapeutic composition is administered only once prior to

improvement of the disease, disorder or condition. In some embodiments the therapeutic composition is administered at intervals greater than two days, such as once every three, four, five or six days, or every week or less frequently than every week. Or the preparation may be administered intermittently according to a set schedule, *e.g.*, once a day, once weekly, or once monthly, or when the subject relapses from the primary illness. In another embodiment, the preparation may be administered on a long-term basis to subjects who are at risk for infection with or who may be carriers of these pathogens, including subjects who will have an invasive medical procedure (such as surgery), who will be hospitalized, who live in a long-term care or rehabilitation facility, who are exposed to pathogens by virtue of their profession (livestock and animal processing workers), or who could be carriers of pathogens (including hospital workers such as physicians, nurses, and other health care professionals).

[0193] In embodiments, the bacterial composition is administered enterically. This preferentially includes oral administration, or by an oral or nasal tube (including nasogastric, nasojejunal, oral gastric, or oral jejunal). In other embodiments, administration includes rectal administration (including enema, suppository, or colonoscopy). The bacterial composition may be administered to at least one region of the gastrointestinal tract, including the mouth, esophagus, stomach, small intestine, large intestine, and rectum. In some embodiments it is administered to all regions of the gastrointestinal tract. The bacterial compositions may be administered orally in the form of medicaments such as powders, capsules, tablets, gels or liquids. The bacterial compositions may also be administered in gel or liquid form by the oral route or through a nasogastric tube, or by the rectal route in a gel or liquid form, by enema or instillation through a colonoscope or by a suppository.

[0194] If the composition is administered colonoscopically and, optionally, if the bacterial composition is administered by other rectal routes (such as an enema or suppository) or even if the subject has an oral administration, the subject may have a colon cleansing preparation. The colon-cleansing preparation can facilitate proper use of the colonoscope or other administration devices, but even when it does not serve a mechanical purpose it can also maximize the proportion of the bacterial composition relative to the other organisms previously residing in the gastrointestinal tract of the subject. Any ordinarily acceptable colon cleansing preparation may be used such as those typically provided when a subject undergoes a colonoscopy.

[0195]

Dosages and Schedule for Administration

[0196] In some embodiments, the bacteria and bacterial compositions are provided in a dosage form. In certain embodiments, the dosage form is designed for administration of at least one OTU or combination thereof disclosed herein, wherein the total amount of bacterial composition administered is selected from 0.1ng to 10g, 10ng to 1g, 100ng to 0.1g, 0.1mg to 500mg, 1mg to 100mg, or from 10-15mg. In other embodiments, the bacterial composition is consumed at a rate of from 0.1ng to 10g a

day, 10ng to 1g a day, 100ng to 0.1g a day, 0.1mg to 500mg a day, 1mg to 100mg a day, or from 10-15mg a day, or more.

[0197] In certain embodiments, the treatment period is at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, or at least 1 year. In some embodiments the treatment period is from 1 day to 1 week, from 1 week to 4 weeks, from 1 month, to 3 months, from 3 months to 6 months, from 6 months to 1 year, or for over a year.

[0198] In one embodiment, 10^5 and 10^{12} microorganisms total can be administered to the patient in a given dosage form. In another embodiment, an effective amount can be provided in from 1 to 500 ml or from 1 to 500 grams of the bacterial composition having from 10^7 to 10^{11} bacteria per ml or per gram, or a capsule, tablet or suppository having from 1 mg to 1000 mg lyophilized powder having from 10^7 to 10^{11} bacteria. Those receiving acute treatment can receive higher doses than those who are receiving chronic administration.

[0199] Any of the preparations described herein can be administered once on a single occasion or on multiple occasions, such as once a day for several days or more than once a day on the day of administration (including twice daily, three times daily, or up to five times daily). In another embodiment, the preparation can be administered intermittently according to a set schedule, *e.g.*, once weekly, once monthly, or when the patient relapses from the primary illness. In one embodiment, the preparation can be administered on a long-term basis to individuals who are at risk for infection with or who may be carriers of these pathogens.

Patient Selection

[0200] Particular bacterial compositions can be selected for individual patients or for patients with particular profiles. For example, 16S sequencing can be performed for a given patient to identify the bacteria present in his or her microbiota. The sequencing can either profile the patient's entire microbiome using 16S sequencing (to the family, genera, or species level), a portion of the patient's microbiome using 16S sequencing, or it can be used to detect the presence or absence of specific candidate bacteria that are biomarkers for health or a particular disease state. Based on the biomarker data, a particular composition can be selected for administration to a patient to supplement or complement a patient's microbiota in order to restore health or treat or prevent disease. In another embodiment, patients can be screened to determine the composition of their microbiota to determine the likelihood of successful treatment.

Combination Therapy

[0201] The bacterial compositions can be administered with other agents in a combination therapy mode, including anti-microbial agents and prebiotics. Administration can be sequential, over a period of hours or days, or simultaneous.

[0202] In one embodiment, the bacterial compositions are included in combination therapy with one or more anti-microbial agents, which include anti-bacterial agents, anti-fungal agents, anti-viral agents and anti-parasitic agents.

[0203] Anti-bacterial agents can include cephalosporin antibiotics (cephalexin, cefuroxime, cefadroxil, cefazolin, cephalothin, cefaclor, cefamandole, cefoxitin, cefprozil, and ceftobiprole); fluoroquinolone antibiotics (cipro, Levaquin, floxin, tequin, avelox, and norflox); tetracycline antibiotics (tetracycline, minocycline, oxytetracycline, and doxycycline); penicillin antibiotics (amoxicillin, ampicillin, penicillin V, dicloxacillin, carbenicillin, vancomycin, and methicillin); and carbapenem antibiotics (ertapenem, doripenem, imipenem/cilastatin, and meropenem).

[0204] Anti-viral agents can include Abacavir, Acyclovir, Adefovir, Amprenavir, Atazanavir, Cidofovir, Darunavir, Delavirdine, Didanosine, Docosanol, Efavirenz, Elvitegravir, Emtricitabine, Enfuvirtide, Etravirine, Famciclovir, Foscarnet, Fomivirsen, Ganciclovir, Indinavir, Idoxuridine, Lamivudine, Lopinavir Maraviroc, MK-2048, Nelfinavir, Nevirapine, Penciclovir, Raltegravir, Rilpivirine, Ritonavir, Saquinavir, Stavudine, Tenofovir Trifluridine, Valaciclovir, Valganciclovir, Vidarabine, Ibacitabine, Amantadine, Oseltamivir, Rimantidine, Tipranavir, Zalcitabine, Zanamivir and Zidovudine.

[0205] Examples of antifungal compounds include, but are not limited to polyene antifungals such as natamycin, rimocidin, filipin, nystatin, amphotericin B, candicin, and hamycin; imidazole antifungals such as miconazole, ketoconazole, clotrimazole, econazole, omoconazole, bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole, sulconazole, and tioconazole; triazole antifungals such as fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazole, terconazole, and albaconazole; thiazole antifungals such as abafungin; allylamine antifungals such as terbinafine, naftifine, and butenafine; and echinocandin antifungals such as anidulafungin, caspofungin, and micafungin. Other compounds that have antifungal properties include, but are not limited to polygodial, benzoic acid, ciclopirox, tolnaftate, undecylenic acid, flucytosine or 5-fluorocytosine, griseofulvin, and haloprogin.

[0206] In one embodiment, the bacterial compositions are included in combination therapy with one or more corticosteroids, mesalazine, mesalamine, sulfasalazine, sulfasalazine derivatives, immunosuppressive drugs, cyclosporin A, mercaptopurine, azathiopurine, prednisone, methotrexate, antihistamines, glucocorticoids, epinephrine, theophylline, cromolyn sodium, anti-leukotrienes, anti-cholinergic drugs for rhinitis, anti-cholinergic decongestants, mast-cell stabilizers, monoclonal anti-IgE antibodies, vaccines, and combinations thereof.

[0207] A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health. Prebiotics can include complex carbohydrates, amino acids, peptides, or other essential nutritional components for the survival of the bacterial composition. Prebiotics include, but

are not limited to, amino acids, biotin, fructooligosaccharide, galactooligosaccharides, inulin, lactulose, mannan oligosaccharides, oligofructose-enriched inulin, oligofructose, oligodextrose, tagatose, trans-galactooligosaccharide, and xylooligosaccharides.

Methods for Testing Bacterial Compositions for Populating Effect

***In Vivo* Assay for Determining Whether a Bacterial Composition Populates a Subject's Gastrointestinal Tract**

[0208] In order to determine that the bacterial composition populates the gastrointestinal tract of a subject, an animal model, such as a mouse model, can be used. The model can begin by evaluating the microbiota of the mice. Qualitative assessments can be accomplished using 16S profiling of the microbial community in the feces of normal mice. It can also be accomplished by full genome sequencing, whole genome shotgun sequencing (WGS), or traditional microbiological techniques. Quantitative assessments can be conducted using quantitative PCR (qPCR), described below, or by using traditional microbiological techniques and counting colony formation.

[0209] Optionally, the mice can receive an antibiotic treatment to mimic the condition of dysbiosis. Antibiotic treatment can decrease the taxonomic richness, diversity, and evenness of the community, including a reduction of abundance of a significant number of bacterial taxa. Dethlefsen *et al.*, The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing, PLoS Biology 6(11):3280 (2008). At least one antibiotic can be used, and antibiotics are well known. Antibiotics can include aminoglycoside antibiotic (amikacin, arbekacin,gentamicin, kanamycin, neomycin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, and apramycin), amoxicillin, ampicillin, Augmentin (an amoxicillin/clavulanate potassium combination), cephalosporin (cefaclor, defadroxil, cefazolin, cefixime, fefoxitin, cefprozil, ceftazimdime, cefuroxime, cephalexin), clavulanate potassium, clindamycin, colistin, gentamycin, kanamycin, metronidazole, or vancomycin. As an individual, nonlimiting specific example, the mice can be provided with drinking water containing a mixture of the antibiotics kanamycin, colistin, gentamycin, metronidazole and vancomycin at 40 mg/kg, 4.2 mg/kg, 3.5 mg/kg, 21.5 mg/kg, and 4.5 mg/kg (mg per average mouse body weight), respectively, for 7 days. Alternatively, mice can be administered ciprofloxacin at a dose of 15-20 mg/kg (mg per average mouse body weight), for 7 days. If the mice are provided with an antibiotic, a wash out period of from one day to three days may be provided with no antibiotic treatment and no bacterial composition treatment.

[0210] Subsequently, the bacterial composition is administered to the mice by oral gavage. The bacterial composition may be administered in a volume of 0.2 ml containing 10^4 CFUs of each type of bacteria in the bacterial composition. Dose-response may be assessed by using a range of doses, including, but not limited to 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, and/or 10^{10}.

[0211] The mice can be evaluated using 16S sequencing, full genome sequencing, whole genome shotgun sequencing (WGS), or traditional microbiological techniques to determine whether the

bacterial composition has populated the gastrointestinal tract of the mice. For example only, one day, three days, one week, two weeks, and one month after administration of the bacterial composition to the mice, 16S profiling is conducted to determine whether the test bacterial composition has populated the gastrointestinal tract of the mice. Quantitative assessments, including qPCR and traditional microbiological techniques such as colony counting, can additionally or alternatively be performed, at the same time intervals.

[0212] Furthermore, the number of sequence counts that correspond exactly to those in the bacterial composition over time can be assessed to determine specifically which components of the bacterial composition reside in the gastrointestinal tract over a particular period of time. In one embodiment, the strains of the bacterial composition persist for a desired period of time. In another embodiment, the components of the bacterial composition persist for a desired period of time, while also increasing the ability of other microbes (such as those present in the environment, food, etc.) to populate the gastrointestinal tract, further increasing overall diversity, as discussed below.

Ability of Bacterial compositions to Populate Different Regions of the Gastrointestinal Tract

[0213] The present bacterial compositions can also be assessed for their ability to populate different regions of the gastrointestinal tract. In one embodiment, a bacterial composition can be chosen for its ability to populate one or more than one region of the gastrointestinal tract, including, but not limited to the stomach, the small intestine (duodenum, jejunum, and ileum), the large intestine (the cecum, the colon (the ascending, transverse, descending, and sigmoid colon), and the rectum).

[0214] An *in vivo* study can be conducted to determine which regions of the gastrointestinal tract a given bacterial composition will populate. A mouse model similar to the one described above can be conducted, except instead of assessing the feces produced by the mice, particular regions of the gastrointestinal tract can be removed and studied individually. For example, at least one particular region of the gastrointestinal tract can be removed and a qualitative or quantitative determination can be performed on the contents of that region of the gastrointestinal tract. In another embodiment, the contents can optionally be removed and the qualitative or quantitative determination may be conducted on the tissue removed from the mouse.

qPCR

[0215] As one quantitative method for determining whether a bacterial composition populates the gastrointestinal tract, quantitative PCR (qPCR) can be performed. Standard techniques can be followed to generate a standard curve for the bacterial composition of interest, either for all of the components of the bacterial composition collectively, individually, or in subsets (if applicable). Genomic DNA can be extracted from samples using commercially-available kits, such as the Mo Bio Powersoil®-htp 96 Well Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), the Mo Bio

Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), or the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

[0216] In some embodiments, qPCR can be conducted using HotMasterMix (5PRIME, Gaithersburg, MD) and primers specific for the bacterial composition of interest, and may be conducted on a MicroAmp® Fast Optical 96-well Reaction Plate with Barcode (0.1mL) (Life Technologies, Grand Island, NY) and performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA), with fluorescent readings of the FAM and ROX channels. The Cq value for each well on the FAM channel is determined by the CFX Manager™ software version 2.1. The $\log_{10}$(cfu/ml) of each experimental sample is calculated by inputting a given sample's Cq value into linear regression model generated from the standard curve comparing the Cq values of the standard curve wells to the known $\log_{10}$(cfu/ml) of those samples. The skilled artisan may employ alternative qPCR modes.

Methods for Characterization of Bacterial Compositions

[0217] In certain embodiments, provided are methods for testing certain characteristics of bacterial compositions. For example, the sensitivity of bacterial compositions to certain environmental variables is determined, *e.g.*, in order to select for particular desirable characteristics in a given composition, formulation and/or use. For example, the constituents in the bacterial composition can be tested for pH resistance, bile acid resistance, and/or antibiotic sensitivity, either individually on a constituent-by-constituent basis or collectively as a bacterial composition comprised of multiple bacterial constituents (collectively referred to in this section as bacterial composition).

pH Sensitivity Testing

[0218] If a bacterial composition will be administered other than to the colon or rectum (*i.e.*, for example, an oral route), optionally testing for pH resistance enhances the selection of bacterial compositions that will survive at the highest yield possible through the varying pH environments of the distinct regions of the GI tract. Understanding how the bacterial compositions react to the pH of the GI tract also assists in formulation, so that the number of bacteria in a dosage form can be increased if beneficial and/or so that the composition may be administered in an enteric-coated capsule or tablet or with a buffering or protective composition. As the pH of the stomach can drop to a pH of 1 to 2 after a high-protein meal for a short time before physiological mechanisms adjust it to a pH of 3 to 4 and often resides at a resting pH of 4 to 5, and as the pH of the small intestine can range from a pH of 6 to 7.4, bacterial compositions can be prepared that survive these varying pH ranges (specifically wherein at least 1%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or as much as 100% of the bacteria can survive gut transit times through various pH ranges). This can be tested by exposing the bacterial composition to varying pH ranges for the expected gut transit times through those pH ranges. Therefore, as a nonlimiting example only, 18-hour cultures of bacterial compositions can be grown in standard media, such as gut microbiota medium ("GMM", see

Goodman et al., Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice, PNAS 108(15):6252-6257 (2011)) or another animal-products-free medium, with the addition of pH adjusting agents for a pH of 1 to 2 for 30 minutes, a pH of 3 to 4 for 1 hour, a pH of 4 to 5 for 1 to 2 hours, and a pH of 6 to 7.4 for 2.5 to 3 hours. An alternative method for testing stability to acid is described in U.S. Patent No. 4,839,281. Survival of bacteria may be determined by culturing the bacteria and counting colonies on appropriate selective or non-selective media.

Bile Acid Sensitivity Testing

[0219] Additionally, in some embodiments, testing for bile-acid resistance enhances the selection of bacterial compositions that will survive exposures to bile acid during transit through the GI tract. Bile acids are secreted into the small intestine and can, like pH, affect the survival of bacterial compositions. This can be tested by exposing the bacterial compositions to bile acids for the expected gut exposure time to bile acids. For example, bile acid solutions can be prepared at desired concentrations using 0.05 mM Tris at pH 9 as the solvent. After the bile acid is dissolved, the pH of the solution may be adjusted to 7.2 with 10% HCl. Bacterial compositions can be cultured in 2.2 ml of a bile acid composition mimicking the concentration and type of bile acids in the patient, 1.0 ml of 10% sterile-filtered feces media and 0.1 ml of an 18-hour culture of the given strain of bacteria. Incubations may be conducted for from 2.5 to 3 hours or longer. An alternative method for testing stability to bile acid is described in U.S. Patent No. 4,839,281. Survival of bacteria can be determined by culturing the bacteria and counting colonies on appropriate selective or non-selective media.

Antibiotic Sensitivity Testing

[0220] As a further optional sensitivity test, bacterial compositions can be tested for sensitivity to antibiotics. In one embodiment, bacterial compositions can be chosen so that the bacterial constituents are sensitive to antibiotics such that if necessary they can be eliminated or substantially reduced from the patient's gastrointestinal tract by at least one antibiotic targeting the bacterial composition.

Adherence to Gastrointestinal Cells

[0221] The bacterial compositions may optionally be tested for the ability to adhere to gastrointestinal cells. A method for testing adherence to gastrointestinal cells is described in U.S. Patent No. 4,839,281.

Methods for Purifying Spores

Solvent Treatments

[0222] To purify the bacterial spores, the fecal material is subjected to one or more solvent treatments. A solvent treatment is a miscible solvent treatment (either partially miscible or fully miscible) or an immiscible solvent treatment. Miscibility is the ability of two liquids to mix with each to form a homogeneous solution. Water and ethanol, for example, are fully miscible such that a

mixture containing water and ethanol in any ratio will show only one phase. Miscibility is provided as a wt/wt%, or weight of one solvent in 100 g of final solution. If two solvents are fully miscible in all proportions, their miscibility is 100%. Provided as fully miscible solutions with water are alcohols, *e.g.*, methanol, ethanol, isopropanol, butanol, etc. The alcohols can be provided already combined with water; *e.g.*, a solution containing10%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 89%, 85%, 90%, 95% or greater than 95% Other solvents are only partially miscible, meaning that only some portion will dissolve in water. Diethyl ether, for example, is partially miscible with water. Up to 7 grams of diethyl ether will dissolve in 93 g of water to give a 7% (wt/wt%) solution. If more diethyl ether is added, a two phase solution will result with a distinct diethyl ether layer above the water. Other miscible materials include ethers, dimethoxyethane, or tetrahydrofuran In contrast, an oil such as an alkane and water are immiscible and form two phases. Further, immiscible treatments are optionally combined with a detergent, either an ionic detergent or a non-ionic detergent. Exemplary detergents include Triton X-100, Tween 20, Tween 80, Nonidet P40, a pluronic, or a polyol.

Chromatography treatments

[0223] To purify spore populations, the fecal materials are subjected to one or more chromatographic treatments, either sequentially or in parallel. In a chromatographic treatment, a solution containing the fecal material is contacted with a solid medium containing a hydrophobic interaction chromatographic (HIC) medium or an affinity chromatographic medium. In an alternative embodiment, a solid medium capable of absorbing a residual habitat product present in the fecal material is contacted with a solid medium that adsorbs a residual habitat product. In certain embodiments, the HIC medium contains sepharose or a derivatized sepharose such as butyl sepharose, octyl sepharose, phenyl sepharose, or butyl-s sepharose. In other embodiments, the affinity chromatographic medium contains material derivatized with mucin type I, II, III, IV, V, or VI, or oligosaccharides derived from or similar to those of mucins type I, II, III, IV, V, or VI. Alternatively, the affinity chromatographic medium contains material derivatized with antibodies that recognize spore-forming bacteria.

Mechanical Treatments

[0224] Provided herein is the physical disruption of the fecal material, particularly by one or more mechanical treatment such as blending, mixing, shaking, vortexing, impact pulverization, and sonication. As provided herein, the mechanical disrupting treatment substantially disrupts a non-spore material present in the fecal material and does not substantially disrupt a spore present in the fecal material. Mechanical treatments optionally include filtration treatments, where the desired spore populations are retained on a filter while the undesirable (non-spore) fecal components to pass through, and the spore fraction is then recovered from the filter medium. Alternatively, undesirable particulates and eukaryotic cells may be retained on a filter while bacterial cells including spores pass through. In some embodiments the spore fraction retained on the filter medium is subjected to a

diafiltration step, wherein the retained spores are contacted with a wash liquid, typically a sterile saline-containing solution or other diluent, in order to further reduce or remove the undesirable fecal components.

Thermal Treatments

[0225] Provided herein is the thermal disruption of the fecal material. Generally, the fecal material is mixed in a saline-containing solution such as phosphate-buffered saline (PBS) and subjected to a heated environment, such as a warm room, incubator, water-bath, or the like, such that efficient heat transfer occurs between the heated environment and the fecal material. Preferably the fecal material solution is mixed during the incubation to enhance thermal conductivity and disrupt particulate aggregates. Thermal treatments can be modulated by the temperature of the environment and/or the duration of the thermal treatment. For example, the fecal material or a liquid comprising the fecal material is subjected to a heated environment, *e.g.*, a hot water bath of at least about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or greater than 100 degrees Celsius, for at least about 1, 5, 10, 15, 20, 30, 45 seconds, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or 50 minutes, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 hours. In certain embodiments the thermal treatment occurs at two different temperatures, such as 30 seconds in a 100 degree Celsius environment followed by 10 minutes in a 50 degree Celsius environment. In preferred embodiments the temperature and duration of the thermal treatment are sufficient to kill or remove pathogenic materials while not substantially damaging or reducing the germination-competency of the spores.

Irradiation Treatments

[0226] Provided are methods of treating the fecal material or separated contents of the fecal material with ionizing radiation, typically gamma irradiation, ultraviolet irradiation or electron beam irradiation provided at an energy level sufficient to kill pathogenic materials while not substantially damaging the desired spore populations. For example, ultraviolet radiation at 254nm provided at an energy level below about 22,000 microwatt seconds per cm^2 will not generally destroy desired spores.

Centrifugation and Density Separation Treatments

[0227] Provided are methods of separating desired spore populations from the other components of the fecal material by centrifugation. A solution containing the fecal material is subjected to one or more centrifugation treatments, *e.g.*, at about 1000 x g, 2000 x g, 3000 x g, 4000 x g, 5000 x g, 6000 x g, 7000 x g, 8000 x g or greater than 8000 x g. Differential centrifugation separates desired spores from undesired non-spore material; at low forces the spores are retained in solution, while at higher forces the spores are pelleted while smaller impurities (*e.g.*, virus particles, phage) are retained in solution. For example, a first low force centrifugation pellets fibrous materials; a second, higher force centrifugation pellets undesired eukaryotic cells, and a third, still higher force centrifugation pellets the desired spores while small contaminants remain in suspension. In some embodiments density or mobility gradients or cushions (*e.g.*, step cushions), such as Percoll, Ficoll, Nycodenz, Histodenz or

sucrose gradients, are used to separate desired spore populations from other materials in the fecal material.

[0228] Also provided herein are methods of producing spore populations that combine two or more of the treatments described herein in order to synergistically purify the desired spores while killing or removing undesired materials and/or activities from the spore population. It is generally desirable to retain the spore populations under non-germinating and non-growth promoting conditions and media, in order to minimize the growth of pathogenic bacteria present in the spore populations and to minimize the germination of spores into vegetative bacterial cells.

PHARMACEUTICAL COMPOSITIONS AND FORMULATIONS OF THE INVENTION

Formulations

[0229] Provided are formulations for administration to humans and other subjects in need thereof. Generally the bacterial compositions are combined with additional active and/or inactive materials in order to produce a final product, which may be in single dosage unit or in a multi-dose format.

[0230] In some embodiments, the composition comprises at least one carbohydrate. A "carbohydrate" refers to a sugar or polymer of sugars. The terms "saccharide," "polysaccharide," "carbohydrate," and "oligosaccharide" may be used interchangeably. Most carbohydrates are aldehydes or ketones with many hydroxyl groups, usually one on each carbon atom of the molecule. Carbohydrates generally have the molecular formula $C_nH_{2n}O_n$. A carbohydrate can be a monosaccharide, a disaccharide, trisaccharide, oligosaccharide, or polysaccharide. The most basic carbohydrate is a monosaccharide, such as glucose, sucrose, galactose, mannose, ribose, arabinose, xylose, and fructose. Disaccharides are two joined monosaccharides. Exemplary disaccharides include sucrose, maltose, cellobiose, and lactose. Typically, an oligosaccharide includes between three and six monosaccharide units (*e.g.*, raffinose, stachyose), and polysaccharides include six or more monosaccharide units. Exemplary polysaccharides include starch, glycogen, and cellulose. Carbohydrates can contain modified saccharide units, such as 2'-deoxyribose wherein a hydroxyl group is removed, 2'-fluororibose wherein a hydroxyl group is replace with a fluorine, or N-acetylglucosamine, a nitrogen-containing form of glucose (*e.g.*, 2'-fluororibose, deoxyribose, and hexose). Carbohydrates can exist in many different forms, for example, conformers, cyclic forms, acyclic forms, stereoisomers, tautomers, anomers, and isomers.

[0231] In some embodiments, the composition comprises at least one lipid. As used herein, a "lipid" includes fats, oils, triglycerides, cholesterol, phospholipids, fatty acids in any form including free fatty acids. Fats, oils and fatty acids can be saturated, unsaturated (cis or trans) or partially unsaturated (cis or trans). In some embodiments, the lipid comprises at least one fatty acid selected from lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), margaric acid (17:0), heptadecenoic acid (17:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2),

linolenic acid (18:3), octadecatetraenoic acid (18:4), arachidic acid (20:0), eicosenoic acid (20:1), eicosadienoic acid (20:2), eicosatetraenoic acid (20:4), eicosapentaenoic acid (20:5) (EPA), docosanoic acid (22:0), docosenoic acid (22:1), docosapentaenoic acid (22:5), docosahexaenoic acid (22:6) (DHA), and tetracosanoic acid (24:0). In other embodiments, the composition comprises at least one modified lipid, for example, a lipid that has been modified by cooking.

[0232] In some embodiments, the composition comprises at least one supplemental mineral or mineral source. Examples of minerals include, without limitation: chloride, sodium, calcium, iron, chromium, copper, iodine, zinc, magnesium, manganese, molybdenum, phosphorus, potassium, and selenium. Suitable forms of any of the foregoing minerals include soluble mineral salts, slightly soluble mineral salts, insoluble mineral salts, chelated minerals, mineral complexes, non-reactive minerals such as carbonyl minerals, and reduced minerals, and combinations thereof.

[0233] In certain embodiments, the composition comprises at least one supplemental vitamin. The at least one vitamin can be fat-soluble or water soluble vitamins. Suitable vitamins include but are not limited to vitamin C, vitamin A, vitamin E, vitamin B12, vitamin K, riboflavin, niacin, vitamin D, vitamin B6, folic acid, pyridoxine, thiamine, pantothenic acid, and biotin. Suitable forms of any of the foregoing are salts of the vitamin, derivatives of the vitamin, compounds having the same or similar activity of the vitamin, and metabolites of the vitamin.

[0234] In other embodiments, the composition comprises an excipient. Non-limiting examples of suitable excipients include a buffering agent, a preservative, a stabilizer, a binder, a compaction agent, a lubricant, a dispersion enhancer, a disintegration agent, a flavoring agent, a sweetener, and a coloring agent.

[0235] In another embodiment, the excipient is a buffering agent. Non-limiting examples of suitable buffering agents include sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate.

[0236] In some embodiments, the excipient comprises a preservative. Non-limiting examples of suitable preservatives include antioxidants, such as alpha-tocopherol and ascorbate, and antimicrobials, such as parabens, chlorobutanol, and phenol.

[0237] In other embodiments, the composition comprises a binder as an excipient. Non-limiting examples of suitable binders include starches, pregelatinized starches, gelatin, polyvinylpyrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, C_{12}-C_{18} fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, and combinations thereof.

[0238] In another embodiment, the composition comprises a lubricant as an excipient. Non-limiting examples of suitable lubricants include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethyleneglycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil.

[0239] In other embodiments, the composition comprises a dispersion enhancer as an excipient. Non-limiting examples of suitable dispersants include starch, alginic acid, polyvinylpyrrolidones, guar gum, kaolin, bentonite, purified wood cellulose, sodium starch glycolate, isoamorphous silicate, and microcrystalline cellulose as high HLB emulsifier surfactants.

[0240] In some embodiments, the composition comprises a disintegrant as an excipient. In other embodiments, the disintegrant is a non-effervescent disintegrant. Non-limiting examples of suitable non-effervescent disintegrants include starches such as corn starch, potato starch, pregelatinized and modified starches thereof, sweeteners, clays, such as bentonite, micro-crystalline cellulose, alginates, sodium starch glycolate, gums such as agar, guar, locust bean, karaya, pecitin, and tragacanth. In another embodiment, the disintegrant is an effervescent disintegrant. Non-limiting examples of suitable effervescent disintegrants include sodium bicarbonate in combination with citric acid, and sodium bicarbonate in combination with tartaric acid.

[0241] In another embodiment, the excipient comprises a flavoring agent. Flavoring agents can be chosen from synthetic flavor oils and flavoring aromatics; natural oils; extracts from plants, leaves, flowers, and fruits; and combinations thereof. In some embodiments the flavoring agent is selected from cinnamon oils; oil of wintergreen; peppermint oils; clover oil; hay oil; anise oil; eucalyptus; vanilla; citrus oil such as lemon oil, orange oil, grape and grapefruit oil; and fruit essences including apple, peach, pear, strawberry, raspberry, cherry, plum, pineapple, and apricot.

[0242] In other embodiments, the excipient comprises a sweetener. Non-limiting examples of suitable sweeteners include glucose (corn syrup), dextrose, invert sugar, fructose, and mixtures thereof (when not used as a carrier); saccharin and its various salts such as the sodium salt; dipeptide sweeteners such as aspartame; dihydrochalcone compounds, glycyrrhizin; Stevia Rebaudiana (Stevioside); chloro derivatives of sucrose such as sucralose; and sugar alcohols such as sorbitol, mannitol, sylitol, and the like. Also contemplated are hydrogenated starch hydrolysates and the synthetic sweetener 3,6-dihydro-6-methyl-1,2,3-oxathiazin-4-one-2,2-dioxide, particularly the potassium salt (acesulfame-K), and sodium and calcium salts thereof.

[0243] In yet other embodiments, the composition comprises a coloring agent. Non-limiting examples of suitable color agents include food, drug and cosmetic colors (FD&C), drug and cosmetic colors (D&C), and external drug and cosmetic colors (Ext. D&C). The coloring agents can be used as dyes or their corresponding lakes.

[0244] The weight fraction of the excipient or combination of excipients in the formulation is usually about 99% or less, such as about 95% or less, about 90% or less, about 85% or less, about 80% or less, about 75% or less, about 70% or less, about 65% or less, about 60% or less, about 55% or less, 50% or less, about 45% or less, about 40% or less, about 35% or less, about 30% or less, about 25% or less, about 20% or less, about 15% or less, about 10% or less, about 5% or less, about 2% or less, or about 1% or less of the total weight of the composition.

[0245] The bacterial compositions disclosed herein can be formulated into a variety of forms and administered by a number of different means. The compositions can be administered orally, rectally, or parenterally, in formulations containing conventionally acceptable carriers, adjuvants, and vehicles as desired. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection and infusion techniques. In an exemplary embodiment, the bacterial composition is administered orally.

[0246] Solid dosage forms for oral administration include capsules, tablets, caplets, pills, troches, lozenges, powders, and granules. A capsule typically comprises a core material comprising a bacterial composition and a shell wall that encapsulates the core material. In some embodiments, the core material comprises at least one of a solid, a liquid, and an emulsion. In other embodiments, the shell wall material comprises at least one of a soft gelatin, a hard gelatin, and a polymer. Suitable polymers include, but are not limited to: cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose (HPMC), methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose succinate and carboxymethylcellulose sodium; acrylic acid polymers and copolymers, such as those formed from acrylic acid, methacrylic acid, methyl acrylate, ammonio methylacrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate (*e.g.*, those copolymers sold under the trade name "Eudragit"); vinyl polymers and copolymers such as polyvinyl pyrrolidone, polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers; and shellac (purified lac). In yet other embodiments, at least one polymer functions as taste-masking agents.

[0247] Tablets, pills, and the like can be compressed, multiply compressed, multiply layered, and/or coated. The coating can be single or multiple. In one embodiment, the coating material comprises at least one of a saccharide, a polysaccharide, and glycoproteins extracted from at least one of a plant, a fungus, and a microbe. Non-limiting examples include corn starch, wheat starch, potato starch, tapioca starch, cellulose, hemicellulose, dextrans, maltodextrin, cyclodextrins, inulins, pectin, mannans, gum arabic, locust bean gum, mesquite gum, guar gum, gum karaya, gum ghatti, tragacanth gum, funori, carrageenans, agar, alginates, chitosans, or gellan gum. In some embodiments the coating material comprises a protein. In another embodiment, the coating material comprises at least one of a fat and an oil. In other embodiments, the at least one of a fat and an oil is high temperature melting. In yet another embodiment, the at least one of a fat and an oil is hydrogenated or partially hydrogenated. In one embodiment, the at least one of a fat and an oil is derived from a plant. In other embodiments, the at least one of a fat and an oil comprises at least one of glycerides, free fatty acids, and fatty acid esters. In some embodiments, the coating material comprises at least one edible wax. The edible wax can be derived from animals, insects, or plants. Non-limiting examples include

beeswax, lanolin, bayberry wax, carnauba wax, and rice bran wax. Tablets and pills can additionally be prepared with enteric coatings.

[0248] Alternatively, powders or granules embodying the bacterial compositions disclosed herein can be incorporated into a food product. In some embodiments, the food product is a drink for oral administration. Non-limiting examples of a suitable drink include fruit juice, a fruit drink, an artificially flavored drink, an artificially sweetened drink, a carbonated beverage, a sports drink, a liquid diary product, a shake, an alcoholic beverage, a caffeinated beverage, infant formula and so forth. Other suitable means for oral administration include aqueous and nonaqueous solutions, emulsions, suspensions and solutions and/or suspensions reconstituted from non-effervescent granules, containing at least one of suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, coloring agents, and flavoring agents.

[0249] In some embodiments, the food product can be a solid foodstuff. Suitable examples of a solid foodstuff include without limitation a food bar, a snack bar, a cookie, a brownie, a muffin, a cracker, an ice cream bar, a frozen yogurt bar, and the like.

[0250] In other embodiments, the compositions disclosed herein are incorporated into a therapeutic food. In some embodiments, the therapeutic food is a ready-to-use food that optionally contains some or all essential macronutrients and micronutrients. In another embodiment, the compositions disclosed herein are incorporated into a supplementary food that is designed to be blended into an existing meal. In one embodiment, the supplemental food contains some or all essential macronutrients and micronutrients. In another embodiment, the bacterial compositions disclosed herein are blended with or added to an existing food to fortify the food's protein nutrition. Examples include food staples (grain, salt, sugar, cooking oil, margarine), beverages (coffee, tea, soda, beer, liquor, sports drinks), snacks, sweets and other foods.

[0251] In one embodiment, the formulations are filled into gelatin capsules for oral administration. An example of an appropriate capsule is a 250 mg gelatin capsule containing from 10 (up to 100 mg) of lyophilized powder (10^8 to 10^{11} bacteria), 160 mg microcrystalline cellulose, 77.5 mg gelatin, and 2.5 mg magnesium stearate. In an alternative embodiment, from 10^5 to 10^{12} bacteria may be used, 10^5 to 10^7, 10^6 to 10^7, or 10^8 to 10^{10}, with attendant adjustments of the excipients if necessary. In an alternative embodiment, an enteric-coated capsule or tablet or with a buffering or protective composition can be used.

EXAMPLES

[0252] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[0253] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.); *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Example 1: Sequence-based Genomic Characterization of Operational Taxonomic Units (OTU) and Functional Genes

Method for Determining 16S rDNA Gene Sequence

[0254] As described above, OTUs are defined either by full 16S sequencing of the rRNA gene, by sequencing of a specific hypervariable region of this gene (*i.e.* V1, V2, V3, V4, V5, V6, V7, V8, or V9), or by sequencing of any combination of hypervariable regions from this gene (*e.g.* V1-3 or V3-5). The bacterial 16S rRNA gene is approximately 1500 nucleotides in length and is used in reconstructing the evolutionary relationships and sequence similarity of one bacterial isolate to another using phylogenetic approaches. 16S sequences are used for phylogenetic reconstruction as they are in general highly conserved, but contain specific hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most microbes. rRNA gene sequencing methods are applicable to both the analysis of non-enriched samples, but also for identification of microbes after enrichment steps that either enrich the microbes of interest from the microbial composition and/or the nucleic acids that harbor the appropriate rDNA gene sequences as described below. For example, enrichment treatments prior to 16S rDNA gene characterization will increase the sensitivity of 16S as well as other molecular-based characterization nucleic acid purified from the microbes.

[0255] Using well known techniques, in order to determine the full 16S sequence or the sequence of any hypervariable region of the 16S rRNA sequence, genomic DNA is extracted from a bacterial sample, the 16S rDNA (full region or specific hypervariable regions) amplified using polymerase chain reaction (PCR), the PCR products cleaned, and nucleotide sequences delineated to determine the genetic composition of 16S gene or subdomain of the gene. If full 16S sequencing is performed, the sequencing method used may be, but is not limited to, Sanger sequencing. If one or more hypervariable regions are used, such as the V4 region, the sequencing may be, but is not limited to being, performed using the Sanger method or using a next-generation sequencing method, such as an Illumina (sequencing by synthesis) method using barcoded primers allowing for multiplex reactions.

Method for Determining 18S rDNA and ITS Gene Sequence

[0256] Methods to assign and identify fungal OTUs by genetic means can be accomplished by analyzing 18S sequences and the internal transcribed spacer (ITS). The rRNA of fungi that forms the core of the ribosome is transcribed as a signal gene and consists of the 8S, 5.8S and 28S regions with ITS4 and 5 between the 8S and 5.8S and 5.8S and 28S regions, respectively. These two intercistronic segments between the 18S and 5.8S and 5.8S and 28S regions are removed by splicing and contain significant variation between species for barcoding purposes as previously described (Schoch et al Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. PNAS 109:6241-6246. 2012). 18S rDNA is traditionally used for phylogenetic reconstruction however the ITS can serve this function as it is generally highly conserved but contains hypervariable regions that harbor sufficient nucleotide diversity to differentiate genera and species of most fungus.

[0257] Using well known techniques, in order to determine the full 18S and ITS sequences or a smaller hypervariable section of these sequences, genomic DNA is extracted from a microbial sample, the rDNA amplified using polymerase chain reaction (PCR), the PCR products cleaned, and nucleotide sequences delineated to determine the genetic composition rDNA gene or subdomain of the gene. The sequencing method used may be, but is not limited to, Sanger sequencing or using a next-generation sequencing method, such as an Illumina (sequencing by synthesis) method using barcoded primers allowing for multiplex reactions.

Method for Determining Other Marker Gene Sequences

[0258] In addition to the 16S and 18S rRNA gene, one may define an OTU by sequencing a selected set of genes that are known to be marker genes for a given species or taxonomic group of OTUs. These genes may alternatively be assayed using a PCR-based screening strategy. As example, various strains of pathogenic Escherichia coli can be distinguished using DNAs from the genes that encode heat-labile (LTI, LTIIa, and LTIIb) and heat-stable (STI and STII) toxins, verotoxin types 1, 2, and 2e (VT1, VT2, and VT2e, respectively), cytotoxic necrotizing factors (CNF1 and CNF2), attaching and effacing mechanisms (eaeA), enteroaggregative mechanisms (Eagg), and enteroinvasive mechanisms (Einv). The optimal genes to utilize for taxonomic assignment of OTUs by use of marker genes will be familiar to one with ordinary skill of the art of sequence based taxonomic identification.

Genomic DNA Extraction

[0259] Genomic DNA is extracted from pure microbial cultures using a hot alkaline lysis method. 1 µl of microbial culture is added to 9 µl of Lysis Buffer (25mM NaOH, 0.2 mM EDTA) and the mixture is incubated at 95°C for 30 minutes. Subsequently, the samples are cooled to 4°C and neutralized by the addition of 10 µl of Neutralization Buffer (40 mM Tris-HCl) and then diluted 10-fold in Elution Buffer (10 mM Tris-HCl). Alternatively, genomic DNA is extracted from pure microbial cultures using commercially available kits such as the Mo Bio Ultraclean® Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) or by standard methods known to those skilled in

the art. For fungal samples, DNA extraction can be performed by methods described previously (US20120135127) for producing lysates from fungal fruiting bodies by mechanical grinding methods.

Amplification of 16S Sequences for Downstream Sanger Sequencing

[0260] To amplify bacterial 16S rDNA (e.g, in Figure 1), 2 µl of extracted gDNA is added to a 20 µl final volume PCR reaction. For full-length 16 sequencing the PCR reaction also contains 1x HotMasterMix (5PRIME, Gaithersburg, MD), 250 nM of 27f (AGRGTTTGATCMTGGCTCAG, IDT, Coralville, IA), and 250 nM of 1492r (TACGGYTACCTTGTTAYGACTT, IDT, Coralville, IA), with PCR Water (Mo Bio Laboratories, Carlsbad, CA) for the balance of the volume.

[0261] Figure 1 shows the hypervariable regions mapped onto a 16s sequence and the sequence regions corresponding to these sequences on a sequence map. A schematic is shown of a 16S rRNA gene and the figure denotes the coordinates of hypervariable regions 1-9 (V1-V9), according to an embodiment of the invention. Coordinates of V1-V9 are 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294, and 1435-1465 respectively, based on numbering using E. coli system of nomenclature defined by Brosius et al., Complete nucleotide sequence of a 16S ribosomal RNA gene (16S rRNA) from Escherichia coli, PNAS 75(10):4801-4805 (1978).

[0262] Alternatively, other universal bacterial primers or thermostable polymerases known to those skilled in the art are used. For example, primers are available to those skilled in the art for the sequencing of the the "V1-V9 regions" of the 16S rRNA (*e.g.*, Figure 1). These regions refer to the first through ninth hypervariable regions of the 16S rRNA gene that are used for genetic typing of bacterial samples. These regions in bacteria are defined by nucleotides 69-99, 137-242, 433-497, 576-682, 822-879, 986-1043, 1117-1173, 1243-1294 and 1435-1465 respectively using numbering based on the E. coli system of nomenclature. *See* Brosius et al., Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli, PNAS 75(10):4801-4805 (1978). In some embodiments, at least one of the V1, V2, V3, V4, V5, V6, V7, V8, and V9 regions are used to characterize an OTU. In one embodiment, the V1, V2, and V3 regions are used to characterize an OTU. In another embodiment, the V3, V4, and V5 regions are used to characterize an OTU. In another embodiment, the V4 region is used to characterize an OTU. A person of ordinary skill in the art can identify the specific hypervariable regions of a candidate 16S rRNA (*e.g.*, Figure 1) by comparing the candidate sequence in question to the reference sequence (as in Figure 2) and identifying the hypervariable regions based on similarity to the reference hypervariable regions. Figure 2 highlights in bold the nucleotide sequences for each hypervariable region in the exemplary reference E. coli 16S sequence described by Brosius et al.

[0263] The PCR is performed on commercially available thermocyclers such as a BioRad MyCycler™ Thermal Cycler (BioRad, Hercules, CA). The reactions are run at 94°C for 2 minutes followed by 30 cycles of 94°C for 30 seconds, 51°C for 30 seconds, and 68°C for 1 minute 30 seconds, followed by a 7 minute extension at 72°C and an indefinite hold at 4°C. Following PCR, gel

electrophoresis of a portion of the reaction products is used to confirm successful amplification of a ~1.5 kb product.

[0264] To remove nucleotides and oligonucleotides from the PCR products, 2 µl of HT ExoSap-IT (Affymetrix, Santa Clara, CA) is added to 5 µl of PCR product followed by a 15 minute incubation at 37°C and then a 15 minute inactivation at 80°C.

Amplification of 16S Sequences for Downstream Characterization By Massively Parallel Sequencing Technologies

[0265] Amplification performed for downstream sequencing by short read technologies such as Illumina require amplification using primers known to those skilled in the art that additionally include a sequence-based barcoded tag. As example, to amplify the 16s hypervariable region V4 region of bacterial 16S rDNA, 2 µl of extracted gDNA is added to a 20 µl final volume PCR reaction. The PCR reaction also contains 1x HotMasterMix (5PRIME, Gaithersburg, MD), 200 nM of V4_515f_adapt (AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCMGCCGCGGTAA, IDT, Coralville, IA), and 200 nM of barcoded 806rbc (CAAGCAGAAGACGGCATACGAGAT_12bpGolayBarcode_AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT, IDT, Coralville, IA), with PCR Water (Mo Bio Laboratories, Carlsbad, CA) for the balance of the volume. These primers incorporate barcoded adapters for Illumina sequencing by synthesis. Optionally, identical replicate, triplicate, or quadruplicate reactions may be performed. Alternatively other universal bacterial primers or thermostable polymerases known to those skilled in the art are used to obtain different amplification and sequencing error rates as well as results on alternative sequencing technologies.

[0266] The PCR amplification is performed on commercially available thermocyclers such as a BioRad MyCycler™ Thermal Cycler (BioRad, Hercules, CA). The reactions are run at 94°C for 3 minutes followed by 25 cycles of 94°C for 45 seconds, 50°C for 1 minute, and 72°C for 1 minute 30 seconds, followed by a 10 minute extension at 72°C and a indefinite hold at 4°C. Following PCR, gel electrophoresis of a portion of the reaction products is used to confirm successful amplification of a ~1.5 kb product. PCR cleanup is performed as described above.

Sanger Sequencing of Target Amplicons from Pure Homogeneous Samples

[0267] To detect nucleic acids for each sample, two sequencing reactions are performed to generate a forward and reverse sequencing read. For full-length 16s sequencing primers 27f and 1492r are used. 40 ng of ExoSap-IT-cleaned PCR products are mixed with 25 pmol of sequencing primer and Mo Bio Molecular Biology Grade Water (Mo Bio Laboratories, Carlsbad, CA) to 15 µl total volume. This reaction is submitted to a commercial sequencing organization such as Genewiz (South Plainfield, NJ) for Sanger sequencing.

Amplification of 18S and ITS regions for Downstream Sequencing

[0268] To amplify the 18S or ITS regions, 2 μL fungal DNA were amplified in a final volume of 30 μL with 15 μL AmpliTaq Gold 360 Mastermix, PCR primers, and water. The forward and reverse primers for PCR of the ITS region are 5'-TCCTCCGCTTATTGATATGC-3' and 5'-GGAAGTAAAAGTCGTAACAAGG-3' and are added at 0.2uM concentration each. The forward and reverse primers for the 18s region are 5'-GTAGTCATATGCTTGTCTC-3' and 5'-CTTCCGTCAATTCCTTTAAG-3' and are added at 0.4uM concentration each. PCR is performed with the following protocol: 95C for 10 min, 35 cycles of 95C for 15 seconds, 52C for 30 seconds, 72C for 1.5s; and finally 72C for 7 minutes followed by storage at 4C. All forward primers contained the M13F-20 sequencing primer, and reverse primers included the M13R-27 sequencing primer. PCR products (3 μL) were enzymatically cleaned before cycle sequencing with 1 μL ExoSap-IT and 1 μL Tris EDTA and incubated at 37 °C for 20 min followed by 80 °C for 15 min. Cycle sequencing reactions contained 5 μL cleaned PCR product, 2 μL BigDye Terminator v3.1 Ready Reaction Mix, 1 μL 5× Sequencing Buffer, 1.6 pmol of appropriate sequencing primers designed by one skilled in the art, and water in a final volume of 10 μL. The standard cycle sequencing protocol is 27 cycles of 10 s at 96 °C, 5 s at 50 °C, 4 min at 60 °C, and hold at 4 °C. Sequencing cleaning is performed with the BigDye XTerminator Purification Kit as recommended by the manufacturer for 10-μL volumes. The genetic sequence of the resulting 18S and ITS sequences is performed using methods familiar to one with ordinary skill in the art using either Sanger sequencing technology or next-generation sequencing technologies such as but not limited to Illumina.

Preparation of Extracted Nucleic Acids for Metagenomic Characterization by Massively Parallel Sequencing Technologies

[0269] Extracted nucleic acids (DNA or RNA) are purified and prepared by downstream sequencing using standard methods familiar to one with ordinary skill in the art and as described by the sequencing technology's manufactures instructions for library preparation. In short, RNA or DNA are purified using standard purification kits such as but not limited to Qiagen's RNeasy Kit or Promega's Genomic DNA purification kit. For RNA, the RNA is converted to cDNA prior to sequence library construction. Following purification of nucleic acids, RNA is converted to cDNA using reverse transcription technology such as but not limited to Nugen Ovation RNA-Seq System or Illumina Truseq as per the manufacturer's instructions. Extracted DNA or transcribed cDNA are sheared using physical (*e.g.*, Hydroshear), acoustic (*e.g.*, Covaris), or molecular (*e.g.*, Nextera) technologies and then size selected as per the sequencing technologies manufacturer's recommendations. Following size selection, nucleic acids are prepared for sequencing as per the manufacturer's instructions for sample indexing and sequencing adapter ligation using methods familiar to one with ordinary skill in the art of genomic sequencing.

Massively Parallel Sequencing of Target Amplicons from Heterogeneous Samples

DNA Quantification & Library Construction

[0270] The cleaned PCR amplification products are quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Following quantification, the barcoded cleaned PCR products are combined such that each distinct PCR product is at an equimolar ratio to create a prepared Illumina library.

Nucleic Acid Detection

[0271] The prepared library is sequenced on Illumina HiSeq or MiSeq sequencers (Illumina, San Diego, CA) with cluster generation, template hybridization, isothermal amplification, linearization, blocking and denaturation and hybridization of the sequencing primers performed according to the manufacturer's instructions. 16SV4SeqFw (TATGGTAATTGTGTGCCAGCMGCCGCGGTAA), 16SV4SeqRev (AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT), and 16SV4Index (ATTAGAWACCCBDGTAGTCCGGCTGACTGACT) (IDT, Coralville, IA) are used for sequencing. Other sequencing technologies can be used such as but not limited to 454, Pacific Biosciences, Helicos, Ion Torrent, and Nanopore using protocols that are standard to someone skilled in the art of genomic sequencing.

Example 2. Sequence Read Annotation

Primary Read Annotation

[0272] Nucleic acid sequences are analyzed and annotated to define taxonomic assignments using sequence similarity and phylogenetic placement methods or a combination of the two strategies. A similar approach can be used to annotate protein names, protein function, transcription factor names, and any other classification schema for nucleic acid sequences. Sequence similarity based methods include those familiar to individuals skilled in the art including, but not limited to BLAST, BLASTx, tBLASTn, tBLASTx, RDP-classifier, DNAclust, and various implementations of these algorithms such as Qiime or Mothur. These methods rely on mapping a sequence read to a reference database and selecting the match with the best score and e-value. Common databases include, but are not limited to the Human Microbiome Project, NCBI non-redundant database, Greengenes, RDP, and Silva for taxonomic assignments. For functional assignments reads are mapped to various functional databases such as but not limited to COG, KEGG, BioCyc, and MetaCyc. Further functional annotations can be derived from 16S taxonomic annotations using programs such as PICRUST (M. Langille, et al 2013. Nature Biotechnology 31, 814-821). Phylogenetic methods can be used in combination with sequence similarity methods to improve the calling accuracy of an annotation or taxonomic assignment. Here tree topologies and nodal structure are used to refine the resolution of the analysis. In this approach we analyze nucleic acid sequences using one of numerous sequence similarity approaches and leverage phylogenetic methods that are well known to those skilled in the art, including but not limited to maximum likelihood phylogenetic reconstruction (see *e.g.* Liu K, Linder CR, and Warnow T. 2011. RAxML and FastTree: Comparing Two Methods for Large-Scale Maximum Likelihood Phylogeny Estimation. PLoS ONE 6: e27731. McGuire G, Denham MC, and

Balding DJ. 2001. Models of sequence evolution for DNA sequences containing gaps. Mol. Biol. Evol 18: 481–490. Wróbel B. 2008. Statistical measures of uncertainty for branches in phylogenetic trees inferred from molecular sequences by using model-based methods. J. Appl. Genet. 49: 49–67.) Sequence reads (*e.g.* 16S, 18S, or ITS) are placed into a reference phylogeny comprised of appropriate reference sequences. Annotations are made based on the placement of the read in the phylogenetic tree. The certainty or significance of the OTU annotation is defined based on the OTU's sequence similarity to a reference nucleic acid sequence and the proximity of the OTU sequence relative to one or more reference sequences in the phylogeny. As an example, the specificity of a taxonomic assignment is defined with confidence at the the level of Family, Genus, Species, or Strain with the confidence determined based on the position of bootstrap supported branches in the reference phylogenetic tree relative to the placement of the OTU sequence being interrogated. Nucleic acid sequences can be assigned functional annotations using the methods described above.

Clade Assignments

[0273] The ability of 16S-V4 OTU identification to assign an OTU as a specific species depends in part on the resolving power of the 16S-V4 region of the 16S gene for a particular species or group of species. Both the density of available reference 16S sequences for different regions of the tree as well as the inherent variability in the 16S gene between different species will determine the definitiveness of a taxonomic annotation. Given the topological nature of a phylogenetic tree and the fact that tree represents hierarchical relationships of OTUs to one another based on their sequence similarity and an underlying evolutionary model, taxonomic annotations of a read can be rolled up to a higher level using a clade-based assignment procedure. Using this approach, clades are defined based on the topology of a phylogenetic tree that is constructed from full-length 16S sequences using maximum likelihood or other phylogenetic models familiar to individuals with ordinary skill in the art of phylogenetics. Clades are constructed to ensure that all OTUs in a given clade are: (i) within a specified number of bootstrap supported nodes from one another (generally, 1-5 bootstraps), and (ii) share a defined percent similarity (for 16S molecular data typically set to 95%-97% sequence similarity). OTUs that are within the same clade can be distinguished as genetically and phylogenetically distinct from OTUs in a different clade based on 16S-V4 sequence data. OTUs falling within the same clade are evolutionarily closely related and may or may not be distinguishable from one another using 16S-V4 sequence data. The power of clade based analysis is that members of the same clade, due to their evolutionary relatedness, are likely to play similar functional roles in a microbial ecology such as that found in the human gut. Compositions substituting one species with another from the same clade are likely to have conserved ecological function and therefore are useful in the present invention. Notably in addition to 16S-V4 sequences, clade-based analysis can be used to analyze 18S, ITS, and other genetic sequences.

[0274] Notably, 16S sequences of isolates of a given OTU are phylogenetically placed within their respective clades, sometimes in conflict with the microbiological-based assignment of species and

genus that may have preceded 16S-based assignment. Discrepancies between taxonomic assignment based on microbiological characteristics versus genetic sequencing are known to exist from the literature.

[0275] For a given network ecology or functional network ecology one can define a set of OTUs from the network's representative clades. As example, if a network was comprised of clade_100 and clade_102 it can be said to be comprised of at least one OTU from the group consisting of Corynebacterium coyleae, Corynebacterium mucifaciens, and Corynebacterium ureicelerivorans, and at least one OTU from the group consisting of Corynebacterium appendicis, Corynebacterium genitalium, Corynebacterium glaucum, Corynebacterium imitans, Corynebacterium riegelii, Corynebacterium sp. L_2012475, Corynebacterium sp. NML 93_0481, Corynebacterium sundsvallense, and Corynebacterium tuscaniae (see Table 1). Conversely as example, if a network was said to consist of Corynebacterium coyleae and/or Corynebacterium mucifaciens and/or Corynebacterium ureicelerivorans, and also consisted of Corynebacterium appendicis and/or Corynebacterium genitalium and/or Corynebacterium glaucum and/or Corynebacterium imitans and/or Corynebacterium riegelii and/or Corynebacterium sp. L_2012475 and/or Corynebacterium sp. NML 93_0481 and/or Corynebacterium sundsvallense and/or Corynebacterium tuscaniae it can be said to be comprised of clade_100 and clade_102.

[0276] The applicants made clade assignments to all OTUs reported in the application using the above described method and these assignments are reported in Table 1. In some embodiments, the network analysis permits substitutions of clade_172 by clade_172i. In another embodiment, the network analysis permits substitutions of clade_198 by clade_198i. In another embodiment, the network analysis permits substitutions of clade_260 by clade_260c, clade_260g or clade_260h. In another embodiment, the network analysis permits substitutions of clade_262 by clade_262i. In another embodiment, the network analysis permits substitutions of clade_309 by clade_309c, clade_309e, clade_309g, clade_309h or clade_309i. In another embodiment, the network analysis permits substitutions of clade_313 by clade_313f. In another embodiment, the network analysis permits substitutions of clade_325 by clade_325f. In another embodiment, the network analysis permits substitutions of clade_335 by clade_335i. In another embodiment, the network analysis permits substitutions of clade_351 by clade_351e. In another embodiment, the network analysis permits substitutions of clade_354 by clade_354e. In another embodiment, the network analysis permits substitutions of clade_360 by clade_360c, clade_360g, clade_360h, or clade_360i. In another embodiment, the network analysis permits substitutions of clade_378 by clade_378e. In another embodiment, the network analysis permits substitutions of clade_38 by clade_38e or clade_38i. In another embodiment, the network analysis permits substitutions of clade_408 by clade_408b, clade_408d, clade_408f, clade_408g or clade_408h. In another embodiment, the network analysis permits substitutions of clade_420 by clade_420f. In another embodiment, the network analysis permits substitutions of clade_444 by clade_444i. In another embodiment, the

network analysis permits substitutions of clade_478 by clade_478i. In another embodiment, the network analysis permits substitutions of clade_479 by clade_479c, by clade_479g or by clade_479h. In another embodiment, the network analysis permits substitutions of clade_481 by clade_481a, clade_481b, clade_481e, clade_481g, clade_481h or by clade_481i. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_497 by clade_497e or by clade_497f. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_512 by clade_512i. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_516 by clade_516c, by clade_516g or by clade_516h. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_522 by clade_522i. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_553 by clade_553i. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_566 by clade_566f. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_572 by clade_572i. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_65 by clade_65e. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_92 by clade_92e or by clade_92i. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_96 by clade_96g or by clade_96h. In another embodiment, the network analysis permits the network analysis permits substitutions of clade_98 by clade_98i. These permitted clade substitutions are described in Table 22.

Metagenomic Read Annotation

[0277] Metagenomic or whole genome shotgun sequence data is annotated as described above, with the additional step that sequences are either clustered or assembled prior to annotation. Following sequence characterization as described above, sequence reads are demultiplexed using the indexing (*i.e.* barcodes). Following demultiplexing sequence reads are either: (i) clustered using a rapid clustering algorithm such as but not limited to UCLUST (http://drive5.com/usearch/manual/uclust_algo.html) or hash methods such VICUNA (Xiao Yang, Patrick Charlebois, Sante Gnerre, Matthew G Coole, Niall J. Lennon, Joshua Z. Levin, James Qu, Elizabeth M. Ryan, Michael C. Zody, and Matthew R. Henn. 2012. De novo assembly of highly diverse viral populations. BMC Genomics 13:475). Following clustering a representative read for each cluster is identified based and analyzed as described above in "Primary Read Annotation". The result of the primary annotation is then applied to all reads in a given cluster. (ii) A second strategy for metagenomic sequence analysis is genome assembly followed by annotation of genomic assemblies using a platform such as but not limited to MetAMOS (TJ. Treangen et al. 2013 Geneome Biology 14:R2), HUMAaN (Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL, Rodriguez-Mueller B, Zucker J, Thiagarajan M, Henrissat B, et al. 2012. Metabolic Reconstruction

for Metagenomic Data and Its Application to the Human Microbiome ed. J.A. Eisen. PLoS Computational Biology 8: e1002358) and other methods familiar to one with ordinary skill in the art.

Example 3. OTU Identification Using Microbial Culturing Techniques

[0278] The identity of the bacterial species which grow up from a complex fraction can be determined in multiple ways. First, individual colonies are picked into liquid media in a 96 well format, grown up and saved as 15% glycerol stocks at -80°C. Aliquots of the cultures are placed into cell lysis buffer and colony PCR methods can be used to amplify and sequence the 16S rDNA gene (Example 1). Alternatively, colonies are streaked to purity in several passages on solid media. Well separated colonies are streaked onto the fresh plates of the same kind and incubated for 48-72 hours at 37°C. The process is repeated multiple times in order to ensure purity. Pure cultures are analyzed by phenotypic- or sequence-based methods, including 16S rDNA amplification and sequencing as described in Example 1. Sequence characterization of pure isolates or mixed communities *e.g.* plate scrapes and spore fractions can also include whole genome shotgun sequencing. The latter is valuable to determine the presence of genes associated with sporulation, antibiotic resistance, pathogenicity, and virulence. Colonies are also scraped from plates en masse and sequenced using a massively parallel sequencing method as described in Example 1, such that individual 16S signatures can be identified in a complex mixture. Optionally, the sample can be sequenced prior to germination (if appropriate DNA isolation procedures are used to lsye and release the DNA from spores) in order to compare the diversity of germinable species with the total number of species in a spore sample. As an alternative or complementary approach to 16S analysis, MALDI-TOF-mass spec is used for species identification (Barreau M, Pagnier I, La Scola B. 2013. Improving the identification of anaerobes in the clinical microbiology laboratory through MALDI-TOF mass spectrometry. Anaerobe 22: 123–125).

Example 4. Microbiological Strain Identification Approaches

[0279] Pure bacterial isolates are identified using microbiological methods as described in Wadsworth-KTL Anaerobic Microbiology Manual (Jouseimies-Somer H, Summanen PH, Citron D, Baron E, Wexler HM, Finegold SM. 2002. Wadsworth-KTL Anaerobic Bacteriology Manual), and The Manual of Clinical Microbiology (ASM Press, 10th Edition). These methods rely on phenotypes of strains and include Gram-staining to confirm Gram positive or negative staining behavior of the cell envelope, observance of colony morphologies on solid media, motility, cell morphology observed microscopically at 60x or 100x magnification including the presence of bacterial endospores and flagella. Biochemical tests that discriminate between genera and species are performed using appropriate selective and differential agars and/or commercially available kits for identification of Gram negative and Gram positive bacteria and yeast, for example, RapID tests (Remel) or API tests (bioMerieux). Similar identification tests can also be performed using instrumentation such as the Vitek 2 system (bioMerieux). Phenotypic tests that discriminate between genera and species and strains (for example the ability to use various carbon and nitrogen sources) can also be performed

using growth and metabolic activity detection methods, for example the Biolog Microbial identification microplates. The profile of short chain fatty acid production during fermentation of particular carbon sources can also be used as a way to discriminate between species (Wadsworth-KTL Anaerobic Microbiology Manual, Jousimies-Somer, et al 2002). MALDI-TOF-mass spectrometry can also be used for species identification (as reviewed in Anaerobe 22:123).

Example 5. Computational Prediction of Network Ecologies

[0280] Source data comprising a genomic-based characterization of a microbiome of individual samples were used as input computationally delineate network ecologies that would have biological properties that are characteristic of a state of health and could catalzye a shift from a state of microbial dysbiosis to a state of health. Applicants obtained 16S and metagenomic sequence datasets from public data repositories (see *e.g.* The Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486: 207–214. Data accessible at URL: hmpdacc.org) and MetaHit Project (Arumugam M, Raes J, Pelletier E, Paslier DL, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto J-M, et al. 2011. Enterotypes of the human gut microbiome. Nature 473: 174–180. Data accessible at URL: metahit.eu) for relevant microbiome studies in multiple disease indications including CDAD, Type 2 Diabetes, Ulcerative Colitis, and Irritable Bowel Disease, or generated data sets from samples directly using the methods described in Examples 1 & 2 and further described in the literature (see *e.g.* Aagaard K, Riehle K, Ma J, Segata N, Mistretta T-A, Coarfa C, Raza S, Rosenbaum S, Van den Veyver I, Milosavljevic A, et al. 2012. A Metagenomic Approach to Characterization of the Vaginal Microbiome Signature in Pregnancy ed. A.J. Ratner. PLoS ONE 7: e36466. Jumpstart Consortium Human Microbiome Project Data Generation Working Group. 2012. Evaluation of 16S rDNA-Based Community Profiling for Human Microbiome Research ed. J. Ravel. PLoS ONE 7: e39315. The Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486: 207–214.). Nucleic acid sequences were analyzed and taxonomic and phylogenetic assignments of specific OTUs were made using sequence similarity and phylogenetic methods that are well known to those skilled in the art, including but not limited to maximum likelihood phylogenetic reconstruction (see *e.g.* Liu K, Linder CR, and Warnow T. 2011. RAxML and FastTree: Comparing Two Methods for Large-Scale Maximum Likelihood Phylogeny Estimation. PLoS ONE 6: e27731. McGuire G, Denham MC, and Balding DJ. 2001. Models of sequence evolution for DNA sequences containing gaps. Mol. Biol. Evol 18: 481–490. Wróbel B. 2008. Statistical measures of uncertainty for branches in phylogenetic trees inferred from molecular sequences by using model-based methods. J. Appl. Genet. 49: 49–67.) From these taxonomic assignments OTUs and clades in the dataset were defined using the method described in Examples 1 and 2. The certainty of the OTU call was defined based on the OTU's sequence similarity to a reference nucleic acid sequence and the proximity of the OTU sequence relative to one or more reference sequences in the phylogeny. The specificity of an OTU's taxonomic and phlylogenetic assignment determines whether the match is assigned at the level of

Family, Genus, Species, or Strain, and the confidence of this assignment is determined based on the position of bootstrap supported branches in the reference phylogenetic tree relative to the placement of the OTU sequence being interrogated. In addition, microbial OTU assignments may be obtained from assignments made in peer-reviewed publications.

[0281] Applicants designated individual subject samples to biologically relevant sample phenotypes such as but not limited to "healthy state," "recurrent Clostridium difficile infection," "Crohn's disease," "Insulin Resistance," "Obesity," "Type 2 diabetes," "Ulcerative Colitis". In one embodiment samples are assigned to "health" and "disease" phenotypes. In another embodiment, samples are assigned higher resolution phenotype such as but not limited to: "health:human", "health:mouse", "health:human microbiome project", "health:microbiota donor", "health:microbiota recipient", "disease:microbiota recipient", or "disease:no treatment", "disease:human", or "disease:mouse". In another embodiment, samples where assigned to higher resolution phenotypes, such as but not limited to those defined in Figure 19 that characterize phenotypes specific to samples from fecal donors and patients who received a fecal microbial transplant from these donors. Figure 19 shows phenotypes assigned to samples for the computational derivation of Network Ecologies that typify microbiome states of health (Hpost, Hdon, & Hgen) and states of disease (DdonF & DpreF).

[0282] In another embodiment, other phenotypes that define a category of disease or health that represents the underlying state of the population under study can be used. Applicants then computationally determined the microbial network ecologies for each phenotype using the OTU and clade assignments that comprise the microbial profile for each sample and the algorithms described above in the Section entitled "Method of Determining Network Ecologies."

[0283] Tables 8, 11, and 14a below provide exemplary network ecologies that define states of health as compared to states of dysbiosis or disease for multiple disease indications. The disease indications for which the network ecologies represent a health state are denoted in Table 8, and Keystone and Non-Keystone OTUs (see Example 6) are delineated in Tables 9-10. Importantly, Network Ecologies that represent a state of health in one disease indication can represent states of health in additional disease states. Additionally, Keystone OTUs found in a network associated with health for different disease indications can overlap. Applicants found that a large number of network ecologies overlapped particularly between those associated with health in the cases of CDAD and Type 2 Diabetes despite the analysis of substantially different genomic data sets for the two diseases.

Example 6. Identification of Network Classes, Keystone OTUs, Clades, and Functional Modalities

Identification of Keystone OTUs, Clades and Functions

[0284] The human body is an ecosystem in which the microbiota and the microbiome play a significant role in the basic healthy function of human systems (*e.g.* metabolic, immunological, and neurological). The microbiota and resulting microbiome comprise an ecology of microorganisms that

co-exist within single subjects interacting with one another and their host (*i.e.*, the mammalian subject) to form a dynamic unit with inherent biodiversity and functional characteristics. Within these networks of interacting microbes (*i.e.* ecologies), particular members can contribute more significantly than others; as such these members are also found in many different ecologies, and the loss of these microbes from the ecology can have a significant impact on the functional capabilities of the specific ecology. Robert Paine coined the concept "Keystone Species" in 1969 (see Paine RT. 1969. A note on trophic complexity and community stability. The American Naturalist 103: 91–93) to describe the existence of such lynchpin species that are integral to a given ecosystem regardless of their abundance in the ecological community. Paine originally describe the role of the starfish Pisaster ochraceus in marine systems and since the concept has been experimentally validated in numerous ecosystems.

[0285] Keystone OTUs (as shown in Table 9), Phylogenetic Clades (a.k.a. Clades), and/or Functions (for example, but not limited to, KEGG Orthology Pathways) are computationally-derived by analysis of network ecologies elucidated from a defined set of samples that share a specific phenotype. Keystone OTUs, Clades and/or Functions are defined as all Nodes within a defined set of networks that meet two or more of the following criteria. Using Criterion 1, the node is frequently observed in networks, and the networks in which the node is observed are found in a large number of individual subjects; the frequency of occurrence of these Nodes in networks and the pervasiveness of the networks in individuals indicates these Nodes perform an important biological function in many individuals. Using Criterion 2, the node is frequently observed in networks, and the Node is observed contains a large number of edges connecting it to other nodes in the network. These Nodes are thus "super-connectors", meaning that they form a nucleus of a majority of networks (See Figure 17) and as such have high biological significance with respect to their functional contributions to a given ecology.

[0286] Figure 17 is a schematic representation of how Keystone OTUs (nodes 2 and 4, shaded circles) are central members of many network ecologies that contain non-Keystone OTUs (nodes 1, 3, and 5-9). Distinct network ecologies include [node 2--node 7], [node--3--node 2--node--4], [node 2--node 4--node 5--node 6--node 7], [node 1--node 2--node 8--node 9], and [node --node 3].

[0287] Using Criterion 3, the Node is found in networks containing a large number of Nodes (*i.e.*, they are large networks), and the networks in which the Node is found occur in a large number of subjects; these networks are potentially of high interest as it is unlikely that large networks occurring in many individuals would occur by chance alone strongly suggesting biological relevance. Optionally, the required thresholds for the frequency at which a Node is observed in network ecologies, the frequency at which a given network is observed across subject samples, and the size of a given network to be considered a Keystone Node are defined by the 50th, 70th, 80th, or 90th percentiles of the distribution of these variables. Optionally, the required thresholds are defined by

the value for a given variable that is significantly different from the mean or median value for a given variable using standard parametric or non-parametric measures of statistical significance. In another embodiment a Keystone Node is defined as one that occurs in a sample phenotype of interest such as but not limited to "health" and simultaneously does not occur in a sample phenotype that is not of interest such as but not limited to "disease." Optionally, a Keystone Node is defined as one that is shown to be significantly different from what is observed using permuted test datasets to measure significance. In another embodiment of Criterion 2 Keystone OTUs, Clades, or Functions can be defined using a hierarchical clustering method that clusters Networks based on their OTU, Clade, or functional pathways. Statistically significant branch points in the hierarchy are defined based on the topological overlap measure; this measure is a highly robust measure of network interconnectedness (Langfelder P, Zhang B, Horvath S. 2008. Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. Bioinformatics 24: 719–720.). Once these branch points are defined the Keystones are delineated as OTUs, clades or functional pathways that are found consistently across all networks in all or a subset of the network clusters.

[0288] Applicants defined the Keystone OTUs and Clades characteristic of health states for the computationally determined networks reported in Table 8 for the various disease indications analyszed using the three criterion defined above. Keystone Clades were defined from the Keystone OTUs using clade definitions as outlined in Example 1. Keystone OTUs are reported in Table 9. Importantly, we identified the absence of Keystone OTUs in multiple particular disease states, indicating that bacterial compositions comprised of specific sets of Keystone OTUs are likely to have utility in multiple disease indications.

Demonstration that Keystone OTUs inhibit *C. difficile* Growth in a Competitive In Vitro Simulation Assay

[0289] To screen the ability of binary combinations comprising at least one Keystone OTU (binary pairs) to inhibit the growth of Clostridium difficile in vitro, vials of -80 °C glycerol stock banks of each OTU were thawed and diluted to 1e8 CFU/mL. Each strain was then diluted 10x (to a final concentration of 1e7 CFU/mL of each strain) into 200 uL of PBS + 15% glycerol in the wells of a 96-well plate. Plates were then frozen at -80 °C. When needed for the assay, plates were removed from -80 °C and thawed at room temperature under anaerobic conditions prior to use.

[0290] An overnight culture of Clostridium difficile was grown under anaerobic conditions in SweetB-FosIn or other suitable media for the growth of C. difficile. SweetB-FosIn is a complex media composed of brain heart infusion, yeast extract, cysteine, cellobiose, maltose, soluble starch, and fructooligosaccharides/inulin, and hemin, and is buffered with morpholino-propane sulphonic acid (MOPS). After 24 hr of growth the culture was diluted 100,000 fold into SweetB-FosIn. The diluted C. difficile mixture was then aliquoted to wells of a 96-well plate (180 uL to each well). 20 uL of a unique binary pair of Keystone OTUs was then added to each well at a final concentration of 1e6

CFU/mL of each species. Alternatively the assay can be tested with binary pairs at different initial concentrations (1e9 CFU/mL, 1e8 CFU/mL, 1e7 CFU/mL, 1e5 CFU/mL, 1e4 CFU/mL, 1e3 CFU/mL, 1e2 CFU/mL). Control wells only inoculated with C. difficile were included for a comparison to the growth of C. difficile without inhibition. Additional wells were used for controls that either inhibit or do not inhibit the growth of C. difficile. Plates were wrapped with parafilm and incubated for 24 hr at 37 °C under anaerobic conditions. After 24 hr the wells containing C. difficile alone were serially diluted and plated to determine titer on selective media such as CCFA (Anaerobe Systems) or CDSA (Becton Dickinson). The 96-well plate was then frozen at -80°C before quantifying C. difficile by qPCR.

[0291] C. difficile in each well was quantified by qPCR. A standard curve was generated from a well on each assay plate containing only pathogenic C. difficile grown in SweetB + FosIn media as provided herein and compare to the microbiological titer determined above. Genomic DNA was extracted from the standard curve samples along with the other wells. Genomic DNA was extracted from 5 µl of each sample using a dilution, freeze/thaw, and heat lysis protocol. 5 µL of thawed samples were added to 45 µL of UltraPure water (Life Technologies, Carlsbad, CA) and mixed by pipetting. The plates with diluted samples were frozen at -20°C until use for qPCR which includes a heated lysis step prior to amplification. Alternatively the genomic DNA could be isolated using the Mo Bio Powersoil®-htp 96 Well Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), Mo Bio Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), or the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

[0292] The qPCR reaction mixture contained 1x SsoAdvanced Universal Probes Supermix, 900 nM of Wr-tcdB-F primer (AGCAGTTGAATATAGTGGTTTAGTTAGAGTTG, IDT, Coralville, IA), 900 nM of Wr-tcdB-R primer (CATGCTTTTTTAGTTTCTGGATTGAA, IDT, Coralville, IA), 250 nM of Wr-tcdB-P probe (6FAM-CATCCAGTCTCAATTGTATATGTTTCTCCA-MGB, Life Technologies, Grand Island, NY), and Molecular Biology Grade Water (Mo Bio Laboratories, Carlsbad, CA) to 18 µl (Primers adapted from: Wroblewski, D. et al., Rapid Molecular Characterization of Clostridium difficile and Assessment of Populations of C. difficile in Stool Specimens, Journal of Clinical Microbiology 47:2142–2148 (2009)). This reaction mixture was aliquoted to wells of a Hard-shell Low-Profile Thin Wall 96-well Skirted PCR Plate (BioRad, Hercules, CA). To this reaction mixture, 2 µl of diluted, frozen, and thawed samples were added and the plate sealed with a Microseal 'B' Adhesive Seal (BioRad, Hercules, CA). The qPCR was performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA). The thermocycling conditions were 95°C for 15 minutes followed by 45 cycles of 95°C for 5 seconds, 60°C for 30 seconds, and fluorescent readings of the FAM channel. Alternatively, the qPCR could be performed with other standard methods known to those skilled in the art.

[0293] The Cq value for each well on the FAM channel was determined by the CFX Manager™ 3.0 software. The log10(cfu/mL) of C. difficile each experimental sample was calculated by inputting a given sample's Cq value into a linear regression model generated from the standard curve comparing the Cq values of the standard curve wells to the known log10(cfu/mL) of those samples. The log inhibition was calculated for each sample by subtracting the log10(cfu/mL) of C. difficile in the sample from the log10(cfu/mL) of C. difficile in the sample on each assay plate used for the generation of the standard curve that has no additional bacteria added. The mean log inhibition was calculated for all replicates for each composition.

[0294] A histogram of the range and standard deviation of each composition was plotted. Ranges or standard deviations of the log inhibitions that were distinct from the overall distribution were examined as possible outliers. If the removal of a single log inhibition datum from one of the binary pairs that were identified in the histograms would bring the range or standard deviation in line with those from the majority of the samples, that datum was removed as an outlier, and the mean log inhibition was recalculated.

[0295] The pooled variance of all samples evaluated in the assay was estimated as the average of the sample variances weighted by the sample's degrees of freedom. The pooled standard error was then calculated as the square root of the pooled variance divided by the square root of the number of samples. Confidence intervals for the null hypothesis were determined by multiplying the pooled standard error to the z score corresponding to a given percentage threshold. Mean log inhibitions outside the confidence interval were considered to be inhibitory if positive or stimulatory if negative with the percent confidence corresponding to the interval used. Samples with mean log inhibition greater than the 99% confidence interval (C.I) of the null hypothesis are reported as ++++, those with a 95% < C.I. <99% as +++, those with a 90% < C.I. <95% as ++, those with a 80% < C.I. <90% as + while samples with mean log inhibition less than than the 99% confidence interval (C.I) of the null hypothesis are reported as ——, those with a 95% < C.I. <99% as —, those with a 90% < C.I. <95% as —, those with a 80% < C.I. <90% as −. Many binary pairs comprising Keystone OTUs inhibit C. difficile as delineated in Table 20.

Assignment of a Network Classes Based On Phylogenetic Diversity and Functional Modalities

[0296] "Network Classes" can be delineated by clustering computationally determined network ecologies into groupings based on the OTUs observed in a given network. In one example, OTUs are treated individualistically with each OTU representing a unique entity within the network. In other examples, the OTUs are clustered according to their phylogenetic relationships defined by a phylogenetic tree, e.g., into clades. In yet another embodiment, functional modules such as but not limited to KEGG Orthology Pathways can be used to cluster the networks, OTUs and Clades according to the biological or biochemical functions they comprise. . A set of ecological networks

from which a Network Class is defined, is selected using one or a combination of the following criteria: (i) networks that are derived from a biological phenotype, (ii) the frequency at which a given network is observed across samples, or (iii) the size of the network. In one embodiment, the required thresholds for the frequency at which a given network is observed across subject samples, and the size of a given network to be considered for further analysis are defined by the 50^{th}, 70^{th}, 80^{th}, or 90^{th} percentiles of the distribution of these variables. In another embodiment, the required thresholds are defined by the value for a given variable that is significantly different from the mean or median value for a given variable using standard parametric or non-parametric measures of statistical significance. In yet another embodiment, ecological networks derived from Network Classes are selected that contain 5 or fewer, 10 or fewer, 15 or fewer, 20 or fewer, 25 or fewer, or 50 or fewer OTUs, Clades, or Functional modalities.

[0297] Network Class ecologies are defined using a heatmap analytical strategy whereby the OTU content of a given network is mapped relative to the networks in which it exists (See, *e.g.*, Figure 18). Figure 18 is a Derivation of Network Ecology Classes, according to an embodiment of the invention. Subsets of networks are selected for use in defining Network Classes based on key biological criteria. Hierarchical Network clusters are defined by the presence (white) and absence (blue (or dark color)) of OTUs and/or Functional Metabolic Pathways and Classes are defined as branches of the hierarchical clustering tree based on the topological overlap measure.

[0298] Both OTUs comprising the network ecologies and the network ecologies themselves are ordered using a dendrogram that represents the relatedness of each OTU to every other OTU, or each Network Ecology to every other Network Ecology. The dendrogram for OTUs can be constructed using various clustering algorithms including but not limited to phylogenetic maximum likelihood, hierarchical clustering, or k-means clustering. In one embodiment, each row in the heatmap represents a single OTU, each column represents a Network Ecology and the color in the heatmap at a given row/column intersection represents whether the given OTU is present or absent in the given network. In another embodiment, the color in the heatmap represents the summed number of occurrences of the OTU in a set of related networks, represented as a cluster in the dendrogram of network ecologies. In another embodiment, the row and column intersections represent a summary variable calculated from the collapse of multiple rows and/or columns at selected nodes in the dendrograms. Network Classes are defined finding significant branch points in the hierarchical dendrogram. In one embodiment these branch points are defined as branches of the hierarchical clustering tree based on the topological overlap measure; this measure is a highly robust measure of network interconnectedness (Langfelder P, Zhang B, Horvath S. 2008. Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. Bioinformatics 24: 719–720.). Network Classes are defined based on OTU presence/absence or presence/absence and frequency patterns in network clusters; these patterns can be defined using specific OTUs, taxonomic groupings, or phylogenetic clades defined by the phylogenetically derived dendrogram (*i.e.* phylogenetic tree).

Network Classes can be defined with the intent of maximizing the phylogenetic diversity of the class, and/or representing specific regions of functional relevance on the phylogenetic tree.
We defined a set of Network Classes for the Network Ecologies reported in Table 8 that were computationally inferred from health and disease datasets tied to CDAD studies using the method described above. We defined six Network Classes for these network ecologies (Figure 18 and Tables 12-13).

Example 7. Biologically-Informed Optimization of Network Ecologies Based on Biological Properties

[0299] Network Ecologies can be optimized to have specific biological properties including but not limited to being of specific size (as example a specific number or OTUs); having a frequency of being observed in a population of healthy individuals (*i.e.* pervasiveness); having a certain percentage of spore forming OTUs as constituents; having a certain percentage of Keystone OTUs, clades or functions; having a defined phylogenetic breadth (as example defined by the total evolutionary distance spanned on a tree by the constituent members, or by the total number of genera or other taxonomic unit); or comprising specific functional capabilities such as but not limited to the ability metabolize secondary bile acids, or produce short chain fatty acids (SCFAs), or the biological intersection in which network ecology falls in a comparative phenotype map (see Figure 19). The constituents of a network ecology can be optimized using both computational means as well as experimental means.

[0300] In one embodiment, we developed a biopriority score for networks that was computationally derived. This algorithm took the form of [F1 * W1] + [F2 * W2] + [F3 * W3] + [F4 * W4] where F is a biological criteria of interest and W is a weighting for that factor based on its importance to the derivation of the target network ecology. As example, if having a network with phylogenetic breadth was important one would weight this factor greater than the other factors. We developed a biopriority score that took into consideration the biological intersection of the network (Figure 19), phylogenetic breadth, the pervasiveness or prevalence of the network in populations of healthy individuals, and the percentage of OTUs in the network that were Keystone OTUs. Network Ecologies reported in Table 8 were ranked based on this scoring and networks with a high score were preferentially screened and in vivo mouse model of C. difficile infection (Table 16).

[0301] In another embodiment we used a phylogenetic method paired with empirical testing to optimize the network ecologies for efficacy for the treatment of CDAD. Based on computational insights from our network analysis (Table 8), applicants defined Keystone Clades that represent specific phylogenetic clusters of OTUs. Applicants constructed various bacterial compositions using the methods described in Example 9 below, whereby applicants varied the phylogenetic breadth of the network ecologies based on the inclusion or exclusion of OTUs from specific clades. To test the effect of these variations on efficacy, 11 networks that feature clade

substitutions, additions, or subtractions were tested at the same target dose of 1e7 CFU per OTU per animal in the mouse model of C. difficile infection experiment SP-376 (see Example 13 and Table 16). Figure 3 provides an overview of the various clade substitutions or removals

[0302] The removal of clades 494 & 537 and the addition of clade 444 from network N1962, which was highly efficacious in protecting from symptoms of C. difficile infection with no mortality, yields network N1991, which was still largely protective of weight loss, but had increased mean maximum clinical scores relative to N1962.

[0303] N1990 adds clades 444 & 478 to N1962, and resulted in decreased mean minimum relative weight and increased mean maximum clinical scores relative to N1962 while remaining efficacious relative to the experiment's vehicle control.

[0304] Removal of clades 252 & 253 and the addition of clades 444 & 478 from N1962 produces N1975, which has increased mortality, decreased mean minimum relative weight and increased mean maximum clinical scores relative to N1962, which is only slightly less efficacious than the vehicle control.

[0305] The optimization of network ecologies to design microbiome therapeutics (as example a composition comprised of bacterial OTUs) with particular biological properties and features is executed using the strategy of having a core Backbone Network Ecology onto which R-Groups are added or subtracted to design toward particular characteristics. The Backbone forms a foundational composition of organisms or functions that are core to efficacy and need be present to observe efficacy. On this backbone one can make various compositional modifications using R-groups. R-Groups can be defined in multiple terms including but not limited to: individual OTUs, individual or multiple OTUs derived from a specific phylogenetic clade, and functional modalities comprised of multiple functional pathways and/or specific metabolic pathways. In other embodiments, R-groups could be considered prebiotics and other co-factors that are design into, or administered with a network ecology to promote specific biological properties.

Example 8. Network Analysis Across Multiple Data Sets and Selection of Target Network Ecologies with Capacity to Sporulate

[0306] One can select Network Ecologies and/or Network Class Ecologies as lead targets by defining networks with a specific biological function or activity such as sporulation. Networks Ecologies or Network Class Ecologies are first selected as described above and in Example 5 and 6. In one example, all Network Ecologies or Network Class Ecologies that contain at least one OTU that is capable of forming spores are targeted. In another example, all Network Ecologies or Network Class Ecologies that contain at least one OTU that is capable of forming spores, and that are comprised of at least 50%, 75%, or 100% Keystone OTUs are targeted. Keystone OTUs are selected as described above and in Example 6. OTUs are defined as spore formers using either phenotypic assays (see *e.g.* Stackebrandt and Hippe. Taxonomy and Systematics. In Clostridia. Biotechnology

and Medical Applications.) or genetic assays (see *e.g.* Abecasis AB, Serrano M, Alves R, Quintais L, Pereira-Leal JB, and Henriques AO. 2013. A genomic signature and the identification of new sporulation genes. J. Bacteriol.; Paredes-Sabja D, Setlow P, and Sarker MR. 2011. Germination of spores of Bacillales and Clostridiales species: mechanisms and proteins involved. Trends Microbiol. 19: 85–94). Exemplary network ecologies that are comprised of spore formers are illustrated in Table11.

Example 9. Construction of Defined Ecobiotic Compositions

[0307] Source of Microbial Cultures. Pure cultures of organisms are isolated from the stool, oral cavity or other niche of the body of clinically qualified donors (as in Example 10) that contains microorganisms of interest using microbiological methods including those described below, and as are known to those skilled in the art. Alternatively, pure cultures are sourced from repositories such as the ATCC (www.atcc.org) or the DSMZ (https://www.dsmz.de/) which preserve and distribute cultures of bacteria, yeasts, phages, cell lines and other biological materials.

[0308] Enrichment and Purification of Bacteria. To purify individual bacterial strains, dilution plates were selected in which the density enables distinct separation of single colonies. Colonies were picked with a sterile implement (either a sterile loop or toothpick) and re-streaked to BBA or other solid media. Plates were incubated at 37°C for 3-7 days. One or more well-isolated single colonies of the major morphology type were re-streaked. This process was repeated at least three times until a single, stable colony morphology is observed. The isolated microbe was then cultured anaerobically in liquid media for 24 hours or longer to obtain a pure culture of 106-1010 cfu/ml. Liquid growth medium might include Brain Heart Infusion-based medium (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract, hemin, cysteine, and carbohydrates (for example, maltose, cellobiose, soluble starch) or other media described previously. The culture was centrifuged at 10,000 x g for 5 min to pellet the bacteria, the spent culture media was removed, and the bacteria were resuspended in sterile PBS. Sterile 75% glycerol was added to a final concentration of 20%. An aliquot of glycerol stock was titered by serial dilution and plating. The remainder of the stock was frozen on dry ice for 10-15 min and then placed at -80°C for long term storage.

Cell Bank Preparation

[0309] Cell banks (RCBs) of bacterial strains were prepared as follows. Bacterial strains were struck from -80°C frozen glycerol stocks to Brucella blood agar with Hemin or Vitamin K (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010), M2GSC (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) or other solid growth media and incubated for 24 to 48 h at 37°C in an anaerobic chamber with a gas mixture of H2:CO2:N2 of 10:10:80. Single colonies were then picked and used to inoculate 250 ml to 1 L of Wilkins-Chalgren broth, Brain-Heart Infusion broth, M2GSC broth or other growth media, and grown to mid to late exponential phase or into the

stationary phase of growth. Alternatively, the single colonies may be used to inoculate a pilot culture of 10 ml, which were then used to inoculate a large volume culture. The growth media and the growth phase at harvest were selected to enhance cell titer, sporulation (if desired) and phenotypes that might be associated desired in vitro or in vivo. Optionally, cultures were grown static or shaking, depending which yielded maximal cell titer. The cultures were then concentrated 10 fold or more by centrifugation at 5000 rpm for 20 min, and resuspended in sterile phosphate buffered saline (PBS) plus 15% glycerol. 1 ml aliquots were transferred into 1.8 ml cryovials which were then frozen on dry ice and stored at -80°C. The identity of a given cell bank was confirmed by PCR amplification of the 16S rDNA gene, followed by Sanger direct cycle sequencing. See Examples 1, 2. Each bank was confirmed to yield colonies of a single morphology upon streaking to Brucella blood agar or M2GSC agar. When more than one morphology was observed, colonies were confirmed to be the expected species by PCR and sequencing analysis of the 16S rDNA gene. Variant colony morphologies can be observed within pure cultures, and in a variety of bacteria the mechanisms of varying colony morphologies have been well described (van der Woude, Clinical Microbiology Reviews, 17:518, 2004), including in Clostridium species (Wadsworth-KTL Anaerobic Bacteriology Manual, 6th Ed, Jousimie-Somer, et al 2002). For obligate anaerobes, RCBs were confirmed to lack aerobic colony forming units at a limit of detection of 10 cfu/ml.

Titer Determination

[0310] The number of viable cells per ml was determined on the freshly harvested, washed and concentrated culture by plating serial dilutions of the RCB to Brucella blood agar or other solid media, and varied from 106 to 1010 cfu/ml. The impact of freezing on viability was determined by titering the banks after one or two freeze-thaw cycles on dry ice or at -80°C, followed by thawing in an anaerobic chamber at room temperature. Some strains displayed a 1-3 log drop in viable cfu/ml after the 1st and/or 2nd freeze thaw, while the viability of others were unaffected.

Preparation of Bacterial Compositions

[0311] Individual strains were typically thawed on ice and combined in an anaerobic chamber to create mixtures, followed by a second freeze at -80°C to preserve the mixed samples. When making combinations of strains for in vitro or in vivo assays, the cfu in the final mixture was estimated based on the second freeze-thaw titer of the individual strains. For experiments in rodents, strains may be combined at equal counts in order to deliver between 1e4 and 1e10 per strain. Additionally, some bacteria may not grow to sufficient titer to yield cell banks that allowed the production of compositions where all bacteria were present at 1e10.

Selection of Media for Growth

[0312] Provided are appropriate media to support growth, including preferred carbon sources. For example, some organisms prefer complex sugars such as cellobiose over simple sugars. Examples of media used in the isolation of sporulating organisms include EYA, BHI, BHIS, and GAM (see below

for complete names and references). Multiple dilutions are plated out to ensure that some plates will have well isolated colonies on them for analysis, or alternatively plates with dense colonies may scraped and suspended in PBS to generate a mixed diverse community.

[0313] Plates are incubated anaerobically or aerobically at 37°C for 48-72 or more hours, targeting anaerobic or aerobic spore formers, respectively.

[0314] Solid plate media include:

- Gifu Anaerobic Medium (GAM, Nissui) without dextrose supplemented with fructooligosaccharides/inulin (0.4%), mannitol (0.4%), inulin (0.4%), or fructose (0.4%), or a combination thereof.
- Sweet GAM [Gifu Anaerobic Medium (GAM, Nissui)] modified, supplemented with glucose, cellobiose, maltose, L-arabinose, fructose, fructooligosaccharides/inulin, mannitol and sodium lactate)
- Brucella Blood Agar (BBA, Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)
- PEA sheep blood (Anaerobe Systems; 5% Sheep Blood Agar with Phenylethyl Alcohol)
- Egg Yolk Agar (EYA) (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)
- Sulfite polymyxin milk agar (Mevissen-Verhage et al., J. Clin. Microbiol. 25:285-289 (1987))
- Mucin agar (Derrien et al., IJSEM 54: 1469-1476 (2004))
- Polygalacturonate agar (Jensen & Canale-Parola, Appl. Environ. Microbiol. 52:880-997 (1986))
- M2GSC (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010)
- M2 agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with starch (1%), mannitol (0.4%), lactate (1.5g/L) or lactose (0.4%)
- Sweet B - Brain Heart Infusion agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract (0.5%), hemin, cysteine (0.1%), maltose (0.1%), cellobiose (0.1%), soluble starch (sigma, 1%), MOPS (50mM, pH 7).
- PY-salicin (peptone-yeast extract agar supplemented with salicin) (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010).
- Modified Brain Heart Infusion (M-BHI) [[sweet and sour]] contains the following per L: 37.5g Brain Heart Infusion powder (Remel), 5g yeast extract, 2.2g meat extract, 1.2g liver extract, 1g cystein HCl, 0.3g sodium thioglycolate, 10mg hemin, 2g soluble starch, 2g FOS/Inulin, 1g cellobiose, 1g L-arabinose, 1g mannitol, 1 Na-lactate, 1mL Tween 80, 0.6g $MgSO_4x7H_2O$, 0.6g $CaCl_2$, 6g $(NH_4)_2SO_4$, 3g KH_2PO_4, 0.5g K_2HPO_4, 33mM Acetic acid, 9mM propionic acid, 1mM Isobutyric acid, 1mM isovaleric acid, 15g agar, and after autoclaving add 50mL of 8% $NaHCO_3$ solution and 50mL 1M MOPS-KOH (pH 7).

- Noack-Blaut Eubacterium agar (See Noack et al. J. Nutr. (1998) 128:1385-1391)
- BHIS az1/ge2 - BHIS az/ge agar (Reeves et. al. Infect. Immun. 80:3786-3794 (2012)) [Brain Heart Infusion agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract 0.5%, cysteine 0.1%, 0.1% cellobiose, 0.1% inulin, 0.1% maltose, aztreonam 1 mg/L, gentamycin 2 mg/L]
- BHIS CInM az1/ge2- BHIS CInM [Brain Heart Infusion agar (Atlas, Handbook of Microbiological Media, 4th ed, ASM Press, 2010) supplemented with yeast extract 0.5%, cysteine 0.1%, 0.1% cellobiose, 0.1% inulin, 0.1% maltose, aztreonam 1 mg/L, gentamycin 2 mg/L].

Method of Preparing the Bacterial Composition for Administration to a Patient

[0315] Two strains for the bacterial composition are independently cultured and mixed together before administration. Both strains are independently be grown at 37°C, pH 7, in a GMM or other animal-products-free medium, pre-reduced with 1 g/L cysteineŸHCl. After each strain reaches a sufficient biomass, it is preserved for banking by adding 15% glycerol and then frozen at -80°C in 1 ml cryotubes.

[0316] Each strain is then be cultivated to a concentration of 1010 CFU/mL, then concentrated 20-fold by tangential flow microfiltration; the spent medium is exchanged by diafiltering with a preservative medium consisting of 2% gelatin, 100 mM trehalose, and 10 mM sodium phosphate buffer, or other suitable preservative medium. The suspension is freeze-dried to a powder and titrated.

[0317] After drying, the powder is blended with microcrystalline cellulose and magnesium stearate and formulated into a 250 mg gelatin capsule containing 10 mg of lyophilized powder (108 to 1011 bacteria), 160 mg microcrystalline cellulose, 77.5 mg gelatin, and 2.5 mg magnesium stearate.

Example 10. Construction and Administration of an Ethanol-treated spore Preparation

[0318] Provision of fecal material. Fresh fecal samples were obtained from healthy human donors who have been screened for general good health and for the absence of infectious diseases, and meet inclusion and exclusion criteria, inclusion criteria include being in good general health, without significant medical history, physical examination findings, or clinical laboratory abnormalities, regular bowel movements with stool appearance typically Type 2, 3, 4, 5 or 6 on the Bristol Stool Scale, and having a BMI $\geq$ 18 kg/m2 and $\leq$ 25 kg/m2. Exclusion criteria generally included significant chronic or acute medical conditions including renal, hepatic, pulmonary, gastrointestinal, cardiovascular, genitourinary, endocrine, immunologic, metabolic, neurologic or hematological disease, a family history of, inflammatory bowel disease including Crohn's disease and ulcerative colitis, Irritable bowel syndrome, colon, stomach or other gastrointestinal malignancies, or gastrointestinal polyposis syndromes, or recent use of yogurt or commercial probiotic materials in

which an organism(s) is a primary component. Non-related donors were screened for general health history for absence of chronic medical conditions (including inflammatory bowel disease; irritable bowel syndrome; Celiac disease; or any history of gastrointestinal malignancy or polyposis), absence of risk factors for transmissible infections, antibiotic non-use in the previous 6 months, and negative results in laboratory assays for blood-borne pathogens (HIV, HTLV, HCV, HBV, CMV, HAV and Treponema pallidum) and fecal bacterial pathogens (Salmonella, Shigella, Yersinia, Campylobacter, E. coli 0157), ova and parasites, and other infectious agents (Giardia, Cryptosporidium, Cyclospora, Isospora) prior to stool donation. Samples were collected directly using a commode specimen collection system, which contains a plastic support placed on the toilet seat and a collection container that rests on the support. Feces were deposited into the container, and the lid was then placed on the container and sealed tightly. The sample was then delivered on ice within 1-4 hours for processing. Samples were mixed with a sterile disposable tool, and 2-4 g aliquots were weighed and placed into tubes and flash frozen in a dry ice/ethanol bath. Aliquots are frozen at -80 degrees Celsius until use.

[0319] Optionally, the fecal material was suspended in a solution, and/or fibrous and/or particulate materials were removed using either filtration or centrifugation. A frozen aliquot containing a known weight of feces was removed from storage at -80°C and allowed to thaw at room temperature. Sterile 1x PBS was added to create a 10% w/v suspension, and vigorous vortexing was performed to suspend the fecal material until the material appeared homogeneous. The material was then left to sit for 10 minutes at room temperature to sediment fibrous and particulate matter. The suspension above the sediment was then carefully removed into a new tube and contains a purified spore population. Optionally, the suspension was then centrifuged at a low speed, *e.g.*, 1000 x g, for 5 minutes to pellet particulate matter including fibers. The pellet was discarded and the supernatant, which contained vegetative organisms and spores, was removed into a new tube. The supernatant was then centrifuged at 6000 x g for 10 minutes to pellet the vegetative organisms and spores. The pellet was then resuspended in 1x PBS with vigorous vortexing until the material appears homogenous.

Generation of a Spore Preparation From Alcohol Treatment of Fecal Material

[0320] A 10% w/v suspension of human fecal material in PBS was filtered, centrifuged at low speed, and the supernate containing spores was mixed with absolute ethanol in a 1:1 ratio and vortexed to mix. The suspension was incubated at room temperature for 1 hour. After incubation the suspension was centrifuged at high speed to concentrate spores into a pellet containing a purified spore-containing preparation. The supernate was discarded and the pellet resuspended in an equal mass of glycerol, and the purified spore preparation was placed into capsules and stored at -80 degrees Celsius.

Characterization of Spores Content in Purified Spore Populations

[0321] In one embodiment, counts of viable spores are determined by performing 10 fold serial dilutions in PBS and plating to Brucella Blood Agar Petri plates or applicable solid media. Plates are

incubated at 37 degrees Celsius for 2 days. Colonies are counted from a dilution plate with 50-400 colonies and used to back-calculate the number of viable spores in the population. The ability to germinate into vegetative bacteria is also demonstrated. Visual counts are determined by phase contrast microscopy. A spore preparation is either diluted in PBS or concentrated by centrifugation, and a 5 microliter aliquot is placed into a Petroff Hauser counting chamber for visualization at 400x magnification. Spores are counted within ten 0.05 mm x 0.05 mm grids and an average spore count per grid is determined and used to calculate a spore count per ml of preparation. Lipopolysaccharide (LPS) reduction in purified spore populations is measured using a Limulus amebocyte lysate (LAL) assay such as the commercially available ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript, Piscataway, NJ) or other standard methods known to those skilled in the art.

[0322] In a second embodiment, counts of spores are determined using a spore germination assay. Germinating a spore fraction increases the number of viable spores that will grow on various media types. To germinate a population of spores, the sample is moved to the anaerobic chamber, resuspended in prereduced PBS, mixed and incubated for 1 hour at 37°C to allow for germination. Germinants can include amino-acids (*e.g.*, alanine, glycine), sugars (*e.g.*, fructose), nucleosides (*e.g.*, inosine), bile salts (*e.g.*, cholate and taurocholate), metal cations (*e.g.*, Mg2+, Ca2+), fatty acids, and long-chain alkyl amines (*e.g.*, dodecylamine, Germination of bacterial spores with alkyl primary amines" J. Bacteriology, 1961.). Mixtures of these or more complex natural mixtures, such as rumen fluid or Oxgall, can be used to induce germination. Oxgall is dehydrated bovine bile composed of fatty acids, bile acids, inorganic salts, sulfates, bile pigments, cholesterol, mucin, lecithin, glycuronic acids, porphyrins, and urea. The germination can also be performed in a growth medium like prereduced BHIS/oxgall germination medium, in which BHIS (Brain heart infusion powder (37 g/L), yeast extract (5 g/L), L-cysteine HCl (1 g/L)) provides peptides, amino acids, inorganic ions and sugars in the complex BHI and yeast extract mixtures and Oxgall provides additional bile acid germinants.

[0323] In addition, pressure may be used to germinate spores. The selection of germinants can vary with the microbe being sought. Different species require different germinants and different isolates of the same species can require different germinants for optimal germination. Finally, it is important to dilute the mixture prior to plating because some germinants are inhibitory to growth of the vegetative-state microorganisms. For instance, it has been shown that alkyl amines must be neutralized with anionic lipophiles in order to promote optimal growth. Bile acids can also inhibit growth of some organisms despite promoting their germination, and must be diluted away prior to plating for viable cells.

[0324] For example, BHIS/oxgall solution is used as a germinant and contains 0.5X BHIS medium with 0.25% oxgall (dehydrated bovine bile) where 1x BHIS medium contains the following per L of solution: 6g Brain Heart Infusion from solids, 7g peptic digest of animal tissue, 14.5g of pancreatic

digest of casein, 5g of yeast extract, 5g sodium chloride, 2g glucose, 2.5g disodium phosphate, and 1g cysteine. Additionally, Ca-DPA is a germinant and contains 40mM CaCl2, and 40mM dipicolinic acid (DPA). Rumen fluid (Bar Diamond, Inc.) is also a germinant. Simulated gastric fluid (Ricca Chemical) is a germinant and is 0.2% (w/v) Sodium Chloride in 0.7% (v/v) Hydrochloric Acid. Mucin medium is a germinant and prepared by adding the following items to 1L of distilled sterile water: 0.4 g KH2PO4, 0.53 g Na2HPO4, 0.3 g NH4Cl, 0.3 g NaCl, 0.1 g MgCl2 x 6H2O, 0.11 g CaCl2, 1 ml alkaline trace element solution, 1 ml acid trace element solution, 1 ml vitamin solution, 0.5 mg resazurin, 4 g NaHCO3, 0.25 g Na2S x 9 H2O. The trace element and vitamin solutions prepared as described previously (Stams et al., 1993). All compounds were autoclaved, except the vitamins, which were filter-sterilized. The basal medium was supplemented with 0.7% (v/v) clarified, sterile rumen fluid and 0.25% (v/v) commercial hog gastric mucin (Type III; Sigma), purified by ethanol precipitation as described previously (Miller & Hoskins, 1981). This medium is referred herein as mucin medium.

[0325] Fetal Bovine Serum (Gibco) can be used as a germinant and contains 5% FBS heat inactivated, in Phosphate Buffered Saline (PBS, Fisher Scientific) containing 0.137M Sodium Chloride, 0.0027M Potassium Chloride, 0.0119M Phosphate Buffer. Thioglycollate is a germinant as described previously (Kamiya et al Journal of Medical Microbiology 1989) and contains 0.25M (pH10) sodium thioglycollate. Dodecylamine solution containing 1mM dodecylamine in PBS is a germinant. A sugar solution can be used as a germinant and contains 0.2% fructose, 0.2% glucose, and 0.2% mannitol. Amino acid solution can also be used as a germinant and contains 5mM alanine, 1mM arginine, 1mM histidine, 1mM lysine, 1mM proline, 1mM asparagine, 1mM aspartic acid, 1mM phenylalanine. A germinant mixture referred to herein as Germix 3 can be a germinant and contains 5mM alanine, 1mM arginine, 1mM histidine, 1mM lysine, 1mM proline, 1mM asparagine, 1mM aspartic acid, 1mM phenylalanine, 0.2%taurocholate, 0.2% fructose, 0.2% mannitol, 0.2% glucose, 1mM inosine, 2.5mM Ca-DPA, and 5mM KCl. BHIS medium + DPA is a germinant mixture and contains BHIS medium and 2mM Ca-DPA. Escherichia coli spent medium supernatant referred to herein as EcSN is a germinant and is prepared by growing E. coli MG1655 in SweetB/Fos inulin medium anaerobically for 48 hr, spinning down cells at 20,000rcf for 20 minutes, collecting the supernatant and heating to 60C for 40 min. Finally, the solution is filter sterilized and used as a germinant solution.

Determination of Bacterial Pathogens In Purified Spore Populations

[0326] Bacterial pathogens present in a purified spore population are determined by qPCR using specific oligonucleotide primers as follows.

Standard Curve Preparation

[0327] The standard curve is generated from wells containing the pathogen of interest at a known concentration or simultaneously quantified by selective spot plating. Serial dilutions of duplicate

cultures are performed in sterile phosphate-buffered saline. Genomic DNA is then extracted from the standard curve samples along with the other samples.

Genomic DNA Extraction

[0328] Genomic DNA may be extracted from 100 µl of fecal samples, fecal-derived samples, or purified spore preparations using the Mo Bio Powersoil®-htp 96 Well Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) according to the manufacturer's instructions with two exceptions: the beadbeating is performed for 2 x 4:40 minutes using a BioSpec Mini-Beadbeater-96 (BioSpec Products, Bartlesville, OK) and the DNA is eluted in 50 µl of Solution C6. Alternatively the genomic DNA could be isolated using the Mo Bio Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA), the Sigma-Aldrich Extract-N-Amp™Plant PCR Kit, the QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions.

qPCR Composition and Conditions

[0329] The qPCR reaction to detect C. difficile contains 1x HotMasterMix (5PRIME, Gaithersburg, MD), 900 nM of Wr-tcdB-F (AGCAGTTGAATATAGTGGTTTAGTTAGAGTTG, IDT, Coralville, IA), 900 nM of Wr-tcdB-R (CATGCTTTTTTAGTTTCTGGATTGAA, IDT, Coralville, IA), 250 nM of We-tcdB-P (6FAM-CATCCAGTCTCAATTGTATATGTTTCTCCA-MGB, Life Technologies, Grand Island, NY), and PCR Water (Mo Bio Laboratories, Carlsbad, CA) to 18 µl (Primers adaped from: Wroblewski, D. et al. Rapid Molecular Characterization of Clostridium difficile and Assessment of Populations of C. difficile in Stool Specimens. Journal of Clinical Microbiology 47:2142–2148 (2009)). This reaction mixture is aliquoted to wells of a MicroAmp® Fast Optical 96-well Reaction Plate with Barcode (0.1mL) (Life Technologies, Grand Island, NY). To this reaction mixture, 2 µl of extracted genomic DNA is added. The qPCR is performed on a BioRad C1000™ Thermal Cycler equipped with a CFX96™ Real-Time System (BioRad, Hercules, CA). The thermocycling conditions are 95°C for 2 minutes followed by 45 cycles of 95°C for 3 seconds, 60°C for 30 seconds, and fluorescent readings of the FAM and ROX channels. Other bacterial pathogens can be detected by using primers and a probe specific for the pathogen of interest.

Data Analysis

[0330] The Cq value for each well on the FAM channel is determined by the CFX Manager™ Software Version 2.1. The log10(cfu/ml) of each experimental sample is calculated by inputting a given sample's Cq value into linear regression model generated from the standard curve comparing the Cq values of the standard curve wells to the known log10(cfu/ml) of those samples. Viral pathogens present in a purified spore population are determined by qPCR as described herein and otherwise known in the art.

Example 11. Characterization of Microbiome in Ethanol-treated spore population and Patients Post-Treatment

Microbial Population Engraftment, Augmentation, and Reduction of Pathogen Carriage in Patients Treated with Spore Compositions.

[0331] Complementary genomic and microbiological methods were used to characterize the composition of the microbiota from Patient 1, 2, 3, 4, and 5, 6, 7, 8, 9, and 10 at pretreatment (pretreatment) and up to 4 weeks post-treatment.

[0332] Table 3 shows bacterial OTUs associated with engraftment and ecological augmentation and establishment of a more diverse microbial ecology in patients treated with an ethanol-treated spore preparation. OTUs that comprise an augmented ecology are below the limit of detection in the patient prior to treatment and/or exist at extremely low frequencies such that they do not comprise a significant fraction of the total microbial carriage and are not detectable by genomic and/or microbiological assay methods in the bacterial composition. OTUs that are members of the engrafting and augmented ecologies were identified by characterizing the OTUs that increase in their relative abundance post treatment and that respectively are: (i) present in the ethanol-treated spore preparation and not detectable in the patient pretreatment (engrafting OTUs), or (ii) absent in the ethanol-treated spore preparation, but increase in their relative abundance in the patient through time post treatment with the preparation due to the formation of favorable growth conditions by the treatment (augmenting OTUs). Notably, the latter OTUs can grow from low frequency reservoirs in the patient, or be introduced from exogenous sources such as diet. OTUs that comprise a "core" augmented or engrafted ecology can be defined by the percentage of total patients in which they are observed to engraft and/or augment; the greater this percentage the more likely they are to be part of a core ecology responsible for catalyzing a shift away from a dysbiotic ecology. The dominant OTUs in an ecology can be identified using several methods including but not limited to defining the OTUs that have the greatest relative abundance in either the augmented or engrafted ecologies and defining a total relative abundance threshold. As example, the dominant OTUs in the augmented ecology of Patient-1 were identified by defining the OTUs with the greatest relative abundance, which together comprise 60% of the microbial carriage in this patient's augmented ecology by day 25 post-treatment..

[0333] Patient treatment with the ethanol-treated spore preparation leads to the population of a microbial ecology that has greater diversity than prior to treatment (See Figure 5 & 6). Genomic-based microbiome characterization confirmed engraftment of a range of OTUs that were not detectable in the patient pretreatment (Table 3). These OTUs comprised both bacterial species that were capable and not capable of forming spores, and OTUs that represent multiple phylogenetic clades. Organisms not detectable in Patient 1 pre-treatment either engraft directly from the ethanol-treated spore fraction or are augmented by the creation of a gut environment favoring a healthy,

diverse microbiota. Microbiological analysis shows that Bacteroides fragilis group species were increased by 4 and 6 logs in patients 1 and 2 (Figure 7).

[0334] Figure 5 shows the microbial diversity measured in the ethanol-treated spore treatment sample and patient pre- and post-treatment samples. Total microbial diversity is defined using the Chao1 Alpha-Diversity Index and is measured at different genomic sampling depths to confirm adequate sequence coverage to assay the microbiome in the target samples. The patient pretreatment (purple) harbored a microbiome that was significantly reduced in total diversity as compared to the ethanol-treated spore product (red) and patient post treatment at days 5 (blue), 14 (orange), and 25 (green).

[0335] Figure 6 shows patient microbial ecology is shifted by treatment with an ethanol-treated spore treatment from a dysbiotic state to a state of health. Principal Coordinates Analysis based on the total diversity and structure of the microbiome (Bray-Curtis Beta-Diversity) of the patient pre- and post-treatment delineates that the engraftment of OTUs from the spore treatment and the augmentation of the patient microbial ecology leads to a microbial ecology that is distinct from both the pretreatment microbiome and the ecology of the ethanol-treated spore treatment (Table 3).

[0336] Figure 7 shows the augmentation of Bacteroides species in patients. Comparing the number of Bacteroides fragilis groups species in feces (cfu/g) pre-treatment and in week 4 post treatment reveals an increase of 4 logs or greater. The ability of 16S-V4 OTU identification to assign an OTU as a specific species depends in part on the resolution of the 16S-V4 region of the 16S gene for a particular species or group of species. Both the density of available reference 16S sequences for different regions of the tree as well as the inherent variability in the 16S gene between different species will determine the definitiveness of a taxonomic annotation to a given sequence read. Given the topological nature of a phylogenetic tree and that the tree represents hierarchical relationships of OTUs to one another based on their sequence similarity and an underlying evolutionary model, taxonomic annotations of a read can be rolled up to a higher level using a clade-based assignment procedure (Table 1). Using this approach, clades are defined based on the topology of a phylogenetic tree that is constructed from full-length 16S sequences using maximum likelihood or other phylogenetic models familiar to individuals with ordinary skill in the art of phylogenetics. Clades are constructed to ensure that all OTUs in a given clade are: (i) within a specified number of bootstrap supported nodes from one another (generally, 1-5 bootstraps), and (ii) within a 5% genetic similarity. OTUs that are within the same clade can be distinguished as genetically and phylogenetically distinct from OTUs in a different clade based on 16S-V4 sequence data. OTUs falling within the same clade are evolutionarily closely related and may or may not be distinguishable from one another using 16S-V4 sequence data. The power of clade based analysis is that members of the same clade, due to their evolutionary relatedness, play similar functional roles in a microbial ecology such as that found in the

human gut. Compositions substituting one species with another from the same clade are likely to have conserved ecological function and therefore are useful in the present invention.

[0337] Stool samples were aliquoted and resuspended 10x vol/wt in either 100% ethanol (for genomic characterization) or PBS containing 15% glycerol (for isolation of microbes) and then stored at -80°C until needed for use. For genomic 16S sequence analysis colonies picked from plate isolates had their full-length 16S sequence characterized as described in Examples 2 and 3, and primary stool samples were prepared targeting the 16S-V4 region using the method for heterogeneous samples described herein.

[0338] Notably, 16S sequences of isolates of a given OTU are phylogenetically placed within their respective clades despite that the actual taxonomic assignment of species and genus may suggest they are taxonomically distinct from other members of the clades in which they fall. Discrepancies between taxonomic names given to an OTU is based on microbiological characteristics versus genetic sequencing are known to exist from the literature. The OTUs footnoted in this table are known to be discrepant between the different methods for assigning a taxonomic name.

[0339] Engraftment of OTUs from the ethanol-treated spore preparation treatment into the patient as well as the resulting augmentation of the resident microbiome led to a significant decrease in and elimination of the carriage of pathogenic species other than C. difficile in the patient. 16S-V4 sequencing of primary stool samples demonstrated that at pretreatment, 20% of reads were from the genus Klebsiella and an additional 19% were assigned to the genus Fusobacterium. These striking data are evidence of a profoundly dysbiotic microbiota associated with recurrent C. difficile infection and chronic antibiotic use. In healthy individuals, Klebsiella is a resident of the human microbiome in only about 2% of subjects based on an analysis of HMP database (www.hmpdacc.org), and the mean relative abundance of Klebsiella is only about 0.09% in the stool of these people. Its surprising presence at 20% relative abundance in Patient 1 before treatment is an indicator of a proinflammatory gut environment enabling a "pathobiont" to overgrow and outcompete the commensal organisms normally found in the gut. Similarly, the dramatic overgrowth of Fusobacterium indicates a profoundly dysbiotic gut microbiota. One species of Fusobacterium, F. nucleatum (an OTU phylogenetically indistinguishable from Fusobacterium sp. 3_1_33 based on 16S-V4), has been termed "an emerging gut pathogen" based on its association with IBD, Crohn's disease, and colorectal cancer in humans and its demonstrated causative role in the development of colorectal cancer in animal models [Allen-Vercoe, Gut Microbes (2011) 2:294-8]. Importantly, neither Klebsiella nor Fusobacterium was detected in the 16S-V4 reads by Day 25 (Table 4).

[0340] To further characterize the colonization of the gut by Klebsiella and other Enterobacteriaceae and to speciate these organisms, pretreatment and Day 25 fecal samples stored at -80C as PBS-glycerol suspensions were plated on a variety of selective media including MacConkey lactose media (selective for gram negative enterobacteria) and Simmons Citrate Inositol media

(selective for Klebsiella spp) [Van Cregten et al, J. Clin. Microbiol. (1984) 20: 936-41]. Enterobacteria identified in the patient samples included K. pneumoniae, Klebsiella sp. Co_9935 and E. coli. Strikingly, each Klebsiella species was reduced by 2-4 logs whereas E. coli, a normal commensal organism present in a healthy microbiota, was reduced by less than 1 log (Table 14 below). This decrease in Klebsiella spp. carriage is consistent across multiple patients. Four separate patients were evaluated for the presence of Klebsiella spp. pre treatment and 4 weeks post treatment. Klebsiella spp. were detected by growth on selective Simmons Citrate Inositol media as previously described. Serial dilution and plating, followed by determining cfu/mL titers of morphologically distinct species and 16S full length sequence identification of representatives of those distinct morphological classes, allowed calculation of titers of specific species.

[0341] The genus Bacteroides is an important member of the gastrointestinal microbiota; 100% of stool samples from the Human Microbiome Project contain at least one species of Bacteroides with total relative abundance in these samples ranging from 0.96% to 93.92% with a median relative abundance of 52.67% (www.hmpdacc.org reference data set HMSMCP). Bacteroides in the gut has been associated with amino acid fermentation and degradation of complex polysaccharides. Its presence in the gut is enhanced by diets rich in animal-derived products as found in the typical western diet [David, L. A. et al, Nature (2013) doi:10.1038/nature12820]. Strikingly, prior to treatment, fewer than 0.008% of the 16S-V4 reads from Patient 1 mapped to the genus Bacteroides strongly suggesting that Bacteroides species were absent or that viable Bacteroides were reduced to an extremely minor component of the patient's gut microbiome. Post treatment, ≥42% of the 16S-V4 reads could be assigned to the genus Bacteroides within 5 days of treatment and by Day 25 post treatment 59.48% of the patients gut microbiome was comprised of Bacteroides. These results were confirmed microbiologically by the absence of detectable Bacteroides in the pretreatment sample plated on two different Bacteroides selective media: Bacteroides Bile Esculin (BBE) agar which is selective for Bacteroides fragilis group species [Livingston, S.J.et al J. Clin. Microbiol (1978). 7: 448-453] and Polyamine Free Arabinose (PFA) agar [Noack et al. J. Nutr. (1998) 128: 1385-1391; modified by replacing glucose with arabinose]. The highly selective BBE agar had a limit of detection of <2x103 cfu/g, while the limit of detection for Bacteroides on PFA agar was approximately 2x107 cfu/g due to the growth of multiple non-Bacteroides species in the pretreatment sample on that medium. Colony counts of Bacteroides species on Day 25 were up to 2x1010 cfu/g, consistent with the 16S-V4 sequencing, demonstrating a profound reconstitution of the gut microbiota in Patient 1 (Table 5 below).

[0342] The significant abundance of Bacteroides in Patient 1 on Day 25 (and as early as Day 5 as shown by 16S-V4 sequencing) is remarkable. Viable Bacteroides fragilis group species were not present in the ethanol-treated spore population based on microbiological plating (limit of detection of 10 cfu/ml). Thus, administration of the ethanol-treated spore population to Patient 1 resulted in microbial population of the patient's GI tract, not only due to the engraftment of bacterial species such

as but not limited to spore forming species, but also the restoration of high levels of non-spore forming species commonly found in healthy individuals through the creation of a niche that allowed for the repopulation of Bacteroides species. These organisms were most likely either present at extremely low abundance in the GI tract of Patient 1, or present in a reservoir in the GI tract from which they could rebound to high titer. Those species may also be reinoculated via oral uptake from food following treatment. We term this healthy repopulation of the gut with OTUs that are not present in the bacterial composition such as but not limited to a spore population or ethanol-treated spore population, "Augmentation." Augmentation is an important phenomenon in that it shows the ability to use an ethanol-treated spore ecology or other bacterial composition to restore a healthy microbiota by seeding a diverse array or commensal organisms beyond the actual component organisms in the bacterial composition such as but not limited to an ethanol-treated spore population itself; specifically the spore composition treatment itself and the engraftment of OTUs from the spore composition create a niche that enables the outgrowth of OTUs required to shift a dysbiotic microbiome to a microbial ecology that is associated with health. The diversity of Bacteroides species and their approximate relative abundance in the gut of Patient 1 is shown in Table 16, comprising at least 8 different species.

[0343] Figure 8 shows species engrafting versus species augmenting in patients microbiomes after treatment with a bacterial composition such as but not limited to an ethanol-treated spore population. Relative abundance of species that engrafted or augmented as described were determined based on the number of 16S sequence reads. Each plot is from a different patient treated with the bacterial composition such as but not limited to an ethanol-treated spore population for recurrent C. difficile.

[0344] The impact of the bacterial composition such as but not limited to an ethanol-treated spore population treatment on carriage of imipenem resistant Enterobacteriaceae was assessed by plating pretreatment and Day 28 clinical samples from Patients 2, 4 and 5 on MacConkey lactose plus 1 ug/mL of imipenem. Resistant organisms were scored by morphology, enumerated and DNA was submitted for full length 16S rDNA sequencing as described above. Isolates were identified as Morganella morganii, Providencia rettgeri and Proteus penneri. Each of these are gut commensal organisms; overgrowth can lead to bacteremia and/or urinary tract infections requiring aggressive antibiotic treatment and, in some cases, hospitalization [Kim, B-N, et al Scan J. Inf Dis (2003) 35: 98-103; Lee, I-K and Liu, J-W J. Microbiol Immunol Infect (2006) 39: 328-334; O'Hara et al, Clin Microbiol Rev (2000) 13: 534]. The titer of organisms at pretreatment and Day 28 by patient is shown in Table 17. Importantly, administration of the bacterial composition such as but not limited to an ethanol-treated spore preparation resulted in greater than 100-fold reduction in 4 of 5 cases of Enterobacteriaceae carriage with multiple imipenem resistant organisms (See Table 17 which shows titers (in cfu/g) of imipenem-resistant M. morganii, P. rettgeri and P. penneri from Patients 2, 4 & 5).

[0345] In addition to speciation and enumeration, multiple isolates of each organism from Patient 4 were grown overnight in 96-well trays containing a 2-fold dilution series of imipenem in order to quantitatively determine the minimum inhibitory concentration (MIC) of antibiotic. Growth of organisms was detected by light scattering at 600 nm on a SpectraMax M5e plate reader. In the clinical setting, these species are considered resistant to imipenem if they have an MIC of 1 ug/mL or greater. M. morganii isolates from pretreatment samples from Patient 4 had MICs of 2-4 ug/mL and P. penneri isolates had MICs of 4-8 ug/mL. Thus, the bacterial composition, such as but not limited to, an ethanol-treated spores administered to Patient 4 caused the clearance of 2 imipenem resistant organisms (Table 4). While this example specifically uses a spore preparation, the methods herein describe how one skilled in the art would use a more general bacterial composition to achieve the same effects. The specific example should not be viewed as a limitation of the scope of this disclosure.

Identifying the Core Ecology From the Bacterial Combination

[0346] Ten different bacterial compositions were made by the ethanol-treated spore preparation methods from 6 different donors (as described above). The spore preparations were used to treat 10 patients, each suffering from recurrent C. difficile infection. Donors were identified using the inclusion/exclusion criteria described above under provision of fecal material. None of the patients experienced a relapse of C. difficile in the 4 weeks of follow up after treatment, whereas the literature would predict that 70-80% of subjects would experience a relapse following cessation of antibiotic [Van Nood, et al, NEJM (2013)]. Thus, the ethanol-treated spore preparations derived from multiple different donors and donations showed remarkable clinical efficacy. These ethanol-treated spore preparations are a subset of the bacterial compositions described herein and the results should not be viewed as a limitation on the scope of the broader set of bacterial compositions.

[0347] To define the Core Ecology underlying the remarkable clinical efficacy of the bacterial compositions *e.g.* ethanol-treated spore preparations, the following analysis was carried out. The OTU composition of the spore preparation was determined by 16S-V4 rDNA sequencing and computational assignment of OTUs per Example 2. A requirement to detect at least ten sequence reads in the ethanol-treated spore preparation was set as a conservative threshold to define only OTUs that were highly unlikely to arise from errors during amplification or sequencing. Methods routinely employed by those familiar to the art of genomic-based microbiome characterization use a read relative abundance threshold of 0.005% (see *e.g.* Bokulich, A. et al. 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nature Methods 10: 57-59), which would equate to ≥2 reads given the sequencing depth obtained for the samples analyzed in this example, as cut-off which is substantially lower than the ≥10 reads used in this analysis. All taxonomic and clade assignments were made for each OTU as described in Example 2. The resulting list of OTUs, clade assignments, and frequency of detection in the spore preparations are shown in

Table 18. Table 18 shows OTUs detected by a minimum of ten 16S-V4 sequence reads in at least one ethanol-treated spore preparatio. OTUs that engraft in a treated patients and the percentage of patients in which they engraft are denoted, as are the clades, spore forming status, and Keystone OTU status. Starred OTUs occur in ≥80% of the ethanol preps and engraft in ≥50% of the treated patients.

[0348] Next, it was reasoned that for an OTU to be considered a member of the Core Ecology of the bacterial composition, that OTU must be shown to engraft in a patient. Engraftment is important for two reasons. First, engraftment is a sine qua non of the mechanism to reshape the microbiome and eliminate C. difficile colonization. OTUs that engraft with higher frequency are highly likely to be a component of the Core Ecology of the spore preparation or broadly speaking a set bacterial composition. Second, OTUs detected by sequencing a bacterial composition (as in Table 6 may include non-viable cells or other contaminant DNA molecules not associated with the composition. The requirement that an OTU must be shown to engraft in the patient eliminates OTUs that represent non-viable cells or contaminating sequences. Table 6 also identifies all OTUs detected in the bacterial composition that also were shown to engraft in at least one patient post-treatment. OTUs that are present in a large percentage of the bacterial composition *e.g.* ethanol spore preparations analyzed and that engraft in a large number of patients represent a subset of the Core Ecology that are highly likely to catalyze the shift from a dysbiotic disease ecology to a healthy microbiome.

[0349] A third lens was applied to further refine insights into the Core Ecology of the bacterial composition (*e.g.* spore preparation). Computational-based, network analysis has enabled the description of microbial ecologies that are present in the microbiota of a broad population of healthy individuals (see Example 5). These network ecologies are comprised of multiple OTUs, some of which are defined as Keystone OTUs. Keystone OTUs are computationally defined as described in Example 6. Keystone OTUs form a foundation to the microbially ecologies in that they are found and as such are central to the function of network ecologies in healthy subjects. Keystone OTUs associated with microbial ecologies associated with healthy subjects are often are missing or exist at reduced levels in subjects with disease. Keystone OTUs may exist in low, moderate, or high abundance in subjects. Table 6 further notes which of the OTUs in the bacterial composition *e.g.* spore preparation are Keystone OTUs exclusively associated with individuals that are healthy and do not harbor disease. The presence of computationally derived Keystone OTUs in the Core Ecology of the doses validates the predictive capacity of computationally derived network ecologies.

[0350] There are several important findings from this data. A relatively small number of species, 16 in total, are detected in all of the spore preparations from 6 donors and 10 donations. This is surprising because the HMP database (www.hmpdacc.org) describes the enormous variability of commensal species across healthy individuals. The presence of a small number of consistent OTUs lends support to the concept of a Core Ecology and Backbone Networks. The engraftment data further supports this conclusion. A regression analysis shows a significant correlation between

frequency of detection in a spore preparation and frequency of engraftment in a donor: $R = 0.43$ ($p < 0.001$). While this may seem obvious, there is no a priori requirement that an OTU detected frequently in the bacterial composition *e.g.* spore preparation will or should engraft. For instance, Lutispora thermophila, a spore former found in all ten spore preparations, did not engraft in any of the patients. Bilophila wadsworthia, a gram negative anaerobe, is present in 9 of 10 donations, yet it does not engraft in any patient, indicating that it is likely a non-viable contaminant in the ethanol-treated spore preparation. Finally, it is worth noting the high preponderance of previously defined Keystone OTUs among the most frequent OTUs in the spore preparations.

[0351] These three factors--prevalence in the bacterial composition such as but not limited to a spore preparation, frequency of engraftment, and designation as a Keystone OTUs--enabled the creation of a "Core Ecology Score" (CES) to rank individual OTUs. CES was defined as follows:

- 40% weighting for presence of OTU in spore preparation
    - multiplier of 1 for presence in 1-3 spore preparations
    - multiplier of 2.5 for presence in 4-8 spore preparations
    - multiplier of 5 for presences in ≥ 9 spore preparations
- 40% weighting for engraftment in a patient
    - multiplier of 1 for engraftment in 1-4 patients
    - multiplier of 2.5 for engraftment in 5-6 patients
    - multiplier of 5 for engraftment in ≥ 7 patients
- 20% weighting to Keystone OTUs
    - multiplier of 1 for a Keystone OTU
    - multiplier of 0 for a non-Keystone OTU

[0352] Using this guide, the CES has a maximum possible score of 5 and a minimum possible score of 0.8. As an example, an OTU found in 8 of the 10 bacterial composition such as but not limited to a spore preparations that engrafted in 3 patients and was a Keystone OTU would be assigned the follow CES:

[0353] $CES = (0.4 \times 2.5) + (0.4 \times 1) + (0.2 \times 1) = 1.6$

[0354] Table 7 ranks the top 20 OTUs by CES with the further requirement that an OTU must be shown to engraft to be a considered an element of a core ecology.

Defining Efficacious Subsets of the Core Ecology

[0355] The number of organisms in the human gastrointestinal tract, as well as the diversity between healthy individuals, is indicative of the functional redundancy of a healthy gut microbiome ecology (see The Human Microbiome Consortia. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486: 207-214). This redundancy makes it highly likely that subsets of the Core Ecology describe therapeutically beneficial components of the bacterial composition such as but not limited to an ethanol-treated spore preparation and that such subsets may

themselves be useful compositions for populating the GI tract and for the treatment of C. difficile infection given the ecologies functional characteristics. Using the CES, individual OTUs can be prioritized for evaluation as an efficacious subset of the Core Ecology.

[0356] Another aspect of functional redundancy is that evolutionarily related organisms (*i.e.* those close to one another on the phylogenetic tree, *e.g.* those grouped into a single clade) will also be effective substitutes in the Core Ecology or a subset thereof for treating C. difficile.

[0357] To one skilled in the art, the selection of appropriate OTU subsets for testing in vitro (see Example 20 below) or in vivo (see Examples 13 or 14) is straightforward. Subsets may be selected by picking any 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 OTUs from Table 6, with a particular emphasis on those with higher CES, such as the OTUs described Table 7. In addition, using the clade relationships defined in Example 2 above and Table 1, related OTUs can be selected as substitutes for OTUs with acceptable CES values. These organisms can be cultured anaerobically in vitro using the appropriate media (selected from those described in Example 5 above), and then combined in a desired ratio. A typical experiment in the mouse C. difficile model utilizes at least 10^4 and preferably at least 10^5, 10^6, 10^7, 10^8, 10^9 or more than 10^9 colony forming units of a each microbe in the composition. Variations in the culture yields may sometimes mean that organisms are combined in unequal ratios, *e.g.* 1:10, 1:100, 1:1,000, 1:10,000, 1:100,000, or greater than 1:100,000. What is important in these compositions is that each strain be provided in a minimum amount so that the strain's contribution to the efficacy of the Core Ecology subset can be measured. Using the principles and instructions described here, it is straightforward for one of skill in the art to make clade-based substitutions to test the efficacy of subsets of the Core Ecology. Table 18 describes the clades for each OTU detected in a spore preparation, and Table 1 describes the OTUs that can be used for substitutions based on clade relationships. Examples of network ecologies empirically screened in vivo are presented in Example 13 below.

Example 12. Presence of Network Ecologies and Keystone OTUs in Clinically prepped Ethanol-treated spore Preparation and CDAD Patients Post Treatment

[0358] Network ecologies computationally determined as described in Example 5 and reported in Table 8 as being networks or subsets of networks characteristic of health states in the context of CDAD or other disease indications (Table 14a-b) are observed in the ethanol-treated spore preparation (a.k.a. the bacterial composition) and the microbiome of patients post treatment (see Example 11) indicating that they play an important role in treatment of CDAD and other indications. For each computationally determined network ecology (Table 8), we determined whether the full network or a subset of the network was observed in the microbiome of (i) each of the 10 ethanol-treated spore preparations used to treat patients with recurrent Clostridium difficile associated diarrhea; (ii) the engrafted ecology of each of the 10 patients (see Example 11); (iii) the augmented ecology of each of the 10 patients (see Example 11); or (iv) of each of the 10 patient's microbiome pretreatment. If the

computationally determined networks are indeed representative of a state of health and not a disease state, one would expect that these networks would be responsible for catalyzing a shift from a disease state to a health state. This can happen either by the network ecology changing the gut environment to favor the growth of OTUs that are required to establish a health state (*i.e.* promoting augmentation) or by the engraftment of OTUs in the bacterial composition or both. Applicants observed that numerous computationally determined networks and/or subsets of these networks were in fact observed both in the bacterial composition used to treat the patients and the microbiota that expanded post-treatment (Table 14b). These same networks or sub-sets of networks were significantly under-represented in the patients pre-treatment. To demonstrate this, we computed the percentage of network OTUs that are found in (i) the treatment bacterial composition, (ii) the post-treatment augmented ecology, (iii) the post-treatment engrafted ecology, and (iv) the pretreatment ecology (*i.e.* patient microbiome prior to administration of the bacterial composition). Applicants observed across all doses of bacterial composition and patient samples that on average 46% ± 19% , 28% ± 14%, 11% ± 8%, and 7% ± 4% of the computed networks OTUs were present in the various microbiome ecologies, respectively (reported here as average ± standard deviation). There was a significant difference ($p<0.0001$, ANOVA) between all of these percentages indicating that prior to treatment, the OTUs found in CDAD patients are significantly under-represented in the networks, and that the network OTUs are significantly over-represented in the bacterial compositions and post-treatment patient samples, affirming the predictive utility of the computational network analysis. These results in combination with those reported in Table 14b demonstrate that, prior to treatment, the patients harbored a significantly lower number of OTUs that comprised network ecologies. In contrast, the ecology of the bacterial composition, as well as the augmenting ecologies whose appearance was catalyzed by the spore population, were significantly overrepresented in patients whose CDAD resolved due to treatment.

[0359] We observed both large and small computationally determined network ecologies characteristic of states of health in the ethanol-treated spore population and the patients post treatment (Table 14a). These observed networks ranged in size from 2-15 OTUs and were comprised of OTUs that represented from 29% to 100% of the OTUs in the computationally determined network ecology. Notably, on average the network ecologies found in the ethanol-treated spore population or the patient ecologies post treatment comprised 72% ± 15% (average ± SD) of the computationally determined network ecology again strongly indicating an important role of the computed network ecologies in catalyzing a shift in a dysbiotic disease ecology to a state of health in these patients with recurrent CDAD. Further, Keystone OTUs in the computationally determined network ecologies were frequently observed in the ethanol-treated spore preparations and in the patients' post-treatment gut ecologies. Clades representing Keystone OTUs where typically more common in the bacterial composition and post-treatment patient ecologies than in the pre-treatment dysbiotic patient ecology (Table 15).

[0360] The computed network ecologies and their respective subsets that are observed in the ethanol-treated spore preparation and the various patient ecologies post-treatment represent both complete and foundational networks (*e.g.*, Backbone Network Ecology). Microbial therapeutics can be comprised of these network ecologies in their entirety, or they can be modified by the addition or subtraction of other OTUs or functional modalities as described in Example 7 and Example 22 to design particular phylogenetic and/or functional characteristics, including metabolic functions such as SCFA production or bile acid metabolism, into the microbial therapeutic.

Example 13. In vivo validation of Network Ecology Bacterial Compositions Efficacy in Clostridium Difficile Infection Prevention Mouse Model

[0361] To test the therapeutic potential of the bacterial composition such as but not limited to a spore population, a prophylactic mouse model of C. difficile infection was used (model based on Chen X, Katchar K, Goldsmith JD, Nanthakumar N, Cheknis A, Gerding DN, Kelly CP. 2008. A mouse model of Clostridium difficile-associated disease. Gastroenterology 135: 1984–1992.). Two cages of five mice each were tested for each arm of the experiment. All mice received an antibiotic cocktail consisting of 10% glucose, kanamycin (0.5 mg/ml), gentamicin (0.044 mg/ml), colistin (1062.5 U/ml), metronidazole (0.269 mg/ml), ciprofloxacin (0.156 mg/ml), ampicillin (0.1 mg/ml) and Vancomycin (0.056 mg/ml) in their drinking water on days -14 through -5 and a dose of 10 mg/kg Clindamycin by oral gavage on day -3. On day -1, test articles are spun for 5 minutes at 12,100 rcf, their supernatants' removed, and the remaining pellets are resuspended in sterile PBS, prereduced if bacterial composition is not in spore form, and delivered via oral gavage. On day 0 they were challenged by administration of approximately 4.5 log10 cfu of C. difficile (ATCC 43255) or sterile PBS (for the Naive arm) via oral gavage. Optionally a positive control group received vancomycin from day -1 through day 3 in addition to the antibiotic protocol and C. difficile challenge specified above. Feces were collected from the cages for analysis of bacterial carriage. Mortality, weight and clinical scoring of C. difficile symptoms based upon a 0-4 scale by combining scores for Appearance (0-2 pts based on normal, hunched, piloerection, or lethargic), and Clinical Signs (0-2 points based on normal, wet tail, cold-to-the-touch, or isolation from other animals) are assessed every day from day -2 through day6. Mean minimum weight relative to day -1 and mean maximum clinical score where a death is assigned a clinical score of 4 as well as average cumulative mortality are calculated. Reduced mortality, increased mean minimum weight relative to day -1, and reduced mean maximum clinical score with death assigned to a score of 4 relative to the vehicle control are used to assess the success of the test article.

[0362] Table 16 reports results for 15 experiments of the prophylactic mouse model of C. difficile infection. In the 15 experiments, 157 of the arms tested network ecologies, with 86 distinct networks ecologies tested (Table 17). Of those 157 arms, 136 of the arms and 73 of the networks performed better than the respective experiment's vehicle control arm by at least one of the following metrics:

cumulative mortality, mean minimum relative weight, and mean maximum clinical score. Examples of efficacious networks include but are not limited to networks N1979 as tested in SP-361 which had 0% cumulative mortality, 0.97 mean minimum relative weight, and 0 mean maximum clinical score or N2007 which had 10% cumulative mortality, 0.91 mean minimum relative weight, and 0.9 mean maximum clinical score with both networks compared to the vehicle control in SP-361 which had 30% cumulative mortality, 0.88 mean minimum relative weight, and 2.4 mean maximum clinical score. In SP-376, N1962 had no cumulative mortality, mean maximum clinical scores of 0 at both target doses tested with mean minimum relative weights of 0.98 and 0.95 for target doses of 1e8 and 1e7 CFU/OTU/mouse respectively. These results confirm that bacterial compositions comprising bacteria identified from computationally determined networks or subsets of these determined networks have utility and efficacy in the mouse model.

Example 14. In Vivo Validation of Network Ecology Bacterial Composition Efficacy in Prophylactic and Relapse Prevention Hamster Model

[0363] Previous studies with hamsters using toxigenic and nontoxigenic strains of C. difficile demonstrated the utility of the hamster model in examining relapse post antibiotic treatment and the effects of prophylaxis treatments with cecal flora in C. difficile infection (Wilson et al. 1981, Wilson et al. 1983, Borriello et al. 1985) and more broadly in gastrointestinal infectious disease. To demonstrate prophylactic use of ethanol-treated spores and ethanol treated, gradient-purified spores to ameliorate C. difficile infection, the following hamster model was used. In the prophylactic model, Clindamycin (10mg/kg s.c.) was given on day -5, the test article or control was administered on day -3, and C. difficile challenge occurred on day 0. In the positive control arm, vancomycin was then administered on day 1-5 (and vehicle control was delivered on day -3). Feces were collected on day -5, -4, -1, 1, 3, 5, 7, 9 and fecal samples were assessed for pathogen carriage and reduction by microbiological methods. 16S sequencing approaches or other methods could also be utilized by one skilled in the art. Mortality was assessed multiple times per day through 21 days post C. difficile challenge. The percentage survival curves showed that ethanol-treated spores and ethanol treated, gradient-purified spores better protected the hamsters compared to the Vancomycin control, and vehicle control.

[0364] Figure 9 shows a prophylaxis model with the ethanol-treated spore preparation and the ethanol treated, gradient-purified spore preparation. In the relapse prevention model, hamsters were challenged with toxigenic C. difficile strains on day 0, and treated with clindamycin by oral gavage on day 1, and vancomycin was dosed on days 2-6. Test or control treatment was then administered on day 7, 8, and 9. The groups of hamsters for each arm consisted of 8 hamsters per group. Fecal material was collected on day -1, 1, 3, 5, 7, 10 and 13 and hamster mortality was assessed throughout. Survival curves were used to assess the efficacy of the test articles, *e.g.*, ethanol treated or ethanol treated, gradient purified spores versus the control treatment in preventing hamster death. The survival

curves demonstrated maximum efficacy for the ethanol treated, gradient-purified spores followed by the ethanol-treated spores. Both treatments improved survival percentage over vancomycin treatment.

[0365] Also in the relapse prevention model, the efficacy of a bacterial community of pure cultures, N1962, was tested. The survival curves demonstrate protection against relapse by N1962 relative to the vancomycin control treatment.

[0366] Figure 10 shows a relapse prevention model with ethanol-treated spores and ethanol treated, gradient purified spores. In particular, it shows an in vivo hamster Clostridium difficile relapse prevention model to validate efficacy of ethanol-treated spores and ethanol treated, gradient purified spores.

[0367] Figure 11 shows a relapse prevention model with a bacterial community. In particular, it shows an in vivo hamster Clostridium difficile relapse prevention model to validate efficacy of network ecology bacterial composition.

Example 15. Derivation of Functional Profile of Individual Microbial OTUs or Consortia of OTUs Representing Specific Network Ecologies

[0368] To generate a functional profile of an OTU, or consortium of OTUs one can leverage multiple -omic data types. These include, but are not limited to functional prediction based on 16S rRNA sequence, functional annotation of metagenomic or full-genome sequences, transcriptomics, and metabolomics. A consortium of OTUs of interest can be defined using numerous criteria including but not limited to: (i) a computationally derived network of OTUs based on the analysis of samples that represent states of health and disease such as those delineated in Example 5 and reported in Table 8, (ii) a consortia of OTUs that are identified in an individual sample or group of samples using either a 16S-based, metagenomic-based, or microbiological-based methods such as delineated in Examples 3, 4 and 16, and (iii) a list of OTUs derived from the assessment of literature.

[0369] For 16S rRNA sequences, phylogenetic investigation of communities by reconstruction of unobserved states, also known as PICRUSt (Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, et al. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol.), enables the prediction of a functional metabolic pathway of an OTU or a consortium of OTUs based on the KEGG database of reference functional pathways and functional ontologies (Kyoto Encyclopedia of Genes and Genomes; www.genome.jp/kegg/). PICRUSt matches the taxonomic annotation of a single 16S sequence read with a reference functional annotation of a genome sequence for a given OTU or set of OTUs. From these reference genome annotations, a functional annotation is assigned to each OTU. PICRUSt is composed of two high-level workflows: gene content inference and metagenome inference. The gene content inference produces gene content predictions for a set of reference OTUs as well as copy number predictions. The metagenome inference then uses these inputs and an OTU table that defines the OTUs in a sample and their relative

abundances to then infer the functional metabolic profile of the OTUs in the OTU table. In an alternative, but related method, one can lookup for all of the OTUs in a consortia the OTU taxonomic identifications in a functional reference database such as IMG (http://img.jgi.doe.gov) and then derive a functional annotation of the network by concatenating the database's metabolic pathway maps (*e.g.* KEGG Pathway Orthology in case of IMG) for each of the OTUs in the consortia (see below for specific example).

[0370] To generate functional annotation from metagenomic or whole genome shotgun sequence data, reads are first clustered and then representative reads are annotated. Sequence annotation is then performed as described in Example 1, with the additional step that sequences are either clustered or assembled prior to annotation. Following sequence characterization as described above using a technology such as but not limited to Illumina, sequence reads are demultiplexed using the indexing barcodes. Following demultiplexing sequence reads are clustered using a rapid clustering algorithm such as but not limited to UCLUST (http://drive5.com/usearch/manual/uclust_algo.html) or hash-based methods such VICUNA (Xiao Yang, Patrick Charlebois, Sante Gnerre, Matthew G Coole, Niall J. Lennon, Joshua Z. Levin, James Qu, Elizabeth M. Ryan, Michael C. Zody, and Matthew R. Henn. 2012. De novo assembly of highly diverse viral populations. BMC Genomics 13:475). Following clustering a representative read for each cluster is identified and analyzed as described above in Example 2 "Primary Read Annotation". The result of the primary annotation is then applied to all reads in a given cluster. In another embodiment, metagenomic sequences are first assembled into contigs and then these assembled contigs are annotated using methods familiar to one with ordinary skill in the art of genome assembly and annotation. Platforms such as but not limited to MetAMOS (TJ. Treangen et al. 2013 Geneome Biology 14:R2), and HUMAaN (Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL, Rodriguez-Mueller B, Zucker J, Thiagarajan M, Henrissat B, et al. 2012. Metabolic Reconstruction for Metagenomic Data and Its Application to the Human Microbiome ed. J.A. Eisen. PLoS Computational Biology 8: e1002358) are suitable for analysis of metagenomic data sets using the methods described above. Tools such as MetAMOS are also suitable for the generation of a functional annotation of complete genome sequence assembled from the sample or obtained from a reference genome database such as but not limited to NCBI's genome database (http://www.ncbi.nlm.nih.gov/genome). In all cases, functional pathways are derived from the sequence read annotations based on the mapping of the sequence annotations to a functional database, such as but not limited to KEGG (http://www.genome.jp/kegg), Biocyc (http://biocyc.org), IMG (http://img.jgi.doe.gov), MetaCyc (http://www.metacyc.org), or Reactome (http://www.reactome.org). Various tools are available for this task that are familiar to one with ordinary skill in the art including, but not limited to, The HMP Unified Metabolic Analysis Network (HUMAnN) (Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL, Rodriguez-Mueller B, Zucker J, Thiagarajan M, Henrissat B, et al. 2012. Metabolic Reconstruction for Metagenomic Data and Its Application to the Human Microbiome ed. J.A. Eisen. PLoS Computational Biology 8:

e1002358). The HUMAnN software recovers the presence, absence, and abundance of microbial gene families and pathways from metagenomic data. Cleaned short DNA reads are aligned to the KEGG Orthology (or any other characterized sequence database with functional annotation assigned to genetic sequences) using accelerated translated BLAST. Gene family abundances are calculated as weighted sums of the alignments from each read, normalized by gene length and alignment quality. Pathway reconstruction is performed using a maximum parsimony approach followed by taxonomic limitation (to remove false positive pathway identifications) and gap filling (to account for rare genes in abundant pathways). The resulting output is a set of matrices of pathway coverages (presence/absence) and abundances, as analyzed here for the seven primary body sites of the Human Microbiome Project.

[0371] Transcriptomic or RNA-Seq data are also a means to generate a functional profile of a sample (Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10: 57–63). Briefly, long RNAs are first converted into a library of cDNA fragments through either RNA fragmentation or DNA fragmentation. Sequencing adaptors appropriate to the sequencing technology being used for downstream sequencing are subsequently added to each cDNA fragment and a short sequence is obtained from each cDNA using high-throughput sequencing technology. The resulting sequence reads are aligned with the reference genome or transcriptome and annotated and mapped to functional pathways as described above. Reads are categorized as three types: exonic reads, junction reads and poly(A) end-reads. These three types of reads in combination with the gene annotation are used to generate a base-resolution expression profile for each gene.

[0372] In yet another method to generate a metabolic profile of a microbial ecology, characterization of metabolites produced by the ecology are analyzed in tissues or fluids. Samples can include, without limitation, blood, urine, serum, feces, ileal fluid, gastric fluid, pulmonary aspirates, tissue culture fluid, or bacterial culture supernatants. Both targeted and untargeted methods can be utilized for metabolomics analysis (Patti GJ, Yanes O, Siuzdak G. 2012. Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol 13: 263–269.). Metabolomic methods utilize LC/MS-based technologies to generate a metabolite profile of sample. In the triple quadrupole (QqQ)-based targeted metabolomic workflow, standard compounds for the metabolites of interest are first used to set up selected reaction monitoring methods. Here, optimal instrument voltages are determined and response curves are generated using reference standards for absolute quantification. After the targeted methods have been established on the basis of standard metabolites, metabolites are extracted from the sample using methods familiar to one with ordinary skill in the art. Extraction methods can include liquid:liquid extraction using organic solvents or two-phase aqueous methods, solid phase extraction using hydrophobic or ion exchange resins, filtrations to remove solid contaminants, centrifugation or other means of clarification, and counter-current techniques. The data output provides quantification only of those metabolites for which standards are available. In the untargeted metabolomic workflow, extracted metabolites are first iseparated by liquid

chromatography followed by mass spectrometry (LC/MS). After data acquisition, the results are processed by using bioinformatic software such as XCMS to perform nonlinear retention time alignment and identify peaks that are changing between the groups of related samples. The m/z values for the peaks of interest are searched in a metabolite databases to obtain putative identifications. Putative identifications are then confirmed by comparing tandem mass spectrometry (MS/MS) data and retention time data to that of standard compounds. The untargeted workflow is global in scope and outputs data related to comprehensive cellular metabolism.

[0373] Applicants generated a functional profile for all of the computationally determined network ecologies delineated in Table 8 and Table 14a that were derived using the methods outlined in Example 5. Table 18 and Table 21 provide written description of the corresponding functional network ecologies respectively. For each network, applicants generated a functional metabolic profile by concatenating the KEGG Orthology Pathways for each OTU available in the IMG functional database (http://img.jgi.doe.gov). The taxonomic annotations of each OTU in the network were mapped to the taxon_display_names in the IMG database. For each taxon_display_name the taxon_iod with the best 16S sequence match to the 16S sequence of the OTU in the computed network ecology was selected (best match based on expectation value and an alignment score). The functional annotation for each OTU in the network was then derived from IMG's KEGG Orthology Pathway (*i.e.* ko_id) for the given taxon_iod. KEGG Orthology Pathways (KO) for all the OTUs in the network were concatenated and then the list was made unique to generate a non-redundant functional profile of the network. In another embodiment, the ko_id list is not made unique and the functional profile of the network is defined based on the relative abundances of the ko_ids not just their presence or absence. It is with the level of ordinary skill using the aforementioned disclosure to construct functional network ecologies that substitute the exemplified OTUs with equivalent OTUs that harbor the orthologous KEGG Orthology Pathways. Such substitiutions are contemplated to be within the scope of the present invention, either literally or as an equivalent to the claimed invention as determined by determined by a court of competent jurisdiction.

[0374] Each functional network ecology was scored for its ability to metabolize bile acids and to produce short chain fatty acids (SCFAs). As described above, both bile acid metabolism and the production of SCFAs by bacterial ecologies plays an important role in human health. Specifically, applicants subsetted the KEGG Orthology Pathways computed for each network ecology to those described to be involved in secondary bile acid biosynthesis, butryrate (a.k.a. butanoate) metabolism, propionate (a.k.a. propanoate) metabolism, or pyruvate metabolism (leads to production of acetate).

We identified and ranked network ecologies for their capacity to metabolize bile acid and produce SCFAs by defining a bile acid and SCFA functional score (F-Score) that defines a network ecologies' capacity to perform these two important metabolic roles. The F-score is defined by the total number of KEGG Orthology Pathways in a given network that mapped to secondary bile acid biosynthesis, a butyrate metabolism, a propionate metabolism, or a pyruvate metabolism (Table 18). A functional

translation of the KEGG Orthology Pathways (*i.e.*, KO numbers) and their respective metabolic ontology classification is provided in Table 19 as reference. Significantly, as shown in Table 18, there are only two computed network ecologies that did not harbor at least one pathway related to secondary bile acid biosynthesis, butyrate metabolism, propionate metabolism, or a pyruvate metabolism, suggesting both likely importance of these pathways to the metabolism of a large number of gastrointestinal ecologies, and the importance of these pathways to catalyzing a shift from a disease to a health state in the example cases of CDAD and Type 2 Diabetes.

Example 16. Use of Biolog assay to Generate a Nutrient Utilization Functional Profile of an OTU or Consortium of OTU

[0375] Metabolic capabilities of individual organisms or a consortia of organisms can be determined using Biolog technology in which metabolic activity is detected by measurement of NADH production using a redox sensitive dye. Carbon source or other metabolic capabilities of a single species can be determined, as described below. Carbon source utilization of an ecology or network can also be assessed using the same methods.

[0376] A screen was performed to test the ability of Clostridium difficile and potential competitor species to utilize a panel of 190 different carbon sources. The screen was carried out using PM1 and PM2 MicroPlates (Biolog #12111, #12112), IF-0a base media (Biolog #72268) and Biolog Redox Dye Mix D (Biolog #74224). For each strain, a 1 uL aliquot from -80° C glycerol stock was streaked out for single colonies to solid Brucella Blood Agar plates (BBA) (Anaerobe Systems #AS-111) and incubated anaerobically at 37° C for 24 hr. A single colony was then re-streaked to a BBA plate and incubated anaerobically at 37° C for 24 hr. The MicroPlates were pre-reduced by incubating for at least 24 hr in a hydrogen free anaerobic environment before use. All liquid media and supplements used were pre-reduced by placing them in an anaerobic chamber with loose lids for at least 24 hr before use. Alternatively, combinations of bacteria can also be tested.

[0377] The base media for inoculation was prepared by adding 0.029 mL of 1M potassium ferricyanide to 0.240 mL of Dye Mix D followed by addition of 19.7 mL of IF-0a, 4 mL sterile water and 0.024 mL 0.5 mM menadione. For some species, the concentrations of potassium ferricyanide and menadione were adjusted to achieve the optimal redox balance or to test multiple redox conditions. Potassium ferricyanide was tested at a final concentration of 0.38, 0.12, 0.038 and 0.06 mM. Menadione was tested at a final concentration of 0.5, 0.16 and 0.05 μM. In total, this yields 9 redox conditions for testing. Reduction of the tetrazolium dye that forms the basis for the endpoint measurement was sensitive to the redox state of each bacterial culture, and thus to the ratio of menadione to potassium ferricyanide. It was therefore important to test various ratios for each bacterial isolate and was also important in some cases to test a species at multiple menadione / potassium ferricyanide ratios in order to detect all conditions in which a possible nutrient utilization

was detectable. Some species were tested beyond the 20 hr time point to detect all conditions resulting in a positive result. In these cases plates were read at 20, 44 or 96 hr.

[0378] Using a sterile, 1 μL microbiological loop, a loopful of biomass was scraped from the BBA plate and resuspended in the base media by vortexing. The OD was adjusted to 0.1 at 600 nm using a SpectraMax M5 plate reader. The bacterial suspension was then aliquoted into each well of the PM1 and PM2 plates (100 μL per well). The plates were incubated at 37°C for 20 hr in a rectangular anaerobic jar (Mitsubishi) with 3 anaerobic, hydrogen-free gas packs (Mitsubishi AnaeroPack). After 20 hr, OD at 550 nm was read using a SpectraMax M5 plate reader. Wells were scored as a weak hit if the value was 1.5x above the negative control well, and a strong hit if the value was 2x above the negative control well. The results are shown in the Table in Figure 4.

[0379] The following list of nutrient sources were tested: L-Arabinose, N-Acetyl-D-Glucosamine, D-Saccharic Acid, SuccinicAcid, D-Galactose, L-AsparticAcid, L-Proline, D-Alanine, D-Trehalose, D-Mannose, Dulcitol, D-Serine, D-Sorbitol, Glycerol, L-Fucose, D-Glucuronic Acid, D-Gluconic Acid, D, L-alpha-Glycerol-Phosphate, D-Xylose, L-Lactic Acid, Formic Acid, D-Mannitol, L-Glutamic Acid, D-Glucose-6-Phosphate, D-Galactonic Acid-gamma-Lactone, D,L-Malic Acid, D-Ribose, Tween 20, L-Rhamnose, D-Fructose, Acetic Acid, alpha-D-Glucose, Maltose, D-Mellibiose, Thymidine, L-Asparagine, D-Aspartic Acid, D-Glucosaminic Acid, 1,2-Propanediol, Tween 40, alpha-Keto-Glutaric Acid, alpha-Keto-ButyricAcid, alpha-Methyl-D-Galactoside, alpha-D-Lactose, Lactulose, Sucrose, Uridine, L-Glutamine, M-Tartaric Acid, D-Glucose-1-Phosphate, D-Fructose-6-Phosphate, Tween 80, alpha-Hydroxy-Glutaric-gamma-lactone, alpha-Hydroxy Butyric Acid, beta-Methyl-D-Glucoside, Adonitol, Maltotriose, 2-Deoxy Adenosine, Adenosine, Glycyl-L-Aspartic Acid, Citric Acid ,M-Inositol ,D-Threonine, Fumaric Acid, Bromo Succinic Acid, Propionic Acid, Mucic Acid, Glycolic Acid, Glyoxylic Acid, D-Cellobiose, Inosine, Glycyl-L-Glutamic Acid, Tricarballylic Acid,L-Serine, L-Threonine, L-Alanine, L-Alanyl-Glycine, Acetoacetic Acid, N-Acetyl-beta-D-Mannosamine, Mono Methyl Succinate, Methyl Pyruvate, D-Malic Acid, L-Malic Acid,Glycyl-L-Proline, p-Hydroxy Phenyl Acetic Acid, m-Hydroxy Phenyl Acetic Acid, Tyramine, D-Psicose, L-Lyxose, Glucuronamide, Pyruvic Acid, L-Galactonic Acid-gamma-Lactone,D-Galacturonic Acid,Pheylethyl-amine,2-aminoethanol, Chondroitin Sulfate C, alpha-Cyclodextrin, beta-Cyclodextrin, gamma-Cyclodextrin, Dextrin, Gelatin, Glycogen, Inulin, Laminarin, Mannan, Pectin, N-Acetyl-D-Galactosamine, N-Acetyl-Neuramic Acid, beta-D-Allose, Amygdalin, D-Arabinose, D-Arabitol, L-Arabitol, Arbutin, 2-Deoxy-D-Ribose, I-Erythritol, D-Fucose, 3-0-beta-D-Galacto-pyranosyl-D-Arabinose, Gentibiose, L-Glucose, Lactitol, D-Melezitose, Maltitol, alpha-Methyl-D-Glucoside, beta-Methyl-D-Galactoside, 3-Methyl Glucose, beta-Methyl-D-GlucoronicAcid, alpha-Methyl-D-Mannoside, beta-Metyl-D-Xyloside, Palatinose, D-Raffinose, Salicin, Sedoheptulosan, L-Sorbose, Stachyose, D-Tagatose, Turanose, Xylitol, N-Acetyl-D-Glucosaminitol, gamma-Amino Butyric Acid, delta-Amino Valeric Acid, Butyric Acid, Capric Acid, Caproic Acid, Citraconic Acid, Citramalic Acid, D-Glucosamine, 2-Hydroxy Benzoic Acid, 4-

Hydroxy Benzoic Acid, beta-Hydroxy Butyric Acid, gamma-Hydroxy Butyric Acid, alpha-Keto Valeric Acid, Itaconic Acid, 5-Keto-D-Gluconic Acid, D-Lactic Acid Methyl Ester, Malonic Acid, Melibionic Acid, Oxalic Acid, Oxalomalic Acid, Quinic Acid, D-Ribino-1,4-Lacton, Sebacic Acid, Sorbic Acid, Succinamic Acid, D-Tartaric Acid, L-Tartaric Acid, Acetamide, L-Alaninamide, N-Acetyl-L-Glutamic Acid, L-Arginine, Glycine, L-Histidine, L-Homserine, Hydroxy-L-Proline, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Ornithine, L-Phenylalanine, L-Pyroglutamic Acid, L-Valine, D,L-Carnithine, Sec-Butylamine, D,L-Octopamine, Putrescine, Dihydroxy Acetone, 2,3-Butanediol, 2,3-Butanone, 3-Hydroxy 2-Butanone.

[0380] Additionally, one of skill in the art could design nutrient utilization assays for a broader set of nutrients using the methods described above including complex polysaccharides or prebiotics.

[0381] A similar screen can be performed to test the utilization of vitamins, amino acids, or cofactors. In these instances, Biolog MicroPlates for screening of vitamins, amino acids or cofactors that are of interest would be used in place of the PM1 and PM2 plates, for example PM5. Table 2 contains a list of representative vitamins, minerals, and cofactors. For each strain tested, a universal carbon source such as glucose will be used as a positive control to demonstrate reduction of the tetrazolium dye under the specific conditions of the assay.

Example 17. In vitro Screening of microbes for 7-alphadehydroxylase activity

[0382] Cultures of individual microbes are grown overnight and frozen for later use as described according to Example 9. The sodium salts of CA, CDCA, GCA, GCDCA, TCA, and TCDCA (Sigma) are obtained and prepared as aqueous stock solutions. For initial screening to define organisms capable of 7-alphadehydroxylation reactions, growth media are prepared containing 0.4 mM of each bile salt. Cultures are inoculated from a 1:100 dilution of the frozen stock into the media and grown in an anaerobic chamber for 24-48 hours, or until the culture is turbid. Two mL of culture is acidified by the addition of 1 mL of 2N HCl and 100 ug of 23-nordeoxycholic acid (Steraloids) as an internal reference standard. The acidified mixture is extracted twice with 6 mL of diethyl ether. The organic extracts are combined and then evaporated and derivatized to methyl esters with diazomethane. Gas chromatography is performed on a 7 ft (ca. 2 m) 3% OV-1 column at 260°C and a 3% OV-17 column at 250°C after trimethylsilylation of the methylated bile acids with Tri-Sil (Pierce, Rockford, Ill.). The retention times of the silylated bile acids are compared with those of reference products representing CA, CDCA, DCA and LCA.

[0383] For strains showing 7-alphadehydroxylase activity, a kinetic assessment is performed by harvesting a growing culture of each organisms of interest, washing and resuspending in fresh media at a concentration of between 108 to 1010 cfu/mL. The sodium salts of CA, CDCA, GCA, GCDCA, TCA, and TCDCA are then added at 0.5 to 5 mM and the resulting culture is sampled at 1, 2, 4 and 8 hours. The sample is analyzed as described above to find organisms with maximal activity. Highly

active strains are selected for further incorporation into microbial compositions that exhibit maximal 7-alphadehydroxylase activity.

Example 18. In vitro Screening of Microbes for Bile Salt Hydrolase Activity

[0384] Cultures of individual microbes are grown overnight and frozen for later use as described according to Example 9. The sodium salts of GCA, GCDCA, TCA, and TCDCA (Sigma) are obtained and prepared as aqueous stock solutions. Overnight, actively growing cultures are combined with 0.5 to 5 mM of conjugated bile acid and allowed to incubate for 24-48 hours. To analyze cultures, 0.5 mL of culture is first centrifuged at 3,000 x g for 10 min to remove the bacteria, and is then acidified with 5 uL of 6 N HCl. This acidified supernatant is combined with an equal volume of methanol containing 4 mM of 23-nordeoxycholic as an internal standard. The samples are vortexed for at least 2 min and clarified by centrifugation at 1000 x g for 15 min. Samples are filtered through a 0.2 um filter prior to HPLC analysis according to the method described by Jones et al 2003 J Med Sci 23: 277-80. Briefly, the isocratic method is performed on a reversed-phase C-18 column (LiChrosorb RP-18, 5m, 250 x 4.6 mm from HiChrom, Novato, CA, USA). Acetate buffer is prepared daily with 0.5 M sodium acetate, adjusted to pH 4.3 with o-phosphoric acid, and filtered through a 0.22m filter. The flow is 1.0 mL/min and the detection is performed at 205 nm. The injection loop is set to 20 uL.

[0385] For strains showing bile salt hydrolase activity, a kinetic assessment is performed by harvesting a growing culture of each organisms of interest, washing and resuspending in fresh media at a concentration of between 108 to 1010 cfu/mL. The sodium salts of GCA, GCDCA, TCA, and TCDCA are then added at 0.5 to 5 mM and the resulting culture is sampled at 1, 2, 4 and 8 hours. The sample is analyzed by HPLC as described above to find organisms with maximal activity. Highly active strains are selected for further incorporation into microbial compositions that exhibit maximal bile salt hydrolase activity.

Example 19. In vitro Screening of Microbial Communities for 7-alpha-dehydroxylase activity

[0386] Measurement of the conversion of 7-alpha-hydroxyl bile salts (primary bile salts) to 7-dehydroxy-bile salts (secondary bile salts) by single bacterial strains or bacterial communities is determined in an in vitro assay, and can be used to screen a library of organisms, whole communities or subsets of communities using limiting dilutions to identify simpler compositions. Communities to be studies include cecal or fecal communities from animals with altered gastrointestinal microbiota due to antibiotics, diet, genetics, enterohepatic metabolism, or other experimental perturbations that cause GI alterations, or from human fecal samples from healthy individuals or those with altered gastrointestinal microbiomes due to antibiotics, diet, enterohepatic metabolism dysfunction, metabolic dysfunction, or gastrointestinal infection. Dilutions or subsets of these communities (such as could be generated by selective culturing for of the whole community to enrich for aerobes, anaerobes, Gram

positives, Gram negatives, spore formers or using other microbiological selections known to one skilled in the art) can be utilized to identify a group of organisms required for a particular multi-step conversion.

[0387] To assay 7-alpha-dehydroxylation activity in vitro, an enzymatic assay is established to quantify the amount of 7-alphahydroxy bile acid in a sample. Recombinant 7-alpha-hydroxysteroid dehydrogenase (7-alpha-HSDH) from E. coli (MyBiosource.com) is an enzyme that oxidizes the 7-hydroxy group to a ketone and simultaneously reduces NAD+ to NADH + H+. The production of NADH is monitored at 340 nm using the extinction co-efficient of 6.2 x 103 M-1 cm-1.

[0388] A community of microbes is prepared according to Example 9 or, alternatively a preparation of cecal or fecal bacteria from mice or from human feces or a dilution thereof, or an enriched community thereof, can be tested after being washed 5 times to remove bile acids from the matrix. To the initial sample, a mixture of one or more primary bile acids including but not limited to CA, CDCA or any of their taurine or glycine conjugates is added to a final total concentration of 0.5-5 mM. An initial 100 uL aliquot is removed and heated at 55°C for 15 min to quench further enzymatic activity. The bacterial composition is then incubated under anaerobic conditions at 37°C, and aliquots are removed sequentially after 30 min, 1 hour, 2 hours, 4 and 8 hours and heated as per above. An assay mix is prepared by combining 0.9 mL glycine-NaOH buffer pH 9.5, 50 uL of 53 mM NAD+ (Sigma), and 20 uL of freshly prepared 7-alpha-HSDH (4 mg/mL in distilled water). 80 uL of assay mix is combined with 20 uL of each aliquot in a 96-well microtiter plate and incubated at 37°C on a SpectraMax m5 plate reader, monitoring A340. The incubation is allowed to proceed until the A340 value achieves its maximum. Total 7-alpha-hydroxyl bile acid is determined using the extinction coefficient for NADH. Changes due to dehydroxylation by the bacterial composition are calculated by subtracting the final value at any timepoint from the initial value.

[0389] Microbial communities of interest can be further fractionated using methods described in Example 9.

Example 20. In vitro evaluation of mixed microbial cultures for bile acid metabolism

[0390] Candidates strains identified in Examples 17, 18 and 19 above are tested using the methods defined for bile salt hydrolase activity and 7-alphadehydroxylase activity are combined in communities to evaluate synergies among strains and define ecologies for further testing in animal models. Synergies include: i) the potential for more rapid conversion from conjugated primary bile salts to unconjugated, dehydryoxylated bile acids; ii) the potential for a broader range of products than determined by the additive combination of activities; iii) equivalent activity at a lower concentration (cfu) of the individual strains. Combinations exhibiting such synergies are particularly favored for subsequent in vivo testing. Another important function of a community is to remove endproducts of a microbial conversion so as to avoid inhibition of growth through product accumulation. For bile acid conversion, communities can optionally include organisms capable of degrading taurine, using it both

as a carbon and nitrogen source and using the sulfonic acid group as an electron acceptor in fermentation.

Example 21. Combinations of Bacterial Compositions For SCFA Production Under Variable Conditions

[0391] Combinations of synergistic bacterial compositions may be selected such that the composition is capable of producing SCFA under a wide range of in vitro conditions when the entire mixture is tested together. That is, a combination of bacterial compositions comprises multiple pairs of organisms that, together with a complex carbon source, are capable of synthesizing SCFA. Combinations may be constructed that are capable of producing a given set of SCFAs, for example butyrate and proprionate, but not acetate, or that produce butyrate, proprionate and acetate, but that the acetate is then used by another organism as a carbon source. A number of specific combinations of final SCFAs may be generated by communities designed by one skilled in the art. Construction of bacterial combinations follows the protocol described in Example 9.

Example 22. De novo Design of Network Ecologies with Specific Functional Properties

[0392] The role of the microbiome in mediating and influencing human metabolic function is well established. Microbes produce secondary bile acids (as example, Louis P, Flint HJ. 2009. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 294: 1–8.), short chain fatty acids (for example, Smith PM et al. 2013 Science. The microbial metabolites, short-chain fatty acid regulate colonic Treg cell homeostasis 341: 569-73) as well as numerous other functional metabolites that influence immunity and metabolic health of the human host.

[0393] To identify consortia of microbes suitable for the use as therapeutics, to influence host metabolic functions, and to treat microbial dysbiosis one can computationally derive in silico network ecologies that possess specific metabolic functions such as, but not limited to, a single or multiple metabolic nodes in the functional pathways involved in secondary bile acid biosynthesis (Figure 12), butyrate metabolism (Figure 13), propionate metabolism (Figure 14), or pyruvate metabolism (Figure 15). As additional examples, network ecologies can be in silico designed to target host genes involved in important host:microbe innate and adaptive immune responses through targets such as the Toll-like receptors (TLRs) and nucleotide-binding oligomerization domains (NOD) (Saleh M, Trinchieri G. 2011. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. Nat Rev Immunol 11: 9–20. and Knight P, Campbell BJ, Rhodes JM. 2008. Host-bacteria interaction in inflammatory bowel disease. Br Med Bull 88: 95–113.). In addition, the functional pathways to target for in silico network ecology design can be empirically defined by comparing the microbiomes of samples derived from different phenotypes such as but not limited to a state of disease and a state of health. For example, one can compare the microbiome and corresponding metabolic functional profile of individuals with and without insulin resistance. Vrieze et al. have shown that treatment

with vancomycin can reduce the diversity of the microbiome and result in a small, but statistically significant change in peripheral insulin sensitivity. Similar changes are not observed following amoxicillin treatment (Vrieze, A et al., 2013, J Hepatol. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity dx.doi.org/10.1016/j.jhep.2013.11.034). Decreased insulin sensitivity was associated with a decrease in the presence of secondary bile acids DCA, LCA and iso-LCA and an increase in primary bile acids CA and CDCA increased. In another example, Applicants can compare the microbiome and metabolomic profile of healthy individuals to those with CDAD disease. In yet another example, Applicants can compare the microbiome and metabolomic profile of healthy individuals to those that harbor IBD, IBS, Ulcerative Colitis, Crohn's Disease, Type-2-Diabetes, or Type-1-Diabetes.

[0394] For both CDAD and insulin resistance, Applicants can define the functional metabolic profile of the respective disease and health microbiomes using the 16S and metagenomic genomic methods outlined in Example 15. In another embodiment, Applicants can use the transcriptomic and metabolomic methods outlined in Example 15. In another embodiment we use functional metabolic information garnered from the literature and derived from functional screens such as but not limited to Biolog MicroPlates (see Example 16). From these profiles, Applicants can generate a metabolic function matrix for both the disease state and the health state. This matrix is comprised of columns of OTUs and rows of KEGG Orthology Pathways delineated as described in Example 15. A metabolic function matrix can be generated for both the disease state and the health state. From these disease and health matrices, Applicants can compute a delta-function matrix (*i.e.* difference matrix) that defines the OTUs, the relative abundance of the metabolic pathways they harbor, and the difference in the relative abundance between the disease and health state. In another embodiment, the relative abundances are discretized to be a binary, ternary, or quaternary factor. This delta-function matrix defines the differences in the microbiome distinguishing the disease state from the health state.

[0395] One can then design a network ecology with the desired functional characteristics described by the delta-function matrix. In one embodiment, one can use a greedy algorithm to optimize for the most parsimonious solution to the delta-function matrix. One can design towards (*i.e.* select) the minimal number of OTUs to capture the full breadth of KEGG Orthology Pathways that are represented in the health state. In short, the greedy algorithm repetitively samples the OTUs spanning the greatest number of health associated KEGGs until the desired breath of KEGGs is obtained to define a functional network ecology comprised of specific OTUs. In another embodiment, one can optimize the greedy algorithm to weigh OTUs that are from specific phylogenetic clades. In another embodiment, one can start with the computationally derived network ecologies derived using the methods defined in Example 5 to both seed and constrain the greedy algorithm to return functional network ecologies that embody the co-occurrence relationships that exist between OTUs. Microbial therapeutic compositions comprised of the OTUs of the computed network ecologies are constructed using the methods defined in Example 9. In one embodiment, constraints around network ecologies

are defined by networks found in specific individuals. In another embodiment, strains of each OTU that are used for construction preferentially are selected from strains isolated from the same individual since these strains are evolutionary co-evolved and have an increased likelihood of functional synergy.

[0396] In another embodiment, Applicants computationally defined in silico a network ecology with the explicit capacity to produce butyrate. In this embodiment, Applicants defined the health state in terms of the metabolic pathways and associated gene products required for the metabolism of non-digestable carbohydrates via fermentation by colonic bacteria and by the gene products leading from mono- and di-saccharides and simple substrates such as acetate and lactate to butyrate (Figures 12-15). We then used the IMG functional database (http://img.jgi.doe.gov) of OTU KEGG Orthology Pathways (*i.e.* ko_id) to generate a metabolic function matrix comprised of columns of OTUs and rows of KEGG Orthology Pathways delineated as described in Example 15. This matrix was restricted to OTUs known to reside in the gastrointestinal tract. From this metabolic function matrix we used the greedy algorithm described above to design network ecologies capable of butyrate production.

Example 23. Identification of Organisms Harboring butyryl-CoA: acetate CoA Transferase Genes

[0397] A panel of putative butyrate forming bacteria can be screened for the presence of butyryl-CoA: acetate CoA transferase genes to define candidates for SCFA production. Bacteria are scraped from isolated colonies on agar plates or from liquid culture (media selected from Example 9) and subjected to DNA isolation in 96-well plates. 1 µl of microbial culture or an amount of a bacterial colony approximately 1 µL in volume is added to 9 µl of Lysis Buffer (25mM NaOH, 0.2 mM EDTA) in each well of a 96 well, thin walled PCR plate, sealed with an adhesive seal, and the mixture is incubated at 95°C for 30 minutes. Subsequently, the samples are cooled to 4°C and neutralized by the addition of 10 µl of Neutralization Buffer (40 mM Tris-HCl) and then diluted 10-fold in Elution Buffer (10 mM Tris-HCl), at which point the genomic DNA is suitable for use in downstream amplifications such as PCR amplification. Alternatively, genomic DNA is extracted from pure microbial cultures using commercially available kits such as the Mo Bio Ultraclean® Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) or by standard methods known to those skilled in the art.

[0398] Degenerate primers are designed to selectively amplify the gene for butyryl-CoA:acetate CoA transferase based on published genomic sequences. Examples of primers are BCoATforward: 5' GCIGAICATTTCACITGGAAYWSITGGCAYATG; and BCoATreverse: 5' CCTGCCTTTGCAATRTCIACRAANGC, where I = inosine; N = any base; W = A or T; Y = T or C; S = C or G. Amplification is as follows: 1 cycle of 95°C for 3 min; 40 cycles of 95°C, 53°C, and 72°C for 30 s each with data acquisition at 72°C; 1 cycle each of 95°C and 55°C for 1 min; and a

stepwise increase of the temperature from 55 to 95°C (at 10 s/0.5°C) to obtain melting curve data and evaluate product complexity. The target amplicon is about 530 nt in length.

Example 24. Identification of Organisms Harboring butyrate-kinase Genes

[0399] Butyrate may be produced by substrate level phosphorylation of butyrylCoA by butyrate-kinase and subsequent phosphorylation of ADP to generate ATP and butyrate. DNA isolation and PCR amplification was performed as in Example 23 with the exception that the following primers were used: BUKfor: 5' GTATAGATTACTIR- YIATHAAYCCNGG; and BUKrev: 5' CAAGCTCRTCIACIACIACNGGRTCNAC, where I = inosine; N = any base; R = A or G.

Example 25. Identification of Organisms with butyryl-CoA: acetate CoA transferase Enzymatic Activity

[0400] Bacterial strains are grown overnight in an anaerobic chamber at 37°C in pre-reduced media selected from those described in Example 9. 10 mL of the bacterial culture is harvested by centrifugation at 10,000 rpm for 10 min, cooled to 4°C on ice, and disrupted by sonication as described (Duncan, S. et al., 2002 Appl Environ Microbiol Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate producing bacteria from the human large intestine 68: 5186-90). ButyrylCoA: acetate CoA transferase activities are determined by the method of Barker, scaled for application to a microtiter plate (Barker HA, et al., 1955 Methods Enzymol 1: 599-600).

Example 26. Identification of organisms with butyrate-kinase, propionate-kinase and acetate-kinase enzymatic activity

[0401] Bacterial strains are grown overnight in an anaerobic chamber at 37°C in pre-reduced media selected from those described in Example 9. 10 mL of the bacterial culture is harvested by centrifugation at 10,000 rpm for 10 min, cooled to 4°C on ice, and disrupted by sonication as described (Duncan, S. et al., 2002 Appl Environ Microbiol Acetate utilization and butyryl coenzyme A (CoA):acetate-CoA transferase in butyrate producing bacteria from the human large intestine 68: 5186-90). Butyrate-, propionate-, and acetate-kinase activities were determined by colorimetric the method of Rose (Rose IA, 1955 Methods Enzymol Acetate kinase of bacteria 1: 591-5).

Example 27. Characterization of propionate or butyrate production from a variety of carbon sources

[0402] Strains identified as having either genes for butyrate or propionate fermentation or having the corresponding enzymatic activities are assayed in vitro using a variety of simple carbon sources for the production of propionate and butyrate. Bacteria are grown overnight in complex media selected from Example 9 in an anaerobic chamber at 37°C. When cultures are visibly turbid, the bacteria are pelleted at 10,000 x g for 10 min, the spent media is removed, and they are resuspended in pre-reduced minimal media containing essential vitamins and cofactors (pyridoxamine, p-aminobenzoic acid, biotin, nicotinic acid, folic acid, nicotinamide, choline, pantothenate, riboflavin or vitamin), divalent mineral salts (including the chloride salts of Mg2+, Ca2+ and Mn2+), and organic

nitrogenous nutrients (especially glycine, glutamate or asparagine) but lacking carbohydrate as a carbon source. Alternatively, strains may be resuspended in a dilution of the original rich media, for instance a 1:10 or 1:100 dilution, such that essential factors are available but a required carbon source is limiting.

[0403] Various carbon sources are added to individual cell suspensions. These include acetate and D and L isomers of lactate, simple sugars including glucose, galactose, mannose, arabinose, xylose or any other naturally sugar, amino sugars such as N-acetyl glucosamine, galactosamine, sialic acid or glucosamine, sugar alcohols such as glycerol, erythritol, threitol, mannitol, inositol or sorbitol. In addition, the cell suspensions are individually incubated with complex carbon sources including di-, tri-, oligo- and polysaccharide carbon sources including fructans, starches, cellulose, galactomannans, xylans, arabinoxylans, pectins, inulin, and fructooligosaccharides. Tested carbon sources also include glycopeptides and glycoproteins, such as mucin. The cell suspension is incubated overnight in a sealed 96-well plate in order prevent the escape of volatile products.

[0404] At the end of the incubation period, the production of propionate, butyrate and other SCFAs is determined according to the following protocol:

[0405] Reagents

- Internal Standard: 2-ethylbutyric acid, 2-EBA (100 mM)
- SCFA Mixed Standard: formic acid 10 mM, acetic acid 30 mM, propionic acid 20 mM, isobutyric acid 5 mM, n-butyric acid 20 mM, n-valeric acid 5 mM, isovaleric acid 5 mM, sodium lactate 10 mM, sodium succinate 10 mM, phenylacetic acid 5 mM
- MTBSTFA Derivatizing Reagent (N-Methyl-N-(tert-butyldimethylsilyl)- trifluoroacetamide), purchased from Regis Technologies
- Concentrated HCl
- Deionized Water
- Diethyl ether (unstabilized)
- Hexane

[0406] Linearity Standards Preparation: Linearity Standard 1 is prepared using straight SCFA Standard. Linearity Standard 2 is prepared using 100 uL of SCFA Standard and 900 uL of water. Linearity Standard 3 is prepared using 100 uL of Linearity Standard 2 and 900 uL of water.

[0407] SCFA Extraction: Extractions of samples (Media and Culture Supernatant), water blanks, and linearity standards were prepared in 4-mL vials using 250 uL of sample, blank, or standard, 250 uL of concentrated HCl, and 50 uL of Internal Standard (2-EBA). Once combined, the sample, standards, and blanks were vortexed and allowed to stand for about 5-10 minutes. Diethyl ether (2000 uL) was added to each of the samples, standards and blanks, and each was liquid-liquid extracted for approximately 2 minutes. The aqueous and organic phases of the extracted samples, standards and blanks were allowed to separate. Once the layers separated, 1000 uL of the ether layer was

transferred to 2-mL micro-centrifuge tubes and centrifuged at 14k for 2 minutes to remove any remaining water.

Sample/Standard/Blank*	250 uL
HCl	250 uL
Internal Standard (2-EBA)	50 uL
Ether	2000 uL

[0408] *substitute deionized water for blank preparations

[0409] Derivatization: Derivatization of all samples, blanks and standards was conducted in HPLC vials using 175 uL of the upper ether layer of samples or standards and 25 uL of MTBSTFA derivatizing reagent. The reaction mixture was vortexed and allowed to sit at RT for 24 hours. After 24 hours, the ether was removed using a gentle stream of nitrogen, and the residual material was dissolved in 50 uL of hexane. (Note: solvent was removed until no further change in volume was apparent, ~ 5 – 10 min). The derivatized solutions were transferred to small-volume inserts for GC-MS analysis.

[0410] An aliquot of the resulting derivatized material is injected into a gas chromatograph (Hewlett Packard 6890) coupled to a mass spectrometer detector (Agilent Technologies 5973). Analyses are completed using DB-5MS (60 m, 0.25 mm i.d., 0.25 mm film coating; P. J. Cobert, St. Louis, MO) and electronic impact (70 eV) for ionization. A linear temperature gradient is used. The initial temperature of 80°C is held for 1 min, then increased to 280°C (15°C/min) and maintained at 280°C for 5 min. The source temperature and emission current are 200°C and 300 mA, respectively. The injector and transfer line temperatures are 250°C. Quantitation is completed in selected ion monitoring acquisition mode by comparison to labeled internal standards [formate was also compared to acetate-13C1,d2]. The m/z ratios of monitored ions for formic acid, acetic acid, propionic acid, butyric acid, acetate, proprionate and butyrate are as follows: 103 (formic acid), 117 (acetic acid), 131 (propionic acid), 145 (butyric acid), 121 ([2H2]- and [1-13C]acetate), 136 ([2H5]propionate), and 149 ([13C4]butyrate).

[0411] At the completion of the experiment, a database is generated for each tested organism defining what carbon sources yield which SCFAs. In each case where a microbe is capable of making propionate or butyrate from acetate, lactate, a simple sugar including a disaccharide, an amino sugar or sugar alcohol it is scored as positive for SCFA production. Also noted is whether organisms are capable of utilizing complex carbon sources such as polysaccharides to produce SCFA and which SCFAs are produced.

Example 28. Identification of Organisms Capable of Metabolizing Complex Carbon Sources Including Polysaccharides and Steroids

[0412] Individual strains are screened for their ability to metabolize complex carbon sources including polysaccharides and steroids (such as bile salts) according to Example 16 to determine bacterial strain nutrient utilization. For a more complete characterization, specialized plates are constructed utilizing polysaccharide carbon sources including fructans, starches, cellulose, galactomannans, xylans, arabinoxylans, pectins, inulin, and fructooligosaccharides as well as carbon sources including glycopeptides and glycoproteins (such as mucin). These can be made to order by Biolog.

[0413] At the end of the experiment, a catalogue of is generated for each tested organism defining what carbon sources it can utilize.

Example 29. Construction of Cross Feeding Compositions

[0414] Data from Examples 27 and 28 are analyzed to determine combinations where one organism can make SCFAs from at least one simple carbon source but not from at least one complex carbon source (a polysaccharide or a glycoprotein), and another organism cannot make SCFAs from a simple carbon source but can utilize at least one complex carbon source as a metabolic substrate.

[0415] In these cases, a bacterial mixture is made combining a washed overnight culture of the SCFA producer and a washed overnight culture of the SCFA non-producer in a minimal media as described in Example 27 with the addition of the at least one complex carbon source. The bacterial mixture is incubated anaerobically overnight at 37°C in minimal media or a 1:10 or 1:100 dilution of rich media, and the next day is worked up according to Example 27 in order to detect whether SCFA has been produced. Control cultures include each microbe cultured individually, and the bacterial mixture cultured overnight without the complex carbon source.

[0416] Bacterial mixtures in which control cultures do not yield SCFA but the complete mixture does define synergistic bacterial compositions. Synergistic bacterial compositions may be tested for further effects in a variety of in vitro or in vivo models, with and without the complex carbon source, which may be considered a component of one embodiment of the synergistic bacterial composition.

Example 30. In vivo Validation of Bacterial Composition Efficacy In For Amelioration of Leaky Gut

[0417] A murine model for "leaky gut syndrome" is constructed by intraperitoneal injection of pregnant C57BL/6N mice (Charles River, Wilmington, MA) with 20 mg/kg poly(I:C) in 200 uL of saline on embryonic day 12.5. Control pregnant mice are injected with 200 uL of saline only (Hsiao EY et al., 2013 Cell Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders 155: 1451-63).

[0418] Pups are randomly selected for treatment with a single bacterial composition or a combination of bacterial compositions at the time of weaning (Day 20-22) and received oral gavage

every other day for 6 days. In addition, groups of animals receive mouse chow supplemented with the complex carbohydrate relevant to the bacterial composition(s) that is (are) dosed. Control groups (saline injections) receive comparable combinations of bacterial compositions, with and without the complex carbohydrate.

[0419] Animals are tested at adolescence (3 weeks post-weaning) and adulthood (8 weeks post weaning) for leaky gut. Mice are fasted for 4 hr before gavage with 0.6 g/kg 4 kDa FITC-dextran (Sigma Aldrich). Four hours later, serum samples are read for fluorescence intensity at 521 nm using an xFluor4 spectrometer (Tecan). Increased fluorescence is taken as evidence of leaky gut, while decreased fluorescence is evidence for amelioration of leaky gut induced by poly(I:C) treatment. Preferred bacterial compositions decrease leak gut in mice.

Example 31. In vivo Validation of Bacterial Composition Efficacy in Germ Free Mice Conventionalized with Human Obese Microbiota

[0420] Ridaura et al. (2013) showed that germ-free (GF) mice conventionalized with microbiota from female twins discordant for obesity showed taxonomic and phenotypic features of the human donor's microbiota. Mice receiving obese twin microbiota (Ob mice) showed significantly greater body mass and adiposity than recipients of lean twin microbiota (Ln mice). Furthermore, they observed that cohousing Ob mice and Ln mice prevented development of the obese phenotype in the Ob mice and showed that the rescue correlated with invasion of members of the microbiota from the Ln mice into the Ob mice.

[0421] Ob and Ln mice prepared as described by Ridaura et al. (2013) can be used to test the therapeutic potential of a bacterial composition for obesity. Ob and Ln mice are generated by introducing via oral gavage fecal samples from twins discordant for obesity into 8-9 week old adult male germ-free C57BL/6J mice. One gnotobiotic isolator is used per microbiota sample and each recipient mouse is individually caged within the isolator. The obese twin must have BMI > 30 kg/m2 and the pair must have a sustained multi-year BMI difference of at least 5.5 kg/m2. Recipient mice are fed a low fat (4% by weight) high in plant polysaccharide (LF-HPP), autoclaved mouse chow (B&K Universal, East Yorkshire, U.K. diet 7378000).

[0422] To prepare the fecal samples for gavage into the GF mice, fecal samples provided by donors are frozen immediately after production, stored at -80°C. Samples are homogenized by mortar and pestle while submerged in liquid nitrogen and a 500 mg aliquot of the pulverized material is diluted in 5 mL of reduced PBS (PBS supplemented with 0.1% Resazurin (w/v), 0.05% L-cysteine-HCl) in an anaerobic Coy chamber (atmosphere, 75% N2, 20% CO2, 5% H2) and then vortexed for 5 min at room temperature. The suspension is settled by gravity for 5 min, and then the clarified supernatant transferred to an anaerobic crimped tube that is transported to a gnotobiotic mouse facility. Prior to transfer of the tube into the gnotobiotic isolator, the outer surface of the tube is sterilized by exposure

for 20 min to chlorine dioxide in the transfer sleeve attached to the isolator. 200 µL aliquot of the suspension is provided into the stomachs of each recipient animal by gavage.

[0423] At day 15 post-colonization, the bacterial composition containing at least 108 CFU/ml per strain is administered daily by oral gavage for 4 weeks to half of the Ob mice and half of the Ln mice. The remaining Ob mice and Ln mice are administered PBS by the same regimen. Optionally, mice can receive 0-3 days of antibiotic pre-treatment prior to administration of the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple doses of test article, and 103 to 1010 CFU/ml per strain of a bacterial composition may be delivered. The bacterial composition may optionally be administered together or co-formulated with prebiotic(s).

[0424] Feces are collected from the cages for analysis of bacterial carriage. Total body weight, fat mass and lean body mass are measured at baseline before colonization, at days 8 and 15 post-colonization, and days 22, 29, 35, and 42 post-colonization (7, 14, 21, and 28 days after administration of the bacterial composition) using quantitative magnetic resonance analysis of body composition (EchoMRI-3in1 instrument). At time of sacrifice, epididymal fat pads are also collected and weighed. Optionally, luminal contents of the stomach, small intestine, cecum, and colon contents as well as the liver, spleen, and mesenteric lymph nodes can be collected for subsequent analysis. Alternative or additional time points may also be collected.

[0425] By the end of the treatment period with the bacterial composition, the Ob mice receiving the bacterial composition is expected to show significant differences in body composition (change in % fat mass; fat pad weight/total body weight) as compared to the Ob group receiving PBS and the Ln groups.

[0426] Optionally, at the end of the treatment period, the body composition is determined for all mice. Bacterial compositions that produce significant changes in body composition in the Ob mice (decrease in % fat mass; decrease in fat pad weight; or decrease in total body weight) as compared to control Ob mice receiving PBS are identified as therapeutic candidates.

Example 32. In vivo Validation of Bacterial Composition Efficacy In Germ Free Mice Conventionalized With Bacterial Composition and Lean/Obese Microbiota Controls

[0427] To test the potential of a bacterial composition's ability to treat obesity, 8-9 week old GF C57BL/6J mice can be conventionalized by introducing by oral gavage a) the bacterial composition, b) fecal samples from an obese female twin discordant for obesity (obese control), or c) fecal samples from the paired lean female twin (lean control). One gnotobiotic isolator is used per microbiota sample and each recipient mouse is individually caged within the isolator. The obese twin donors must have BMI > 30 kg/m2 and the donating pair must have a sustained multi-year BMI difference of at least 5.5 kg/m2. Recipient mice are fed a low fat (4% by weight) high in plant polysaccharide (LF-HPP), autoclaved mouse chow (B&K Universal, East Yorkshire, U.K. diet 7378000).

[0428] To prepare the fecal samples for gavage into the GF mice, fecal samples provided by donors are frozen immediately after production, stored at -80°C. Samples are homogenized by mortar and pestle while submerged in liquid nitrogen and a 500 mg aliquot of the pulverized material is diluted in 5 mL of reduced PBS (PBS supplemented with 0.1% Resazurin (w/v), 0.05% L-cysteine-HCl) in an anaerobic Coy chamber (atmosphere, 75% N2, 20% CO2, 5% H2) and then vortexed for 5 min at room temperature. The suspension is settled by gravity for 5 min, and then the clarified supernatant transferred to an anaerobic crimped tube that is transported to a gnotobiotic mouse facility.

[0429] To prepare the bacterial composition for gavage into the GF mice, see Example 9. Prior to transfer of tubes into the gnotobiotic isolator, the outer surface of the tube is sterilized by exposure for 20 min to chlorine dioxide in the transfer sleeve attached to the isolator. 200 µL aliquots of the fecal suspensions are provided into the stomachs of the recipient animals by gavage.

[0430] Feces are collected from the cages for analysis of bacterial carriage. Total body weight, fat mass and lean body mass are measured at baseline before colonization, at days 8, 15, 22, 29, and 35. At time of sacrifice, epididymal fat pads are also collected and weighed. Optionally, luminal contents of the stomach, small intestine, cecum, and colon contents as well as the liver, spleen, and mesenteric lymph nodes can be collected for subsequent analysis. Alternative or additional timepoints may also be collected.

[0431] By the end of the treatment period with the bacterial composition, the mice receiving the bacterial composition is expected to show body composition (change in % fat mass; fat pad weight/total body weight) and microbial composition that is similar to the lean control and that is statistically different from the obese control.

Example 33. In vivo Validation of Bacterial Composition Efficacy In Dietary Induced Obesity Mouse Model

[0432] Male C57BL/6 mice fed a high fat diet can be used to test bacterial compositions' ability to treat obesity in a diet-induced obesity (DIO mouse) prevention model. To do so, eight groups of mice (n=8) are used, with all combinations of +/- antibiotic pretreatment, bacterial composition vs. vehicle, and high fat vs. standard diet.

[0433] 4 week old male C57BL/6 mice are group housed (2-5 mice per cage) in filter top cages with autoclaved bedding, and free access to autoclaved irradiated food (LabDiet 5053, LabDiet, St. Louis, MO 63144) and autoclaved water. For groups receiving antibiotic pretreatment, drinking water is replaced by an antibiotic cocktail consisting of 10% glucose, kanamycin (0.5 mg/mL), gentamicin (0.044 mg/mL), colistin (1062.5 U/mL), metronidazole (0.269 mg/mL), ciprofloxacin (0.156 mg/mL), ampicillin (0.1 mg/mL) and vancomycin (0.056 mg/mL) (all constituents from Sigma-Aldrich, St. Louis MO) for 1 week, after which autoclaved water is returned to all cages. The mice are dosed daily with a volume of 0.2 ml containing at least 1 x 108 cfu/ml per strain daily or an equal volume of sterile PBS. Optionally, the dose may range from 5 x 106 to 5 x 1010 cfu/ml per strain and or dosing

may occur three times a week. After one week of dosing, a group (n=10) of mice dosed with vehicle and one with the bacterial composition are switched to a high fat diet (Research Diet D12492) and dosing is continued for all groups. Treatment is continued for 15 weeks following the diet shift. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple doses of test article, and 103 to 1010 CFU/ml per strain of a bacterial composition may be delivered. The bacterial composition may be optionally be administered together or co-formulated with prebiotic(s).

[0434] Body weight will be measured three times per week throughout the study. Blood will be drawn by submandibular bleed every three weeks, from which serum cholesterol and triglycerides will be measured. Fasting blood glucose will be measured in weeks 12 and 15 following the diet shift. At sacrifice, total body, gastrocnemius, liver, epididymal fat pad, and cecum weights are measured, and the contents of the cecum as well as one lobe of the liver are stored at -80°C. By the end of the experiment, successful treatments will have statistically significant differences in total body weight, epididymal fat pad mass, or cholesterol.

Example 34. In vivo Validation of Bacterial Composition Efficacy In Nonobese Diabetic Mouse Model of Type-1-Diabetes

[0435] To demonstrate the efficacy of the microbial composition for improving the incidence of type 1 diabetes, a type 1 diabetes mouse model described previously is utilized (*e.g.* see Markle et al 2013. Sex differences in the gut microbiome drive hormone dependent regulation of autoimmunity. Science 339: 1084). Briefly, nonobese diabetic (NOD)/Jsd (NOD) Specific Pathogen Free (SPF) female mice are housed in sterilized static caging. The animals receive a standard mouse diet (LabDiet #5015, PMI Nutrition International) and autoclaved water. All staff uses autoclaved gowns, caps, masks, shoe covers, and sterile gloves. Animal handling and cage changes are done under HEPA filtered air. The pathogen status is determined by weekly exposure of CD-1 sentinel mice to soiled bedding from the cages in the room. Quarterly serological testing of sentinels confirmed the NOD mice are negative for: Mouse Hepatitis Virus, Minute Virus of Mice, Mouse Parvovirus, Murine Norovirus, Sendai Virus, Theiler's Murine Encephalomyelitis, Retrovirus and for endo- and ectoparasites. In addition, live animals are subjected to additional, annual comprehensive testing, including necropsy, histopathology, bacteriology and parasitology testing.

[0436] To test the microbial composition for prophylactic ability to reduce, delay or prevent disease appearance, weanling NOD females (aged 22-26 days) are gavaged with 250uL the microbial bacterial composition using a 24G round tip gavage needle. Recipients are rested for 24 hours, and this procedure is repeated once. Optionally, mice can receive 0-3 days of antibiotic pre-treatment prior to administration with the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple doses of test article, and 103 to 1010

CFU/ml per strain of a bacterial composition may be delivered. The bacterial composition may be optionally be administered together or co-formulated with prebiotic(s).

[0437] As a negative control, a group of female weanling NOD mice are gavaged with cecal contents from a female NOD mouse, and as a positive control a third group of female NOD mice are given cecal contents from a male NOD diluted 50x (v/v) and delivered in 250ul. Spontaneous development of T1D assessment is assessed biweekly by measuring glucose levels in blood and urine. Animals are checked daily and are classified as diabetic when blood glucose exceeds 16mmol/L or urine glucose exceeds 250mg/dL. Additionally, serum insulin autoantibody (IAA) is measured by a micro-IAA assay (mIAA). Briefly, 125-I labeled human insulin (Perkin Elmer) is incubated with NOD serum with and without cold (unlabeled) human insulin and the immune complex is isolated by binding to protein A and G Sepharose. The assay is performed on a 96-well filtration plate to retain Sepharose beads and radioactivity is counted on a Topcount 96-well plate beta counter or similar instrument. An index is calculated by taking the difference of cpm between wells without and with cold insulin. A positive is defined by any conventional cut-off measure including a value greater than the 99th percentile of control values, or a value 3 standard deviations beyond the mean of the control values. Furthermore Insulitis is assessed. Briefly, pancreata are dissected and immediately immersed in OCT media (Tissue-Tek, Torrance, CA), frozen in -20°C 2-methylbutane, and stored at –70°C. Preparation of frozen sections is performed with a Leica CM 3050 Cryostat (Leica Canada). To maximize analysis of independent islet infiltrates, three 5-μm sections are cut at least 400 μm apart. Pancreatic sections are stained with Mayer's hematoxylin and eosin Y (H+E, Sigma) to visualize leukocyte infiltration. Assessment of insulitis severity in pancreatic sections is performed by one skilled in the art. Briefly, islets are graded according to the following criteria: 0, no visible infiltrates; 1, peri-insulitis as indicated by peri-vascular and peri-islet infiltrates; 2, <25% of the islet interior 9 occluded by leukocytes displaying invasive infiltrates; 3, >25% but <50% of the islet interior invaded by leukocytes; or 4, invasive insulitis involving 50%-100% of the islet field.

[0438] To evaluate the microbial composition for treatment of disease, the procedure above is repeated whereby NOD nonobese diabetic (NOD)/Jsd (NOD) Specific Pathogen Free (SPF) female mice are housed and evaluated for development of diabetes by the criteria described above. Once a mouse develops diabetes it is gavaged with the microbial composition, and blood glucose, urine glucose, and insulin serum levels are evaluated by ELISA weekly to determine disease progression. 7 weeks later animals are sacrificed and insulitis is evaluated by methods described above. Optionally, mice can receive 0-3 days of antibiotic pre-treatment prior to administration with the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple doses of test article, and 103 to 1010 CFU/ml per strain of a bacterial composition may be delivered.

Example 35. In vivo Validation of Bacterial Composition Efficacy In Nile Rat Model of Type-2-Diabetes

[0439] To test the efficacy of a microbial composition for delaying, treating or preventing the symptoms of type 2 diabetes, a Nile grass rat (Arvicanthis niloticus) model described previously is utilized (*e.g.* see Noda, K., et al. (2010). An animal model of spontaneous metabolic syndrome: Nile grass rat. The FASEB Journal 24, 2443–2453. or Chaabo, F., et al. (2010). Nutritional correlates and dynamics of diabetes in the Nile rat (Arvicanthis niloticus): a novel model for diet-induced type 2 diabetes and the metabolic syndrome. Nutrition & Metabolism 7, 29.). Nile rats, which spontaneously develop symptoms of type 2 diabetes and metabolic syndrome, are individually housed and have free access to autoclaved water and autoclaved standard laboratory chow (Lab Diet 5021; PMI Nutrition, St. Louis, MO, USA). At 5 weeks of age, thrice-weekly dosing of the Nile rats with about 5 x 108 cfu/ml per strain of the microbial composition or an equal volume of sterile PBS by oral gavage while under light sedation with 50%/50% O2/CO2 is initiated. Optionally, the dose may range from 5 x 106 to 5 x 1010 cfu/ml per strain and/or dosing may occur once weekly. Dosing will continue for 20 weeks post initiation, optionally lasting 15, 30, 40, or 50 weeks. The model could be modified to address prediabetes by shortening the duration to about 3 to 10 weeks post initiation of dosing.

[0440] Body weight will be measured three times per week throughout the study. Blood glucose, cholesterol, triglycerides, and hemoglobin A1C will be measured after obtaining blood by tail bleed while under light sedation with 50%/50% O2/CO2 every three weeks following initiation of dosing. At sacrifice, total body, liver, kidney, epididymal fat pad, and cecum weights are measured. Terminal plasma samples are used for measurement of insulin, blood glucose, cholesterol, triglycerides, and hemoglobin A1C. Following perfusion with PBS under deep anesthesia, the liver and kidneys are excised and fixed in 4% paraformaldehyde. Subsequently, 15 μm sections are stained with Oil-Red-O and counterstained with Mayer's hematoxylin to facilitate the identification of stores of hydrophobic lipids. The contents of the cecum are flash frozen in liquid nitrogen and stored at -80°C.

[0441] Animals treated with successful compositions will have statistically significant differences in terminal body weight, blood glucose, hemoglobin A1C, liver or kidney accumulation of lipid, and/or insulin from control animals.

Example 36. In vivo Validation Of Bacterial Composition For Prophylactic Use And Treatment In A Mouse Model Of Vancomycin Resistant Enterococcus (VRE) colonization

[0442] The emergence and spread of highly antibiotic-resistant bacteria represent a major clinical challenge (Snitkin et al Science Translational Medicine, 2012). In recent years, the numbers of infections caused by organisms such as methicillin- resistant Staphylococcus aureus, carbapenem-resistant Enterobacteriaceae, vancomycin-resistant Enterococcus (VRE), and Clostridium difficile have increased markedly, and many of these strains are acquiring resistance to the few remaining active antibiotics. Most infections produced by highly antibiotic-resistant bacteria are acquired during hospitalizations, and preventing patient- to-patient transmission of these pathogens is one of the major

challenges confronting hospitals and clinics. Most highly antibiotic-resistant bacterial strains belong to genera that colonize mucosal surfaces, usually at low densities. The highly complex microbiota that normally colonizes mucosal surfaces inhibits expansion of and domination by bacteria such as Enterobacteriaceae and Enterococcaceae. Destruction of the normal flora by antibiotic administration, however, leads to disinhibition antibiotic-resistant members of these bacterial families, enabling to their expansion to very high densities (Ubeda et al Journal of Clinical Investigation 2010). High-density colonization by these organisms can be calamitous for the susceptible patient, resulting in bacteremia and sepsis (Taur et al, Clinical Infectious Disease, 2012).

[0443] To test prophylactic use and treatment of a bacterial composition, a VRE infection mouse model is used as previously described (Ubeda et al, Infectious Immunity 2013, Ubeda et al, Journal of Clinical Investigation, 2010). Briefly, experiments are done with 7-week-old C57BL/6J female mice purchased from Jackson Laboratory, housed with irradiated food, and provided with acidified water. Mice are individually housed to avoid exchange of microbiota between mice due to coprophagia. For experimental infections with VRE, mice are treated with ampicillin (0.5 g/liter) in their drinking water, which is changed every 3 days.

[0444] In the treatment model, on day 1, mice are infected by means of oral gavage with 108 CFU of the vancomycin-resistant Enterococcus faecium strain purchased from ATCC (ATCC 700221). One day after infection (day 1), antibiotic treatment is stopped and VRE levels are determined at different time points by plating serial dilutions of fecal pellets on Enterococcosel agar plates (Difco) with vancomycin (8 ug/ml; Sigma). VRE colonies are identified by appearance and confirmed by Gram staining or other methods previously described (*e.g.* see Examples 1,2,3, and 4). In addition, as previously described (Ubeda et al, Journal of Clinical Investigation 2010), PCR of the vanA gene, which confers resistance to vancomycin, confirms the presence of VRE in infected mice. The bacterial composition test article such as but not limited to an ethanol treated, gradient purified spore preparation (as described herein), fecal suspension, or a Network Ecology is delivered in PBS on days 1-3 while the negative control contains only PBS and is also delivered on days 1-3 by oral gavage. Fresh fecal stool pellets are obtained daily for the duration of the experiment from days -7 to day 10. The samples are immediately frozen and stored at -80°C. DNA is extracted using standard techniques and analyzed with 16S or comparable methods (*e.g.* see Examples 1 and 2).

[0445] In the colonization model, ampicillin is administered as described above for day -7 to day 1, treatment with the bacterial composition or vehicle control is administered on day 0-2 and the VRE resistant bacteria at 108 CFU are administered on day 14. Fecal samples are taken throughout the experiment daily from -7 to day 21 and submitted for 16S sequencing as previously described (*e.g.* see Examples 1 and 2).

[0446] In both models, titers of VRE in feces are used to evaluate the success of the bacterial composition versus the negative control. A preferred bacterial composition either prevents or reduces

colonization by VRE compared to the negative control, or it accelerates the decrease in colonization after cessation of antibiotics. Furthermore, each bacterial composition is assessed for the ability of the bacterial composition test article to induce a healthy microbiome, as measured by engraftment, augmentation and increase in microbiota diversity.

Example 37. In vivo Validation of A Bacterial Composition For Prophylactic Use And Treatment of a Mouse Model of Carbapenem Resistant Klebsiella (CRKp) Colonization

[0447] The emergence of Klebsiella pneumoniae strains with decreased susceptibility to carbapenems is a significant threat to hospitalized patients. Resistance to carbapenems in these organisms is most frequently mediated by K. pneumoniae carbapenemase (KPC), a class A beta-lactamase that also confers resistance to broad-spectrum cephalosporins and commercially available beta-lactam/beta-lactamase inhibitor combinations (Queenan et al, Clinical Microbiology Review, 2007). KPC-producing K. pneumoniae (KPC-Kp) strains often harbor resistance determinants against several other classes of antimicrobials, including aminoglycosides and fluoroquinolones, resulting in truly multidrug-resistant (MDR) organisms (Hirsch et al, Journal of Antimicrobial Chemotherapy, 2009). Considering the limited antimicrobial options, infections caused by KPC-Kp pose a tremendous therapeutic challenge and are associated with poor clinical outcomes.

[0448] A treatment protocol in a mouse model previously described in mice sensitive to KCP-Kp (*e.g.* Perez et al, Antimicrobial Agents Chemotherapy, 2011) is used to evaluate the bacterial composition (test article) for treating carbapenem resistant Klebsiella and reducing carriage in the GI tract. Female CF1 mice (Harlan Sprague-Dawley, Indianapolis, IN) are used and are individually housed and weighed between 25 and 30 g. The bacterial composition includes without limitation an ethanol treated, gradient purified spore preparation (as described herein), fecal suspension, or a Network Ecology.

[0449] The thoroughly characterized strain of K. pneumoniae, VA-367 (8, 9, 25) is used. This clinical isolate is genetically related to the KPC-Kp strain circulating in the Eastern United States. Characterization of the resistance mechanisms in K. pneumoniae VA-367 with PCR and DNA sequence analysis revealed the presence of blaKPC-3, blaTEM-1, blaSHV-11, and blaSHV-12 as well as qnrB19 and aac(6')-Ib. Additionally, PCR and DNA sequencing revealed disruptions in the coding sequences of the following outer membrane protein genes: ompK35, ompK36, and ompK37. Antibiotic susceptibility testing (AST) was performed with the agar dilution method and interpreted according to current recommendations from the Clinical and Laboratory Standards Institute (CLSI). A modified Hodge test were performed, according to a method described previously (*e.g.* see Anderson et al, Journal of Clinical Microbiology, 2007) with ertapenem, meropenem, and imipenem. Tigecycline and polymyxin E were evaluated by Etest susceptibility assays (AB bioMerieux, Solna, Sweden). Results for tigecycline were interpreted as suggested by the U.S. Food and Drug

Administration (FDA) and according to CLSI recommendations (criteria for Pseudomonas) for polymyxin E.

[0450] In a prophylactic design, mice (10 per group) are assigned to receive either a bacterial composition (test article; *e.g.* see Example 9 or 10), or control group receiving only the vehicle. After 3 days of subcutaneous clindamycin treatment (Day -6,-5, -4) to sensitize them to KPC-Kp, mice are administered the test article or vehicle daily from day -10 to day 0, On day 0, 103 CFU of KPC-Kp VA-367 diluted in 0.5 ml phosphate-buffered saline (PBS) is administered by oral gavage. Fecal samples are collected 1, 4, 6, and 11 days after the administration of KPC-Kp to measure the concentration of carbapenem-resistant K. pneumoniae. Fecal samples (100 mg diluted in 800 ml of PBS) are plated onto MacConkey agar with 0.5 ug/ml of imipenem, and the number of CFU per gram of stool is determined. Efficacy of test articles is apparent as a reduction in KPC-Kp burden.

[0451] Alternatively other methods may be used to measure the levels of carbapenem-resistant K. pneumoniae *e.g.* PCR, antigen testing, as one who is skilled in the art could perform.

[0452] In a treatment design, mice are treated with subcutaneous clindamycin to reduce the normal intestinal flora 1 day before receiving 104 CFU of KPC-Kp VA-367 by oral gavage. For 7 days after oral gavage with KPC-Kp, mice receive oral gavage of normal saline (control group), or the bacterial composition. Fecal samples are collected at baseline and at 3, 6, 8, 11, 16, and 21 days after KPC-Kp VA-367 was given by gavage. The level of CRKp in feces is determined by plating serial dilutions of fecal suspensions to MacConkey agar with 0.5 ug/ml of imipenem, and the number of CFU per gram of stool is determined. Alternatively other methods may be used to measure the levels of carbapenem-resistant K. pneumoniae *e.g.* PCR, antigen testing, as one who's skilled in the art could perform. Efficacy of test articles is apparent as a reduction in KPC-Kp burden.

Example 38. In vivo validation of Bacterial Composition For Efficacy in For the Prophylactic Use or Treatment of Pathogenic Fungus in Mice Models

[0453] The bacterial compositions of the invention can be utilized for prophylaxis or treatment of pathogenic fungus in a mouse colonized with one of several Candida species. Adult male CD-1 (ICR) mice are intragastrically inoculated with C. albicans, C. tropicalis or C. parapsilosis as previously described (Mellado et al., Diagnostic Microbiology and Infectious Disease 2000). Tetracycline-HCl at 1g/L and 5% glucose are included in the drinking water starting on Day -2, 2 days before Candida dosing on Day 0, and throughout the experiment, to enhance colonization. 5 x 107 Candida are dosed in 0.1 mL on Day 0. By Day 4 all mice are colonized as detected by fecal cfu assay described below. Test articles are used in both prophylactic and treatment regimens. Prophylactic dosing with a bacterial composition including without limitation an ethanol treated, gradient purified spore preparation (as described herein), fecal suspension, or a Network Ecology occurs on Day -1 with a dose between 104 and 1010 bacteria, while treatment dosing occurs on Days 1, 2 and 3 with a similar dose. Negative control groups in both regimes are dosed with PBS administered in a similar manner.

All test article dosing is by oral gavage. Treated and untreated mice are kept separate in independently ventilated cages for all of the experiments. Sterilized food, bedding and bottles are used throughout the experiment. Sterilized tap water with or without supplements are also used to avoid contamination. Starting at day -1 postinfection (p.i.), mice are weighed daily and stool samples are collected from each animal and scored for consistency (0, normal feces; 1, mixed stool samples containing both solid and pasty feces; 2, pasty feces; 3, semiliquid feces; 4, liquid feces).

[0454] Feces are cultured for yeasts. Dilutions of fecal samples are titrated on Sabouraud Dextrose Chloramphenicol agar (Neogen cat #(7306) agar plates which are selective for fungi. After 24 – 48 h of incubation at 37°C, quantification of the cultures is achieved by counting the plates visually or by scanning the plates on a Colony Image Analysis Scanner (Spiral Biotech) and processed by the computer software CASBA 4 (Spiral Biotech). The results are noted as colony forming units (CFU) per gram of feces. Effect of bacterial composition on Candida colonization and quality of feces of infected mice is thus analyzed by comparing to placebo, and representative colonies are submitted for 16S/18S/ITS microbial identification before and after infection as previously described (*e.g.* See Example 1 and 2).

[0455] Using this model, the ability of test articles to prevent fungal dissemination and death is also tested. Starting on Day 4 in the above regimen, animals colonized with fungi are treated with immunosuppressive agents to induce deep neutropenia [defined as >500 polymorphonuclear cell per ml. Total white cells counts are performed using a hemacytometer Neubauer improved (Brand, Werthheim/Main, Germany)]. The immunosuppressive agents (150 mg/kg of cyclophosphamide (Sigma) and 65 mg/kg of 6-methyl-prednisolone (Sigma) are both administered intraperitoneally (i.p.) every 72–96 h until deep neutropenia is obtained and continue for 10 days. Test articles are delivered either on Day 4, 5 and 6, in parallel with the start of immunosuppression or for 3 consecutive days after deep neurtopenia is confirmed. Control animals are treated with PBS in each mode of treatment (Day 4-6, or 3 days post neutropenia. Mortality, dissemination and histology are monitored. When animals are severely ill, they are humanely euthanized with pentobarbital (Nembutal) or similar acceptable methods. Dissemination is quantified in kidneys, liver and spleen is quantified by suspending tissue separately in 2 mL of cooled PBS, and homogenizing using a lab-blender (Stomacher 80, Madrid, Spain). Aliquots of the homogenates are cultured for yeasts and bacterial flora. Results are expressed as CFU per gram of tissues. Candida dissemination is defined as positive cultures of at least two cultured organs. Positive culture is defined as plates yielding a value of > 1.5 log10 CFU/g of tissues. Histologic studies are also performed on five cut sections of liver, kidneys and spleen to detect yeasts.

Example 39. In vivo Validation of Bacterial Composition for Efficacy for Prophylaxis or Treatment in a Mouse Model of Methicillin Resistant Staphylococcus Aureus (MRSA)

[0456] Methicillin resistant Staphylococcus aureus (MRSA) is a Gram positive pathogen that is a major cause of nosocomial infections including sepsis, pneumonia and surgical site infections. Both nasal and gastrointestinal carriage of MRSA are implicated as sources of organisms associated with nosocomial infections. Rectal carriage of MRSA is common in patients in intensive care units and patients with both rectal and nasal colonization had significantly higher rates of MRSA infection than did patients with nasal colonization alone (Squier et al Staphylococcus aureus rectal carriage and its association with infections in patients in a surgical intensive care unit and a liver transplant unit. Infect Control Hosp Epidemiol 2002; 23:495–501.)

[0457] MRSA is also associated with gastrointestinal disease, including antibiotic associated diarrhea (Boyce and Havill, Nosocomial antibiotic-associated diarrhea associated with enterotoxin-producing strains of methicillin-resistant Staphylococcus aureus. Am J Gastroenterol. 2005 Aug;100(8):1828-34; Lo and Bourchardt, Antibiotic-associated diarrhea due to methicillin-resistant Staphylococcus aureus, Diagnostic Microbiology & Infectious Disease 63:388-389, 2009).

[0458] A mouse model of MRSA colonization is used to test the efficacy of bacterial compositions in treating MRSA colonization of the gut. CF1 mice are treated with streptomycin (1mg/ml), delivered in drinking water, for 5 days, after which they are orally inoculated with 1e7 cfu MRSA daily from Day 0 to Day 5 via their drinking water (Gries et al, Growth in Cecal Mucus Facilitates Colonization of the Mouse Intestinal Tract by Methicillin-Resistant Staphylococcus aureus, JID 2005;192:1621–7). Drinking water is prepared fresh each day. Colonization by MRSA is monitored by determining cfu/ml in feces each day starting on the day prior to the first day of MRSA inoculation. Feces are homogenized in sterile PBS and serial dilutions are plated to Mannitol salt agar and incubated aerobically for 48 h at 37°C. Presumptive MRSA colonies are confirmed by 16S rDNA PCR and sequencing as in (Examples 1 and 2). Bacterial compositions, control PBS or vancomycin are delivered by oral gavage starting on Day 6 for 3 days. Efficacy is observed as a reduction in MRSA cfu/g in feces, and/or faster time to a reduction of MRSA cfu/g, in the animals treated with bacterial compositions but not in control animals. Efficacy is compared to that of the positive control vancomycin, which clears the colonization.

[0459] The efficacy of bacterial compositions in preventing MRSA colonization is tested in a mouse model of prophylaxis in which CF1 mice are treated with streptomycin, delivered in drinking water, for 5 days. After 2 days without streptomycin, the mice are treated with bacterial compositions or control PBS by oral gavage for 3 days, and then inoculated with 1e7 cfu MRSA by oral gavage. Colonization by MRSA is monitored by determining cfu/ml in feces each day starting on the day prior to the first day of MRSA inoculation. Feces are homogenized in sterile PBS and serial dilutions are plated to Mannitol salt agar and incubated aerobically for 48 h at 37°C. Presumptive MRSA colonies

are confirmed by 16S rDNA PCR and sequencing as in Examples 1 and 2 for 16S sequencing. Efficacy is observed as a reduction in MRSA cfu/g in feces, and/or faster reduction of MRSA cfu/g, in the animals treated with bacterial compositions compared to control animals.

Example 40. Clinical Validation of Bacterial Composition for Efficacy in Obesity

[0460] To demonstrate a bacterial composition's ability to treat obesity, a group of 400 obese human subjects can be prospectively recruited. Inclusion criteria include BMI 30-45 kg/m^2. Exclusion criteria include Type 1 or Type 2 diabetes, treatment with any kind of anti-diabetic, anti-hyperglycemic or anti-obesity medication or surgical procedure (*e.g.* bariatric surgery), significant co-morbidities, participation in a formal weight loss program, either systolic blood pressure > 160 mm Hg or diastolic blood pressure >100 mmHg, subjects whose body weight is not stable, as judged by the Investigator (*e.g.* >5% change within 3 months prior to screening).

[0461] During a double blind treatment period of 12 weeks, the experimental treatment group (n=200) receives a daily oral dose of about 1x109 CFUs of viable bacteria either in the form of vegetative organisms or spores or both, whereas the control group (n=200) is administered a placebo at an identical frequency. The composition can be formulated in a delayed release enteric coated capsule or co-administered with bicarbonate buffer to aid passage of viable organisms through the stomach. The bacterial composition may be optionally be administered together or co-formulated with prebiotic(s).

[0462] Patients can be optionally treated with a broad spectrum antibiotic 0-10 days prior to first administration of the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple daily doses of test article, and a range of 103 to 1010 CFU of a given composition may be delivered.

[0463] At baseline and 6, 12, and 24 weeks after the beginning of the treatment period, change in body weight, waist and hip circumference, and waist/hip ratio will be measured. By the end of the 24 week treatment challenge period, the experimental group is expected to show significant differences from the control group in weight loss and/or waist and hip circumference, optionally 5% or greater weight loss.

[0464] Optionally, in the event an effect is detected at the end of the 24 week treatment period, the durability of the effect may be tested. All subjects will be taken off the experimental treatment and change in weight measured after 2 weeks, 4 weeks, 8 weeks, 16 weeks, and 52 weeks.

Example 41. Clinical Validation of Bacterial Composition for Efficacy in Weight Loss

[0465] To demonstrate a bacterial composition's ability to cause weight loss, a group of 400 human subjects with BMI > 25 kg/m^2 is prospectively recruited. Inclusion criteria include BMI > 25 kg/m^2. Exclusion criteria include Type 1 or Type 2 diabetes, treatment with any kind of anti-diabetic, anti-hyperglycemic or anti-obesity medication or surgical procedure (*e.g.* bariatric surgery), significant co-morbidities, participation in a formal weight loss program, either systolic blood

pressure > 160 mm Hg or diastolic blood pressure >100 mmHg, subjects who do not show stable body weight as judged by PI (*e.g.* >5% change within 3 months prior to screening).

[0466] During a double blind treatment period of 24 weeks, the experimental treatment group (n=200) receives a daily oral dose of about 1x109 CFUs of viable bacteria either in the form of vegetative organisms or spores or both, whereas the control group (n=200) is administered a placebo at an identical frequency. The composition can be formulated in a delayed release enteric coated capsule or co-administered with bicarbonate buffer to aid passage of viable organisms through the stomach. The bacterial composition may be optionally be administered together or co-formulated with prebiotic(s).

[0467] Patients may be optionally treated with a broad spectrum antibiotic 0-10 days prior to first administration of the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple daily doses of test article, and a range of 103 to 1010 CFU of a given composition may be delivered.

[0468] At baseline and 6, 12, and 24 weeks after the beginning of the treatment period, change in body weight will be measured. By the end of the 24 week treatment challenge period, the experimental group are expected to show significant differences from the control group in weight loss.

[0469] Optionally, in the event an effect is detected at the end of the 24 week treatment period, the durability of the effect may be tested. All subjects will be taken off the experimental treatment and change in weight measured after 2 weeks, 4 weeks, 8 weeks, 16 weeks, and 52 weeks.

Example 42. Clinical Validation of Bacterial Composition for Efficacy In Prediabetes

[0470] To demonstrate a bacterial composition's ability to treat prediabetes by exerting beneficial effects on markers associated with the onset of diabetes, a group of 60 human subjects with metabolic syndrome/prediabetes is prospectively recruited. Inclusion criteria include either (a) fasting plasma glucose between 5.6 and 6.9 mmol/L and 2 hr post-glucose load plasma glucose < 7.8 mmol/L, and/or (b) 2 hr post-glucose load plasma glucose in oral glucose tolerance test (OGTT) between 7.8 and 11.0 mmol/L. Exclusion criteria include established gestational, Type 1 or Type 2 diabetes, treatment with any kind of anti-diabetic, anti-hyperglycemic or anti-obesity medication or surgical procedure, use of systemic long-acting corticosteroids or prolonged use (greater than 10 days) of systemic corticosteroids, or any significant medical condition that would complicate the measurement of the endpoint or put the patient at risk.

[0471] Optionally, the study can be performed specifically in obese patients meeting the above inclusion criteria with the additional inclusion criteria of BMI 30-45 kg/m^2 as well as waist circumference > 88 cm in women and > 102 cm in men. Additional exclusion criteria include: 1) a history of surgical procedures for weight loss; 2) 2 repeat laboratory values at the screening visit of triglycerides > 4.52 mmol; and 3) either systolic blood pressure > 160 mm Hg or diastolic blood pressure >100 mmHg.

[0472] During a double blind treatment period of 12 weeks, the experimental treatment group (n=30) receives a daily oral dose of about 1x109 CFUs of viable bacteria either in the form of vegetative organisms or spores or both, whereas the control group is administered a placebo at an identical frequency (n=30). The composition can be formulated in a delayed release enteric coated capsule or co-administered with bicarbonate buffer to aid passage of viable organisms through the stomach. The bacterial composition may be optionally be administered together or co-formulated with prebiotic(s).

[0473] Patients can be optionally treated with a broad spectrum antibiotic 0-3 days prior to first administration of the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple daily doses of test article, and a range of 103 to 1011 CFU of a given composition may be delivered.

[0474] At baseline and 4, 8 and 12 weeks after the beginning of the treatment period, glucose tolerance is tested by OGTT and HbA1c (glycosylated hemoglobin) levels measured. At the same timepoints, insulin secretion will be assessed by plasma insulin levels measured during the oral glucose tolerance tests. Homeostatic model assessment beta (HOMA-beta) will be used to quantify beta cell function and HOMA-IR for insulin sensitivity. In addition, subjects will perform home blood glucose testing once weekly at home.

[0475] By the end of the 12 week treatment period, the experimental group is expected to show significant differences from the control group in glucose tolerance, insulin sensitivity, and/or insulin secretion reflecting improved insulin sensitivity, decreased pre-diabetes symptoms and improvement in metabolic syndrome.

[0476] Optionally, in the event an effect is detected at the end of the 12 week treatment period, the durability of the effect may be tested. All subjects will be taken off their respective treatment and return for oral glucose tolerance tests after 2 weeks, 4 weeks, 8 weeks, 16 weeks, and 52 weeks to measure HbA1c, insulin secretion, HOMA-beta, and HOMA-IR.

[0477] Optionally, the treatment period can be extended to collect an additional endpoint of progression to type 2 diabetes at 6 months and 12 months after the beginning of the treatment period.

Example 43. Clinical validation of bacterial composition for efficacy in type-2-diabetes

[0478] To demonstrate a bacterial composition's ability to treat type 2 diabetes, a group of 60 human subjects with type 2 diabetes is prospectively recruited. Inclusion criteria include diagnosis of type 2 diabetes with inadequate glycemic control on diet and exercise, glycosylated hemoglobin between 7.5% and 10.0% at screening, BMI $\leq$ 45 kg/m2.

[0479] Exclusion criteria include gestational diabetes, type 1 diabetes, treatment with any kind of anti-diabetic medication in the 12 weeks prior to screening, use of anti-obesity medication/surgical procedure, use of systemic long-acting corticosteroids or prolonged use (greater than 10 days) of systemic corticosteroids, or any significant co-morbidities related to the underlying diabetic condition.

[0480] Optionally, the study can be done in non insulin dependent type 2 diabetics who have inadequate glycemic control who are taking oral medications such as metformin, sulfonylureas, DPP-4 inhibitors, GLP-1 agonists, and SGLT2 inhibitors. Optionally, the study can be done in newly diagnosed non insulin dependent type 2 diabetics who are completely treatment naive.

[0481] During a double-blinded treatment period of 18 weeks, the experimental treatment group (n=30) receives a daily oral dose of about 1x109 CFUs of viable bacteria either in the form of vegetative organisms or spores or both, whereas the control group (n=30) is administered a placebo at an identical frequency. The composition can be formulated in a delayed release enteric coated capsule or co-administered with bicarbonate buffer to aid passage of viable organisms through the stomach. The bacterial composition may be optionally be administered together or co-formulated with prebiotic(s).

[0482] Patients may be optionally treated with a broad spectrum antibiotic 0-10 days prior to first administration of the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple daily doses of test article, and a range of 103 to 1010 CFUs of a given composition may be delivered.

[0483] At baseline and 6, 12 and 18 weeks after the beginning of the treatment period, HbA1c (glycosylated hemoglobin) levels, fasting plasma glucose, fasting insulin, HOMA-beta, and HOMA-IR, In addition, subjects will perform home blood glucose testing once weekly at home.

[0484] Optionally high sensitivity C-reactive protein, adiponectin, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, systolic and diastolic blood pressure can also be measured at the same timepoints.

[0485] By the end of the 18 week treatment period, the experimental group are expected to show significant differences from the control group in change in HbA1c, fasting plasma glucose, insulin sensitivity, and/or insulin secretion from baseline.

[0486] Optionally, in the event an effect is detected at the end of the 18 week treatment period, the durability of the effect may be tested. All subjects will be taken off the experimental treatment and return for measurement of HbA1c, fasting plasma glucose, fasting insulin, HOMA-beta, and HOMA-IR after 2 weeks, 4 weeks, 8 weeks, 16 weeks, and 52 weeks.

Example 44. Clinical Validation of Bacterial Composition for Efficacy in Recent Onset Type-1-Diabetes

[0487] To demonstrate a bacterial composition's ability to slow progression of recent onset type 1 diabetes, a group of 60 human subjects with recent onset type 1 diabetes is prospectively assembled.

[0488] Inclusion criteria include diagnosis of type 1 diabetes within 40 days prior to screening, positive test for at least one diabetes-related autoantibody such as GAD, IA-2, ZnT8, and/or anti-insulin (obtained within 10 days of onset of insulin therapy), peak stimulated C-peptide level ≥ 0.2 pmol/mL following mixed meal tolerance test (MMTT), and evidence of some fraction of residual

(normal) pancreatic function. Exclusion criteria include any form of diabetes other than type 1 (*e.g.* type 2 diabetes), prior or current treatment with corticosteroids, significant co-morbidities.

[0489] During a double-blind treatment period of 18 weeks, the experimental treatment group (n=30) receives a daily oral dose of about 1x109 CFUs of viable bacteria either in the form of vegetative organisms or spores or both, whereas the control group (n=30) is administered a placebo at an identical frequency. The composition can be formulated in a delayed release enteric coated capsule or co-administered with bicarbonate buffer to aid passage of viable organisms through the stomach. The bacterial composition may be optionally be administered together or co-formulated with prebiotic(s).

[0490] Patients can be optionally treated with a broad spectrum antibiotic 0-10 days prior to first administration of the bacterial composition. Alternative dosing schedules and routes of administration (*e.g.* rectal) may be employed, including multiple daily doses of test article, and a range of 103 to 1010 CFUs of a given composition are delivered.

[0491] At baseline and 6, 12 and 18 weeks after the beginning of the treatment period, stimulated C-peptide released in 2 hours during a standard mixed meal tolerance test (MMTT) and HbA1c levels will be measured. In addition, subjects will record total daily dose of insulin in a diary.

[0492] By the end of the 18 week treatment period, the experimental group is expected to show significant differences from the control group in change in stimulated C-peptide, HbA1c, and/or insulin dosage from baseline.

[0493] Optionally, in the event an effect is detected at the end of the 18 week treatment period, the durability of the effect may be tested. All subjects will be taken off the experimental treatment and return for measurement of stimulated C-peptide in response to MMTT and HbA1c levels after 2 weeks, 4 weeks, 8 weeks, 16 weeks, and 52 weeks.

Example 45. Clinical Validation of Bacterial Composition for Efficacy in Reduction of Opportunistic Pathogenic Fungus in Humans

[0494] The dimorphic yeast, Candida albicans, is the leading fungal pathogen in normal hosts and in patients with damaged immune systems. In immunocompromised hosts such as cancer patients, transplant patients, post-operative surgical patients, premature newborns, or HIV-infected people, C. albicans ranks as the leading fungal pathogen. Invasion leading to systemic infection may also develop in neutropenic patients whose T cell function is comprised. (Hostetter MK, Clinical Microbiology Reviews, Jan 1994, pp. 29-42.) In this population, disease ranges from aggressive local infections such as periodontitis, oral ulceration, or esophagitis in HIV-infected patients, to complex and potentially lethal infections of the bloodstream with subsequent dissemination to brain, eye, heart, liver, spleen, kidneys, or bone. Recently, the incidence of systemic candidiasis, which is caused by Candida spp., predominantly Candida albicans, has increased. This increase over the last two decades has caused a rise in the use of antifungal drugs, including azoles, such as fluconazole or ketoconazol,

leading to emergence of resistant organisms and thus increasing the need for alternative therapies (Looi et al., FEMS Microbiol Lett 2005).

[0495] In a prophylactic, randomized, double-blind study, healthy volunteers who have been prescreened as colonized with Candida albicans at >104 cfu/g by fecal culturing are randomized to receive either a placebo or a bacterial composition daily. Study volunteers are asked to avoid taking probiotics in any form in the week prior to dosing. The dosing of bacterial composition may, optionally, be modified to daily, every-other-day, weekly or any other frequency, and doses may range from 105 to 1010 CFU/mL. The subjects provide faecal and vaginal fluid samples pretreatment and on Days 7, 14 and 28 post-treatment that are cultivated on agar plates within 3 hours after delivery to the laboratory. Complementary genomic and microbiological methods are used to characterize the composition of the microbiota from each of the samples. C. albicans is detected by microbiological methods, for example by serial dilution and plating to fungal selective media CHROMagar Candida (BD cat#254093) which selects for fungal organisms, and against bacterial growth, or another fungal selective media, and also by using Taqman PCR based assay using similar methods as described previously (Maaroufi et al., J Clin Microbiol. 2003). A reduction in C. albicans levels in feces indicates efficacy in reducing colonization.

Summary

[0496] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification, including claims, are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical parameters are approximations and may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0497] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series.

[0498] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[0499] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

[0500] The foregoing description of the embodiments of the invention has been presented for the purpose of illustration; it is not intended to be exhaustive or to limit the invention to the precise forms disclosed. Persons skilled in the relevant art can appreciate that many modifications and variations are possible in light of the above disclosure.

[0501] Some portions of this description describe the embodiments of the invention in terms of algorithms and symbolic representations of operations on information. These algorithmic descriptions and representations are commonly used by those skilled in the data processing arts to convey the substance of their work effectively to others skilled in the art. These operations, while described functionally, computationally, or logically, are understood to be implemented by computer programs or equivalent electrical circuits, microcode, or the like. Furthermore, it has also proven convenient at times, to refer to these arrangements of operations as modules, without loss of generality. The described operations and their associated modules may be embodied in software, firmware, hardware, or any combinations thereof.

[0502] Any of the steps, operations, or processes described herein may be performed or implemented with one or more hardware or software modules, alone or in combination with other devices. In one embodiment, a software module is implemented with a computer program product comprising a computer-readable medium containing computer program code, which can be executed by a computer processor for performing any or all of the steps, operations, or processes described.

[0503] Embodiments of the invention may also relate to an apparatus for performing the operations herein. This apparatus may be specially constructed for the required purposes, and/or it may comprise a general-purpose computing device selectively activated or reconfigured by a computer program stored in the computer. Such a computer program may be stored in a tangible computer readable storage medium or any type of media suitable for storing electronic instructions, and coupled to a computer system bus. Furthermore, any computing systems referred to in the specification may include a single processor or may be architectures employing multiple processor designs for increased computing capability.

[0504] Embodiments of the invention may also relate to a computer data signal embodied in a carrier wave, where the computer data signal includes any embodiment of a computer program product or other data combination described herein. The computer data signal is a product that is presented in a tangible medium or carrier wave and modulated or otherwise encoded in the carrier wave, which is tangible, and transmitted according to any suitable transmission method.

[0505] Finally, the language used in the specification has been principally selected for readability and instructional purposes, and it may not have been selected to delineate or circumscribe the inventive subject matter. It is therefore intended that the scope of the invention be limited not by this detailed description, but rather by any claims that issue on an application based hereon. Accordingly, the disclosure of the embodiments of the invention is intended to be illustrative, but not limiting, of the scope of the invention, which is set forth in the following claims.

TABLES

Table 1.

List of Operational Taxonomic Units (OTU) with taxonomic assignments made to Genus, Species, and Phylogenetic Clade. Clade membership of bacterial OTUs is based on 16S sequence data. Clades are defined based on the topology of a phylogenetic tree that is constructed from full-length 16S sequences using maximum likelihood methods familiar to individuals with ordinary skill in the art of phylogenetics. Clades are constructed to ensure that all OTUs in a given clade are: (i) within a specified number of bootstrap supported nodes from one another, and (ii) within 5% genetic similarity. OTUs that are within the same clade can be distinguished as genetically and phylogenetically distinct from OTUs in a different clade based on 16S-V4 sequence data, while OTUs falling within the same clade are closely related. OTUs falling within the same clade are evolutionarily closely related and may or may not be distinguishable from one another using 16S-V4 sequence data. Members of the same clade, due to their evolutionary relatedness, play similar functional roles in a microbial ecology such as that found in the human gut. Compositions substituting one species with another from the same clade are likely to have conserved ecological function and therefore are useful in the present invention. All OTUs are denoted as to their putative capacity to form spores and whether they are a Pathogen or Pathobiont (see Definitions for description of "Pathobiont"). NIAID Priority Pathogens are denoted as 'Category-A', 'Category-B', or 'Category-C', and Opportunistic Pathogens are denoted as 'OP'. OTUs that are not pathogenic or for which their ability to exist as a pathogen is unknown are denoted as 'N'. The 'SEQ ID Number' denotes the identifier of the OTU in the Sequence Listing File and 'Public DB Accession' denotes the identifier of the OTU in a public sequence repository.

OTU	SEQ ID Number	Public DB Accession	Phylogenetic Clade	Spore Former	Pathogen Status
Corynebacterium coyleae	692	X96497	clade_100	N	N
Corynebacterium mucifaciens	711	NR_026396	clade_100	N	N
Corynebacterium ureicelerivorans	733	AM397636	clade_100	N	N
Corynebacterium appendicis	684	NR_028951	clade_102	N	N
Corynebacterium genitalium	698	ACLJ01000031	clade_102	N	N
Corynebacterium glaucum	699	NR_028971	clade_102	N	N
Corynebacterium imitans	703	AF537597	clade_102	N	N
Corynebacterium riegelii	719	EU848548	clade_102	N	N
Corynebacterium sp. L_2012475	723	HE575405	clade_102	N	N
Corynebacterium sp. NML 93_0481	724	GU238409	clade_102	N	N
Corynebacterium sundsvallense	728	Y09655	clade_102	N	N
Corynebacterium tuscaniae	730	AY677186	clade_102	N	N
Prevotella maculosa	1504	AGEK01000035	clade_104	N	N
Prevotella oris	1513	ADDV01000091	clade_104	N	N
Prevotella salivae	1517	AB108826	clade_104	N	N
Prevotella sp. ICM55	1521	HQ616399	clade_104	N	N
Prevotella sp. oral clone AA020	1528	AY005057	clade_104	N	N

Prevotella sp. oral clone GI032	1538	AY349396	clade_104	N	N
Prevotella sp. oral taxon G70	1558	GU432179	clade_104	N	N
Prevotella corporis	1491	L16465	clade_105	N	N
Bacteroides sp. 4_1_36	312	ACTC01000133	clade_110	N	N
Bacteroides sp. AR20	315	AF139524	clade_110	N	N
Bacteroides sp. D20	319	ACPT01000052	clade_110	N	N
Bacteroides sp. F_4	322	AB470322	clade_110	N	N
Bacteroides uniformis	329	AB050110	clade_110	N	N
Prevotella nanceiensis	1510	JN867228	clade_127	N	N
Prevotella sp. oral taxon 299	1548	ACWZ01000026	clade_127	N	N
Prevotella bergensis	1485	ACKS01000100	clade_128	N	N
Prevotella buccalis	1489	JN867261	clade_129	N	N
Prevotella timonensis	1564	ADEF01000012	clade_129	N	N
Prevotella oralis	1512	AEPE01000021	clade_130	N	N
Prevotella sp. SEQ072	1525	JN867238	clade_130	N	N
Leuconostoc carnosum	1177	NR_040811	clade_135	N	N
Leuconostoc gasicomitatum	1179	FN822744	clade_135	N	N
Leuconostoc inhae	1180	NR_025204	clade_135	N	N
Leuconostoc kimchii	1181	NR_075014	clade_135	N	N
Edwardsiella tarda	777	CP002154	clade_139	N	N
Photorhabdus asymbiotica	1466	Z76752	clade_139	N	N
Psychrobacter arcticus	1607	CP000082	clade_141	N	N
Psychrobacter cibarius	1608	HQ698586	clade_141	N	N
Psychrobacter cryohalolentis	1609	CP000323	clade_141	N	N
Psychrobacter faecalis	1610	HQ698566	clade_141	N	N
Psychrobacter nivimaris	1611	HQ698587	clade_141	N	N
Psychrobacter pulmonis	1612	HQ698582	clade_141	N	N
Pseudomonas aeruginosa	1592	AABQ07000001	clade_154	N	N
Pseudomonas sp. 2_1_26	1600	ACWU01000257	clade_154	N	N
Corynebacterium confusum	691	Y15886	clade_158	N	N
Corynebacterium propinquum	712	NR_037038	clade_158	N	N
Corynebacterium pseudodiphtheriticum	713	X84258	clade_158	N	N
Bartonella bacilliformis	338	NC_008783	clade_159	N	N
Bartonella grahamii	339	CP001562	clade_159	N	N
Bartonella henselae	340	NC_005956	clade_159	N	N
Bartonella quintana	341	BX897700	clade_159	N	N
Bartonella tamiae	342	EF672728	clade_159	N	N
Bartonella washoensis	343	FJ719017	clade_159	N	N
Brucella abortus	430	ACBJ01000075	clade_159	N	Category-B
Brucella canis	431	NR_044652	clade_159	N	Category-B
Brucella ceti	432	ACJD01000006	clade_159	N	Category-B
Brucella melitensis	433	AE009462	clade_159	N	Category-B
Brucella microti	434	NR_042549	clade_159	N	Category-B

Brucella ovis	435	NC_009504	clade_159	N	Category-B
Brucella sp. 83_13	436	ACBQ01000040	clade_159	N	Category-B
Brucella sp. BO1	437	EU053207	clade_159	N	Category-B
Brucella suis	438	ACBK01000034	clade_159	N	Category-B
Ochrobactrum anthropi	1360	NC_009667	clade_159	N	N
Ochrobactrum intermedium	1361	ACQA01000001	clade_159	N	N
Ochrobactrum pseudintermedium	1362	DQ365921	clade_159	N	N
Prevotella genomosp. C2	1496	AY278625	clade_164	N	N
Prevotella multisaccharivorax	1509	AFJE01000016	clade_164	N	N
Prevotella sp. oral clone IDR_CEC_0055	1543	AY550997	clade_164	N	N
Prevotella sp. oral taxon 292	1547	GQ422735	clade_164	N	N
Prevotella sp. oral taxon 300	1549	GU409549	clade_164	N	N
Prevotella marshii	1505	AEEI01000070	clade_166	N	N
Prevotella sp. oral clone IK053	1544	AY349401	clade_166	N	N
Prevotella sp. oral taxon 781	1554	GQ422744	clade_166	N	N
Prevotella stercorea	1562	AB244774	clade_166	N	N
Prevotella brevis	1487	NR_041954	clade_167	N	N
Prevotella ruminicola	1516	CP002006	clade_167	N	N
Prevotella sp. sp24	1560	AB003384	clade_167	N	N
Prevotella sp. sp34	1561	AB003385	clade_167	N	N
Prevotella albensis	1483	NR_025300	clade_168	N	N
Prevotella copri	1490	ACBX02000014	clade_168	N	N
Prevotella oulorum	1514	L16472	clade_168	N	N
Prevotella sp. BI_42	1518	AJ581354	clade_168	N	N
Prevotella sp. oral clone P4PB_83 P2	1546	AY207050	clade_168	N	N
Prevotella sp. oral taxon G60	1557	GU432133	clade_168	N	N
Prevotella amnii	1484	AB547670	clade_169	N	N
Bacteroides caccae	268	EU136686	clade_170	N	N
Bacteroides finegoldii	277	AB222699	clade_170	N	N
Bacteroides intestinalis	283	ABJL02000006	clade_171	N	N
Bacteroides sp. XB44A	326	AM230649	clade_171	N	N
Bifidobacteriaceae genomosp. C1	345	AY278612	clade_172	N	N
Bifidobacterium adolescentis	346	AAXD02000018	clade_172	N	N
Bifidobacterium angulatum	347	ABYS02000004	clade_172	N	N
Bifidobacterium animalis	348	CP001606	clade_172	N	N
Bifidobacterium breve	350	CP002743	clade_172	N	N
Bifidobacterium catenulatum	351	ABXY01000019	clade_172	N	N
Bifidobacterium dentium	352	CP001750	clade_172	N	OP
Bifidobacterium gallicum	353	ABXB03000004	clade_172	N	N
Bifidobacterium infantis	354	AY151398	clade_172	N	N
Bifidobacterium kashiwanohense	355	AB491757	clade_172	N	N
Bifidobacterium longum	356	ABQQ01000041	clade_172	N	N
Bifidobacterium pseudocatenulatum	357	ABXX02000002	clade_172	N	N
Bifidobacterium pseudolongum	358	NR_043442	clade_172	N	N

Bifidobacterium scardovii	359	AJ307005	clade_172	N	N
Bifidobacterium sp. HM2	360	AB425276	clade_172	N	N
Bifidobacterium sp. HMLN12	361	JF519685	clade_172	N	N
Bifidobacterium sp. M45	362	HM626176	clade_172	N	N
Bifidobacterium sp. MSX5B	363	HQ616382	clade_172	N	N
Bifidobacterium sp. TM_7	364	AB218972	clade_172	N	N
Bifidobacterium thermophilum	365	DQ340557	clade_172	N	N
Leuconostoc citreum	1178	AM157444	clade_175	N	N
Leuconostoc lactis	1182	NR_040823	clade_175	N	N
Eubacterium saburreum	858	AB525414	clade_178	Y	N
Eubacterium sp. oral clone IR009	866	AY349376	clade_178	Y	N
Lachnospiraceae bacterium ICM62	1061	HQ616401	clade_178	Y	N
Lachnospiraceae bacterium MSX33	1062	HQ616384	clade_178	Y	N
Lachnospiraceae bacterium oral taxon 107	1063	ADDS01000069	clade_178	Y	N
Alicyclobacillus acidocaldarius	122	NR_074721	clade_179	Y	N
Alicyclobacillus acidoterrestris	123	NR_040844	clade_179	N	N
Alicyclobacillus cycloheptanicus	125	NR_024754	clade_179	N	N
Acinetobacter baumannii	27	ACYQ01000014	clade_181	N	N
Acinetobacter calcoaceticus	28	AM157426	clade_181	N	N
Acinetobacter genomosp. C1	29	AY278636	clade_181	N	N
Acinetobacter haemolyticus	30	ADMT01000017	clade_181	N	N
Acinetobacter johnsonii	31	ACPL01000162	clade_181	N	N
Acinetobacter junii	32	ACPM01000135	clade_181	N	N
Acinetobacter lwoffii	33	ACPN01000204	clade_181	N	N
Acinetobacter parvus	34	AIEB01000124	clade_181	N	N
Acinetobacter schindleri	36	NR_025412	clade_181	N	N
Acinetobacter sp. 56A1	37	GQ178049	clade_181	N	N
Acinetobacter sp. CIP 101934	38	JQ638573	clade_181	N	N
Acinetobacter sp. CIP 102143	39	JQ638578	clade_181	N	N
Acinetobacter sp. M16_22	41	HM366447	clade_181	N	N
Acinetobacter sp. RUH2624	42	ACQF01000094	clade_181	N	N
Acinetobacter sp. SH024	43	ADCH01000068	clade_181	N	N
Lactobacillus jensenii	1092	ACQD01000066	clade_182	N	N
Alcaligenes faecalis	119	AB680368	clade_183	N	N
Alcaligenes sp. CO14	120	DQ643040	clade_183	N	N
Alcaligenes sp. S3	121	HQ262549	clade_183	N	N
Oligella ureolytica	1366	NR_041998	clade_183	N	N
Oligella urethralis	1367	NR_041753	clade_183	N	N
Eikenella corrodens	784	ACEA01000028	clade_185	N	N
Kingella denitrificans	1019	AEWV01000047	clade_185	N	N
Kingella genomosp. P1 oral cone MB2_C20	1020	DQ003616	clade_185	N	N
Kingella kingae	1021	AFHS01000073	clade_185	N	N
Kingella oralis	1022	ACJW02000005	clade_185	N	N
Kingella sp. oral clone ID059	1023	AY349381	clade_185	N	N
Neisseria elongata	1330	ADBF01000003	clade_185	N	N
Neisseria genomosp. P2 oral clone MB5_P15	1332	DQ003630	clade_185	N	N
Neisseria sp. oral clone JC012	1345	AY349388	clade_185	N	N
Neisseria sp. SMC_A9199	1342	FJ763637	clade_185	N	N

Simonsiella muelleri	1731	ADCY01000105	clade_185	N	N
Corynebacterium glucuronolyticum	700	ABYP01000081	clade_193	N	N
Corynebacterium pyruviciproducens	716	FJ185225	clade_193	N	N
Rothia aeria	1649	DQ673320	clade_194	N	N
Rothia dentocariosa	1650	ADDW01000024	clade_194	N	N
Rothia sp. oral taxon 188	1653	GU470892	clade_194	N	N
Corynebacterium accolens	681	ACGD01000048	clade_195	N	N
Corynebacterium macginleyi	707	AB359393	clade_195	N	N
Corynebacterium pseudogenitalium	714	ABYQ01000237	clade_195	N	N
Corynebacterium tuberculostearicum	729	ACVP01000009	clade_195	N	N
Lactobacillus casei	1074	CP000423	clade_198	N	N
Lactobacillus paracasei	1106	ABQV01000067	clade_198	N	N
Lactobacillus zeae	1143	NR_037122	clade_198	N	N
Prevotella dentalis	1492	AB547678	clade_205	N	N
Prevotella sp. oral clone ASCG10	1529	AY923148	clade_206	N	N
Prevotella sp. oral clone HF050	1541	AY349399	clade_206	N	N
Prevotella sp. oral clone ID019	1542	AY349400	clade_206	N	N
Prevotella sp. oral clone IK062	1545	AY349402	clade_206	N	N
Prevotella genomosp. P9 oral clone MB7_G16	1499	DQ003633	clade_207	N	N
Prevotella sp. oral clone AU069	1531	AY005062	clade_207	N	N
Prevotella sp. oral clone CY006	1532	AY005063	clade_207	N	N
Prevotella sp. oral clone FL019	1534	AY349392	clade_207	N	N
Actinomyces genomosp. C1	56	AY278610	clade_212	N	N
Actinomyces genomosp. C2	57	AY278611	clade_212	N	N
Actinomyces genomosp. P1 oral clone MB6_C03	58	DQ003632	clade_212	N	N
Actinomyces georgiae	59	GU561319	clade_212	N	N
Actinomyces israelii	60	AF479270	clade_212	N	N
Actinomyces massiliensis	61	AB545934	clade_212	N	N
Actinomyces meyeri	62	GU561321	clade_212	N	N
Actinomyces odontolyticus	66	ACYT01000123	clade_212	N	N
Actinomyces orihominis	68	AJ575186	clade_212	N	N
Actinomyces sp. CCUG 37290	71	AJ234058	clade_212	N	N
Actinomyces sp. ICM34	75	HQ616391	clade_212	N	N
Actinomyces sp. ICM41	76	HQ616392	clade_212	N	N
Actinomyces sp. ICM47	77	HQ616395	clade_212	N	N
Actinomyces sp. ICM54	78	HQ616398	clade_212	N	N
Actinomyces sp. oral clone IP081	87	AY349366	clade_212	N	N
Actinomyces sp. oral taxon 178	91	AEUH01000060	clade_212	N	N
Actinomyces sp. oral taxon 180	92	AEPP01000041	clade_212	N	N
Actinomyces sp. TeJ5	80	GU561315	clade_212	N	N
Haematobacter sp. BC14248	968	GU396991	clade_213	N	N
Paracoccus denitrificans	1424	CP000490	clade_213	N	N
Paracoccus marcusii	1425	NR_044922	clade_213	N	N
Grimontia hollisae	967	ADAQ01000013	clade_216	N	N
Shewanella putrefaciens	1723	CP002457	clade_216	N	N
Afipia genomosp. 4	111	EU117385	clade_217	N	N
Rhodopseudomonas palustris	1626	CP000301	clade_217	N	N
Methylobacterium extorquens	1223	NC_010172	clade_218	N	N

Methylobacterium podarium	1224	AY468363	clade_218	N	N
Methylobacterium radiotolerans	1225	GU294320	clade_218	N	N
Methylobacterium sp. 1sub	1226	AY468371	clade_218	N	N
Methylobacterium sp. MM4	1227	AY468370	clade_218	N	N
Clostridium baratii	555	NR_029229	clade_223	Y	N
Clostridium colicanis	576	FJ957863	clade_223	Y	N
Clostridium paraputrificum	611	AB536771	clade_223	Y	N
Clostridium sardiniense	621	NR_041006	clade_223	Y	N
Eubacterium budayi	837	NR_024682	clade_223	Y	N
Eubacterium moniliforme	851	HF558373	clade_223	Y	N
Eubacterium multiforme	852	NR_024683	clade_223	Y	N
Eubacterium nitritogenes	853	NR_024684	clade_223	Y	N
Achromobacter denitrificans	18	NR_042021	clade_224	N	N
Achromobacter piechaudii	19	ADMS01000149	clade_224	N	N
Achromobacter xylosoxidans	20	ACRC01000072	clade_224	N	N
Bordetella bronchiseptica	384	NR_025949	clade_224	N	OP
Bordetella holmesii	385	AB683187	clade_224	N	OP
Bordetella parapertussis	386	NR_025950	clade_224	N	OP
Bordetella pertussis	387	BX640418	clade_224	N	OP
Microbacterium chocolatum	1230	NR_037045	clade_225	N	N
Microbacterium flavescens	1231	EU714363	clade_225	N	N
Microbacterium lacticum	1233	EU714351	clade_225	N	N
Microbacterium oleivorans	1234	EU714381	clade_225	N	N
Microbacterium oxydans	1235	EU714348	clade_225	N	N
Microbacterium paraoxydans	1236	AJ491806	clade_225	N	N
Microbacterium phyllosphaerae	1237	EU714359	clade_225	N	N
Microbacterium schleiferi	1238	NR_044936	clade_225	N	N
Microbacterium sp. 768	1239	EU714378	clade_225	N	N
Microbacterium sp. oral strain C24KA	1240	AF287752	clade_225	N	N
Microbacterium testaceum	1241	EU714365	clade_225	N	N
Corynebacterium atypicum	686	NR_025540	clade_229	N	N
Corynebacterium mastitidis	708	AB359395	clade_229	N	N
Corynebacterium sp. NML 97_0186	725	GU238411	clade_229	N	N
Mycobacterium elephantis	1275	AF385898	clade_237	N	OP
Mycobacterium paraterrae	1288	EU919229	clade_237	N	OP
Mycobacterium phlei	1289	GU142920	clade_237	N	OP
Mycobacterium sp. 1776	1293	EU703152	clade_237	N	N
Mycobacterium sp. 1781	1294	EU703147	clade_237	N	N
Mycobacterium sp. AQ1GA4	1297	HM210417	clade_237	N	N
Mycobacterium sp. GN_10546	1299	FJ497243	clade_237	N	N
Mycobacterium sp. GN_10827	1300	FJ497247	clade_237	N	N
Mycobacterium sp. GN_11124	1301	FJ652846	clade_237	N	N
Mycobacterium sp. GN_9188	1302	FJ497240	clade_237	N	N
Mycobacterium sp. GR_2007_210	1303	FJ555538	clade_237	N	N
Anoxybacillus contaminans	172	NR_029006	clade_238	N	N
Anoxybacillus flavithermus	173	NR_074667	clade_238	Y	N
Bacillus aeolius	195	NR_025557	clade_238	N	N
Bacillus aerophilus	196	NR_042339	clade_238	Y	N

Bacillus aestuarii	197	GQ980243	clade_238	Y	N
Bacillus amyloliquefaciens	199	NR_075005	clade_238	Y	N
Bacillus anthracis	200	AAEN01000020	clade_238	Y	Category-A
Bacillus atrophaeus	201	NR_075016	clade_238	Y	OP
Bacillus badius	202	NR_036893	clade_238	Y	OP
Bacillus cereus	203	ABDJ01000015	clade_238	Y	OP
Bacillus circulans	204	AB271747	clade_238	Y	OP
Bacillus firmus	207	NR_025842	clade_238	Y	OP
Bacillus flexus	208	NR_024691	clade_238	Y	OP
Bacillus fordii	209	NR_025786	clade_238	Y	OP
Bacillus halmapalus	211	NR_026144	clade_238	Y	OP
Bacillus herbersteinensis	213	NR_042286	clade_238	Y	OP
Bacillus idriensis	215	NR_043268	clade_238	Y	OP
Bacillus lentus	216	NR_040792	clade_238	Y	OP
Bacillus licheniformis	217	NC_006270	clade_238	Y	OP
Bacillus megaterium	218	GU252124	clade_238	Y	OP
Bacillus nealsonii	219	NR_044546	clade_238	Y	OP
Bacillus niabensis	220	NR_043334	clade_238	Y	OP
Bacillus niacini	221	NR_024695	clade_238	Y	OP
Bacillus pocheonensis	222	NR_041377	clade_238	Y	OP
Bacillus pumilus	223	NR_074977	clade_238	Y	OP
Bacillus safensis	224	JQ624766	clade_238	Y	OP
Bacillus simplex	225	NR_042136	clade_238	Y	OP
Bacillus sonorensis	226	NR_025130	clade_238	Y	OP
Bacillus sp. 10403023 MM10403188	227	CAET01000089	clade_238	Y	OP
Bacillus sp. 2_A_57_CT2	230	ACWD01000095	clade_238	Y	OP
Bacillus sp. 2008724126	228	GU252108	clade_238	Y	OP
Bacillus sp. 2008724139	229	GU252111	clade_238	Y	OP
Bacillus sp. 7_16AIA	231	FN397518	clade_238	Y	OP
Bacillus sp. AP8	233	JX101689	clade_238	Y	OP
Bacillus sp. B27(2008)	234	EU362173	clade_238	Y	OP
Bacillus sp. BT1B_CT2	235	ACWC01000034	clade_238	Y	OP
Bacillus sp. GB1.1	236	FJ897765	clade_238	Y	OP
Bacillus sp. GB9	237	FJ897766	clade_238	Y	OP
Bacillus sp. HU19.1	238	FJ897769	clade_238	Y	OP
Bacillus sp. HU29	239	FJ897771	clade_238	Y	OP
Bacillus sp. HU33.1	240	FJ897772	clade_238	Y	OP
Bacillus sp. JC6	241	JF824800	clade_238	Y	OP
Bacillus sp. oral taxon F79	248	HM099654	clade_238	Y	OP
Bacillus sp. SRC_DSF1	243	GU797283	clade_238	Y	OP
Bacillus sp. SRC_DSF10	242	GU797292	clade_238	Y	OP
Bacillus sp. SRC_DSF2	244	GU797284	clade_238	Y	OP
Bacillus sp. SRC_DSF6	245	GU797288	clade_238	Y	OP
Bacillus sp. tc09	249	HQ844242	clade_238	Y	OP
Bacillus sp. zh168	250	FJ851424	clade_238	Y	OP
Bacillus sphaericus	251	DQ286318	clade_238	Y	OP
Bacillus sporothermodurans	252	NR_026010	clade_238	Y	OP

Bacillus subtilis	253	EU627588	clade_238	Y	OP
Bacillus thermoamylovorans	254	NR_029151	clade_238	Y	OP
Bacillus thuringiensis	255	NC_008600	clade_238	Y	OP
Bacillus weihenstephanensis	256	NR_074926	clade_238	Y	OP
Brevibacterium frigoritolerans	422	NR_042639	clade_238	N	N
Geobacillus kaustophilus	933	NR_074989	clade_238	Y	N
Geobacillus sp. E263	934	DQ647387	clade_238	N	N
Geobacillus sp. WCH70	935	CP001638	clade_238	N	N
Geobacillus stearothermophilus	936	NR_040794	clade_238	Y	N
Geobacillus thermocatenulatus	937	NR_043020	clade_238	N	N
Geobacillus thermodenitrificans	938	NR_074976	clade_238	Y	N
Geobacillus thermoglucosidasius	939	NR_043022	clade_238	Y	N
Geobacillus thermoleovorans	940	NR_074931	clade_238	N	N
Lysinibacillus fusiformis	1192	FN397522	clade_238	N	N
Lysinibacillus sphaericus	1193	NR_074883	clade_238	Y	N
Planomicrobium koreense	1468	NR_025011	clade_238	N	N
Sporosarcina newyorkensis	1754	AFPZ01000142	clade_238	N	N
Sporosarcina sp. 2681	1755	GU994081	clade_238	N	N
Ureibacillus composti	1968	NR_043746	clade_238	N	N
Ureibacillus suwonensis	1969	NR_043232	clade_238	N	N
Ureibacillus terrenus	1970	NR_025394	clade_238	N	N
Ureibacillus thermophilus	1971	NR_043747	clade_238	N	N
Ureibacillus thermosphaericus	1972	NR_040961	clade_238	N	N
Prevotella micans	1507	AGWK01000061	clade_239	N	N
Prevotella sp. oral clone DA058	1533	AY005065	clade_239	N	N
Prevotella sp. SEQ053	1523	JN867222	clade_239	N	N
Treponema socranskii	1937	NR_024868	clade_240	N	OP
Treponema sp. 6:H:D15A_4	1938	AY005083	clade_240	N	N
Treponema sp. oral taxon 265	1953	GU408850	clade_240	N	N
Treponema sp. oral taxon G85	1958	GU432215	clade_240	N	N
Porphyromonas endodontalis	1472	ACNN01000021	clade_241	N	N
Porphyromonas sp. oral clone BB134	1478	AY005068	clade_241	N	N
Porphyromonas sp. oral clone F016	1479	AY005069	clade_241	N	N
Porphyromonas sp. oral clone P2PB_52 P1	1480	AY207054	clade_241	N	N
Porphyromonas sp. oral clone P4GB_100 P2	1481	AY207057	clade_241	N	N
Acidovorax sp. 98_63833	26	AY258065	clade_245	N	N
Comamonadaceae bacterium NML000135	663	JN585335	clade_245	N	N
Comamonadaceae bacterium NML790751	664	JN585331	clade_245	N	N
Comamonadaceae bacterium NML910035	665	JN585332	clade_245	N	N
Comamonadaceae bacterium NML910036	666	JN585333	clade_245	N	N
Comamonas sp. NSP5	668	AB076850	clade_245	N	N
Delftia acidovorans	748	CP000884	clade_245	N	N
Xenophilus aerolatus	2018	JN585329	clade_245	N	N
Clostridiales sp. SS3/4	543	AY305316	clade_246	Y	N
Oribacterium sp. oral taxon 078	1380	ACIQ02000009	clade_246	N	N
Oribacterium sp. oral taxon 102	1381	GQ422713	clade_246	N	N
Weissella cibaria	2007	NR_036924	clade_247	N	N
Weissella confusa	2008	NR_040816	clade_247	N	N

Weissella hellenica	2009	AB680902	clade_247	N	N
Weissella kandleri	2010	NR_044659	clade_247	N	N
Weissella koreensis	2011	NR_075058	clade_247	N	N
Weissella paramesenteroides	2012	ACKU01000017	clade_247	N	N
Weissella sp. KLDS 7.0701	2013	EU600924	clade_247	N	N
Mobiluncus curtisii	1251	AEPZ01000013	clade_249	N	N
Clostridium beijerinckii	557	NR_074434	clade_252	Y	N
Clostridium botulinum	560	NC_010723	clade_252	Y	Category-A
Clostridium butyricum	561	ABDT01000017	clade_252	Y	N
Clostridium chauvoei	568	EU106372	clade_252	Y	N
Clostridium favososporum	582	X76749	clade_252	Y	N
Clostridium histolyticum	592	HF558362	clade_252	Y	N
Clostridium isatidis	597	NR_026347	clade_252	Y	N
Clostridium limosum	602	FR870444	clade_252	Y	N
Clostridium sartagoforme	622	NR_026490	clade_252	Y	N
Clostridium septicum	624	NR_026020	clade_252	Y	N
Clostridium sp. 7_2_43FAA	626	ACDK01000101	clade_252	Y	N
Clostridium sporogenes	645	ABKW02000003	clade_252	Y	N
Clostridium tertium	653	Y18174	clade_252	Y	N
Clostridium carnis	564	NR_044716	clade_253	Y	N
Clostridium celatum	565	X77844	clade_253	Y	N
Clostridium disporicum	579	NR_026491	clade_253	Y	N
Clostridium gasigenes	585	NR_024945	clade_253	Y	N
Clostridium quinii	616	NR_026149	clade_253	Y	N
Enhydrobacter aerosaccus	785	ACYI01000081	clade_256	N	N
Moraxella osloensis	1262	JN175341	clade_256	N	N
Moraxella sp. GM2	1264	JF837191	clade_256	N	N
Brevibacterium casei	420	JF951998	clade_257	N	N
Brevibacterium epidermidis	421	NR_029262	clade_257	N	N
Brevibacterium sanguinis	426	NR_028016	clade_257	N	N
Brevibacterium sp. H15	427	AB177640	clade_257	N	N
Clostridium hylemonae	593	AB023973	clade_260	Y	N
Clostridium scindens	623	AF262238	clade_260	Y	N
Lachnospiraceae bacterium 5_1_57FAA	1054	ACTR01000020	clade_260	Y	N
Acinetobacter radioresistens	35	ACVR01000010	clade_261	N	N
Clostridium glycyrrhizinilyticum	588	AB233029	clade_262	Y	N
Clostridium nexile	607	X73443	clade_262	Y	N
Coprococcus comes	674	ABVR01000038	clade_262	Y	N
Lachnospiraceae bacterium 1_1_57FAA	1048	ACTM01000065	clade_262	Y	N
Lachnospiraceae bacterium 1_4_56FAA	1049	ACTN01000028	clade_262	Y	N
Lachnospiraceae bacterium 8_1_57FAA	1057	ACWQ01000079	clade_262	Y	N
Ruminococcus lactaris	1663	ABOU02000049	clade_262	Y	N
Ruminococcus torques	1670	AAVP02000002	clade_262	Y	N
Lactobacillus alimentarius	1068	NR_044701	clade_263	N	N
Lactobacillus farciminis	1082	NR_044707	clade_263	N	N
Lactobacillus kimchii	1097	NR_025045	clade_263	N	N
Lactobacillus nodensis	1101	NR_041629	clade_263	N	N

Lactobacillus tucceti	1138	NR_042194	clade_263	N	N
Pseudomonas mendocina	1595	AAUL01000021	clade_265	N	N
Pseudomonas pseudoalcaligenes	1598	NR_037000	clade_265	N	N
Pseudomonas sp. NP522b	1602	EU723211	clade_265	N	N
Pseudomonas stutzeri	1603	AM905854	clade_265	N	N
Paenibacillus barcinonensis	1390	NR_042272	clade_270	N	N
Paenibacillus barengoltzii	1391	NR_042756	clade_270	N	N
Paenibacillus chibensis	1392	NR_040885	clade_270	N	N
Paenibacillus cookii	1393	NR_025372	clade_270	N	N
Paenibacillus durus	1394	NR_037017	clade_270	N	N
Paenibacillus glucanolyticus	1395	D78470	clade_270	N	N
Paenibacillus lactis	1396	NR_025739	clade_270	N	N
Paenibacillus lautus	1397	NR_040882	clade_270	Y	N
Paenibacillus pabuli	1398	NR_040853	clade_270	N	N
Paenibacillus polymyxa	1399	NR_037006	clade_270	Y	N
Paenibacillus popilliae	1400	NR_040888	clade_270	N	N
Paenibacillus sp. CIP 101062	1401	HM212646	clade_270	N	N
Paenibacillus sp. HGF5	1402	AEXS01000095	clade_270	Y	N
Paenibacillus sp. HGF7	1403	AFDH01000147	clade_270	Y	N
Paenibacillus sp. JC66	1404	JF824808	clade_270	N	N
Paenibacillus sp. R_27413	1405	HE586333	clade_270	N	N
Paenibacillus sp. R_27422	1406	HE586338	clade_270	N	N
Paenibacillus timonensis	1408	NR_042844	clade_270	N	N
Rothia mucilaginosa	1651	ACVO01000020	clade_271	N	N
Rothia nasimurium	1652	NR_025310	clade_271	N	N
Prevotella sp. oral taxon 302	1550	ACZK01000043	clade_280	N	N
Prevotella sp. oral taxon F68	1556	HM099652	clade_280	N	N
Prevotella tannerae	1563	ACIJ02000018	clade_280	N	N
Prevotellaceae bacterium P4P_62 P1	1566	AY207061	clade_280	N	N
Porphyromonas asaccharolytica	1471	AENO01000048	clade_281	N	N
Porphyromonas gingivalis	1473	AE015924	clade_281	N	N
Porphyromonas macacae	1475	NR_025908	clade_281	N	N
Porphyromonas sp. UQD 301	1477	EU012301	clade_281	N	N
Porphyromonas uenonis	1482	ACLR01000152	clade_281	N	N
Leptotrichia buccalis	1165	CP001685	clade_282	N	N
Leptotrichia hofstadii	1168	ACVB02000032	clade_282	N	N
Leptotrichia sp. oral clone HE012	1173	AY349386	clade_282	N	N
Leptotrichia sp. oral taxon 223	1176	GU408547	clade_282	N	N
Bacteroides fluxus	278	AFBN01000029	clade_285	N	N
Bacteroides helcogenes	281	CP002352	clade_285	N	N
Parabacteroides johnsonii	1419	ABYH01000014	clade_286	N	N
Parabacteroides merdae	1420	EU136685	clade_286	N	N
Treponema denticola	1926	ADEC01000002	clade_288	N	OP
Treponema genomosp. P5 oral clone MB3_P23	1929	DQ003624	clade_288	N	N
Treponema putidum	1935	AJ543428	clade_288	N	OP
Treponema sp. oral clone P2PB_53 P3	1942	AY207055	clade_288	N	N
Treponema sp. oral taxon 247	1949	GU408748	clade_288	N	N
Treponema sp. oral taxon 250	1950	GU408776	clade_288	N	N

Treponema sp. oral taxon 251	1951	GU408781	clade_288	N	N
Anaerococcus hydrogenalis	144	ABXA01000039	clade_289	N	N
Anaerococcus sp. 8404299	148	HM587318	clade_289	N	N
Anaerococcus sp. gpac215	156	AM176540	clade_289	N	N
Anaerococcus vaginalis	158	ACXU01000016	clade_289	N	N
Propionibacterium acidipropionici	1569	NC_019395	clade_290	N	N
Propionibacterium avidum	1571	AJ003055	clade_290	N	N
Propionibacterium granulosum	1573	FJ785716	clade_290	N	N
Propionibacterium jensenii	1574	NR_042269	clade_290	N	N
Propionibacterium propionicum	1575	NR_025277	clade_290	N	N
Propionibacterium sp. H456	1577	AB177643	clade_290	N	N
Propionibacterium thoenii	1581	NR_042270	clade_290	N	N
Bifidobacterium bifidum	349	ABQP01000027	clade_293	N	N
Leuconostoc mesenteroides	1183	ACKV01000113	clade_295	N	N
Leuconostoc pseudomesenteroides	1184	NR_040814	clade_295	N	N
Eubacterium sp. oral clone JI012	868	AY349379	clade_298	Y	N
Johnsonella ignava	1016	X87152	clade_298	N	N
Propionibacterium acnes	1570	ADJM01000010	clade_299	N	N
Propionibacterium sp. 434_HC2	1576	AFIL01000035	clade_299	N	N
Propionibacterium sp. LG	1578	AY354921	clade_299	N	N
Propionibacterium sp. S555a	1579	AB264622	clade_299	N	N
Alicyclobacillus contaminans	124	NR_041475	clade_301	Y	N
Alicyclobacillus herbarius	126	NR_024753	clade_301	Y	N
Alicyclobacillus pomorum	127	NR_024801	clade_301	Y	N
Alicyclobacillus sp. CCUG 53762	128	HE613268	clade_301	N	N
Actinomyces cardiffensis	53	GU470888	clade_303	N	N
Actinomyces funkei	55	HQ906497	clade_303	N	N
Actinomyces sp. HKU31	74	HQ335393	clade_303	N	N
Actinomyces sp. oral taxon C55	94	HM099646	clade_303	N	N
Kerstersia gyiorum	1018	NR_025669	clade_307	N	N
Pigmentiphaga daeguensis	1467	JN585327	clade_307	N	N
Aeromonas allosaccharophila	104	S39232	clade_308	N	N
Aeromonas enteropelogenes	105	X71121	clade_308	N	N
Aeromonas hydrophila	106	NC_008570	clade_308	N	N
Aeromonas jandaei	107	X60413	clade_308	N	N
Aeromonas salmonicida	108	NC_009348	clade_308	N	N
Aeromonas trota	109	X60415	clade_308	N	N
Aeromonas veronii	110	NR_044845	clade_308	N	N
Blautia coccoides	373	AB571656	clade_309	Y	N
Blautia glucerasea	374	AB588023	clade_309	Y	N
Blautia glucerasei	375	AB439724	clade_309	Y	N
Blautia hansenii	376	ABYU02000037	clade_309	Y	N
Blautia luti	378	AB691576	clade_309	Y	N
Blautia producta	379	AB600998	clade_309	Y	N
Blautia schinkii	380	NR_026312	clade_309	Y	N
Blautia sp. M25	381	HM626178	clade_309	Y	N
Blautia stercoris	382	HM626177	clade_309	Y	N
Blautia wexlerae	383	EF036467	clade_309	Y	N

Bryantella formatexigens	439	ACCL02000018	clade_309	Y	N
Clostridium coccoides	573	EF025906	clade_309	Y	N
Eubacterium cellulosolvens	839	AY178842	clade_309	Y	N
Lachnospiraceae bacterium 6_1_63FAA	1056	ACTV01000014	clade_309	Y	N
Marvinbryantia formatexigens	1196	AJ505973	clade_309	N	N
Ruminococcus hansenii	1662	M59114	clade_309	Y	N
Ruminococcus obeum	1664	AY169419	clade_309	Y	N
Ruminococcus sp. 5_1_39BFAA	1666	ACII01000172	clade_309	Y	N
Ruminococcus sp. K_1	1669	AB222208	clade_309	Y	N
Syntrophococcus sucromutans	1911	NR_036869	clade_309	Y	N
Rhodobacter sp. oral taxon C30	1620	HM099648	clade_310	N	N
Rhodobacter sphaeroides	1621	CP000144	clade_310	N	N
Lactobacillus antri	1071	ACLL01000037	clade_313	N	N
Lactobacillus coleohominis	1076	ACOH01000030	clade_313	N	N
Lactobacillus fermentum	1083	CP002033	clade_313	N	N
Lactobacillus gastricus	1085	AICN01000060	clade_313	N	N
Lactobacillus mucosae	1099	FR693800	clade_313	N	N
Lactobacillus oris	1103	AEKL01000077	clade_313	N	N
Lactobacillus pontis	1111	HM218420	clade_313	N	N
Lactobacillus reuteri	1112	ACGW02000012	clade_313	N	N
Lactobacillus sp. KLDS 1.0707	1127	EU600911	clade_313	N	N
Lactobacillus sp. KLDS 1.0709	1128	EU600913	clade_313	N	N
Lactobacillus sp. KLDS 1.0711	1129	EU600915	clade_313	N	N
Lactobacillus sp. KLDS 1.0713	1131	EU600917	clade_313	N	N
Lactobacillus sp. KLDS 1.0716	1132	EU600921	clade_313	N	N
Lactobacillus sp. KLDS 1.0718	1133	EU600922	clade_313	N	N
Lactobacillus sp. oral taxon 052	1137	GQ422710	clade_313	N	N
Lactobacillus vaginalis	1140	ACGV01000168	clade_313	N	N
Brevibacterium aurantiacum	419	NR_044854	clade_314	N	N
Brevibacterium linens	423	AJ315491	clade_314	N	N
Lactobacillus pentosus	1108	JN813103	clade_315	N	N
Lactobacillus plantarum	1110	ACGZ02000033	clade_315	N	N
Lactobacillus sp. KLDS 1.0702	1123	EU600906	clade_315	N	N
Lactobacillus sp. KLDS 1.0703	1124	EU600907	clade_315	N	N
Lactobacillus sp. KLDS 1.0704	1125	EU600908	clade_315	N	N
Lactobacillus sp. KLDS 1.0705	1126	EU600909	clade_315	N	N
Agrobacterium radiobacter	115	CP000628	clade_316	N	N
Agrobacterium tumefaciens	116	AJ389893	clade_316	N	N
Corynebacterium argentoratense	685	EF463055	clade_317	N	N
Corynebacterium diphtheriae	693	NC_002935	clade_317	N	OP
Corynebacterium pseudotuberculosis	715	NR_037070	clade_317	N	N
Corynebacterium renale	717	NR_037069	clade_317	N	N
Corynebacterium ulcerans	731	NR_074467	clade_317	N	N
Aurantimonas coralicida	191	AY065627	clade_318	N	N
Aureimonas altamirensis	192	FN658986	clade_318	N	N
Lactobacillus acidipiscis	1066	NR_024718	clade_320	N	N
Lactobacillus salivarius	1117	AEBA01000145	clade_320	N	N
Lactobacillus sp. KLDS 1.0719	1134	EU600923	clade_320	N	N

Lactobacillus buchneri	1073	ACGH01000101	clade_321	N	N
Lactobacillus genomosp. C1	1086	AY278619	clade_321	N	N
Lactobacillus genomosp. C2	1087	AY278620	clade_321	N	N
Lactobacillus hilgardii	1089	ACGP01000200	clade_321	N	N
Lactobacillus kefiri	1096	NR_042230	clade_321	N	N
Lactobacillus parabuchneri	1105	NR_041294	clade_321	N	N
Lactobacillus parakefiri	1107	NR_029039	clade_321	N	N
Lactobacillus curvatus	1079	NR_042437	clade_322	N	N
Lactobacillus sakei	1116	DQ989236	clade_322	N	N
Aneurinibacillus aneurinilyticus	167	AB101592	clade_323	N	N
Aneurinibacillus danicus	168	NR_028657	clade_323	N	N
Aneurinibacillus migulanus	169	NR_036799	clade_323	N	N
Aneurinibacillus terranovensis	170	NR_042271	clade_323	N	N
Staphylococcus aureus	1757	CP002643	clade_325	N	Category-B
Staphylococcus auricularis	1758	JQ624774	clade_325	N	N
Staphylococcus capitis	1759	ACFR01000029	clade_325	N	N
Staphylococcus caprae	1760	ACRH01000033	clade_325	N	N
Staphylococcus carnosus	1761	NR_075003	clade_325	N	N
Staphylococcus cohnii	1762	JN175375	clade_325	N	N
Staphylococcus condimenti	1763	NR_029345	clade_325	N	N
Staphylococcus epidermidis	1764	ACHE01000056	clade_325	N	N
Staphylococcus equorum	1765	NR_027520	clade_325	N	N
Staphylococcus haemolyticus	1767	NC_007168	clade_325	N	N
Staphylococcus hominis	1768	AM157418	clade_325	N	N
Staphylococcus lugdunensis	1769	AEQA01000024	clade_325	N	N
Staphylococcus pasteuri	1770	FJ189773	clade_325	N	N
Staphylococcus pseudintermedius	1771	CP002439	clade_325	N	N
Staphylococcus saccharolyticus	1772	NR_029158	clade_325	N	N
Staphylococcus saprophyticus	1773	NC_007350	clade_325	N	N
Staphylococcus sp. clone bottae7	1777	AF467424	clade_325	N	N
Staphylococcus sp. H292	1775	AB177642	clade_325	N	N
Staphylococcus sp. H780	1776	AB177644	clade_325	N	N
Staphylococcus succinus	1778	NR_028667	clade_325	N	N
Staphylococcus warneri	1780	ACPZ01000009	clade_325	N	N
Staphylococcus xylosus	1781	AY395016	clade_325	N	N
Cardiobacterium hominis	490	ACKY01000036	clade_326	N	N
Cardiobacterium valvarum	491	NR_028847	clade_326	N	N
Pseudomonas fluorescens	1593	AY622220	clade_326	N	N
Pseudomonas gessardii	1594	FJ943496	clade_326	N	N
Pseudomonas monteilii	1596	NR_024910	clade_326	N	N
Pseudomonas poae	1597	GU188951	clade_326	N	N
Pseudomonas putida	1599	AF094741	clade_326	N	N
Pseudomonas sp. G1229	1601	DQ910482	clade_326	N	N
Pseudomonas tolaasii	1604	AF320988	clade_326	N	N
Pseudomonas viridiflava	1605	NR_042764	clade_326	N	N
Bacillus alcalophilus	198	X76436	clade_327	Y	N
Bacillus clausii	205	FN397477	clade_327	Y	OP

Bacillus gelatini	210	NR_025595	clade_327	Y	OP
Bacillus halodurans	212	AY144582	clade_327	Y	OP
Bacillus sp. oral taxon F26	246	HM099642	clade_327	Y	OP
Listeria grayi	1185	ACCR02000003	clade_328	N	OP
Listeria innocua	1186	JF967625	clade_328	N	N
Listeria ivanovii	1187	X56151	clade_328	N	N
Listeria monocytogenes	1188	CP002003	clade_328	N	Category-B
Listeria welshimeri	1189	AM263198	clade_328	N	OP
Capnocytophaga sp. oral clone ASCH05	484	AY923149	clade_333	N	N
Capnocytophaga sputigena	489	ABZV01000054	clade_333	N	N
Leptotrichia genomosp. C1	1166	AY278621	clade_334	N	N
Leptotrichia shahii	1169	AY029806	clade_334	N	N
Leptotrichia sp. neutropenicPatient	1170	AF189244	clade_334	N	N
Leptotrichia sp. oral clone GT018	1171	AY349384	clade_334	N	N
Leptotrichia sp. oral clone GT020	1172	AY349385	clade_334	N	N
Bacteroides sp. 20_3	296	ACRQ01000064	clade_335	N	N
Bacteroides sp. 3_1_19	307	ADCJ01000062	clade_335	N	N
Bacteroides sp. 3_2_5	311	ACIB01000079	clade_335	N	N
Parabacteroides distasonis	1416	CP000140	clade_335	N	N
Parabacteroides goldsteinii	1417	AY974070	clade_335	N	N
Parabacteroides gordonii	1418	AB470344	clade_335	N	N
Parabacteroides sp. D13	1421	ACPW01000017	clade_335	N	N
Capnocytophaga genomosp. C1	477	AY278613	clade_336	N	N
Capnocytophaga ochracea	480	AEOH01000054	clade_336	N	N
Capnocytophaga sp. GEJ8	481	GU561335	clade_336	N	N
Capnocytophaga sp. oral strain A47ROY	486	AY005077	clade_336	N	N
Capnocytophaga sp. S1b	482	U42009	clade_336	N	N
Paraprevotella clara	1426	AFFY01000068	clade_336	N	N
Bacteroides heparinolyticus	282	JN867284	clade_338	N	N
Prevotella heparinolytica	1500	GQ422742	clade_338	N	N
Treponema genomosp. P4 oral clone MB2_G19	1928	DQ003618	clade_339	N	N
Treponema genomosp. P6 oral clone MB4_G11	1930	DQ003625	clade_339	N	N
Treponema sp. oral taxon 254	1952	GU408803	clade_339	N	N
Treponema sp. oral taxon 508	1956	GU413616	clade_339	N	N
Treponema sp. oral taxon 518	1957	GU413640	clade_339	N	N
Chlamydia muridarum	502	AE002160	clade_341	N	OP
Chlamydia trachomatis	504	U68443	clade_341	N	OP
Chlamydia psittaci	503	NR_036864	clade_342	N	Category-B
Chlamydophila pneumoniae	509	NC_002179	clade_342	N	OP
Chlamydophila psittaci	510	D85712	clade_342	N	OP
Anaerococcus octavius	146	NR_026360	clade_343	N	N
Anaerococcus sp. 8405254	149	HM587319	clade_343	N	N
Anaerococcus sp. 9401487	150	HM587322	clade_343	N	N
Anaerococcus sp. 9403502	151	HM587325	clade_343	N	N
Gardnerella vaginalis	923	CP001849	clade_344	N	N
Campylobacter lari	466	CP000932	clade_346	N	OP

Anaerobiospirillum succiniciproducens	142	NR_026075	clade_347	N	N
Anaerobiospirillum thomasii	143	AJ420985	clade_347	N	N
Ruminobacter amylophilus	1654	NR_026450	clade_347	N	N
Succinatimonas hippei	1897	AEVO01000027	clade_347	N	N
Actinomyces europaeus	54	NR_026363	clade_348	N	N
Actinomyces sp. oral clone GU009	82	AY349361	clade_348	N	N
Moraxella catarrhalis	1260	CP002005	clade_349	N	N
Moraxella lincolnii	1261	FR822735	clade_349	N	N
Moraxella sp. 16285	1263	JF682466	clade_349	N	N
Psychrobacter sp. 13983	1613	HM212668	clade_349	N	N
Actinobaculum massiliae	49	AF487679	clade_350	N	N
Actinobaculum schaalii	50	AY957507	clade_350	N	N
Actinobaculum sp. BM#101342	51	AY282578	clade_350	N	N
Actinobaculum sp. P2P_19 P1	52	AY207066	clade_350	N	N
Actinomyces sp. oral clone IO076	84	AY349363	clade_350	N	N
Actinomyces sp. oral taxon 848	93	ACUY01000072	clade_350	N	N
Clostridium innocuum	595	M23732	clade_351	Y	N
Clostridium sp. HGF2	628	AENW01000022	clade_351	Y	N
Actinomyces neuii	65	X71862	clade_352	N	N
Mobiluncus mulieris	1252	ACKW01000035	clade_352	N	N
Clostridium perfringens	612	ABDW01000023	clade_353	Y	Category-B
Sarcina ventriculi	1687	NR_026146	clade_353	Y	N
Clostridium bartlettii	556	ABEZ02000012	clade_354	Y	N
Clostridium bifermentans	558	X73437	clade_354	Y	N
Clostridium ghonii	586	AB542933	clade_354	Y	N
Clostridium glycolicum	587	FJ384385	clade_354	Y	N
Clostridium mayombei	605	FR733682	clade_354	Y	N
Clostridium sordellii	625	AB448946	clade_354	Y	N
Clostridium sp. MT4 E	635	FJ159523	clade_354	Y	N
Eubacterium tenue	872	M59118	clade_354	Y	N
Clostridium argentinense	553	NR_029232	clade_355	Y	N
Clostridium sp. JC122	630	CAEV01000127	clade_355	Y	N
Clostridium sp. NMBHI_1	636	JN093130	clade_355	Y	N
Clostridium subterminale	650	NR_041795	clade_355	Y	N
Clostridium sulfidigenes	651	NR_044161	clade_355	Y	N
Blastomonas natatoria	372	NR_040824	clade_356	N	N
Novosphingobium aromaticivorans	1357	AAAV03000008	clade_356	N	N
Sphingomonas sp. oral clone FI012	1745	AY349411	clade_356	N	N
Sphingopyxis alaskensis	1749	CP000356	clade_356	N	N
Oxalobacter formigenes	1389	ACDQ01000020	clade_357	N	N
Veillonella atypica	1974	AEDS01000059	clade_358	N	N
Veillonella dispar	1975	ACIK02000021	clade_358	N	N
Veillonella genomosp. P1 oral clone MB5_P17	1976	DQ003631	clade_358	N	N
Veillonella parvula	1978	ADFU01000009	clade_358	N	N
Veillonella sp. 3_1_44	1979	ADCV01000019	clade_358	N	N
Veillonella sp. 6_1_27	1980	ADCW01000016	clade_358	N	N
Veillonella sp. ACP1	1981	HQ616359	clade_358	N	N

Veillonella sp. AS16	1982	HQ616365	clade_358	N	N
Veillonella sp. BS32b	1983	HQ616368	clade_358	N	N
Veillonella sp. ICM51a	1984	HQ616396	clade_358	N	N
Veillonella sp. MSA12	1985	HQ616381	clade_358	N	N
Veillonella sp. NVG 100cf	1986	EF108443	clade_358	N	N
Veillonella sp. OK11	1987	JN695650	clade_358	N	N
Veillonella sp. oral clone ASCG01	1990	AY923144	clade_358	N	N
Veillonella sp. oral clone ASCG02	1991	AY953257	clade_358	N	N
Veillonella sp. oral clone OH1A	1992	AY947495	clade_358	N	N
Veillonella sp. oral taxon 158	1993	AENU01000007	clade_358	N	N
Dorea formicigenerans	773	AAXA02000006	clade_360	Y	N
Dorea longicatena	774	AJ132842	clade_360	Y	N
Lachnospiraceae bacterium 2_1_46FAA	1050	ADLB01000035	clade_360	Y	N
Lachnospiraceae bacterium 2_1_58FAA	1051	ACTO01000052	clade_360	Y	N
Lachnospiraceae bacterium 4_1_37FAA	1053	ADCR01000030	clade_360	Y	N
Lachnospiraceae bacterium 9_1_43BFAA	1058	ACTX01000023	clade_360	Y	N
Ruminococcus gnavus	1661	X94967	clade_360	Y	N
Ruminococcus sp. ID8	1668	AY960564	clade_360	Y	N
Kocuria marina	1040	GQ260086	clade_365	N	N
Kocuria rhizophila	1042	AY030315	clade_365	N	N
Kocuria rosea	1043	X87756	clade_365	N	N
Kocuria varians	1044	AF542074	clade_365	N	N
Blautia hydrogenotrophica	377	ACBZ01000217	clade_368	Y	N
Clostridiaceae bacterium END_2	531	EF451053	clade_368	N	N
Lactonifactor longoviformis	1147	DQ100449	clade_368	Y	N
Robinsoniella peoriensis	1633	AF445258	clade_368	Y	N
Micrococcus antarcticus	1242	NR_025285	clade_371	N	N
Micrococcus luteus	1243	NR_075062	clade_371	N	N
Micrococcus lylae	1244	NR_026200	clade_371	N	N
Micrococcus sp. 185	1245	EU714334	clade_371	N	N
Lactobacillus brevis	1072	EU194349	clade_372	N	N
Lactobacillus parabrevis	1104	NR_042456	clade_372	N	N
Pediococcus acidilactici	1436	ACXB01000026	clade_372	N	N
Pediococcus pentosaceus	1437	NR_075052	clade_372	N	N
Lactobacillus dextrinicus	1081	NR_036861	clade_373	N	N
Lactobacillus perolens	1109	NR_029360	clade_373	N	N
Lactobacillus rhamnosus	1113	ABWJ01000068	clade_373	N	N
Lactobacillus saniviri	1118	AB602569	clade_373	N	N
Lactobacillus sp. BT6	1121	HQ616370	clade_373	N	N
Mycobacterium mageritense	1282	FR798914	clade_374	N	OP
Mycobacterium neoaurum	1286	AF268445	clade_374	N	OP
Mycobacterium smegmatis	1291	CP000480	clade_374	N	OP
Mycobacterium sp. HE5	1304	AJ012738	clade_374	N	N
Dysgonomonas gadei	775	ADLV01000001	clade_377	N	N
Dysgonomonas mossii	776	ADLW01000023	clade_377	N	N
Porphyromonas levii	1474	NR_025907	clade_377	N	N
Porphyromonas somerae	1476	AB547667	clade_377	N	N
Bacteroides barnesiae	267	NR_041446	clade_378	N	N

Bacteroides coprocola	272	ABIY02000050	clade_378	N	N
Bacteroides coprophilus	273	ACBW01000012	clade_378	N	N
Bacteroides dorei	274	ABWZ01000093	clade_378	N	N
Bacteroides massiliensis	284	AB200226	clade_378	N	N
Bacteroides plebeius	289	AB200218	clade_378	N	N
Bacteroides sp. 3_1_33FAA	309	ACPS01000085	clade_378	N	N
Bacteroides sp. 3_1_40A	310	ACRT01000136	clade_378	N	N
Bacteroides sp. 4_3_47FAA	313	ACDR02000029	clade_378	N	N
Bacteroides sp. 9_1_42FAA	314	ACAA01000096	clade_378	N	N
Bacteroides sp. NB_8	323	AB117565	clade_378	N	N
Bacteroides vulgatus	331	CP000139	clade_378	N	N
Bacteroides ovatus	287	ACWH01000036	clade_38	N	N
Bacteroides sp. 1_1_30	294	ADCL01000128	clade_38	N	N
Bacteroides sp. 2_1_22	297	ACPQ01000117	clade_38	N	N
Bacteroides sp. 2_2_4	299	ABZZ01000168	clade_38	N	N
Bacteroides sp. 3_1_23	308	ACRS01000081	clade_38	N	N
Bacteroides sp. D1	318	ACAB02000030	clade_38	N	N
Bacteroides sp. D2	321	ACGA01000077	clade_38	N	N
Bacteroides sp. D22	320	ADCK01000151	clade_38	N	N
Bacteroides xylanisolvens	332	ADKP01000087	clade_38	N	N
Treponema lecithinolyticum	1931	NR_026247	clade_380	N	OP
Treponema parvum	1933	AF302937	clade_380	N	OP
Treponema sp. oral clone JU025	1940	AY349417	clade_380	N	N
Treponema sp. oral taxon 270	1954	GQ422733	clade_380	N	N
Parascardovia denticolens	1428	ADEB01000020	clade_381	N	N
Scardovia inopinata	1688	AB029087	clade_381	N	N
Scardovia wiggsiae	1689	AY278626	clade_381	N	N
Clostridiales bacterium 9400853	533	HM587320	clade_384	N	N
Eubacterium infirmum	849	U13039	clade_384	Y	N
Eubacterium sp. WAL 14571	864	FJ687606	clade_384	Y	N
Mogibacterium diversum	1254	NR_027191	clade_384	N	N
Mogibacterium neglectum	1255	NR_027203	clade_384	N	N
Mogibacterium pumilum	1256	NR_028608	clade_384	N	N
Mogibacterium timidum	1257	Z36296	clade_384	N	N
Erysipelotrichaceae bacterium 5_2_54FAA	823	ACZW01000054	clade_385	Y	N
Eubacterium biforme	835	ABYT01000002	clade_385	Y	N
Eubacterium cylindroides	842	FP929041	clade_385	Y	N
Eubacterium dolichum	844	L34682	clade_385	Y	N
Eubacterium sp. 3_1_31	861	ACTL01000045	clade_385	Y	N
Eubacterium tortuosum	873	NR_044648	clade_385	Y	N
Borrelia burgdorferi	389	ABGI01000001	clade_386	N	OP
Borrelia garinii	392	ABJV01000001	clade_386	N	OP
Borrelia sp. NE49	397	AJ224142	clade_386	N	OP
Caldimonas manganoxidans	457	NR_040787	clade_387	N	N
Comamonadaceae bacterium oral taxon F47	667	HM099651	clade_387	N	N
Lautropia mirabilis	1149	AEQP01000026	clade_387	N	N
Lautropia sp. oral clone AP009	1150	AY005030	clade_387	N	N
Bulleidia extructa	441	ADFR01000011	clade_388	Y	N

Solobacterium moorei	1739	AECQ01000039	clade_388	Y	N
Peptoniphilus asaccharolyticus	1441	D14145	clade_389	N	N
Peptoniphilus duerdenii	1442	EU526290	clade_389	N	N
Peptoniphilus harei	1443	NR_026358	clade_389	N	N
Peptoniphilus indolicus	1444	AY153431	clade_389	N	N
Peptoniphilus lacrimalis	1446	ADDO01000050	clade_389	N	N
Peptoniphilus sp. gpac077	1450	AM176527	clade_389	N	N
Peptoniphilus sp. JC140	1447	JF824803	clade_389	N	N
Peptoniphilus sp. oral taxon 386	1452	ADCS01000031	clade_389	N	N
Peptoniphilus sp. oral taxon 836	1453	AEAA01000090	clade_389	N	N
Peptostreptococcaceae bacterium ph1	1454	JN837495	clade_389	N	N
Dialister pneumosintes	765	HM596297	clade_390	N	N
Dialister sp. oral taxon 502	767	GQ422739	clade_390	N	N
Cupriavidus metallidurans	741	GU230889	clade_391	N	N
Herbaspirillum seropedicae	1001	CP002039	clade_391	N	N
Herbaspirillum sp. JC206	1002	JN657219	clade_391	N	N
Janthinobacterium sp. SY12	1015	EF455530	clade_391	N	N
Massilia sp. CCUG 43427A	1197	FR773700	clade_391	N	N
Ralstonia pickettii	1615	NC_010682	clade_391	N	N
Ralstonia sp. 5_7_47FAA	1616	ACUF01000076	clade_391	N	N
Francisella novicida	889	ABSS01000002	clade_392	N	N
Francisella philomiragia	890	AY928394	clade_392	N	N
Francisella tularensis	891	ABAZ01000082	clade_392	N	Category-A
Ignatzschineria indica	1009	HQ823562	clade_392	N	N
Ignatzschineria sp. NML 95_0260	1010	HQ823559	clade_392	N	N
Coprococcus catus	673	EU266552	clade_393	Y	N
Lachnospiraceae bacterium oral taxon F15	1064	HM099641	clade_393	Y	N
Streptococcus mutans	1814	AP010655	clade_394	N	N
Clostridium cochlearium	574	NR_044717	clade_395	Y	N
Clostridium malenominatum	604	FR749893	clade_395	Y	N
Clostridium tetani	654	NC_004557	clade_395	Y	N
Acetivibrio ethanolgignens	6	FR749897	clade_396	Y	N
Anaerosporobacter mobilis	161	NR_042953	clade_396	Y	N
Bacteroides pectinophilus	288	ABVQ01000036	clade_396	Y	N
Clostridium aminovalericum	551	NR_029245	clade_396	Y	N
Clostridium phytofermentans	613	NR_074652	clade_396	Y	N
Eubacterium hallii	848	L34621	clade_396	Y	N
Eubacterium xylanophilum	875	L34628	clade_396	Y	N
Lactobacillus gasseri	1084	ACOZ01000018	clade_398	N	N
Lactobacillus hominis	1090	FR681902	clade_398	N	N
Lactobacillus iners	1091	AEKJ01000002	clade_398	N	N
Lactobacillus johnsonii	1093	AE017198	clade_398	N	N
Lactobacillus senioris	1119	AB602570	clade_398	N	N
Lactobacillus sp. oral clone HT002	1135	AY349382	clade_398	N	N
Weissella beninensis	2006	EU439435	clade_398	N	N
Sphingomonas echinoides	1744	NR_024700	clade_399	N	N
Sphingomonas sp. oral taxon A09	1747	HM099639	clade_399	N	N

Sphingomonas sp. oral taxon F71	1748	HM099645	clade_399	N	N
Zymomonas mobilis	2032	NR_074274	clade_399	N	N
Arcanobacterium haemolyticum	174	NR_025347	clade_400	N	N
Arcanobacterium pyogenes	175	GU585578	clade_400	N	N
Trueperella pyogenes	1962	NR_044858	clade_400	N	N
Lactococcus garvieae	1144	AF061005	clade_401	N	N
Lactococcus lactis	1145	CP002365	clade_401	N	N
Brevibacterium mcbrellneri	424	ADNU01000076	clade_402	N	N
Brevibacterium paucivorans	425	EU086796	clade_402	N	N
Brevibacterium sp. JC43	428	JF824806	clade_402	N	N
Selenomonas artemidis	1692	HM596274	clade_403	N	N
Selenomonas sp. FOBRC9	1704	HQ616378	clade_403	N	N
Selenomonas sp. oral taxon 137	1715	AENV01000007	clade_403	N	N
Desmospora activa	751	AM940019	clade_404	N	N
Desmospora sp. 8437	752	AFHT01000143	clade_404	N	N
Paenibacillus sp. oral taxon F45	1407	HM099647	clade_404	N	N
Corynebacterium ammoniagenes	682	ADNS01000011	clade_405	N	N
Corynebacterium aurimucosum	687	ACLH01000041	clade_405	N	N
Corynebacterium bovis	688	AF537590	clade_405	N	N
Corynebacterium canis	689	GQ871934	clade_405	N	N
Corynebacterium casei	690	NR_025101	clade_405	N	N
Corynebacterium durum	694	Z97069	clade_405	N	N
Corynebacterium efficiens	695	ACLI01000121	clade_405	N	N
Corynebacterium falsenii	696	Y13024	clade_405	N	N
Corynebacterium flavescens	697	NR_037040	clade_405	N	N
Corynebacterium glutamicum	701	BA000036	clade_405	N	N
Corynebacterium jeikeium	704	ACYW01000001	clade_405	N	OP
Corynebacterium kroppenstedtii	705	NR_026380	clade_405	N	N
Corynebacterium lipophiloflavum	706	ACHJ01000075	clade_405	N	N
Corynebacterium matruchotii	709	ACSH02000003	clade_405	N	N
Corynebacterium minutissimum	710	X82064	clade_405	N	N
Corynebacterium resistens	718	ADGN01000058	clade_405	N	N
Corynebacterium simulans	720	AF537604	clade_405	N	N
Corynebacterium singulare	721	NR_026394	clade_405	N	N
Corynebacterium sp. 1 ex sheep	722	Y13427	clade_405	N	N
Corynebacterium sp. NML 99_0018	726	GU238413	clade_405	N	N
Corynebacterium striatum	727	ACGE01000001	clade_405	N	OP
Corynebacterium urealyticum	732	X81913	clade_405	N	OP
Corynebacterium variabile	734	NR_025314	clade_405	N	N
Ruminococcus callidus	1658	NR_029160	clade_406	Y	N
Ruminococcus champanellensis	1659	FP929052	clade_406	Y	N
Ruminococcus sp. 18P13	1665	AJ515913	clade_406	Y	N
Ruminococcus sp. 9SE51	1667	FM954974	clade_406	Y	N
Aerococcus sanguinicola	98	AY837833	clade_407	N	N
Aerococcus urinae	99	CP002512	clade_407	N	N
Aerococcus urinaeequi	100	NR_043443	clade_407	N	N
Aerococcus viridans	101	ADNT01000041	clade_407	N	N
Anaerostipes caccae	162	ABAX03000023	clade_408	Y	N

Anaerostipes sp. 3_2_56FAA	163	ACWB01000002	clade_408	Y	N
Clostridiales bacterium 1_7_47FAA	541	ABQR01000074	clade_408	Y	N
Clostridiales sp. SM4_1	542	FP929060	clade_408	Y	N
Clostridiales sp. SSC_2	544	FP929061	clade_408	Y	N
Clostridium aerotolerans	546	X76163	clade_408	Y	N
Clostridium aldenense	547	NR_043680	clade_408	Y	N
Clostridium algidixylanolyticum	550	NR_028726	clade_408	Y	N
Clostridium amygdalinum	552	AY353957	clade_408	Y	N
Clostridium asparagiforme	554	ACCJ01000522	clade_408	Y	N
Clostridium bolteae	559	ABCC02000039	clade_408	Y	N
Clostridium celerecrescens	566	JQ246092	clade_408	Y	N
Clostridium citroniae	569	ADLJ01000059	clade_408	Y	N
Clostridium clostridiiformes	571	M59089	clade_408	Y	N
Clostridium clostridioforme	572	NR_044715	clade_408	Y	N
Clostridium hathewayi	590	AY552788	clade_408	Y	N
Clostridium indolis	594	AF028351	clade_408	Y	N
Clostridium lavalense	600	EF564277	clade_408	Y	N
Clostridium saccharolyticum	620	CP002109	clade_408	Y	N
Clostridium sp. M62_1	633	ACFX02000046	clade_408	Y	N
Clostridium sp. SS2_1	638	ABGC03000041	clade_408	Y	N
Clostridium sphenoides	643	X73449	clade_408	Y	N
Clostridium symbiosum	652	ADLQ01000114	clade_408	Y	N
Clostridium xylanolyticum	658	NR_037068	clade_408	Y	N
Eubacterium hadrum	847	FR749933	clade_408	Y	N
Fusobacterium naviforme	898	HQ223106	clade_408	N	N
Lachnospiraceae bacterium 3_1_57FAA	1052	ACTP01000124	clade_408	Y	N
Lachnospiraceae bacterium 5_1_63FAA	1055	ACTS01000081	clade_408	Y	N
Lachnospiraceae bacterium A4	1059	DQ789118	clade_408	Y	N
Lachnospiraceae bacterium DJF VP30	1060	EU728771	clade_408	Y	N
Lachnospiraceae genomosp. C1	1065	AY278618	clade_408	Y	N
Moryella indoligenes	1268	AF527773	clade_408	N	N
Clostridium difficile	578	NC_013315	clade_409	Y	OP
Selenomonas genomosp. P5	1697	AY341820	clade_410	N	N
Selenomonas sp. oral clone IQ048	1710	AY349408	clade_410	N	N
Selenomonas sputigena	1717	ACKP02000033	clade_410	N	N
Hyphomicrobium sulfonivorans	1007	AY468372	clade_411	N	N
Methylocella silvestris	1228	NR_074237	clade_411	N	N
Legionella pneumophila	1153	NC_002942	clade_412	N	OP
Lactobacillus coryniformis	1077	NR_044705	clade_413	N	N
Arthrobacter agilis	178	NR_026198	clade_414	N	N
Arthrobacter arilaitensis	179	NR_074608	clade_414	N	N
Arthrobacter bergerei	180	NR_025612	clade_414	N	N
Arthrobacter globiformis	181	NR_026187	clade_414	N	N
Arthrobacter nicotianae	182	NR_026190	clade_414	N	N
Mycobacterium abscessus	1269	AGQU01000002	clade_418	N	OP
Mycobacterium chelonae	1273	AB548610	clade_418	N	OP
Bacteroides salanitronis	291	CP002530	clade_419	N	N
Paraprevotella xylaniphila	1427	AFBR01000011	clade_419	N	N

Barnesiella intestinihominis	336	AB370251	clade_420	N	N
Barnesiella viscericola	337	NR_041508	clade_420	N	N
Parabacteroides sp. NS31_3	1422	JN029805	clade_420	N	N
Porphyromonadaceae bacterium NML 060648	1470	EF184292	clade_420	N	N
Tannerella forsythia	1913	CP003191	clade_420	N	N
Tannerella sp. 6_1_58FAA_CT1	1914	ACWX01000068	clade_420	N	N
Mycoplasma amphoriforme	1311	AY531656	clade_421	N	N
Mycoplasma genitalium	1317	L43967	clade_421	N	N
Mycoplasma pneumoniae	1322	NC_000912	clade_421	N	N
Mycoplasma penetrans	1321	NC_004432	clade_422	N	N
Ureaplasma parvum	1966	AE002127	clade_422	N	N
Ureaplasma urealyticum	1967	AAYN01000002	clade_422	N	N
Treponema genomosp. P1	1927	AY341822	clade_425	N	N
Treponema sp. oral taxon 228	1943	GU408580	clade_425	N	N
Treponema sp. oral taxon 230	1944	GU408603	clade_425	N	N
Treponema sp. oral taxon 231	1945	GU408631	clade_425	N	N
Treponema sp. oral taxon 232	1946	GU408646	clade_425	N	N
Treponema sp. oral taxon 235	1947	GU408673	clade_425	N	N
Treponema sp. ovine footrot	1959	AJ010951	clade_425	N	N
Treponema vincentii	1960	ACYH01000036	clade_425	N	OP
Eubacterium sp. AS15b	862	HQ616364	clade_428	Y	N
Eubacterium sp. OBRC9	863	HQ616354	clade_428	Y	N
Eubacterium sp. oral clone OH3A	871	AY947497	clade_428	Y	N
Eubacterium yurii	876	AEES01000073	clade_428	Y	N
Clostridium acetobutylicum	545	NR_074511	clade_430	Y	N
Clostridium algidicarnis	549	NR_041746	clade_430	Y	N
Clostridium cadaveris	562	AB542932	clade_430	Y	N
Clostridium carboxidivorans	563	FR733710	clade_430	Y	N
Clostridium estertheticum	580	NR_042153	clade_430	Y	N
Clostridium fallax	581	NR_044714	clade_430	Y	N
Clostridium felsineum	583	AF270502	clade_430	Y	N
Clostridium frigidicarnis	584	NR_024919	clade_430	Y	N
Clostridium kluyveri	598	NR_074165	clade_430	Y	N
Clostridium magnum	603	X77835	clade_430	Y	N
Clostridium putrefaciens	615	NR_024995	clade_430	Y	N
Clostridium sp. HPB_46	629	AY862516	clade_430	Y	N
Clostridium tyrobutyricum	656	NR_044718	clade_430	Y	N
Burkholderiales bacterium 1_1_47	452	ADCQ01000066	clade_432	N	OP
Parasutterella excrementihominis	1429	AFBP01000029	clade_432	N	N
Parasutterella secunda	1430	AB491209	clade_432	N	N
Sutterella morbirenis	1898	AJ832129	clade_432	N	N
Sutterella parvirubra	1899	AB300989	clade_432	Y	N
Sutterella sanguinus	1900	AJ748647	clade_432	N	N
Sutterella sp. YIT 12072	1901	AB491210	clade_432	N	N
Sutterella stercoricanis	1902	NR_025600	clade_432	N	N
Sutterella wadsworthensis	1903	ADMF01000048	clade_432	N	N
Propionibacterium freudenreichii	1572	NR_036972	clade_433	N	N
Propionibacterium sp. oral taxon 192	1580	GQ422728	clade_433	N	N

Tessaracoccus sp. oral taxon F04	1917	HM099640	clade_433	N	N
Peptoniphilus ivorii	1445	Y07840	clade_434	N	N
Peptoniphilus sp. gpac007	1448	AM176517	clade_434	N	N
Peptoniphilus sp. gpac018A	1449	AM176519	clade_434	N	N
Peptoniphilus sp. gpac148	1451	AM176535	clade_434	N	N
Flexispira rappini	887	AY126479	clade_436	N	N
Helicobacter bilis	993	ACDN01000023	clade_436	N	N
Helicobacter cinaedi	995	ABQT01000054	clade_436	N	N
Helicobacter sp. None	998	U44756	clade_436	N	N
Brevundimonas subvibrioides	429	CP002102	clade_438	N	N
Hyphomonas neptunium	1008	NR_074092	clade_438	N	N
Phenylobacterium zucineum	1465	AY628697	clade_438	N	N
Acetanaerobacterium elongatum	4	NR_042930	clade_439	Y	N
Clostridium cellulosi	567	NR_044624	clade_439	Y	N
Ethanoligenens harbinense	832	AY675965	clade_439	Y	N
Streptococcus downei	1793	AEKN01000002	clade_441	N	N
Streptococcus sp. SHV515	1848	Y07601	clade_441	N	N
Acinetobacter sp. CIP 53.82	40	JQ638584	clade_443	N	N
Halomonas elongata	990	NR_074782	clade_443	N	N
Halomonas johnsoniae	991	FR775979	clade_443	N	N
Butyrivibrio fibrisolvens	456	U41172	clade_444	N	N
Eubacterium rectale	856	FP929042	clade_444	Y	N
Eubacterium sp. oral clone GI038	865	AY349374	clade_444	Y	N
Lachnobacterium bovis	1045	GU324407	clade_444	Y	N
Roseburia cecicola	1634	GU233441	clade_444	Y	N
Roseburia faecalis	1635	AY804149	clade_444	Y	N
Roseburia faecis	1636	AY305310	clade_444	Y	N
Roseburia hominis	1637	AJ270482	clade_444	Y	N
Roseburia intestinalis	1638	FP929050	clade_444	Y	N
Roseburia inulinivorans	1639	AJ270473	clade_444	Y	N
Roseburia sp. 11SE37	1640	FM954975	clade_444	N	N
Roseburia sp. 11SE38	1641	FM954976	clade_444	N	N
Shuttleworthia satelles	1728	ACIP02000004	clade_444	N	N
Shuttleworthia sp. MSX8B	1729	HQ616383	clade_444	N	N
Shuttleworthia sp. oral taxon G69	1730	GU432167	clade_444	N	N
Bdellovibrio sp. MPA	344	AY294215	clade_445	N	N
Desulfobulbus sp. oral clone CH031	755	AY005036	clade_445	N	N
Desulfovibrio desulfuricans	757	DQ092636	clade_445	N	N
Desulfovibrio fairfieldensis	758	U42221	clade_445	N	N
Desulfovibrio piger	759	AF192152	clade_445	N	N
Desulfovibrio sp. 3_1_syn3	760	ADDR01000239	clade_445	N	N
Geobacter bemidjiensis	941	CP001124	clade_445	N	N
Brachybacterium alimentarium	401	NR_026269	clade_446	N	N
Brachybacterium conglomeratum	402	AB537169	clade_446	N	N
Brachybacterium tyrofermentans	403	NR_026272	clade_446	N	N
Dermabacter hominis	749	FJ263375	clade_446	N	N
Aneurinibacillus thermoaerophilus	171	NR_029303	clade_448	N	N
Brevibacillus agri	409	NR_040983	clade_448	N	N

Brevibacillus brevis	410	NR_041524	clade_448	Y	N
Brevibacillus centrosporus	411	NR_043414	clade_448	N	N
Brevibacillus choshinensis	412	NR_040980	clade_448	N	N
Brevibacillus invocatus	413	NR_041836	clade_448	N	N
Brevibacillus laterosporus	414	NR_037005	clade_448	Y	N
Brevibacillus parabrevis	415	NR_040981	clade_448	N	N
Brevibacillus reuszeri	416	NR_040982	clade_448	N	N
Brevibacillus sp. phR	417	JN837488	clade_448	N	N
Brevibacillus thermoruber	418	NR_026514	clade_448	N	N
Lactobacillus murinus	1100	NR_042231	clade_449	N	N
Lactobacillus oeni	1102	NR_043095	clade_449	N	N
Lactobacillus ruminis	1115	ACGS02000043	clade_449	N	N
Lactobacillus vini	1141	NR_042196	clade_449	N	N
Gemella haemolysans	924	ACDZ02000012	clade_450	N	N
Gemella morbillorum	925	NR_025904	clade_450	N	N
Gemella morbillorum	926	ACRX01000010	clade_450	N	N
Gemella sanguinis	927	ACRY01000057	clade_450	N	N
Gemella sp. oral clone ASCE02	929	AY923133	clade_450	N	N
Gemella sp. oral clone ASCF04	930	AY923139	clade_450	N	N
Gemella sp. oral clone ASCF12	931	AY923143	clade_450	N	N
Gemella sp. WAL 19451	928	EU427463	clade_450	N	N
Bacillus coagulans	206	DQ297928	clade_451	Y	OP
Sporolactobacillus inulinus	1752	NR_040962	clade_451	Y	N
Sporolactobacillus nakayamae	1753	NR_042247	clade_451	N	N
Gluconacetobacter entanii	945	NR_028909	clade_452	N	N
Gluconacetobacter europaeus	946	NR_026513	clade_452	N	N
Gluconacetobacter hansenii	947	NR_026133	clade_452	N	N
Gluconacetobacter oboediens	949	NR_041295	clade_452	N	N
Gluconacetobacter xylinus	950	NR_074338	clade_452	N	N
Auritibacter ignavus	193	FN554542	clade_453	N	N
Dermacoccus sp. Ellin185	750	AEIQ01000090	clade_453	N	N
Janibacter limosus	1013	NR_026362	clade_453	N	N
Janibacter melonis	1014	EF063716	clade_453	N	N
Kocuria palustris	1041	EU333884	clade_453	Y	N
Acetobacter aceti	7	NR_026121	clade_454	N	N
Acetobacter fabarum	8	NR_042678	clade_454	N	N
Acetobacter lovaniensis	9	NR_040832	clade_454	N	N
Acetobacter malorum	10	NR_025513	clade_454	N	N
Acetobacter orientalis	11	NR_028625	clade_454	N	N
Acetobacter pasteurianus	12	NR_026107	clade_454	N	N
Acetobacter pomorum	13	NR_042112	clade_454	N	N
Acetobacter syzygii	14	NR_040868	clade_454	N	N
Acetobacter tropicalis	15	NR_036881	clade_454	N	N
Gluconacetobacter azotocaptans	943	NR_028767	clade_454	N	N
Gluconacetobacter diazotrophicus	944	NR_074292	clade_454	N	N
Gluconacetobacter johannae	948	NR_024959	clade_454	N	N
Nocardia brasiliensis	1351	AIHV01000038	clade_455	N	N
Nocardia cyriacigeorgica	1352	HQ009486	clade_455	N	N

Nocardia farcinica	1353	NC_006361	clade_455	Y	N
Nocardia puris	1354	NR_028994	clade_455	N	N
Nocardia sp. 01_Je_025	1355	GU574059	clade_455	N	N
Rhodococcus equi	1623	ADNW01000058	clade_455	N	N
Bacillus sp. oral taxon F28	247	HM099650	clade_456	Y	OP
Oceanobacillus caeni	1358	NR_041533	clade_456	N	N
Oceanobacillus sp. Ndiop	1359	CAER01000083	clade_456	N	N
Ornithinibacillus bavariensis	1384	NR_044923	clade_456	N	N
Ornithinibacillus sp. 7_10AIA	1385	FN397526	clade_456	N	N
Virgibacillus proomii	2005	NR_025308	clade_456	N	N
Corynebacterium amycolatum	683	ABZU01000033	clade_457	N	OP
Corynebacterium hansenii	702	AM946639	clade_457	N	N
Corynebacterium xerosis	735	FN179330	clade_457	N	OP
Staphylococcaceae bacterium NML 92_0017	1756	AY841362	clade_458	N	N
Staphylococcus fleurettii	1766	NR_041326	clade_458	N	N
Staphylococcus sciuri	1774	NR_025520	clade_458	N	N
Staphylococcus vitulinus	1779	NR_024670	clade_458	N	N
Stenotrophomonas maltophilia	1782	AAVZ01000005	clade_459	N	N
Stenotrophomonas sp. FG_6	1783	EF017810	clade_459	N	N
Mycobacterium africanum	1270	AF480605	clade_46	N	OP
Mycobacterium alsiensis	1271	AJ938169	clade_46	N	OP
Mycobacterium avium	1272	CP000479	clade_46	N	OP
Mycobacterium colombiense	1274	AM062764	clade_46	N	OP
Mycobacterium gordonae	1276	GU142930	clade_46	N	OP
Mycobacterium intracellulare	1277	GQ153276	clade_46	N	OP
Mycobacterium kansasii	1278	AF480601	clade_46	N	OP
Mycobacterium lacus	1279	NR_025175	clade_46	N	OP
Mycobacterium leprae	1280	FM211192	clade_46	N	OP
Mycobacterium lepromatosis	1281	EU203590	clade_46	N	OP
Mycobacterium mantenii	1283	FJ042897	clade_46	N	OP
Mycobacterium marinum	1284	NC_010612	clade_46	N	OP
Mycobacterium microti	1285	NR_025234	clade_46	N	OP
Mycobacterium parascrofulaceum	1287	ADNV01000350	clade_46	N	OP
Mycobacterium seoulense	1290	DQ536403	clade_46	N	OP
Mycobacterium sp. 1761	1292	EU703150	clade_46	N	N
Mycobacterium sp. 1791	1295	EU703148	clade_46	N	N
Mycobacterium sp. 1797	1296	EU703149	clade_46	N	N
Mycobacterium sp. B10_07.09.0206	1298	HQ174245	clade_46	N	N
Mycobacterium sp. NLA001000736	1305	HM627011	clade_46	N	N
Mycobacterium sp. W	1306	DQ437715	clade_46	N	N
Mycobacterium tuberculosis	1307	CP001658	clade_46	N	Category-C
Mycobacterium ulcerans	1308	AB548725	clade_46	N	OP
Mycobacterium vulneris	1309	EU834055	clade_46	N	OP
Xanthomonas campestris	2016	EF101975	clade_461	N	N
Xanthomonas sp. kmd_489	2017	EU723184	clade_461	N	N
Dietzia natronolimnaea	769	GQ870426	clade_462	N	N
Dietzia sp. BBDP51	770	DQ337512	clade_462	N	N

Dietzia sp. CA149	771	GQ870422	clade_462	N	N
Dietzia timorensis	772	GQ870424	clade_462	N	N
Gordonia bronchialis	951	NR_027594	clade_463	N	N
Gordonia polyisoprenivorans	952	DQ385609	clade_463	N	N
Gordonia sp. KTR9	953	DQ068383	clade_463	N	N
Gordonia sputi	954	FJ536304	clade_463	N	N
Gordonia terrae	955	GQ848239	clade_463	N	N
Leptotrichia goodfellowii	1167	ADAD01000110	clade_465	N	N
Leptotrichia sp. oral clone IK040	1174	AY349387	clade_465	N	N
Leptotrichia sp. oral clone P2PB_51 P1	1175	AY207053	clade_465	N	N
Bacteroidales genomosp. P7 oral clone MB3_P19	264	DQ003623	clade_466	N	N
Butyricimonas virosa	454	AB443949	clade_466	N	N
Odoribacter laneus	1363	AB490805	clade_466	N	N
Odoribacter splanchnicus	1364	CP002544	clade_466	N	N
Capnocytophaga gingivalis	478	ACLQ01000011	clade_467	N	N
Capnocytophaga granulosa	479	X97248	clade_467	N	N
Capnocytophaga sp. oral clone AH015	483	AY005074	clade_467	N	N
Capnocytophaga sp. oral strain S3	487	AY005073	clade_467	N	N
Capnocytophaga sp. oral taxon 338	488	AEXX01000050	clade_467	N	N
Capnocytophaga canimorsus	476	CP002113	clade_468	N	N
Capnocytophaga sp. oral clone ID062	485	AY349368	clade_468	N	N
Catenibacterium mitsuokai	495	AB030224	clade_469	Y	N
Clostridium sp. TM_40	640	AB249652	clade_469	Y	N
Coprobacillus cateniformis	670	AB030218	clade_469	Y	N
Coprobacillus sp. 29_1	671	ADKX01000057	clade_469	Y	N
Lactobacillus catenaformis	1075	M23729	clade_469	N	N
Lactobacillus vitulinus	1142	NR_041305	clade_469	N	N
Cetobacterium somerae	501	AJ438155	clade_470	N	N
Clostridium rectum	618	NR_029271	clade_470	Y	N
Fusobacterium gonidiaformans	896	ACET01000043	clade_470	N	N
Fusobacterium mortiferum	897	ACDB02000034	clade_470	N	N
Fusobacterium necrogenes	899	X55408	clade_470	N	N
Fusobacterium necrophorum	900	AM905356	clade_470	N	N
Fusobacterium sp. 12_1B	905	AGWJ01000070	clade_470	N	N
Fusobacterium sp. 3_1_5R	911	ACDD01000078	clade_470	N	N
Fusobacterium sp. D12	918	ACDG02000036	clade_470	N	N
Fusobacterium ulcerans	921	ACDH01000090	clade_470	N	N
Fusobacterium varium	922	ACIE01000009	clade_470	N	N
Mycoplasma arthritidis	1312	NC_011025	clade_473	N	N
Mycoplasma faucium	1314	NR_024983	clade_473	N	N
Mycoplasma hominis	1318	AF443616	clade_473	N	N
Mycoplasma orale	1319	AY796060	clade_473	N	N
Mycoplasma salivarium	1324	M24661	clade_473	N	N
Mitsuokella jalaludinii	1247	NR_028840	clade_474	N	N
Mitsuokella multacida	1248	ABWK02000005	clade_474	N	N
Mitsuokella sp. oral taxon 521	1249	GU413658	clade_474	N	N
Mitsuokella sp. oral taxon G68	1250	GU432166	clade_474	N	N
Selenomonas genomosp. C1	1695	AY278627	clade_474	N	N

Selenomonas genomosp. P8 oral clone MB5_P06	1700	DQ003628	clade_474	N	N
Selenomonas ruminantium	1703	NR_075026	clade_474	N	N
Veillonellaceae bacterium oral taxon 131	1994	GU402916	clade_474	N	N
Alloscardovia omnicolens	139	NR_042583	clade_475	N	N
Alloscardovia sp. OB7196	140	AB425070	clade_475	N	N
Bifidobacterium urinalis	366	AJ278695	clade_475	N	N
Eubacterium nodatum	854	U13041	clade_476	Y	N
Eubacterium saphenum	859	NR_026031	clade_476	Y	N
Eubacterium sp. oral clone JH012	867	AY349373	clade_476	Y	N
Eubacterium sp. oral clone JS001	870	AY349378	clade_476	Y	N
Faecalibacterium prausnitzii	880	ACOP02000011	clade_478	Y	N
Gemmiger formicilis	932	GU562446	clade_478	Y	N
Subdoligranulum variabile	1896	AJ518869	clade_478	Y	N
Clostridiaceae bacterium JC13	532	JF824807	clade_479	Y	N
Clostridium sp. MLG055	634	AF304435	clade_479	Y	N
Erysipelotrichaceae bacterium 3_1_53	822	ACTJ01000113	clade_479	Y	N
Prevotella loescheii	1503	JN867231	clade_48	N	N
Prevotella sp. oral clone ASCG12	1530	DQ272511	clade_48	N	N
Prevotella sp. oral clone GU027	1540	AY349398	clade_48	N	N
Prevotella sp. oral taxon 472	1553	ACZS01000106	clade_48	N	N
Selenomonas dianae	1693	GQ422719	clade_480	N	N
Selenomonas flueggei	1694	AF287803	clade_480	N	N
Selenomonas genomosp. C2	1696	AY278628	clade_480	N	N
Selenomonas genomosp. P6 oral clone MB3_C41	1698	DQ003636	clade_480	N	N
Selenomonas genomosp. P7 oral clone MB5_C08	1699	DQ003627	clade_480	N	N
Selenomonas infelix	1701	AF287802	clade_480	N	N
Selenomonas noxia	1702	GU470909	clade_480	N	N
Selenomonas sp. oral clone FT050	1705	AY349403	clade_480	N	N
Selenomonas sp. oral clone GI064	1706	AY349404	clade_480	N	N
Selenomonas sp. oral clone GT010	1707	AY349405	clade_480	N	N
Selenomonas sp. oral clone HU051	1708	AY349406	clade_480	N	N
Selenomonas sp. oral clone IK004	1709	AY349407	clade_480	N	N
Selenomonas sp. oral clone JI021	1711	AY349409	clade_480	N	N
Selenomonas sp. oral clone JS031	1712	AY349410	clade_480	N	N
Selenomonas sp. oral clone OH4A	1713	AY947498	clade_480	N	N
Selenomonas sp. oral clone P2PA_80 P4	1714	AY207052	clade_480	N	N
Selenomonas sp. oral taxon 149	1716	AEEJ01000007	clade_480	N	N
Veillonellaceae bacterium oral taxon 155	1995	GU470897	clade_480	N	N
Clostridium cocleatum	575	NR_026495	clade_481	Y	N
Clostridium ramosum	617	M23731	clade_481	Y	N
Clostridium saccharogumia	619	DQ100445	clade_481	Y	N
Clostridium spiroforme	644	X73441	clade_481	Y	N
Coprobacillus sp. D7	672	ACDT01000199	clade_481	Y	N
Clostridiales bacterium SY8519	535	AB477431	clade_482	Y	N
Clostridium sp. SY8519	639	AP012212	clade_482	Y	N
Eubacterium ramulus	855	AJ011522	clade_482	Y	N
Agrococcus jenensis	117	NR_026275	clade_484	N	N
Microbacterium gubbeenense	1232	NR_025098	clade_484	N	N

Pseudoclavibacter sp. Timone	1590	FJ375951	clade_484	N	N
Tropheryma whipplei	1961	BX251412	clade_484	N	N
Zimmermannella bifida	2031	AB012592	clade_484	N	N
Erysipelothrix inopinata	819	NR_025594	clade_485	Y	N
Erysipelothrix rhusiopathiae	820	ACLK01000021	clade_485	Y	N
Erysipelothrix tonsillarum	821	NR_040871	clade_485	Y	N
Holdemania filiformis	1004	Y11466	clade_485	Y	N
Mollicutes bacterium pACH93	1258	AY297808	clade_485	Y	N
Coxiella burnetii	736	CP000890	clade_486	Y	Category-B
Legionella hackeliae	1151	M36028	clade_486	N	OP
Legionella longbeachae	1152	M36029	clade_486	N	OP
Legionella sp. D3923	1154	JN380999	clade_486	N	OP
Legionella sp. D4088	1155	JN381012	clade_486	N	OP
Legionella sp. H63	1156	JF831047	clade_486	N	OP
Legionella sp. NML 93L054	1157	GU062706	clade_486	N	OP
Legionella steelei	1158	HQ398202	clade_486	N	OP
Tatlockia micdadei	1915	M36032	clade_486	N	N
Clostridium hiranonis	591	AB023970	clade_487	Y	N
Clostridium irregulare	596	NR_029249	clade_487	Y	N
Helicobacter pullorum	996	ABQU01000097	clade_489	N	N
Acetobacteraceae bacterium AT_5844	16	AGEZ01000040	clade_490	N	N
Roseomonas cervicalis	1643	ADVL01000363	clade_490	N	N
Roseomonas mucosa	1644	NR_028857	clade_490	N	N
Roseomonas sp. NML94_0193	1645	AF533357	clade_490	N	N
Roseomonas sp. NML97_0121	1646	AF533359	clade_490	N	N
Roseomonas sp. NML98_0009	1647	AF533358	clade_490	N	N
Roseomonas sp. NML98_0157	1648	AF533360	clade_490	N	N
Rickettsia akari	1627	CP000847	clade_492	N	OP
Rickettsia conorii	1628	AE008647	clade_492	N	OP
Rickettsia prowazekii	1629	M21789	clade_492	N	Category-B
Rickettsia rickettsii	1630	NC_010263	clade_492	N	OP
Rickettsia slovaca	1631	L36224	clade_492	N	OP
Rickettsia typhi	1632	AE017197	clade_492	N	OP
Anaeroglobus geminatus	160	AGCJ01000054	clade_493	N	N
Megasphaera genomosp. C1	1201	AY278622	clade_493	N	N
Megasphaera micronuciformis	1203	AECS01000020	clade_493	N	N
Clostridium orbiscindens	609	Y18187	clade_494	Y	N
Clostridium sp. NML 04A032	637	EU815224	clade_494	Y	N
Flavonifractor plautii	886	AY724678	clade_494	Y	N
Pseudoflavonifractor capillosus	1591	AY136666	clade_494	Y	N
Ruminococcaceae bacterium D16	1655	ADDX01000083	clade_494	Y	N
Acetivibrio cellulolyticus	5	NR_025917	clade_495	Y	N
Clostridiales genomosp. BVAB3	540	CP001850	clade_495	N	N
Clostridium aldrichii	548	NR_026099	clade_495	Y	N
Clostridium clariflavum	570	NR_041235	clade_495	Y	N
Clostridium stercorarium	647	NR_025100	clade_495	Y	N

Clostridium straminisolvens	649	NR_024829	clade_495	Y	N
Clostridium thermocellum	655	NR_074629	clade_495	Y	N
Tsukamurella paurometabola	1963	X80628	clade_496	N	N
Tsukamurella tyrosinosolvens	1964	AB478958	clade_496	N	N
Abiotrophia para_adiacens	2	AB022027	clade_497	N	N
Carnobacterium divergens	492	NR_044706	clade_497	N	N
Carnobacterium maltaromaticum	493	NC_019425	clade_497	N	N
Enterococcus avium	800	AF133535	clade_497	N	N
Enterococcus caccae	801	AY943820	clade_497	N	N
Enterococcus casseliflavus	802	AEWT01000047	clade_497	N	N
Enterococcus durans	803	AJ276354	clade_497	N	N
Enterococcus faecalis	804	AE016830	clade_497	N	N
Enterococcus faecium	805	AM157434	clade_497	N	N
Enterococcus gallinarum	806	AB269767	clade_497	N	N
Enterococcus gilvus	807	AY033814	clade_497	N	N
Enterococcus hawaiiensis	808	AY321377	clade_497	N	N
Enterococcus hirae	809	AF061011	clade_497	N	N
Enterococcus italicus	810	AEPV01000109	clade_497	N	N
Enterococcus mundtii	811	NR_024906	clade_497	N	N
Enterococcus raffinosus	812	FN600541	clade_497	N	N
Enterococcus sp. BV2CASA2	813	JN809766	clade_497	N	N
Enterococcus sp. CCRI 16620	814	GU457263	clade_497	N	N
Enterococcus sp. F95	815	FJ463817	clade_497	N	N
Enterococcus sp. RfL6	816	AJ133478	clade_497	N	N
Enterococcus thailandicus	817	AY321376	clade_497	N	N
Fusobacterium canifelinum	893	AY162222	clade_497	N	N
Fusobacterium genomosp. C1	894	AY278616	clade_497	N	N
Fusobacterium genomosp. C2	895	AY278617	clade_497	N	N
Fusobacterium nucleatum	901	ADVK01000034	clade_497	Y	N
Fusobacterium periodonticum	902	ACJY01000002	clade_497	N	N
Fusobacterium sp. 1_1_41FAA	906	ADGG01000053	clade_497	N	N
Fusobacterium sp. 11_3_2	904	ACUO01000052	clade_497	N	N
Fusobacterium sp. 2_1_31	907	ACDC02000018	clade_497	N	N
Fusobacterium sp. 3_1_27	908	ADGF01000045	clade_497	N	N
Fusobacterium sp. 3_1_33	909	ACQE01000178	clade_497	N	N
Fusobacterium sp. 3_1_36A2	910	ACPU01000044	clade_497	N	N
Fusobacterium sp. AC18	912	HQ616357	clade_497	N	N
Fusobacterium sp. ACB2	913	HQ616358	clade_497	N	N
Fusobacterium sp. AS2	914	HQ616361	clade_497	N	N
Fusobacterium sp. CM1	915	HQ616371	clade_497	N	N
Fusobacterium sp. CM21	916	HQ616375	clade_497	N	N
Fusobacterium sp. CM22	917	HQ616376	clade_497	N	N
Fusobacterium sp. oral clone ASCF06	919	AY923141	clade_497	N	N
Fusobacterium sp. oral clone ASCF11	920	AY953256	clade_497	N	N
Granulicatella adiacens	959	ACKZ01000002	clade_497	N	N
Granulicatella elegans	960	AB252689	clade_497	N	N
Granulicatella paradiacens	961	AY879298	clade_497	N	N
Granulicatella sp. oral clone ASC02	963	AY923126	clade_497	N	N

Granulicatella sp. oral clone ASCA05	964	DQ341469	clade_497	N	N
Granulicatella sp. oral clone ASCB09	965	AY953251	clade_497	N	N
Granulicatella sp. oral clone ASCG05	966	AY923146	clade_497	N	N
Tetragenococcus halophilus	1918	NR_075020	clade_497	N	N
Tetragenococcus koreensis	1919	NR_043113	clade_497	N	N
Vagococcus fluvialis	1973	NR_026489	clade_497	N	N
Chryseobacterium anthropi	514	AM982793	clade_498	N	N
Chryseobacterium gleum	515	ACKQ02000003	clade_498	N	N
Chryseobacterium hominis	516	NR_042517	clade_498	N	N
Treponema refringens	1936	AF426101	clade_499	N	OP
Treponema sp. oral clone JU031	1941	AY349416	clade_499	N	N
Treponema sp. oral taxon 239	1948	GU408738	clade_499	N	N
Treponema sp. oral taxon 271	1955	GU408871	clade_499	N	N
Alistipes finegoldii	129	NR_043064	clade_500	N	N
Alistipes onderdonkii	131	NR_043318	clade_500	N	N
Alistipes putredinis	132	ABFK02000017	clade_500	N	N
Alistipes shahii	133	FP929032	clade_500	N	N
Alistipes sp. HGB5	134	AENZ01000082	clade_500	N	N
Alistipes sp. JC50	135	JF824804	clade_500	N	N
Alistipes sp. RMA 9912	136	GQ140629	clade_500	N	N
Mycoplasma agalactiae	1310	AF010477	clade_501	N	N
Mycoplasma bovoculi	1313	NR_025987	clade_501	N	N
Mycoplasma fermentans	1315	CP002458	clade_501	N	N
Mycoplasma flocculare	1316	X62699	clade_501	N	N
Mycoplasma ovipneumoniae	1320	NR_025989	clade_501	N	N
Arcobacter butzleri	176	AEPT01000071	clade_502	N	N
Arcobacter cryaerophilus	177	NR_025905	clade_502	N	N
Campylobacter curvus	461	NC_009715	clade_502	N	OP
Campylobacter rectus	467	ACFU01000050	clade_502	N	OP
Campylobacter showae	468	ACVQ01000030	clade_502	N	OP
Campylobacter sp. FOBRC14	469	HQ616379	clade_502	N	OP
Campylobacter sp. FOBRC15	470	HQ616380	clade_502	N	OP
Campylobacter sp. oral clone BB120	471	AY005038	clade_502	N	OP
Campylobacter sputorum	472	NR_044839	clade_502	N	OP
Bacteroides ureolyticus	330	GQ167666	clade_504	N	N
Campylobacter gracilis	463	ACYG01000026	clade_504	N	OP
Campylobacter hominis	464	NC_009714	clade_504	N	OP
Dialister invisus	762	ACIM02000001	clade_506	N	N
Dialister micraerophilus	763	AFBB01000028	clade_506	N	N
Dialister microaerophilus	764	AENT01000008	clade_506	N	N
Dialister propionicifaciens	766	NR_043231	clade_506	N	N
Dialister succinatiphilus	768	AB370249	clade_506	N	N
Megasphaera elsdenii	1200	AY038996	clade_506	N	N
Megasphaera genomosp. type_1	1202	ADGP01000010	clade_506	N	N
Megasphaera sp. BLPYG_07	1204	HM990964	clade_506	N	N
Megasphaera sp. UPII 199_6	1205	AFIJ01000040	clade_506	N	N
Chromobacterium violaceum	513	NC_005085	clade_507	N	N
Laribacter hongkongensis	1148	CP001154	clade_507	N	N

Methylophilus sp. ECd5	1229	AY436794	clade_507	N	N
Finegoldia magna	883	ACHM02000001	clade_509	N	N
Parvimonas micra	1431	AB729072	clade_509	N	N
Parvimonas sp. oral taxon 110	1432	AFII01000002	clade_509	N	N
Peptostreptococcus micros	1456	AM176538	clade_509	N	N
Peptostreptococcus sp. oral clone FJ023	1460	AY349390	clade_509	N	N
Peptostreptococcus sp. P4P_31 P3	1458	AY207059	clade_509	N	N
Helicobacter pylori	997	CP000012	clade_510	N	OP
Anaplasma marginale	165	ABOR01000019	clade_511	N	N
Anaplasma phagocytophilum	166	NC_007797	clade_511	N	N
Ehrlichia chaffeensis	783	AAIF01000035	clade_511	N	OP
Neorickettsia risticii	1349	CP001431	clade_511	N	N
Neorickettsia sennetsu	1350	NC_007798	clade_511	N	N
Eubacterium barkeri	834	NR_044661	clade_512	Y	N
Eubacterium callanderi	838	NR_026330	clade_512	Y	N
Eubacterium limosum	850	CP002273	clade_512	Y	N
Pseudoramibacter alactolyticus	1606	AB036759	clade_512	N	N
Veillonella montpellierensis	1977	AF473836	clade_513	N	N
Veillonella sp. oral clone ASCA08	1988	AY923118	clade_513	N	N
Veillonella sp. oral clone ASCB03	1989	AY923122	clade_513	N	N
Inquilinus limosus	1012	NR_029046	clade_514	N	N
Sphingomonas sp. oral clone FZ016	1746	AY349412	clade_514	N	N
Anaerococcus lactolyticus	145	ABYO01000217	clade_515	N	N
Anaerococcus prevotii	147	CP001708	clade_515	N	N
Anaerococcus sp. gpac104	152	AM176528	clade_515	N	N
Anaerococcus sp. gpac126	153	AM176530	clade_515	N	N
Anaerococcus sp. gpac155	154	AM176536	clade_515	N	N
Anaerococcus sp. gpac199	155	AM176539	clade_515	N	N
Anaerococcus tetradius	157	ACGC01000107	clade_515	N	N
Bacteroides coagulans	271	AB547639	clade_515	N	N
Clostridiales bacterium 9403326	534	HM587324	clade_515	N	N
Clostridiales bacterium ph2	539	JN837487	clade_515	N	N
Peptostreptococcus sp. 9succ1	1457	X90471	clade_515	N	N
Peptostreptococcus sp. oral clone AP24	1459	AB175072	clade_515	N	N
Tissierella praeacuta	1924	NR_044860	clade_515	N	N
Anaerotruncus colihominis	164	ABGD02000021	clade_516	Y	N
Clostridium methylpentosum	606	ACEC01000059	clade_516	Y	N
Clostridium sp. YIT 12070	642	AB491208	clade_516	Y	N
Hydrogenoanaerobacterium saccharovorans	1005	NR_044425	clade_516	Y	N
Ruminococcus albus	1656	AY445600	clade_516	Y	N
Ruminococcus flavefaciens	1660	NR_025931	clade_516	Y	N
Clostridium haemolyticum	589	NR_024749	clade_517	Y	N
Clostridium novyi	608	NR_074343	clade_517	Y	N
Clostridium sp. LMG 16094	632	X95274	clade_517	Y	N
Helicobacter canadensis	994	ABQS01000108	clade_518	N	N
Eubacterium ventriosum	874	L34421	clade_519	Y	N
Peptostreptococcus anaerobius	1455	AY326462	clade_520	N	N
Peptostreptococcus stomatis	1461	ADGQ01000048	clade_520	N	N

Bilophila wadsworthia	367	ADCP01000166	clade_521	N	N
Desulfovibrio vulgaris	761	NR_074897	clade_521	N	N
Bacteroides galacturonicus	280	DQ497994	clade_522	Y	N
Eubacterium eligens	845	CP001104	clade_522	Y	N
Lachnospira multipara	1046	FR733699	clade_522	Y	N
Lachnospira pectinoschiza	1047	L14675	clade_522	Y	N
Lactobacillus rogosae	1114	GU269544	clade_522	Y	N
Actinomyces nasicola	64	AJ508455	clade_523	N	N
Cellulosimicrobium funkei	500	AY501364	clade_523	N	N
Lactococcus raffinolactis	1146	NR_044359	clade_524	N	N
Bacillus horti	214	NR_036860	clade_527	Y	OP
Bacillus sp. 9_3AIA	232	FN397519	clade_527	Y	OP
Bacteroidales genomosp. P1	258	AY341819	clade_529	N	N
Bacteroidales genomosp. P2 oral clone MB1_G13	259	DQ003613	clade_529	N	N
Bacteroidales genomosp. P3 oral clone MB1_G34	260	DQ003615	clade_529	N	N
Bacteroidales genomosp. P4 oral clone MB2_G17	261	DQ003617	clade_529	N	N
Bacteroidales genomosp. P5 oral clone MB2_P04	262	DQ003619	clade_529	N	N
Bacteroidales genomosp. P6 oral clone MB3_C19	263	DQ003634	clade_529	N	N
Bacteroidales genomosp. P8 oral clone MB4_G15	265	DQ003626	clade_529	N	N
Bacteroidetes bacterium oral taxon D27	333	HM099638	clade_530	N	N
Bacteroidetes bacterium oral taxon F31	334	HM099643	clade_530	N	N
Bacteroidetes bacterium oral taxon F44	335	HM099649	clade_530	N	N
Flavobacterium sp. NF2_1	885	FJ195988	clade_530	N	N
Myroides odoratimimus	1326	NR_042354	clade_530	N	N
Myroides sp. MY15	1327	GU253339	clade_530	N	N
Chlamydiales bacterium NS16	507	JN606076	clade_531	N	N
Chlamydophila pecorum	508	D88317	clade_531	N	OP
Parachlamydia sp. UWE25	1423	BX908798	clade_531	N	N
Fusobacterium russii	903	NR_044687	clade_532	N	N
Streptobacillus moniliformis	1784	NR_027615	clade_532	N	N
Eubacteriaceae bacterium P4P_50 P4	833	AY207060	clade_533	N	N
Eubacterium brachy	836	U13038	clade_533	Y	N
Filifactor alocis	881	CP002390	clade_533	Y	N
Filifactor villosus	882	NR_041928	clade_533	Y	N
Abiotrophia defectiva	1	ACIN02000016	clade_534	N	N
Abiotrophia sp. oral clone P4PA_155 P1	3	AY207063	clade_534	N	N
Catonella genomosp. P1 oral clone MB5_P12	496	DQ003629	clade_534	N	N
Catonella morbi	497	ACIL02000016	clade_534	N	N
Catonella sp. oral clone FL037	498	AY349369	clade_534	N	N
Eremococcus coleocola	818	AENN01000008	clade_534	N	N
Facklamia hominis	879	Y10772	clade_534	N	N
Granulicatella sp. M658_99_3	962	AJ271861	clade_534	N	N
Campylobacter coli	459	AAFL01000004	clade_535	N	OP
Campylobacter concisus	460	CP000792	clade_535	N	OP
Campylobacter fetus	462	ACLG01001177	clade_535	N	OP
Campylobacter jejuni	465	AL139074	clade_535	N	Category-B
Campylobacter upsaliensis	473	AEPU01000040	clade_535	N	OP

Clostridium leptum	601	AJ305238	clade_537	Y	N
Clostridium sp. YIT 12069	641	AB491207	clade_537	Y	N
Clostridium sporosphaeroides	646	NR_044835	clade_537	Y	N
Eubacterium coprostanoligenes	841	HM037995	clade_537	Y	N
Ruminococcus bromii	1657	EU266549	clade_537	Y	N
Eubacterium siraeum	860	ABCA03000054	clade_538	Y	N
Atopobium minutum	183	HM007583	clade_539	N	N
Atopobium parvulum	184	CP001721	clade_539	N	N
Atopobium rimae	185	ACFE01000007	clade_539	N	N
Atopobium sp. BS2	186	HQ616367	clade_539	N	N
Atopobium sp. F0209	187	EU592966	clade_539	N	N
Atopobium sp. ICM42b10	188	HQ616393	clade_539	N	N
Atopobium sp. ICM57	189	HQ616400	clade_539	N	N
Atopobium vaginae	190	AEDQ01000024	clade_539	N	N
Coriobacteriaceae bacterium BV3Ac1	677	JN809768	clade_539	N	N
Actinomyces naeslundii	63	X81062	clade_54	N	N
Actinomyces oricola	67	NR_025559	clade_54	N	N
Actinomyces oris	69	BABV01000070	clade_54	N	N
Actinomyces sp. 7400942	70	EU484334	clade_54	N	N
Actinomyces sp. ChDC B197	72	AF543275	clade_54	N	N
Actinomyces sp. GEJ15	73	GU561313	clade_54	N	N
Actinomyces sp. M2231_94_1	79	AJ234063	clade_54	N	N
Actinomyces sp. oral clone GU067	83	AY349362	clade_54	N	N
Actinomyces sp. oral clone IO077	85	AY349364	clade_54	N	N
Actinomyces sp. oral clone IP073	86	AY349365	clade_54	N	N
Actinomyces sp. oral clone JA063	88	AY349367	clade_54	N	N
Actinomyces sp. oral taxon 170	89	AFBL01000010	clade_54	N	N
Actinomyces sp. oral taxon 171	90	AECW01000034	clade_54	N	N
Actinomyces urogenitalis	95	ACFH01000038	clade_54	N	N
Actinomyces viscosus	96	ACRE01000096	clade_54	N	N
Clostridium viride	657	NR_026204	clade_540	Y	N
Oscillibacter sp. G2	1386	HM626173	clade_540	Y	N
Oscillibacter valericigenes	1387	NR_074793	clade_540	Y	N
Oscillospira guilliermondii	1388	AB040495	clade_540	Y	N
Orientia tsutsugamushi	1383	AP008981	clade_541	N	OP
Megamonas funiformis	1198	AB300988	clade_542	N	N
Megamonas hypermegale	1199	AJ420107	clade_542	N	N
Butyrivibrio crossotus	455	ABWN01000012	clade_543	Y	N
Clostridium sp. L2_50	631	AAYW02000018	clade_543	Y	N
Coprococcus eutactus	675	EF031543	clade_543	Y	N
Coprococcus sp. ART55_1	676	AY350746	clade_543	Y	N
Eubacterium ruminantium	857	NR_024661	clade_543	Y	N
Aeromicrobium marinum	102	NR_025681	clade_544	N	N
Aeromicrobium sp. JC14	103	JF824798	clade_544	N	N
Luteococcus sanguinis	1190	NR_025507	clade_544	N	N
Propionibacteriaceae bacterium NML 02_0265	1568	EF599122	clade_544	N	N
Rhodococcus corynebacterioides	1622	X80615	clade_546	N	N
Rhodococcus erythropolis	1624	ACNO01000030	clade_546	N	N

Rhodococcus fascians	1625	NR_037021	clade_546	N	N
Segniliparus rotundus	1690	CP001958	clade_546	N	N
Segniliparus rugosus	1691	ACZI01000025	clade_546	N	N
Exiguobacterium acetylicum	878	FJ970034	clade_547	N	N
Macrococcus caseolyticus	1194	NR_074941	clade_547	N	N
Streptomyces sp. 1 AIP_2009	1890	FJ176782	clade_548	N	N
Streptomyces sp. SD 524	1892	EU544234	clade_548	N	N
Streptomyces sp. SD 528	1893	EU544233	clade_548	N	N
Streptomyces thermoviolaceus	1895	NR_027616	clade_548	N	N
Borrelia afzelii	388	ABCU01000001	clade_549	N	OP
Borrelia crocidurae	390	DQ057990	clade_549	N	OP
Borrelia duttonii	391	NC_011229	clade_549	N	OP
Borrelia hermsii	393	AY597657	clade_549	N	OP
Borrelia hispanica	394	DQ057988	clade_549	N	OP
Borrelia persica	395	HM161645	clade_549	N	OP
Borrelia recurrentis	396	AF107367	clade_549	N	OP
Borrelia spielmanii	398	ABKB01000002	clade_549	N	OP
Borrelia turicatae	399	NC_008710	clade_549	N	OP
Borrelia valaisiana	400	ABCY01000002	clade_549	N	OP
Providencia alcalifaciens	1586	ABXW01000071	clade_55	N	N
Providencia rettgeri	1587	AM040492	clade_55	N	N
Providencia rustigianii	1588	AM040489	clade_55	N	N
Providencia stuartii	1589	AF008581	clade_55	N	N
Treponema pallidum	1932	CP001752	clade_550	N	OP
Treponema phagedenis	1934	AEFH01000172	clade_550	N	N
Treponema sp. clone DDKL_4	1939	Y08894	clade_550	N	N
Acholeplasma laidlawii	17	NR_074448	clade_551	N	N
Mycoplasma putrefaciens	1323	U26055	clade_551	N	N
Mycoplasmataceae genomosp P1 oral clone	1325	DQ003614	clade_551	N	N
Spiroplasma insolitum	1750	NR_025705	clade_551	N	N
Collinsella aerofaciens	659	AAVN02000007	clade_553	Y	N
Collinsella intestinalis	660	ABXH02000037	clade_553	N	N
Collinsella stercoris	661	ABXJ01000150	clade_553	N	N
Collinsella tanakaei	662	AB490807	clade_553	N	N
Alkaliphilus metalliredigenes	137	AY137848	clade_554	Y	N
Alkaliphilus oremlandii	138	NR_043674	clade_554	Y	N
Caminicella sporogenes	458	NR_025485	clade_554	N	N
Clostridium sticklandii	648	L04167	clade_554	Y	N
Turicibacter sanguinis	1965	AF349724	clade_555	Y	N
Acidaminococcus fermentans	21	CP001859	clade_556	N	N
Acidaminococcus intestini	22	CP003058	clade_556	N	N
Acidaminococcus sp. D21	23	ACGB01000071	clade_556	N	N
Phascolarctobacterium faecium	1462	NR_026111	clade_556	N	N
Phascolarctobacterium sp. YIT 12068	1463	AB490812	clade_556	N	N
Phascolarctobacterium succinatutens	1464	AB490811	clade_556	N	N
Acidithiobacillus ferrivorans	25	NR_074660	clade_557	N	N
Fulvimonas sp. NML 060897	892	EF589680	clade_557	Y	N
Xanthomonadaceae bacterium NML 03_0222	2015	EU313791	clade_557	N	N

Catabacter hongkongensis	494	AB671763	clade_558	N	N
Christensenella minuta	512	AB490809	clade_558	N	N
Clostridiales bacterium oral clone P4PA	536	AY207065	clade_558	N	N
Clostridiales bacterium oral taxon 093	537	GQ422712	clade_558	N	N
Desulfitobacterium frappieri	753	AJ276701	clade_560	Y	N
Desulfitobacterium hafniense	754	NR_074996	clade_560	Y	N
Desulfotomaculum nigrificans	756	NR_044832	clade_560	Y	N
Heliobacterium modesticaldum	1000	NR_074517	clade_560	N	N
Alistipes indistinctus	130	AB490804	clade_561	N	N
Bacteroidales bacterium ph8	257	JN837494	clade_561	N	N
Candidatus Sulcia muelleri	475	CP002163	clade_561	N	N
Cytophaga xylanolytica	742	FR733683	clade_561	N	N
Flavobacteriaceae genomosp. C1	884	AY278614	clade_561	N	N
Gramella forsetii	958	NR_074707	clade_561	N	N
Sphingobacterium faecium	1740	NR_025537	clade_562	N	N
Sphingobacterium mizutaii	1741	JF708889	clade_562	N	N
Sphingobacterium multivorum	1742	NR_040953	clade_562	N	N
Sphingobacterium spiritivorum	1743	ACHA02000013	clade_562	N	N
Jonquetella anthropi	1017	ACOO02000004	clade_563	N	N
Pyramidobacter piscolens	1614	AY207056	clade_563	N	N
Synergistes genomosp. C1	1904	AY278615	clade_563	N	N
Synergistes sp. RMA 14551	1905	DQ412722	clade_563	N	N
Synergistetes bacterium ADV897	1906	GQ258968	clade_563	N	N
Candidatus Arthromitus sp. SFB_mouse_Yit	474	NR_074460	clade_564	N	N
Gracilibacter thermotolerans	957	NR_043559	clade_564	N	N
Lutispora thermophila	1191	NR_041236	clade_564	Y	N
Brachyspira aalborgi	404	FM178386	clade_565	N	N
Brachyspira pilosicoli	405	NR_075069	clade_565	Y	N
Brachyspira sp. HIS3	406	FM178387	clade_565	N	N
Brachyspira sp. HIS4	407	FM178388	clade_565	N	N
Brachyspira sp. HIS5	408	FM178389	clade_565	N	N
Adlercreutzia equolifaciens	97	AB306661	clade_566	N	N
Coriobacteriaceae bacterium JC110	678	CAEM01000062	clade_566	N	N
Coriobacteriaceae bacterium phl	679	JN837493	clade_566	N	N
Cryptobacterium curtum	740	GQ422741	clade_566	N	N
Eggerthella lenta	778	AF292375	clade_566	Y	N
Eggerthella sinensis	779	AY321958	clade_566	N	N
Eggerthella sp. 1_3_56FAA	780	ACWN01000099	clade_566	N	N
Eggerthella sp. HGA1	781	AEXR01000021	clade_566	N	N
Eggerthella sp. YY7918	782	AP012211	clade_566	N	N
Gordonibacter pamelaeae	680	AM886059	clade_566	N	N
Gordonibacter pamelaeae	956	FP929047	clade_566	N	N
Slackia equolifaciens	1732	EU377663	clade_566	N	N
Slackia exigua	1733	ACUX01000029	clade_566	N	N
Slackia faecicanis	1734	NR_042220	clade_566	N	N
Slackia heliotrinireducens	1735	NR_074439	clade_566	N	N
Slackia isoflavoniconvertens	1736	AB566418	clade_566	N	N
Slackia piriformis	1737	AB490806	clade_566	N	N

Slackia sp. NATTS	1738	AB505075	clade_566	N	N
Streptomyces albus	1888	AJ697941	clade_566	Y	N
Chlamydiales bacterium NS11	505	JN606074	clade_567	Y	N
Chlamydiales bacterium NS13	506	JN606075	clade_567	N	N
Victivallaceae bacterium NML 080035	2003	FJ394915	clade_567	N	N
Victivallis vadensis	2004	ABDE02000010	clade_567	N	N
Anaerofustis stercorihominis	159	ABIL02000005	clade_570	Y	N
Butyricicoccus pullicaecorum	453	HH793440	clade_572	Y	N
Eubacterium desmolans	843	NR_044644	clade_572	Y	N
Papillibacter cinnamivorans	1415	NR_025025	clade_572	Y	N
Sporobacter termitidis	1751	NR_044972	clade_572	Y	N
Streptomyces griseus	1889	NR_074787	clade_573	N	N
Streptomyces sp. SD 511	1891	EU544231	clade_573	N	N
Streptomyces sp. SD 534	1894	EU544232	clade_573	N	N
Cloacibacillus evryensis	530	GQ258966	clade_575	N	N
Deferribacteres sp. oral clone JV001	743	AY349370	clade_575	N	N
Deferribacteres sp. oral clone JV006	744	AY349371	clade_575	Y	N
Deferribacteres sp. oral clone JV023	745	AY349372	clade_575	N	N
Synergistetes bacterium LBVCM1157	1907	GQ258969	clade_575	N	N
Synergistetes bacterium oral taxon 362	1909	GU410752	clade_575	N	N
Synergistetes bacterium oral taxon D48	1910	GU430992	clade_575	N	N
Clostridium colinum	577	NR_026151	clade_576	Y	N
Clostridium lactatifermentans	599	NR_025651	clade_576	Y	N
Clostridium piliforme	614	D14639	clade_576	Y	N
Peptococcus sp. oral clone JM048	1439	AY349389	clade_576	N	N
Helicobacter winghamensis	999	ACDO01000013	clade_577	N	N
Wolinella succinogenes	2014	BX571657	clade_577	N	N
Olsenella genomosp. C1	1368	AY278623	clade_578	N	N
Olsenella profusa	1369	FN178466	clade_578	N	N
Olsenella sp. F0004	1370	EU592964	clade_578	N	N
Olsenella sp. oral taxon 809	1371	ACVE01000002	clade_578	N	N
Olsenella uli	1372	CP002106	clade_578	N	N
Nocardiopsis dassonvillei	1356	CP002041	clade_579	N	N
Saccharomonospora viridis	1671	X54286	clade_579	Y	N
Thermobifida fusca	1921	NC_007333	clade_579	Y	N
Peptococcus niger	1438	NR_029221	clade_580	N	N
Peptococcus sp. oral taxon 167	1440	GQ422727	clade_580	N	N
Akkermansia muciniphila	118	CP001071	clade_583	N	N
Opitutus terrae	1373	NR_074978	clade_583	N	N
Clostridiales bacterium oral taxon F32	538	HM099644	clade_584	N	N
Leptospira borgpetersenii	1161	NC_008508	clade_585	N	OP
Leptospira broomii	1162	NR_043200	clade_585	N	OP
Leptospira interrogans	1163	NC_005823	clade_585	N	OP
Leptospira licerasiae	1164	EF612284	clade_585	Y	OP
Methanobrevibacter gottschalkii	1213	NR_044789	clade_587	N	N
Methanobrevibacter millerae	1214	NR_042785	clade_587	N	N
Methanobrevibacter oralis	1216	HE654003	clade_587	N	N
Methanobrevibacter thaueri	1219	NR_044787	clade_587	N	N

Methanobrevibacter smithii	1218	ABYV02000002	clade_588	N	N
Deinococcus radiodurans	746	AE000513	clade_589	N	N
Deinococcus sp. R_43890	747	FR682752	clade_589	N	N
Thermus aquaticus	1923	NR_025900	clade_589	N	N
Actinomyces sp. c109	81	AB167239	clade_590	N	N
Moorella thermoacetica	1259	NR_075001	clade_590	Y	N
Syntrophomonadaceae genomosp. P1	1912	AY341821	clade_590	N	N
Thermoanaerobacter pseudethanolicus	1920	CP000924	clade_590	Y	N
Anaerobaculum hydrogeniformans	141	ACJX02000009	clade_591	N	N
Flexistipes sinusarabici	888	NR_074881	clade_591	Y	N
Microcystis aeruginosa	1246	NC_010296	clade_592	N	N
Prochlorococcus marinus	1567	CP000551	clade_592	N	N
Methanobrevibacter acididurans	1208	NR_028779	clade_593	N	N
Methanobrevibacter arboriphilus	1209	NR_042783	clade_593	N	N
Methanobrevibacter curvatus	1210	NR_044796	clade_593	N	N
Methanobrevibacter cuticularis	1211	NR_044776	clade_593	N	N
Methanobrevibacter filiformis	1212	NR_044801	clade_593	N	N
Methanobrevibacter woesei	1220	NR_044788	clade_593	N	N
Roseiflexus castenholzii	1642	CP000804	clade_594	N	N
Methanobrevibacter olleyae	1215	NR_043024	clade_595	N	N
Methanobrevibacter ruminantium	1217	NR_042784	clade_595	N	N
Methanobrevibacter wolinii	1221	NR_044790	clade_595	N	N
Methanosphaera stadtmanae	1222	AY196684	clade_595	N	N
Chloroflexi genomosp. P1	511	AY331414	clade_596	N	N
Gloeobacter violaceus	942	NR_074282	clade_596	Y	N
Halorubrum lipolyticum	992	AB477978	clade_597	N	N
Methanobacterium formicicum	1207	NR_025028	clade_597	N	N
Acidilobus saccharovorans	24	AY350586	clade_598	N	N
Hyperthermus butylicus	1006	CP000493	clade_598	N	N
Ignicoccus islandicus	1011	X99562	clade_598	N	N
Metallosphaera sedula	1206	D26491	clade_598	N	N
Thermofilum pendens	1922	X14835	clade_598	N	N
Prevotella melaninogenica	1506	CP002122	clade_6	N	N
Prevotella sp. ICM1	1520	HQ616385	clade_6	N	N
Prevotella sp. oral clone FU048	1535	AY349393	clade_6	N	N
Prevotella sp. oral clone GI030	1537	AY349395	clade_6	N	N
Prevotella sp. SEQ116	1526	JN867246	clade_6	N	N
Streptococcus anginosus	1787	AECT01000011	clade_60	N	N
Streptococcus milleri	1812	X81023	clade_60	N	N
Streptococcus sp. 16362	1829	JN590019	clade_60	N	N
Streptococcus sp. 69130	1832	X78825	clade_60	N	N
Streptococcus sp. AC15	1833	HQ616356	clade_60	N	N
Streptococcus sp. CM7	1839	HQ616373	clade_60	N	N
Streptococcus sp. OBRC6	1847	HQ616352	clade_60	N	N
Burkholderia ambifaria	442	AAUZ01000009	clade_61	N	OP
Burkholderia cenocepacia	443	AAHI01000060	clade_61	N	OP
Burkholderia cepacia	444	NR_041719	clade_61	N	OP
Burkholderia mallei	445	CP000547	clade_61	N	Category-

					B
Burkholderia multivorans	446	NC_010086	clade_61	N	OP
Burkholderia oklahomensis	447	DQ108388	clade_61	N	OP
Burkholderia pseudomallei	448	CP001408	clade_61	N	Category-B
Burkholderia rhizoxinica	449	HQ005410	clade_61	N	OP
Burkholderia sp. 383	450	CP000151	clade_61	N	OP
Burkholderia xenovorans	451	U86373	clade_61	N	OP
Prevotella buccae	1488	ACRB01000001	clade_62	N	N
Prevotella genomosp. P8 oral clone MB3_P13	1498	DQ003622	clade_62	N	N
Prevotella sp. oral clone FW035	1536	AY349394	clade_62	N	N
Prevotella bivia	1486	ADFO01000096	clade_63	N	N
Prevotella disiens	1494	AEDO01000026	clade_64	N	N
Bacteroides faecis	276	GQ496624	clade_65	N	N
Bacteroides fragilis	279	AP006841	clade_65	N	N
Bacteroides nordii	285	NR_043017	clade_65	N	N
Bacteroides salyersiae	292	EU136690	clade_65	N	N
Bacteroides sp. 1_1_14	293	ACRP01000155	clade_65	N	N
Bacteroides sp. 1_1_6	295	ACIC01000215	clade_65	N	N
Bacteroides sp. 2_1_56FAA	298	ACWI01000065	clade_65	N	N
Bacteroides sp. AR29	316	AF139525	clade_65	N	N
Bacteroides sp. B2	317	EU722733	clade_65	N	N
Bacteroides thetaiotaomicron	328	NR_074277	clade_65	N	N
Actinobacillus minor	45	ACFT01000025	clade_69	N	N
Haemophilus parasuis	978	GU226366	clade_69	N	N
Vibrio cholerae	1996	AAUR01000095	clade_71	N	Category-B
Vibrio fluvialis	1997	X76335	clade_71	N	Category-B
Vibrio furnissii	1998	CP002377	clade_71	N	Category-B
Vibrio mimicus	1999	ADAF01000001	clade_71	N	Category-B
Vibrio parahaemolyticus	2000	AAWQ01000116	clade_71	N	Category-B
Vibrio sp. RC341	2001	ACZT01000024	clade_71	N	Category-B
Vibrio vulnificus	2002	AE016796	clade_71	N	Category-B
Lactobacillus acidophilus	1067	CP000033	clade_72	N	N
Lactobacillus amylolyticus	1069	ADNY01000006	clade_72	N	N
Lactobacillus amylovorus	1070	CP002338	clade_72	N	N
Lactobacillus crispatus	1078	ACOG01000151	clade_72	N	N
Lactobacillus delbrueckii	1080	CP002341	clade_72	N	N
Lactobacillus helveticus	1088	ACLM01000202	clade_72	N	N
Lactobacillus kalixensis	1094	NR_029083	clade_72	N	N
Lactobacillus kefiranofaciens	1095	NR_042440	clade_72	N	N
Lactobacillus leichmannii	1098	JX986966	clade_72	N	N
Lactobacillus sp. 66c	1120	FR681900	clade_72	N	N

Lactobacillus sp. KLDS 1.0701	1122	EU600905	clade_72	N	N
Lactobacillus sp. KLDS 1.0712	1130	EU600916	clade_72	N	N
Lactobacillus sp. oral clone HT070	1136	AY349383	clade_72	N	N
Lactobacillus ultunensis	1139	ACGU01000081	clade_72	N	N
Prevotella intermedia	1502	AF414829	clade_81	N	N
Prevotella nigrescens	1511	AFPX01000069	clade_81	N	N
Prevotella pallens	1515	AFPY01000135	clade_81	N	N
Prevotella sp. oral taxon 310	1551	GQ422737	clade_81	N	N
Prevotella genomosp. C1	1495	AY278624	clade_82	N	N
Prevotella sp. CM38	1519	HQ610181	clade_82	N	N
Prevotella sp. oral taxon 317	1552	ACQH01000158	clade_82	N	N
Prevotella sp. SG12	1527	GU561343	clade_82	N	N
Prevotella denticola	1493	CP002589	clade_83	N	N
Prevotella genomosp. P7 oral clone MB2_P31	1497	DQ003620	clade_83	N	N
Prevotella histicola	1501	JN867315	clade_83	N	N
Prevotella multiformis	1508	AEWX01000054	clade_83	N	N
Prevotella sp. JCM 6330	1522	AB547699	clade_83	N	N
Prevotella sp. oral clone GI059	1539	AY349397	clade_83	N	N
Prevotella sp. oral taxon 782	1555	GQ422745	clade_83	N	N
Prevotella sp. oral taxon G71	1559	GU432180	clade_83	N	N
Prevotella sp. SEQ065	1524	JN867234	clade_83	N	N
Prevotella veroralis	1565	ACVA01000027	clade_83	N	N
Bacteroides acidifaciens	266	NR_028607	clade_85	N	N
Bacteroides cellulosilyticus	269	ACCH01000108	clade_85	N	N
Bacteroides clarus	270	AFBM01000011	clade_85	N	N
Bacteroides eggerthii	275	ACWG01000065	clade_85	N	N
Bacteroides oleiciplenus	286	AB547644	clade_85	N	N
Bacteroides pyogenes	290	NR_041280	clade_85	N	N
Bacteroides sp. 315_5	300	FJ848547	clade_85	N	N
Bacteroides sp. 31SF15	301	AJ583248	clade_85	N	N
Bacteroides sp. 31SF18	302	AJ583249	clade_85	N	N
Bacteroides sp. 35AE31	303	AJ583244	clade_85	N	N
Bacteroides sp. 35AE37	304	AJ583245	clade_85	N	N
Bacteroides sp. 35BE34	305	AJ583246	clade_85	N	N
Bacteroides sp. 35BE35	306	AJ583247	clade_85	N	N
Bacteroides sp. WH2	324	AY895180	clade_85	N	N
Bacteroides sp. XB12B	325	AM230648	clade_85	N	N
Bacteroides stercoris	327	ABFZ02000022	clade_85	N	N
Actinobacillus pleuropneumoniae	46	NR_074857	clade_88	N	N
Actinobacillus ureae	48	AEVG01000167	clade_88	N	N
Haemophilus aegyptius	969	AFBC01000053	clade_88	N	N
Haemophilus ducreyi	970	AE017143	clade_88	N	OP
Haemophilus haemolyticus	973	JN175335	clade_88	N	N
Haemophilus influenzae	974	AADP01000001	clade_88	N	OP
Haemophilus parahaemolyticus	975	GU561425	clade_88	N	N
Haemophilus parainfluenzae	976	AEWU01000024	clade_88	N	N
Haemophilus paraphrophaemolyticus	977	M75076	clade_88	N	N
Haemophilus somnus	979	NC_008309	clade_88	N	N

Haemophilus sp. 70334	980	HQ680854	clade_88	N	N
Haemophilus sp. HK445	981	FJ685624	clade_88	N	N
Haemophilus sp. oral clone ASCA07	982	AY923117	clade_88	N	N
Haemophilus sp. oral clone ASCG06	983	AY923147	clade_88	N	N
Haemophilus sp. oral clone BJ021	984	AY005034	clade_88	N	N
Haemophilus sp. oral clone BJ095	985	AY005033	clade_88	N	N
Haemophilus sp. oral taxon 851	987	AGRK01000004	clade_88	N	N
Haemophilus sputorum	988	AFNK01000005	clade_88	N	N
Histophilus somni	1003	AF549387	clade_88	N	N
Mannheimia haemolytica	1195	ACZX01000102	clade_88	N	N
Pasteurella bettyae	1433	L06088	clade_88	N	N
Moellerella wisconsensis	1253	JN175344	clade_89	N	N
Morganella morganii	1265	AJ301681	clade_89	N	N
Morganella sp. JB_T16	1266	AJ781005	clade_89	N	N
Proteus mirabilis	1582	ACLE01000013	clade_89	N	N
Proteus penneri	1583	ABVP01000020	clade_89	N	N
Proteus sp. HS7514	1584	DQ512963	clade_89	N	N
Proteus vulgaris	1585	AJ233425	clade_89	N	N
Eubacterium sp. oral clone JN088	869	AY349377	clade_90	Y	N
Oribacterium sinus	1374	ACKX01000142	clade_90	N	N
Oribacterium sp. ACB1	1375	HM120210	clade_90	N	N
Oribacterium sp. ACB7	1376	HM120211	clade_90	N	N
Oribacterium sp. CM12	1377	HQ616374	clade_90	N	N
Oribacterium sp. ICM51	1378	HQ616397	clade_90	N	N
Oribacterium sp. OBRC12	1379	HQ616355	clade_90	N	N
Oribacterium sp. oral taxon 108	1382	AFIH01000001	clade_90	N	N
Actinobacillus actinomycetemcomitans	44	AY362885	clade_92	N	N
Actinobacillus succinogenes	47	CP000746	clade_92	N	N
Aggregatibacter actinomycetemcomitans	112	CP001733	clade_92	N	N
Aggregatibacter aphrophilus	113	CP001607	clade_92	N	N
Aggregatibacter segnis	114	AEPS01000017	clade_92	N	N
Averyella dalhousiensis	194	DQ481464	clade_92	N	N
Bisgaard Taxon	368	AY683487	clade_92	N	N
Bisgaard Taxon	369	AY683489	clade_92	N	N
Bisgaard Taxon	370	AY683491	clade_92	N	N
Bisgaard Taxon	371	AY683492	clade_92	N	N
Buchnera aphidicola	440	NR_074609	clade_92	N	N
Cedecea davisae	499	AF493976	clade_92	N	N
Citrobacter amalonaticus	517	FR870441	clade_92	N	N
Citrobacter braakii	518	NR_028687	clade_92	N	N
Citrobacter farmeri	519	AF025371	clade_92	N	N
Citrobacter freundii	520	NR_028894	clade_92	N	N
Citrobacter gillenii	521	AF025367	clade_92	N	N
Citrobacter koseri	522	NC_009792	clade_92	N	N
Citrobacter murliniae	523	AF025369	clade_92	N	N
Citrobacter rodentium	524	NR_074903	clade_92	N	N
Citrobacter sedlakii	525	AF025364	clade_92	N	N
Citrobacter sp. 30_2	526	ACDJ01000053	clade_92	N	N

Citrobacter sp. KMSI_3	527	GQ468398	clade_92	N	N
Citrobacter werkmanii	528	AF025373	clade_92	N	N
Citrobacter youngae	529	ABWL02000011	clade_92	N	N
Cronobacter malonaticus	737	GU122174	clade_92	N	N
Cronobacter sakazakii	738	NC_009778	clade_92	N	N
Cronobacter turicensis	739	FN543093	clade_92	N	N
Enterobacter aerogenes	786	AJ251468	clade_92	N	N
Enterobacter asburiae	787	NR_024640	clade_92	N	N
Enterobacter cancerogenus	788	Z96078	clade_92	N	N
Enterobacter cloacae	789	FP929040	clade_92	N	N
Enterobacter cowanii	790	NR_025566	clade_92	N	N
Enterobacter hormaechei	791	AFHR01000079	clade_92	N	N
Enterobacter sp. 247BMC	792	HQ122932	clade_92	N	N
Enterobacter sp. 638	793	NR_074777	clade_92	N	N
Enterobacter sp. JC163	794	JN657217	clade_92	N	N
Enterobacter sp. SCSS	795	HM007811	clade_92	N	N
Enterobacter sp. TSE38	796	HM156134	clade_92	N	N
Enterobacteriaceae bacterium 9_2_54FAA	797	ADCU01000033	clade_92	N	N
Enterobacteriaceae bacterium CF01Ent_1	798	AJ489826	clade_92	N	N
Enterobacteriaceae bacterium Smarlab 3302238	799	AY538694	clade_92	N	N
Escherichia albertii	824	ABKX01000012	clade_92	N	N
Escherichia coli	825	NC_008563	clade_92	N	Category-B
Escherichia fergusonii	826	CU928158	clade_92	N	N
Escherichia hermannii	827	HQ407266	clade_92	N	N
Escherichia sp. 1_1_43	828	ACID01000033	clade_92	N	N
Escherichia sp. 4_1_40B	829	ACDM02000056	clade_92	N	N
Escherichia sp. B4	830	EU722735	clade_92	N	N
Escherichia vulneris	831	NR_041927	clade_92	N	N
Ewingella americana	877	JN175329	clade_92	N	N
Haemophilus genomosp. P2 oral clone MB3_C24	971	DQ003621	clade_92	N	N
Haemophilus genomosp. P3 oral clone MB3_C38	972	DQ003635	clade_92	N	N
Haemophilus sp. oral clone JM053	986	AY349380	clade_92	N	N
Hafnia alvei	989	DQ412565	clade_92	N	N
Klebsiella oxytoca	1024	AY292871	clade_92	N	OP
Klebsiella pneumoniae	1025	CP000647	clade_92	N	OP
Klebsiella sp. AS10	1026	HQ616362	clade_92	N	N
Klebsiella sp. Co9935	1027	DQ068764	clade_92	N	N
Klebsiella sp. enrichment culture clone SRC_DSD25	1036	HM195210	clade_92	N	N
Klebsiella sp. OBRC7	1028	HQ616353	clade_92	N	N
Klebsiella sp. SP_BA	1029	FJ999767	clade_92	N	N
Klebsiella sp. SRC_DSD1	1033	GU797254	clade_92	N	N
Klebsiella sp. SRC_DSD11	1030	GU797263	clade_92	N	N
Klebsiella sp. SRC_DSD12	1031	GU797264	clade_92	N	N
Klebsiella sp. SRC_DSD15	1032	GU797267	clade_92	N	N
Klebsiella sp. SRC_DSD2	1034	GU797253	clade_92	N	N
Klebsiella sp. SRC_DSD6	1035	GU797258	clade_92	N	N
Klebsiella variicola	1037	CP001891	clade_92	N	N

Kluyvera ascorbata	1038	NR_028677	clade_92	N	N
Kluyvera cryocrescens	1039	NR_028803	clade_92	N	N
Leminorella grimontii	1159	AJ233421	clade_92	N	N
Leminorella richardii	1160	HF558368	clade_92	N	N
Pantoea agglomerans	1409	AY335552	clade_92	N	N
Pantoea ananatis	1410	CP001875	clade_92	N	N
Pantoea brenneri	1411	EU216735	clade_92	N	N
Pantoea citrea	1412	EF688008	clade_92	N	N
Pantoea conspicua	1413	EU216737	clade_92	N	N
Pantoea septica	1414	EU216734	clade_92	N	N
Pasteurella dagmatis	1434	ACZR01000003	clade_92	N	N
Pasteurella multocida	1435	NC_002663	clade_92	N	N
Plesiomonas shigelloides	1469	X60418	clade_92	N	N
Raoultella ornithinolytica	1617	AB364958	clade_92	N	N
Raoultella planticola	1618	AF129443	clade_92	N	N
Raoultella terrigena	1619	NR_037085	clade_92	N	N
Salmonella bongori	1683	NR_041699	clade_92	N	Category-B
Salmonella enterica	1672	NC_011149	clade_92	N	Category-B
Salmonella enterica	1673	NC_011205	clade_92	N	Category-B
Salmonella enterica	1674	DQ344532	clade_92	N	Category-B
Salmonella enterica	1675	ABEH02000004	clade_92	N	Category-B
Salmonella enterica	1676	ABAK02000001	clade_92	N	Category-B
Salmonella enterica	1677	NC_011080	clade_92	N	Category-B
Salmonella enterica	1678	EU118094	clade_92	N	Category-B
Salmonella enterica	1679	NC_011094	clade_92	N	Category-B
Salmonella enterica	1680	AE014613	clade_92	N	Category-B
Salmonella enterica	1682	ABFH02000001	clade_92	N	Category-B
Salmonella enterica	1684	ABEM01000001	clade_92	N	Category-B
Salmonella enterica	1685	ABAM02000001	clade_92	N	Category-B
Salmonella typhimurium	1681	DQ344533	clade_92	N	Category-B
Salmonella typhimurium	1686	AF170176	clade_92	N	Category-B
Serratia fonticola	1718	NR_025339	clade_92	N	N
Serratia liquefaciens	1719	NR_042062	clade_92	N	N
Serratia marcescens	1720	GU826157	clade_92	N	N

Serratia odorifera	1721	ADBY01000001	clade_92	N	N
Serratia proteamaculans	1722	AAUN01000015	clade_92	N	N
Shigella boydii	1724	AAKA01000007	clade_92	N	Category-B
Shigella dysenteriae	1725	NC_007606	clade_92	N	Category-B
Shigella flexneri	1726	AE005674	clade_92	N	Category-B
Shigella sonnei	1727	NC_007384	clade_92	N	Category-B
Tatumella ptyseos	1916	NR_025342	clade_92	N	N
Trabulsiella guamensis	1925	AY373830	clade_92	N	N
Yersinia aldovae	2019	AJ871363	clade_92	N	OP
Yersinia aleksiciae	2020	AJ627597	clade_92	N	OP
Yersinia bercovieri	2021	AF366377	clade_92	N	OP
Yersinia enterocolitica	2022	FR729477	clade_92	N	Category-B
Yersinia frederiksenii	2023	AF366379	clade_92	N	OP
Yersinia intermedia	2024	AF366380	clade_92	N	OP
Yersinia kristensenii	2025	ACCA01000078	clade_92	N	OP
Yersinia mollaretii	2026	NR_027546	clade_92	N	OP
Yersinia pestis	2027	AE013632	clade_92	N	Category-A
Yersinia pseudotuberculosis	2028	NC_009708	clade_92	N	OP
Yersinia rohdei	2029	ACCD01000071	clade_92	N	OP
Yokenella regensburgei	2030	AB273739	clade_92	N	N
Conchiformibius kuhniae	669	NR_041821	clade_94	N	N
Morococcus cerebrosus	1267	JN175352	clade_94	N	N
Neisseria bacilliformis	1328	AFAY01000058	clade_94	N	N
Neisseria cinerea	1329	ACDY01000037	clade_94	N	N
Neisseria flavescens	1331	ACQV01000025	clade_94	N	N
Neisseria gonorrhoeae	1333	CP002440	clade_94	N	OP
Neisseria lactamica	1334	ACEQ01000095	clade_94	N	N
Neisseria macacae	1335	AFQE01000146	clade_94	N	N
Neisseria meningitidis	1336	NC_003112	clade_94	N	OP
Neisseria mucosa	1337	ACDX01000110	clade_94	N	N
Neisseria pharyngis	1338	AJ239281	clade_94	N	N
Neisseria polysaccharea	1339	ADBE01000137	clade_94	N	N
Neisseria sicca	1340	ACKO02000016	clade_94	N	N
Neisseria sp. KEM232	1341	GQ203291	clade_94	N	N
Neisseria sp. oral clone AP132	1344	AY005027	clade_94	N	N
Neisseria sp. oral strain B33KA	1346	AY005028	clade_94	N	N
Neisseria sp. oral taxon 014	1347	ADEA01000039	clade_94	N	N
Neisseria sp. TM10_1	1343	DQ279352	clade_94	N	N
Neisseria subflava	1348	ACEO01000067	clade_94	N	N
Clostridium oroticum	610	FR749922	clade_96	Y	N
Clostridium sp. D5	627	ADBG01000142	clade_96	Y	N
Eubacterium contortum	840	FR749946	clade_96	Y	N
Eubacterium fissicatena	846	FR749935	clade_96	Y	N

Okadaella gastrococcus	1365	HQ699465	clade_98	N	N
Streptococcus agalactiae	1785	AAJO01000130	clade_98	N	N
Streptococcus alactolyticus	1786	NR_041781	clade_98	N	N
Streptococcus australis	1788	AEQR01000024	clade_98	N	N
Streptococcus bovis	1789	AEEL01000030	clade_98	N	N
Streptococcus canis	1790	AJ413203	clade_98	N	N
Streptococcus constellatus	1791	AY277942	clade_98	N	N
Streptococcus cristatus	1792	AEVC01000028	clade_98	N	N
Streptococcus dysgalactiae	1794	AP010935	clade_98	N	N
Streptococcus equi	1795	CP001129	clade_98	N	N
Streptococcus equinus	1796	AEVB01000043	clade_98	N	N
Streptococcus gallolyticus	1797	FR824043	clade_98	N	N
Streptococcus genomosp. C1	1798	AY278629	clade_98	N	N
Streptococcus genomosp. C2	1799	AY278630	clade_98	N	N
Streptococcus genomosp. C3	1800	AY278631	clade_98	N	N
Streptococcus genomosp. C4	1801	AY278632	clade_98	N	N
Streptococcus genomosp. C5	1802	AY278633	clade_98	N	N
Streptococcus genomosp. C6	1803	AY278634	clade_98	N	N
Streptococcus genomosp. C7	1804	AY278635	clade_98	N	N
Streptococcus genomosp. C8	1805	AY278609	clade_98	N	N
Streptococcus gordonii	1806	NC_009785	clade_98	N	N
Streptococcus infantarius	1807	ABJK02000017	clade_98	N	N
Streptococcus infantis	1808	AFNN01000024	clade_98	N	N
Streptococcus intermedius	1809	NR_028736	clade_98	N	N
Streptococcus lutetiensis	1810	NR_037096	clade_98	N	N
Streptococcus massiliensis	1811	AY769997	clade_98	N	N
Streptococcus mitis	1813	AM157420	clade_98	N	N
Streptococcus oligofermentans	1815	AY099095	clade_98	N	N
Streptococcus oralis	1816	ADMV01000001	clade_98	N	N
Streptococcus parasanguinis	1817	AEKM01000012	clade_98	N	N
Streptococcus pasteurianus	1818	AP012054	clade_98	N	N
Streptococcus peroris	1819	AEVF01000016	clade_98	N	N
Streptococcus pneumoniae	1820	AE008537	clade_98	N	N
Streptococcus porcinus	1821	EF121439	clade_98	N	N
Streptococcus pseudopneumoniae	1822	FJ827123	clade_98	N	N
Streptococcus pseudoporcinus	1823	AENS01000003	clade_98	N	N
Streptococcus pyogenes	1824	AE006496	clade_98	N	OP
Streptococcus ratti	1825	X58304	clade_98	N	N
Streptococcus salivarius	1826	AGBV01000001	clade_98	N	N
Streptococcus sanguinis	1827	NR_074974	clade_98	N	N
Streptococcus sinensis	1828	AF432857	clade_98	N	N
Streptococcus sp. 2_1_36FAA	1831	ACOI01000028	clade_98	N	N
Streptococcus sp. 2285_97	1830	AJ131965	clade_98	N	N
Streptococcus sp. ACS2	1834	HQ616360	clade_98	N	N
Streptococcus sp. AS20	1835	HQ616366	clade_98	N	N
Streptococcus sp. BS35a	1836	HQ616369	clade_98	N	N
Streptococcus sp. C150	1837	ACRI01000045	clade_98	N	N
Streptococcus sp. CM6	1838	HQ616372	clade_98	N	N

Streptococcus sp. ICM10	1840	HQ616389	clade_98	N	N
Streptococcus sp. ICM12	1841	HQ616390	clade_98	N	N
Streptococcus sp. ICM2	1842	HQ616386	clade_98	N	N
Streptococcus sp. ICM4	1844	HQ616387	clade_98	N	N
Streptococcus sp. ICM45	1843	HQ616394	clade_98	N	N
Streptococcus sp. M143	1845	ACRK01000025	clade_98	N	N
Streptococcus sp. M334	1846	ACRL01000052	clade_98	N	N
Streptococcus sp. oral clone ASB02	1849	AY923121	clade_98	N	N
Streptococcus sp. oral clone ASCA03	1850	DQ272504	clade_98	N	N
Streptococcus sp. oral clone ASCA04	1851	AY923116	clade_98	N	N
Streptococcus sp. oral clone ASCA09	1852	AY923119	clade_98	N	N
Streptococcus sp. oral clone ASCB04	1853	AY923123	clade_98	N	N
Streptococcus sp. oral clone ASCB06	1854	AY923124	clade_98	N	N
Streptococcus sp. oral clone ASCC04	1855	AY923127	clade_98	N	N
Streptococcus sp. oral clone ASCC05	1856	AY923128	clade_98	N	N
Streptococcus sp. oral clone ASCC12	1857	DQ272507	clade_98	N	N
Streptococcus sp. oral clone ASCD01	1858	AY923129	clade_98	N	N
Streptococcus sp. oral clone ASCD09	1859	AY923130	clade_98	N	N
Streptococcus sp. oral clone ASCD10	1860	DQ272509	clade_98	N	N
Streptococcus sp. oral clone ASCE03	1861	AY923134	clade_98	N	N
Streptococcus sp. oral clone ASCE04	1862	AY953253	clade_98	N	N
Streptococcus sp. oral clone ASCE05	1863	DQ272510	clade_98	N	N
Streptococcus sp. oral clone ASCE06	1864	AY923135	clade_98	N	N
Streptococcus sp. oral clone ASCE09	1865	AY923136	clade_98	N	N
Streptococcus sp. oral clone ASCE10	1866	AY923137	clade_98	N	N
Streptococcus sp. oral clone ASCE12	1867	AY923138	clade_98	N	N
Streptococcus sp. oral clone ASCF05	1868	AY923140	clade_98	N	N
Streptococcus sp. oral clone ASCF07	1869	AY953255	clade_98	N	N
Streptococcus sp. oral clone ASCF09	1870	AY923142	clade_98	N	N
Streptococcus sp. oral clone ASCG04	1871	AY923145	clade_98	N	N
Streptococcus sp. oral clone BW009	1872	AY005042	clade_98	N	N
Streptococcus sp. oral clone CH016	1873	AY005044	clade_98	N	N
Streptococcus sp. oral clone GK051	1874	AY349413	clade_98	N	N
Streptococcus sp. oral clone GM006	1875	AY349414	clade_98	N	N
Streptococcus sp. oral clone P2PA_41 P2	1876	AY207051	clade_98	N	N
Streptococcus sp. oral clone P4PA_30 P4	1877	AY207064	clade_98	N	N
Streptococcus sp. oral taxon 071	1878	AEEP01000019	clade_98	N	N
Streptococcus sp. oral taxon G59	1879	GU432132	clade_98	N	N
Streptococcus sp. oral taxon G62	1880	GU432146	clade_98	N	N
Streptococcus sp. oral taxon G63	1881	GU432150	clade_98	N	N
Streptococcus suis	1882	FM252032	clade_98	N	N
Streptococcus thermophilus	1883	CP000419	clade_98	N	N
Streptococcus uberis	1884	HQ391900	clade_98	N	N
Streptococcus urinalis	1885	DQ303194	clade_98	N	N
Streptococcus vestibularis	1886	AEKO01000008	clade_98	N	N
Streptococcus viridans	1887	AF076036	clade_98	N	N
Synergistetes bacterium oral clone 03 5 D05	1908	GU227192	clade_98	N	N

Table 2.

Representative vitamins, minerals, and cofactors

L-glutamine
nickel chloride
$BaCl_2$
hemin
potassium tellurite
Fibrinogen
Bacto Vitamin-Free Casamino Acids
cocarboxylase
bovine albumin fraction V
$FeCl_2 \cdot H_2O$
L-cystine·2HCl
Bacto Casamino Acids
Agar
$CuSO_4$
pyridoxine
$SnCl_2 \cdot 2H_2O$
sodium selenite
$CaCl_2$
NaCl
albumin fraction V
vitamin B_{12}
folic acid
$ZnCl_2$
$FeSO_4$
oleic acid
$Co(NO_3)_2 \cdot 6H_2O$
L-cystine
$Na_2B_4O_7 \cdot 10H_2O$
$CaSO_4 \cdot 2H_2O$
$AlCl_3$
$SeCl_4$
$Na_2MoO_4 \cdot 2H_2O$
thiamine pyrophosphate
Pyridoxine·HCl
$MnCl_2 \cdot 4H_2O$
aluminum sulphate
Na_2HPO_4
H_3BO_3
L-cysteine·HCl·H_2O
adenine sulfate

long-chain fatty acids
KNO_3
sodium metabisulfite
sodium molybdate
$CoCl_2 \cdot 6H_2O$
Na_2MoO_4
Castenholz Salts
$NaNO_3$
HCl
L-cysteine
copper sulfate
L-cysteine·HCl
thiamine·HCl
biotin
sodium chloride
thallium acetate
$NiCl_2 \cdot 6H_2O$
$NaVO_3 \cdot H_2O$
nicotinamide adenine dinucleotide
nicotinic acid
$Na_2MoO_4 \cdot H_2O$
$CuCl_2 \cdot 2H_2O$
$FeCl_2 \cdot 4H_2O$
$(NH_4)_2MoO_4$
$MnSO_4$
guanine·HCl
H_2SO_4
$CoCl_2$
cholesterol
LiCl
pyridoxine·2HCl
Disodium ethylenediamine tetraacetic acid
Vitamin K1
KBr
alkalinized oleic acid
$ZnSO_4 \cdot 7H_2O$
trypsin inhibitor
KI
ethanol
cobalt nitrate
Ethylenediamine tetraacetic acid
$CuSO_4 \cdot 5H_2O$
calcium-D-pathothenate

$Fe(NO_3)_3$
$CaCl_2 \cdot 2H_2O$
Sodium pyruvate
NaOH
p-aminobenzoic acid
a-ketoglutarate
boric acid
casein
Pyridoxine hydrochloride
Dried bovine hemoglobin
$ZnSO_4$
Nicotinamide
$FeCl_3$
$Fe(NO_3)_3 \cdot 6H_2O$
calcium pantothenate
cyanocobalamin
nitrilotriacetic acid
Adenine
sodium tartrate
magnesium sulfate
zinc sulfate
$NaHCO_3$
Glucose
$MgSO_4 \cdot 7H_2O$
$Na_2S \cdot 9H_2O$
riboflavin
ferric pyrophosphate
Essential growth factors V and X
Peptone
$FeSO_4 \cdot 7H_2O$
catalase
$MnSO_4 \cdot 7H_2O$
$CuCl_2$
$Na_2SeO_3 \cdot 5H_2O$
thiamine
$NiCl_2$
sodium tungstate
iron sulfate
calcium chloride
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
ACES buffer/KOH
Thioctic acid
succinate
formate

lactate
butyrate
acetate
Vitamin K
Mercaptoethane-sulfonic acid
Lipoic acid
ammonia
heme
S-Adenosylmethionine

Table 3.

Bacterial OTUs associated with engraftment and ecological augmentation and establishment of a more diverse microbial ecology in patients treated with an ethanol-treated spore preparation. OTUs that comprise an augmented ecology are not present in the patient prior to treatment and/or exist at extremely low frequencies such that they do not comprise a significant fraction of the total microbial carriage and are not detectable by genomic and/or microbiological assay methods. OTUs that are members of the engrafting and augmented ecologies were identified by characterizing the OTUs that increase in their relative abundance post treatment and that respectively are: (i) present in the ethanol-treated spore preparation and absent in the patient pretreatment, or (ii) absent in the ethanol-treated spore preparation, but increase in their relative abundance through time post treatment with the preparation due to the formation of favorable growth conditions by the treatment. Notably, the latter OTUs can grow from low frequency reservoirs in the patient, or be introduced from exogenous sources such as diet. OTUs that comprise a "core" augmented or engrafted ecology can be defined by the percentage of total patients in which they are observed to engraft and/or augment; the greater this percentage the more likely they are to be part of a core ecology responsible for catalyzing a shift away from a dysbiotic ecology. The dominant OTUs in an ecology can be identified using several methods including but not limited to defining the OTUs that have the greatest relative abundance in either the augmented or engrafted ecologies and defining a total relative abundance threshold. As example, the dominant OTUs in the augmented ecology of Patient-1 were identified by defining the OTUs with the greatest relative abundance, which together comprise 60% of the microbial carriage in this patient's augmented ecology.

OTU	Phylogenetic clade	Spore Fraction OTU	Dominant OTU in Augmented Ecology
Bacteroides sp. 2_1_22	clade38	N	Y
Streptococcus anginosus	clade60	N	
Prevotella intermedia	clade81	N	
Prevotella nigrescens	clade81	N	
Oribacterium sp. ACB7	clade90	N	
Prevotella salivae	clade104	N	
Bacteroides intestinalis	clade171	N	Y
Bifidobacterium dentium	clade172	N	
Alcaligenes faecalis	clade183	N	

MH1	*Phylogenetic Clade*	*Strain Resolution MH1*	*Investigated MH1 in Augmented Ecology*
Rothia dentocariosa	clade194	N	
Peptoniphilus lacrimalis	clade291	N	
Anaerococcus sp. gpac155	clade294	N	
Sutterella stercoricanis	clade302	N	Y
Bacteroides sp. 3_1_19	clade335	N	Y
Parabacteroides goldsteinii	clade335	N	
Bacteroides dorei	clade378	N	Y
Bacteroides massiliensis	clade378	N	
Lactobacillus iners	clade398	N	
Granulicatella adiacens	clade460	N	
Eggerthella sp. 1_3_56FAA	clade477	N	
Gordonibacter pamelaeae	clade477	N	
Finegoldia magna	clade509	N	
Actinomyces nasicola	clade523	N	
Streptobacillus moniliformis	clade532	N	
Oscillospira guilliermondii	clade540	N	
Orientia tsutsugamushi	clade541	N	
Christensenella minuta	clade558	N	
Clostridium oroticum	clade96	Y	
Clostridium sp. D5	clade96	Y	
Clostridium glycyrrhizinilyticum	clade147	Y	
Coprococcus comes	clade147	Y	
Ruminococcus lactaris	clade147	Y	
Ruminococcus torques	clade147	Y	Y
Clostridiales sp. SS3/4	clade246	Y	
Clostridium hylemonae	clade260	Y	

16S	Phylogenetic Clade	Spore Forming 16S	Dominant 16S in Augmented Ecology
Clostridium aerotolerans	clade269	Y	
Clostridium asparagiforme	clade300	Y	Y
Clostridium sp. M62/1	clade300	Y	
Clostridium symbiosum	clade300	Y	
Lachnospiraceae genomosp. C1	clade300	Y	
Blautia sp. M25	clade304	Y	Y
Blautia stercoris	clade304	Y	
Ruminococcus hansenii	clade304	Y	
Ruminococcus obeum	clade304	Y	
Ruminococcus sp. 5_1_39BFAA	clade304	Y	
Bryantella formatexigens	clade309	Y	
Eubacterium cellulosolvens	clade309	Y	
Clostridium sp. HGF2	clade351	Y	
Clostridium bartlettii	clade354	Y	
Clostridium bifermentans	clade354	Y	
Clostridium glycolicum	clade354	Y	
Eubacterium tenue	clade354	Y	
Dorea formicigenerans	clade360	Y	
Dorea longicatena	clade360	Y	
Lachnospiraceae bacterium 2_1_46FAA	clade360	Y	
Lachnospiraceae bacterium 9_1_43BFAA	clade360	Y	Y
Ruminococcus gnavus	clade360	Y	
Clostridium hathewayi	clade362	Y	
Blautia hydrogenotrophica	clade368	Y	
Clostridiaceae bacterium END-2	clade368	Y	
Roseburia faecis	clade369	Y	

MH1	*Phylogenetic Clade*	*Strain Resolution MH1*	*Dominant MH1 in Augmented Ecology*
Roseburia hominis	clade370	Y	
Roseburia intestinalis	clade370	Y	
Eubacterium sp. WAL 14571	clade384	Y	
Erysipelotrichaceae bacterium 5_2_54FAA	clade385	Y	
Eubacterium biforme	clade385	Y	
Eubacterium dolichum	clade385	Y	
Coprococcus catus	clade393	Y	
Acetivibrio ethanolgignens	clade396	Y	
Anaerosporobacter mobilis	clade396	Y	
Bacteroides pectinophilus	clade396	Y	
Eubacterium hallii	clade396	Y	
Eubacterium xylanophilum	clade396	Y	
Anaerostipes caccae	clade408	Y	
Clostridiales bacterium 1_7_47FAA	clade408	Y	
Clostridium aldenense	clade408	Y	
Clostridium citroniae	clade408	Y	
Eubacterium hadrum	clade408	Y	Y
Acetanaerobacterium elongatum	clade439	Y	
Faecalibacterium prausnitzii	clade478	Y	
Gemmiger formicilis	clade478	Y	Y
Eubacterium ramulus	clade482	Y	
Lachnospiraceae bacterium 3_1_57FAA_CT1	clade483	Y	
Lachnospiraceae bacterium A4	clade483	Y	Y
Lachnospiraceae bacterium DJF VP30	clade483	Y	
Holdemania filiformis	clade485	Y	
Clostridium orbiscindens	clade494	Y	

16S	Phylogenetic Clade	Super Keystone 16S	Dominant 16S in Augmented Ecology
Pseudoflavonifractor capillosus	clade494	Y	
Ruminococcaceae bacterium D16	clade494	Y	
Acetivibrio cellulolyticus	clade495	Y	
Eubacterium limosum	clade512	Y	
Anaerotruncus colihominis	clade516	Y	
Clostridium methylpentosum	clade516	Y	
Clostridium sp. YIT 12070	clade516	Y	
Hydrogenoanaerobacterium saccharovorans	clade516	Y	
Eubacterium ventriosum	clade519	Y	
Eubacterium eligens	clade522	Y	
Lachnospira pectinoschiza	clade522	Y	
Lactobacillus rogosae	clade522	Y	Y
Clostridium leptum	clade537	Y	
Eubacterium coprostanoligenes	clade537	Y	
Ruminococcus bromii	clade537	Y	
Clostridium viride	clade540	Y	
Butyrivibrio crossotus	clade543	Y	
Coprococcus eutactus	clade543	Y	
Eubacterium ruminantium	clade543	Y	
Eubacterium rectale	clade568	Y	Y
Roseburia inulinivorans	clade568	Y	
Butyricicoccus pullicaecorum	clade572	Y	
Eubacterium desmolans	clade572	Y	
Papillibacter cinnamivorans	clade572	Y	
Sporobacter termitidis	clade572	Y	
Clostridium lactatifermentans	clade576	Y	

Table 4.

Reduction in pathogen carriage post treatment with bacterial composition treatment of Patient 1.

	Pretreatment	Day 5	Day 14	Day 25
Klebsiella (% of total reads)	20.27%	1.32%	7.62%	0.00%
Fusobacterium (% total of reads)	19.14%	3.01%	0.01%	0.00%

Table 5.

Augmentation of Bacteroides as a function of bacterial composition treatment of Patient 1.

Media	Bacteroides species	Pretreatment titer (cfu/g)	Day 25 titer (cfu/g)
BBE	*B. fragilis* group	$<2x10^4$	$3x10^8$
PFA	All Bacteroides	$<2x10^7$	$2x10^{10}$

Table 6.

OTUs detected by a minimum of ten 16S-V4 sequence reads in at least a one ethanol-treated spore preparation. OTUs that engraft in a treated patients and the percentage of patients in which they engraft are denoted, as are the clades, spore forming status, and Keystone OTU status. Starred OTUs occur in ≥80% of the ethanol preps and engraft in ≥50% of the treated patients.

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Prevotella_maculosa	clade_104	10%	0%	N	N
Prevotella_copri	clade_168	20%	0%	N	N
Bacteroides_caccae	clade_170	30%	0%	N	Y
Bifidobacterium_sp_TM_7*	**clade_172**	**90%**	**60%**	**N**	**N**
Bifidobacterium_gallicum	clade_172	70%	20%	N	N
Bifidobacterium_dentium	clade_172	50%	0%	N	N
Lactobacillus_casei	clade_198	20%	10%	N	N
Actinomyces_odontolyticus	clade_212	20%	30%	N	N
Clostridium_colicanis	clade_223	10%	10%	Y	N
Clostridiales_sp_SS3_4*	**clade_246**	**100%**	**70%**	**Y**	**N**
Clostridium_sporogenes	clade_252	40%	40%	Y	N
Clostridium_butyricum	clade_252	20%	20%	Y	N
Clostridium_disporicum	clade_253	40%	30%	Y	N
Clostridium_hylemonae*	**clade_260**	**100%**	**50%**	**Y**	**N**
Clostridium_scindens	clade_260	10%	60%	Y	N
Coprococcus_comes*	**clade_262**	**90%**	**80%**	**Y**	**Y**
Lachnospiraceae_bacterium_1_4_56FAA*	**clade_262**	**90%**	**80%**	**Y**	**Y**
Ruminococcus_torques	clade_262	30%	70%	Y	Y
Parabacteroides_merdae	clade_286	30%	20%	N	Y
Bifidobacterium_bifidum	clade_293	10%	0%	N	N

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Johnsonella_ignava	clade_298	10%	10%	N	N
Blautia_glucerasea*	**clade_309**	**100%**	**80%**	**Y**	**N**
Blautia_sp_M25*	**clade_309**	**100%**	**70%**	**Y**	**Y**
Lachnospiraceae_bacterium_6_1_63FAA*	**clade_309**	**100%**	**60%**	**Y**	**N**
Eubacterium_cellulosolvens	clade_309	10%	30%	Y	Y
Lactobacillus_fermentum	clade_313	10%	0%	N	N
Sarcina_ventriculi	clade_353	10%	10%	Y	N
Clostridium_bartlettii*	**clade_354**	**90%**	**70%**	**Y**	**N**
Clostridium_bifermentans	clade_354	70%	70%	Y	N
Clostridium_mayombei	clade_354	50%	50%	Y	N
Dorea_longicatena*	**clade_360**	**100%**	**60%**	**Y**	**Y**
Lachnospiraceae_bacterium_9_1_43BFAA	clade_360	100%	30%	Y	N
Lachnospiraceae_bacterium_2_1_58FAA*	**clade_360**	**80%**	**80%**	**Y**	**N**
Lachnospiraceae_bacterium_2_1_46FAA	clade_360	50%	50%	Y	N
Lactobacillus_perolens	clade_373	10%	0%	N	N
Bacteroides_dorei	clade_378	60%	50%	N	Y
Eubacterium_biforme	clade_385	10%	0%	Y	N
Peptoniphilus_sp_gpac077	clade_389	10%	20%	N	N
Coprococcus_catus*	**clade_393**	**100%**	**70%**	**Y**	**Y**
Eubacterium_hallii*	**clade_396**	**90%**	**60%**	**Y**	**Y**
Anaerosporobacter_mobilis	clade_396	40%	60%	Y	N
Bacteroides_pectinophilus	clade_396	10%	60%	Y	N
Lactobacillus_hominis	clade_398	10%	0%	N	N
Lactococcus_lactis	clade_401	40%	40%	N	N
Ruminococcus_champanellensis*	**clade_406**	**80%**	**50%**	**Y**	**N**

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Ruminococcus_callidus	clade_406	10%	10%	Y	N
Clostridium_clostridioforme*	**clade_408**	**100%**	**60%**	**Y**	**Y**
Eubacterium_hadrum*	**clade_408**	**100%**	**90%**	**Y**	**Y**
Clostridium_symbiosum	clade_408	30%	50%	Y	Y
Anaerostipes_caccae	clade_408	10%	50%	Y	N
Parasutterella_excrementihominis	clade_432	10%	0%	N	N
Sutterella_stercoricanis	clade_432	10%	0%	N	N
Eubacterium_rectale*	**clade_444**	**100%**	**80%**	**Y**	**Y**
Lachnobacterium_bovis*	**clade_444**	**100%**	**80%**	**Y**	**N**
Desulfovibrio_desulfuricans	clade_445	10%	0%	N	Y
Eubacterium_sp_oral_clone_JS001*	**clade_476**	**80%**	**70%**	**Y**	**N**
Faecalibacterium_prausnitzii*	**clade_478**	**100%**	**60%**	**Y**	**Y**
Subdoligranulum_variabile*	**clade_478**	**100%**	**80%**	**Y**	**Y**
Coprobacillus_sp_D7*	**clade_481**	**90%**	**60%**	**Y**	**N**
Clostridium_cocleatum	clade_481	60%	20%	Y	N
Clostridium_spiroforme	clade_481	40%	50%	Y	N
Eubacterium_ramulus*	**clade_482**	**80%**	**60%**	**Y**	**N**
Flavonifractor_plautii	clade_494	70%	60%	Y	Y
Pseudoflavonifractor_capillosus	clade_494	60%	60%	Y	Y
Ruminococcaceae_bacterium_D16	clade_494	30%	50%	Y	Y
Acetivibrio_cellulolyticus*	**clade_495**	**70%**	**80%**	**Y**	**N**
Clostridium_stercorarium	clade_495	40%	50%	Y	N
Enterococcus_durans	clade_497	10%	10%	N	N
Enterococcus_faecium	clade_497	10%	10%	N	N
Dialister_invisus	clade_506	50%	10%	N	N

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Eubacterium_limosum	clade_512	20%	0%	Y	N
Ruminococcus_flavefaciens	clade_516	60%	60%	Y	N
Eubacterium_ventriosum	clade_519	30%	60%	Y	Y
Bilophila_wadsworthia	clade_521	90%	0%	N	Y
Lachnospira_pectinoschiza	clade_522	40%	60%	Y	N
Eubacterium_eligens	clade_522	30%	50%	Y	Y
Catonella_morbi	clade_534	20%	0%	N	N
Clostridium_sporosphaeroides*	**clade_537**	**100%**	**80%**	**Y**	**N**
Ruminococcus_bromii	clade_537	60%	30%	Y	Y
Clostridium_leptum	clade_537	40%	70%	Y	Y
Clostridium_sp_YIT_12069	clade_537	40%	60%	Y	N
Clostridium_viride	clade_540	10%	10%	Y	N
Megamonas_funiformis	clade_542	50%	0%	N	N
Eubacterium_ruminantium*	**clade_543**	**80%**	**90%**	**Y**	**N**
Coprococcus_eutactus	clade_543	20%	20%	Y	N
Collinsella_aerofaciens	clade_553	50%	10%	Y	Y
Alkaliphilus_metalliredigenes	clade_554	40%	10%	Y	N
Turicibacter_sanguinis	clade_555	80%	40%	Y	N
Phascolarctobacterium_faecium	clade_556	20%	0%	N	N
Clostridiales_bacterium_oral_clone_P4PA*	**clade_558**	**80%**	**50%**	**N**	**N**
Lutispora_thermophila	clade_564	100%	0%	Y	N
Coriobacteriaceae_bacterium_JC110	clade_566	70%	0%	N	N
Eggerthella_sp_1_3_56FAA	clade_566	70%	30%	N	N
Adlercreutzia_equolifaciens	clade_566	40%	0%	N	N
Gordonibacter_pamelaeae	clade_566	30%	0%	N	Y

OTU	Clade	% of Spore Preps with OTU	% of Patients OTU Engrafts	Spore Former	Keystone OTU
Slackia_isoflavoniconvertens	clade_566	10%	0%	N	N
Eubacterium_desmolans*	**clade_572**	**90%**	**70%**	**Y**	**N**
Papillibacter_cinnamivorans*	**clade_572**	**90%**	**80%**	**Y**	**N**
Clostridium_colinum	clade_576	30%	30%	Y	N
Akkermansia_muciniphila	clade_583	60%	10%	N	Y
Clostridiales_bacterium_oral_taxon_F32	clade_584	60%	30%	N	N
Prochlorococcus_marinus	clade_592	30%	0%	N	N
Methanobrevibacter_wolinii	clade_595	30%	0%	N	N
Bacteroides_fragilis	clade_65	20%	30%	N	Y
Lactobacillus_delbrueckii	clade_72	10%	0%	N	N
Escherichia_coli	clade_92	50%	0%	N	Y
Clostridium_sp_D5	clade_96	80%	60%	Y	N
Streptococcus_thermophilus	clade_98	90%	20%	N	Y
Streptococcus_sp_CM6	clade_98	20%	10%	N	N
Streptococcus_sp_oral_clone_ASCE05	clade_98	10%	0%	N	N

Table 7.

Top 20 OTUs ranked by CES

OTU	Clade	CES	Spore Former	Keystone OTU
Eubacterium_hadrum	clade_408	4.2	Y	Y
Eubacterium_rectale	clade_444	4.2	Y	Y
Subdoligranulum_variabile	clade_478	4.2	Y	Y
Blautia_sp_M25	clade_309	4.2	Y	Y
Coprococcus_catus	clade_393	4.2	Y	Y
Lachnospiraceae_bacterium_1_4_56FAA	clade_262	4.2	Y	Y
Coprococcus_comes	clade_262	4.2	Y	Y
Blautia_glucerasea	clade_309	4.0	Y	N
Lachnobacterium_bovis	clade_444	4.0	Y	N
Clostridium_sporosphaeroides	clade_537	4.0	Y	N
Clostridiales_sp_SS3_4	clade_246	4.0	Y	N
Papillibacter_cinnamivorans	clade_572	4.0	Y	N
Clostridium_bartlettii	clade_354	4.0	Y	N
Eubacterium_desmolans	clade_572	4.0	Y	N
Clostridium_clostridioforme	clade_408	3.2	Y	Y
Dorea_longicatena	clade_360	3.2	Y	Y
Faecalibacterium_prausnitzii	clade_478	3.2	Y	Y
Eubacterium_hallii	clade_396	3.2	Y	Y
Clostridium_leptum	clade_537	3.2	Y	Y
Lachnospiraceae_bacterium_6_1_63FAA	clade_309	3.0	Y	N

Table 8.

Computationally derived Network Ecologies and associated disease indications for which bacterial composition represents a state of health. Network Ecologies are ordered based on their size defined by the number of OTUs in the network.

Network Ecology ID	Disease Indication for which Network Ecology is Health State	Number of Clades in Network Ecology	Number of OTUs in Network Ecology	Percent of OTUs that are Spore Forming	Percent of Keystone OTUs in Network Ecology	Exemplary Network clades	Exemplary Network OTUs	Exemplary Keystone OTUs
N249	CDAD	29	37	27	16.2	clade_141, clade_195, clade_256, (clade_262 or clade_262i), (clade_325 or clade_325f), clade_333, clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_393, (clade_444 or clade_444i), clade_476, (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i), (clade_497 or clade_497e or	Akkermansia muciniphila, Alistipes indistinctus, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bacteroides xylanisolvens, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Capnocytophaga sp. oral clone ASCH05, Chlamydiales bacterium NS13, Chromobacterium violaceum, Clostridium cocleatum, Coprobacillus sp. D7, Coprococcus catus, Corynebacterium pseudogenitalium, Enhydrobacter aerosaccus, Enterococcus raffinosus, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Lachnobacterium bovis, Lachnospiraceae bacterium 1_1_57FAA,	Akkermansia muciniphila, Bacteroides xylanisolvens, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

						clade_497f), clade_507, clade_515, clade_521, (clade_522 or clade_522i), clade_535, clade_556, clade_561, clade_567, clade_575, clade_583, clade_94, (clade_98 or clade_98i)	Neisseria meningitidis, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Psychrobacter pulmonis, Ruminococcus sp. ID8, Staphylococcus warneri, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07, Streptococcus suis, Synergistetes bacterium oral taxon D48, Tissierella praeacuta	

N250	CDAD	30	37	24.3	16.2	clade_195, clade_256, (clade_262 or clade_262i), (clade_325 or clade_325f), clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), (clade_38 or clade_38e or clade_38i)1, clade_393, (clade_420 or clade_420f), (clade_444 or clade_444i), clade_453, clade_467, clade_476, (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i), (clade_497 or clade_497e or clade_497f), clade_507, clade_521, (clade_522 or clade_522i), clade_535, (clade_553 or clade_553i), clade_556, clade_558, clade_561, clade_583,	Akkermansia muciniphila, Alistipes indistinctus, Auritibacter ignavus, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bacteroides xylanisolvens, Barnesiella viscericola, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Capnocytophaga sp. oral taxon 338, Chromobacterium violaceum, Clostridiales bacterium oral clone P4PA, Clostridium cocleatum, Collinsella tanakaei, Coprobacillus sp. D7, Coprococcus catus, Corynebacterium pseudogenitalium, Enhydrobacter aerosaccus, Enterococcus sp. CCRI 16620, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Lachnospiraceae bacterium 1_1_57FAA, Neisseria meningitidis, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Ruminococcus sp. ID8, Scardovia inopinata, Staphylococcus warneri, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07,	Akkermansia muciniphila, Bacteroides xylanisolvens, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

						clade_94, (clade_98 or clade_98i)	Streptococcus suis	

N251	CDAD	29	34	20.6	14.7	clade_141, clade_181, clade_195, clade_256, (clade_262 or clade_262i), clade_293, clade_333, clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_393, (clade_420 or clade_420f), (clade_444 or clade_444i), clade_476, clade_507, clade_515, clade_521, (clade_522 or clade_522i), clade_535, clade_551, (clade_553 or clade_553i), clade_556, clade_558, clade_583, clade_597, (clade_98 or clade_98i)	Acinetobacter johnsonii, Akkermansia muciniphila, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bifidobacterium bifidum, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Capnocytophaga sp. oral clone ASCH05, Chromobacterium violaceum, Clostridiales bacterium oral clone P4PA, Collinsella tanakaei, Coprococcus catus, Corynebacterium pseudogenitalium, Enhydrobacter aerosaccus, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Halorubrum lipolyticum, Lachnospiraceae bacterium 1_1_57FAA, Mycoplasmataceae genomosp P1 oral clone, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Psychrobacter pulmonis, Ruminococcus sp. ID8, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07, Streptococcus suis, Tannerella sp. 6_1_58FAA_CT1, Tissierella praeacuta	Akkermansia muciniphila, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

N252	CDAD	25	32	28.1	18.8	clade_181, clade_195, clade_256, (clade_262 or clade_262i), (clade_325 or clade_325f), clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_393, (clade_444 or clade_444i), clade_476, (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i), clade_507, clade_515, clade_521, (clade_522 or clade_522i), clade_535, clade_556, clade_561, clade_583, clade_94, (clade_98 or clade_98i)	Acinetobacter johnsonii, Akkermansia muciniphila, Alistipes indistinctus, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bacteroides xylanisolvens, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Chromobacterium violaceum, Clostridium cocleatum, Coprobacillus sp. D7, Coprococcus catus, Corynebacterium pseudogenitalium, Enhydrobacter aerosaccus, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Lachnospiraceae bacterium 1_1_57FAA, Neisseria meningitidis, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Ruminococcus sp. ID8, Staphylococcus warneri, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07, Streptococcus suis, Tissierella praeacuta	Akkermansia muciniphila, Bacteroides xylanisolvens, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

N253	CDAD	24	31	29	19.4	clade_195, clade_256, (clade_262 or clade_262i), (clade_325 or clade_325f), clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_393, (clade_444 or clade_444i), clade_476, (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i), clade_507, clade_515, clade_521, (clade_522 or clade_522i), clade_535, clade_556, clade_561, clade_583, clade_94, (clade_98 or clade_98i)	Akkermansia muciniphila, Alistipes indistinctus, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bacteroides xylanisolvens, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Chromobacterium violaceum, Clostridium cocleatum, Coprobacillus sp. D7, Coprococcus catus, Corynebacterium pseudogenitalium, Enhydrobacter aerosaccus, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Lachnospiraceae bacterium 1_1_57FAA, Neisseria meningitidis, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Ruminococcus sp. ID8, Staphylococcus warneri, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07, Streptococcus suis, Tissierella praeacuta	Akkermansia muciniphila, Bacteroides xylanisolvens, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

N254	CDAD	24	30	23.3	20	clade_141, clade_181, clade_195, (clade_262 or clade_262i), clade_293, clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_393, (clade_420 or clade_420f), (clade_444 or clade_444i), clade_476, clade_507, clade_521, (clade_522 or clade_522i), clade_535, clade_556, clade_567, clade_575, clade_583, (clade_98 or clade_98i)	Acinetobacter johnsonii, Akkermansia muciniphila, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bacteroides xylanisolvens, Barnesiella viscericola, Bifidobacterium bifidum, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Chlamydiales bacterium NS13, Chromobacterium violaceum, Coprococcus catus, Corynebacterium pseudogenitalium, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Lachnospiraceae bacterium 1_1_57FAA, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Psychrobacter pulmonis, Ruminococcus sp. ID8, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07, Streptococcus suis, Synergistetes bacterium oral taxon D48	Akkermansia muciniphila, Bacteroides xylanisolvens, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

N255	CDAD	23	29	34.5	17.2	clade_141, clade_181, clade_195, (clade_262 or clade_262i), clade_293, clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_393, (clade_444 or clade_444i), clade_476, (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i), clade_507, (clade_516 or clade_516c or clade_516g or clade_516h), clade_521, (clade_522 or clade_522i), clade_535, clade_556, clade_583, (clade_98 or clade_98i)	Acinetobacter johnsonii, Akkermansia muciniphila, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bifidobacterium bifidum, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Chromobacterium violaceum, Coprobacillus sp. D7, Coprococcus catus, Corynebacterium pseudogenitalium, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Lachnobacterium bovis, Lachnospiraceae bacterium 1_1_57FAA, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Psychrobacter pulmonis, Ruminococcus albus, Ruminococcus sp. ID8, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07, Streptococcus suis	Akkermansia muciniphila, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

N256	CDAD	19	26	76.9	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h), clade_519, clade_521, (clade_522 or clade_522i), clade_537, clade_538	Alistipes putredinis, Alistipes shahii, Bacteroides pectinophilus, Bacteroides xylanisolvens, Bilophila wadsworthia, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus albus, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Bilophila wadsworthia, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N257	CDAD	21	26	26.9	19.2	clade_141, clade_181, clade_195, (clade_262 or clade_262i), clade_293, clade_344, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_393, (clade_444 or clade_444i), clade_476, clade_507, clade_521, (clade_522 or clade_522i), clade_535, clade_556, clade_583, (clade_98 or clade_98i)	Acinetobacter johnsonii, Akkermansia muciniphila, Bacteroides coprocola, Bacteroides sp. 3_1_40A, Bacteroides sp. D2, Bifidobacterium bifidum, Bilophila wadsworthia, Blautia hydrogenotrophica, Campylobacter upsaliensis, Chromobacterium violaceum, Coprococcus catus, Corynebacterium pseudogenitalium, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale, Gardnerella vaginalis, Lachnospiraceae bacterium 1_1_57FAA, Peptoniphilus sp. gpac077, Phascolarctobacterium sp. YIT 12068, Psychrobacter pulmonis, Ruminococcus sp. ID8, Streptococcus dysgalactiae, Streptococcus peroris, Streptococcus pyogenes, Streptococcus sp. oral clone ASCF07, Streptococcus suis	Akkermansia muciniphila, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

N258	CDAD	18	24	75	91.7	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, clade_474, (clade_478 or clade_478i), clade_494, clade_500, clade_519, (clade_522 or clade_522i), clade_537, clade_538, (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Mitsuokella multacida, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N259	CDAD	17	24	75	91.7	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h), clade_519, (clade_522 or clade_522i), clade_537, clade_556	Acidaminococcus fermentans, Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Bacteroides dorei, Clostridium leptum, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides dorei, Clostridium leptum, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N260	CDAD	15	22	72.7	95.5	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, clade_519, clade_537	Alistipes putredinis, Alistipes shahii, Bifidobacterium catenulatum, Bifidobacterium longum, Clostridium leptum, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bifidobacterium longum, Clostridium leptum, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N261	CDAD	14	21	81	90.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_449, clade_466, (clade_478 or clade_478i), clade_485,	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Lactobacillus ruminis, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques,	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques,

						clade_500, clade_519, (clade_522 or clade_522i), (clade_553 or clade_553i)	Subdoligranulum variabile	Subdoligranulum variabile
N262	CDAD	13	21	71.4	95.2	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i), (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Bifidobacterium dentium, Bifidobacterium longum, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bifidobacterium longum, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Subdoligranulum variabile

N263	CDAD,T2D	13	21	76.2	90.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h), (clade_522 or clade_522i), clade_537	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Marvinbryantia formatexigens, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N264	CDAD,T2D	14	21	76.2	95.2	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466,	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Marvinbryantia formatexigens, Odoribacter splanchnicus,	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia

						(clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i), clade_537	Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N265	CDAD,T2D	15	21	71.4	90.5	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, clade_474, (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Mitsuokella multacida, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N266	CDAD,T2D	13	21	76.2	90.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h), (clade_522 or clade_522i), clade_537	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Marvinbryantia formatexigens, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N267	CDAD,T2D	14	21	81	95.2	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466,	Alistipes putredinis, Alistipes shahii, Bacteroides pectinophilus, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia

						(clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i), clade_537	intestinalis, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	inulinivorans, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N268	CDAD,T2D	14	21	76.2	95.2	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i), clade_537	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Marvinbryantia formatexigens, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N269	CDAD,T2D	15	21	71.4	90.5	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, clade_474, (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Mitsuokella multacida, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N270	CDAD,T2D	14	21	81	95.2	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i),	Alistipes putredinis, Alistipes shahii, Bacteroides pectinophilus, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans,	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus bromii,

						clade_485, clade_494, clade_500, (clade_522 or clade_522i), clade_537	Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N271	CDAD	11	20	70	90	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Streptococcus salivarius, Streptococcus vestibularis, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Subdoligranulum variabile

N272	CDAD	14	20	70	95	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_494, clade_500, clade_538	Alistipes putredinis, Alistipes shahii, Bacteroides coprophilus, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N273	CDAD,T2D	13	20	65	95	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_500,	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Methanobrevibacter smithii, Parabacteroides merdae, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum,	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Parabacteroides merdae, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques,

						clade_519, (clade_522 or clade_522i), clade_537, clade_588	Ruminococcus torques, Subdoligranulum variabile	Subdoligranulum variabile
N274	CDAD,T2D	13	20	65	95	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_500, clade_519, (clade_522 or clade_522i), clade_537, clade_588	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Methanobrevibacter smithii, Parabacteroides merdae, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Bacteroides xylanisolvens, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Parabacteroides merdae, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N275	CDAD	13	19	73.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_357, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, clade_519	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Oxalobacter formigenes, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Oxalobacter formigenes, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N276	CDAD	13	19	78.9	94.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Clostridium methylpentosum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						clade_516c or clade_516g or clade_516h), clade_537		
N277	CDAD,T2D	13	19	73.7	94.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_494, clade_500, clade_538	Alistipes putredinis, Alistipes shahii, Bacteroides coprophilus, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N278	CDAD,T2D	13	19	73.7	94.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_393, clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_494, clade_500, clade_538	Alistipes putredinis, Alistipes shahii, Bacteroides coprophilus, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N279	CDAD	12	18	77.8	94.4	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i)8, clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i),	Alistipes putredinis, Alistipes shahii, Bifidobacterium longum, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Solobacterium moorei, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bifidobacterium longum, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						clade_485, clade_494, clade_500		
N280	CDAD	11	18	77.8	94.4	(clade_262 or clade_262i), clade_271, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Rothia mucilaginosa, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N281	CDAD	11	18	72.2	94.4	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_500, clade_538, (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Coprococcus comes, Desulfovibrio desulfuricans, Desulfovibrio piger, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Desulfovibrio piger, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N282	CDAD	11	18	77.8	94.4	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_401, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Lactococcus lactis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N283	CDAD	11	18	83.3	94.4	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Blautia hansenii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N284	CDAD,T2D	12	18	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500, clade_519, clade_538, clade_543	Alistipes putredinis, Alistipes shahii, Butyrivibrio crossotus, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Butyrivibrio crossotus, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N285	CDAD,T2D	12	18	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500, clade_519, clade_538, clade_543	Alistipes putredinis, Alistipes shahii, Butyrivibrio crossotus, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Butyrivibrio crossotus, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N286	CDAD	13	18	72.2	94.4	clade_129, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500,	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Prevotella buccalis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						clade_519		
N287	CDAD	11	17	76.5	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N288	CDAD	11	17	76.5	94.1	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus mitis, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N289	CDAD	11	17	76.5	100	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N290	CDAD,T2D	11	17	88.2	94.1	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), clade_543	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Coprococcus eutactus, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N291	CDAD	11	17	76.5	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_494, clade_500, clade_567	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Victivallis vadensis	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Victivallis vadensis

N292	CDAD	12	17	82.4	88.2	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Clostridium symbiosum, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N293	CDAD,T2D	12	17	70.6	94.1	clade_129, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396,	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Prevotella buccalis, Pseudoflavonifractor capillosus, Roseburia inulinivorans,	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum,

						(clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Ruminococcus torques, Subdoligranulum variabile
N294	CDAD,T2D	12	17	70.6	94.1	clade_129, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Prevotella buccalis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N295	CDAD,T2D	10	17	70.6	94.1	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Coprococcus comes, Desulfovibrio desulfuricans, Desulfovibrio piger, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Desulfovibrio piger, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N296	CDAD,T2D	11	17	70.6	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_357, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Oxalobacter formigenes, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Oxalobacter formigenes, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N297	CDAD,T2D	11	17	88.2	94.1	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), clade_543	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Coprococcus eutactus, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N298	CDAD,T2D	10	17	70.6	94.1	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Coprococcus comes, Desulfovibrio desulfuricans, Desulfovibrio piger, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Desulfovibrio piger, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N299	CDAD,T2D	11	17	70.6	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_357, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Oxalobacter formigenes, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Oxalobacter formigenes, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N300	CDAD	11	16	81.3	93.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_566 or clade_566f)	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eggerthella lenta, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N301	CDAD	10	16	75	93.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_432, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques, Sutterella wadsworthensis	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques
N302	CDAD	10	16	81.3	93.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_519, clade_588	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Methanobrevibacter smithii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N303	CDAD	11	16	68.8	93.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides plebeius, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N304	CDAD,T2D	11	16	81.3	100	clade_171, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Coprococcus comes, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Coprococcus comes, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N305	CDAD,T2D	10	16	81.3	93.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), (clade_522 or clade_522i), clade_543	Bacteroides ovatus, Bacteroides xylanisolvens, Coprococcus comes, Coprococcus eutactus, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Bacteroides ovatus, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N306	CDAD	12	16	100	31.3	clade_252, (clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_351 or clade_351e), (clade_38 or clade_38e or clade_38i)4, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or	Blautia producta, Clostridium hylemonae, Clostridium innocuum, Clostridium orbiscindens, Clostridium symbiosum, Clostridium tertium, Collinsella aerofaciens, Coprobacillus sp. D7, Coprococcus comes, Eubacterium rectale, Eubacterium sp. WAL 14571, Faecalibacterium prausnitzii, Lachnospiraceae bacterium 5_1_57FAA, Roseburia faecalis, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

						clade_444i), (clade_478 or clade_478i), (clade_481 or clade_481a or clade_481b or clade_481c or clade_481g or clade_481h or clade_481i), clade_494, (clade_553 or clade_553i)		
N307	CDAD	13	16	75	68.8	clade_170, (clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), clade_485,	Alistipes shahii, Bacteroides caccae, Bacteroides stercoris, Blautia producta, Clostridium hathewayi, Clostridium symbiosum, Collinsella aerofaciens, Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Holdemania filiformis, Lachnospiraceae bacterium 5_1_57FAA, Parabacteroides merdae, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides caccae, Bacteroides stercoris, Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Holdemania filiformis, Parabacteroides merdae, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques

						clade_500, clade_537, (clade_553 or clade_553i), clade_85		
N308	CDAD,T2D	10	16	81.3	93.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), (clade_522 or clade_522i), clade_543	Bacteroides ovatus, Bacteroides xylanisolvens, Coprococcus comes, Coprococcus eutactus, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Bacteroides ovatus, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N309	CDAD,T2D	11	16	81.3	100	clade_171, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Coprococcus comes, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Coprococcus comes, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N310	CDAD,T2D	10	16	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_494, clade_500, clade_567	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Victivallis vadensis	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Victivallis vadensis

N311	CDAD,T2D	10	16	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_494, clade_500, clade_567	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Victivallis vadensis	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Victivallis vadensis
N312	CDAD,T2D	10	15	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, clade_469, (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Catenibacterium mitsuokai, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Catenibacterium mitsuokai, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N313	CDAD,T2D	11	15	80	86.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), (clade_553 or clade_553i), clade_62	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Prevotella buccae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N314	CDAD	10	15	73.3	93.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_335 or clade_335i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Parabacteroides distasonis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N315	CDAD	9	15	80	93.3	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium pseudocatenulatum, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N316	CDAD	10	15	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Clostridium nexile, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Clostridium nexile, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N317	CDAD,T2D	11	15	80	93.3	clade_168, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_519, (clade_522 or clade_522i)	Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Prevotella copri, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N318	CDAD,T2D	11	15	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_357, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_500, clade_519,	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Oxalobacter formigenes, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Oxalobacter formigenes, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						(clade_522 or clade_522i)		
N319	CDAD,T2D	11	15	80	86.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), (clade_553 or clade_553i), clade_62	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Prevotella buccae, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N320	CDAD,T2D	11	15	80	93.3	clade_168, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_519, (clade_522 or clade_522i)	Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Prevotella copri, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N321	CDAD,T2D	10	15	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, clade_469, (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Catenibacterium mitsuokai, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Catenibacterium mitsuokai, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N322	CDAD,T2D	10	15	66.7	93.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides plebeius, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N323	CDAD,T2D	9	15	80	93.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_588	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Methanobrevibacter smithii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N324	CDAD,T2D	11	15	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_357, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_500, clade_519, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Oxalobacter formigenes, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Oxalobacter formigenes, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N325	CDAD	11	15	73.3	86.7	(clade_262 or clade_262i), clade_281, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500,	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Bacteroides xylanisolvens, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Porphyromonas asaccharolytica, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						(clade_516 or clade_516c or clade_516g or clade_516h)		
N326	CDAD	12	15	66.7	93.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_500, clade_504, clade_519, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Campylobacter hominis, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N327	CDAD,T2D	10	15	66.7	93.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_396, (clade_444 or clade_444i), clade_445, clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides plebeius, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N328	CDAD,T2D	9	15	80	93.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_588	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Methanobrevibacter smithii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N329	CDAD,T2D	10	14	71.4	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i), (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Subdoligranulum variabile, Veillonella dispar	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Subdoligranulum variabile
N330	CDAD	9	14	78.6	92.9	clade_168, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Prevotella copri, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N331	CDAD	8	14	85.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_538	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N332	CDAD	10	14	78.6	92.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus thermophilus, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N333	CDAD	11	14	78.6	92.9	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Clostridium scindens, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N334	CDAD	11	14	78.6	92.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), clade_445, (clade_478 or	Alistipes putredinis, Alistipes shahii, Clostridium hathewayi, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						clade_478i), clade_485, clade_494, clade_500		
N335	CDAD,T2D	10	14	71.4	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i), (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Subdoligranulum variabile, Veillonella dispar	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Subdoligranulum variabile

N336	CDAD,T2D	9	14	71.4	92.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_335 or clade_335i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides distasonis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N337	CDAD,T2D	9	14	71.4	92.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_335 or clade_335i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides distasonis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N338	CDAD	9	13	76.9	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i), (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus australis, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N339	CDAD	9	13	76.9	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_566 or clade_566f)	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N340	CDAD	9	13	84.6	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_354 or clade_354e), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Clostridium bartlettii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N341	CDAD	9	13	84.6	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N342	CDAD,T2D	9	13	69.2	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_500, clade_583	Akkermansia muciniphila, Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Akkermansia muciniphila, Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N343	CDAD	9	13	69.2	100	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N344	CDAD	9	13	69.2	92.3	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium adolescentis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N345	CDAD	10	13	84.6	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Clostridium asparagiforme, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N346	CDAD	9	13	84.6	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus albus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N347	CDAD	9	13	69.2	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides cellulosilyticus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides cellulosilyticus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N348	CDAD	8	13	76.9	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_65 or clade_65e)	Alistipes putredinis, Alistipes shahii, Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N349	CDAD,T2D	9	13	69.2	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_500, clade_583	Akkermansia muciniphila, Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Akkermansia muciniphila, Alistipes putredinis, Alistipes shahii, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N350	CDAD,T2D	11	13	61.5	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_38 or clade_38e or clade_38i), clade_396, clade_445, clade_466, (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Blautia hydrogenotrophica, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N351	CDAD,T2D	11	13	61.5	92.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_368, (clade_38 or clade_38e or clade_38i), clade_396, clade_445, clade_466, (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Blautia hydrogenotrophica, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Coprococcus comes, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N352	CDAD	8	12	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Veillonella parvula	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Veillonella parvula
N353	CDAD	9	12	75	91.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, clade_506	Alistipes putredinis, Alistipes shahii, Dialister invisus, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N354	CDAD	8	12	75	91.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_88	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Haemophilus parainfluenzae, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N355	CDAD,T2D	8	12	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_519	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N356	CDAD	8	12	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_537	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N357	CDAD	9	12	75	100	clade_170, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides finegoldii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides finegoldii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N358	CDAD	8	12	75	100	clade_110, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides uniformis, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides uniformis, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N359	CDAD	8	12	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides eggerthii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Bacteroides eggerthii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N360	CDAD,T2D	10	12	58.3	100	clade_110, (clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_519	Bacteroides ovatus, Bacteroides uniformis, Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Bacteroides ovatus, Bacteroides uniformis, Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N361	CDAD,T2D	8	12	83.3	91.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus albus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N362	CDAD,T2D	8	12	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_519	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N363	CDAD,T2D	10	12	66.7	91.7	(clade_262 or clade_262i), clade_271, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Rothia mucilaginosa, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N364	CDAD,T2D	10	12	58.3	100	clade_110, (clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_519	Bacteroides ovatus, Bacteroides uniformis, Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Bacteroides ovatus, Bacteroides uniformis, Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N365	CDAD,T2D	10	12	66.7	91.7	(clade_262 or clade_262i), clade_271, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Rothia mucilaginosa, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N366	CDAD,T2D	8	12	83.3	91.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus albus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N367	CDAD	8	11	72.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Veillonella atypica	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile, Veillonella atypica

N368	CDAD	8	11	72.7	90.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus salivarius	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N369	CDAD	8	11	81.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N370	CDAD	8	11	72.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N371	CDAD	7	11	81.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N372	CDAD	8	11	81.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum,	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus obeum,

						clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Ruminococcus torques, Subdoligranulum variabile	Ruminococcus torques, Subdoligranulum variabile
N373	CDAD	8	11	81.8	90.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus gnavus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N374	CDAD	8	11	72.7	100	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or	Alistipes putredinis, Alistipes shahii, Bifidobacterium longum, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bifidobacterium longum, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

						clade_478i), clade_500		
N375	CDAD	8	11	81.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_537	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Clostridium leptum, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N376	CDAD	8	11	72.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i),	Alistipes putredinis, Alistipes shahii, Bacteroides stercoris, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides stercoris, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

						clade_500, clade_85		
N377	CDAD	8	11	72.7	100	clade_170, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides caccae, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides caccae, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N378	CDAD	9	11	72.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_396, (clade_444 or clade_444i), (clade_478 or	Alistipes putredinis, Alistipes shahii, Bacteroides dorei, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides dorei, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

						clade_478i), clade_485, clade_500		
N379	CDAD	8	11	72.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N380	CDAD	9	11	81.8	81.8	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or	Bacteroides ovatus, Bifidobacterium adolescentis, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia faecalis, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

						clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_519, (clade_522 or clade_522i)		
N381	CDAD,T2D	9	11	90.9	81.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, (clade_516 or clade_516c or clade_516g or clade_516h), (clade_522 or clade_522i), clade_537	Anaerotruncus colihominis, Bacteroides xylanisolvens, Clostridium leptum, Clostridium methylpentosum, Eubacterium eligens, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Bacteroides xylanisolvens, Clostridium leptum, Eubacterium eligens, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N382	CDAD	10	11	100	18.2	clade_252, (clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_351 or clade_351e), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494, clade_537, (clade_553 or clade_553i)	Blautia producta, Clostridium hylemonae, Clostridium innocuum, Clostridium orbiscindens, Clostridium symbiosum, Clostridium tertium, Collinsella aerofaciens, Coprococcus comes, Lachnospiraceae bacterium 5_1_57FAA, Ruminococcus bromii, Ruminococcus gnavus	Coprococcus comes, Ruminococcus bromii
N383	CDAD,T2D	9	11	90.9	81.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, (clade_516 or clade_516c or clade_516g or clade_516h),	Anaerotruncus colihominis, Bacteroides xylanisolvens, Clostridium leptum, Clostridium methylpentosum, Eubacterium eligens, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Bacteroides xylanisolvens, Clostridium leptum, Eubacterium eligens, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

						(clade_522 or clade_522i), clade_537		
N384	CDAD,T2D	10	11	63.6	90.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), clade_419, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes shahii, Bacteroides ovatus, Bacteroides salanitronis, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides ovatus, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N385	CDAD,T2D	10	11	63.6	90.9	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), clade_419, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485,	Alistipes shahii, Bacteroides ovatus, Bacteroides salanitronis, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides ovatus, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

						clade_494, clade_500		
N386	CDAD	8	10	70	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis
N387	CDAD	8	10	80	90	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500,	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

						(clade_553 or clade_553i)		
N388	CDAD	7	10	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques
N389	CDAD	8	10	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i),	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus catus, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

						clade_500		
N390	CDAD	8	10	70	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_521	Alistipes putredinis, Alistipes shahii, Bilophila wadsworthia, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bilophila wadsworthia, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N391	CDAD	8	10	70	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i),	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

						clade_500		
N392	CDAD	7	10	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N393	CDAD	7	10	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N394	CDAD	7	10	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N395	CDAD	8	10	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N396	CDAD	8	10	70	90	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_65 or clade_65e)	Alistipes putredinis, Alistipes shahii, Bacteroides fragilis, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N397	CDAD	8	10	70	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides ovatus, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques

N398	CDAD,T2D	8	10	70	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_519, clade_567	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Subdoligranulum variabile, Victivallis vadensis	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Subdoligranulum variabile, Victivallis vadensis
N399	CDAD,T2D	10	10	70	90	clade_110, (clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes shahii, Bacteroides uniformis, Bifidobacterium catenulatum, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides uniformis, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N400	CDAD	10	10	100	50	clade_253, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_351 or clade_351e), (clade_354 or clade_354e), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i)	Clostridium celatum, Clostridium glycolicum, Clostridium innocuum, Clostridium ramosum, Clostridium symbiosum, Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N401	CDAD	8	10	60	70	(clade_262 or clade_262i), (clade_335 or clade_335i), (clade_38 or clade_38e or clade_38i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i),	Bacteroides ovatus, Bacteroides sp. 1_1_30, Bacteroides sp. 20_3, Bilophila wadsworthia, Clostridium leptum, Eubacterium desmolans, Lachnospiraceae bacterium 3_1_57FAA, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus torques	Bacteroides ovatus, Bilophila wadsworthia, Clostridium leptum, Roseburia inulinivorans, Ruminococcus lactaris, Ruminococcus torques

						clade_521, clade_537, (clade_572 or clade_572i)		
N402	CDAD	8	10	70	100	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides merdae, Ruminococcus obeum, Ruminococcus torques
N403	CDAD	7	10	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_65 or clade_65e)	Alistipes shahii, Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

N404	CDAD,T2D	8	10	70	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_519, clade_567	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Subdoligranulum variabile, Victivallis vadensis	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Subdoligranulum variabile, Victivallis vadensis
N405	CDAD,T2D	7	10	70	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Bacteroides coprocola, Bacteroides vulgatus, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N406	CDAD,T2D	10	10	70	90	clade_110, (clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes shahii, Bacteroides uniformis, Bifidobacterium catenulatum, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides uniformis, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N407	CDAD,T2D	9	10	80	90	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes shahii, Clostridium methylpentosum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques

N408	CDAD,T2D	9	10	60	90	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), clade_556	Acidaminococcus fermentans, Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N409	CDAD,T2D	9	10	80	90	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes shahii, Clostridium methylpentosum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques

N410	CDAD,T2D	9	10	60	90	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), clade_556	Acidaminococcus fermentans, Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N411	CDAD,T2D	7	10	70	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Bacteroides coprocola, Bacteroides vulgatus, Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Desulfovibrio desulfuricans, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N412	CDAD	6	9	88.9	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N413	CDAD,T2D	7	9	77.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N414	CDAD	7	9	88.9	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i),	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques

						(clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), (clade_522 or clade_522i)		
N415	CDAD	7	9	77.8	100	clade_170, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_65 or clade_65e)	Bacteroides caccae, Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides caccae, Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N416	CDAD,T2D	8	9	66.7	77.8	(clade_262 or clade_262i), clade_281, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or clade_444i), clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Eubacterium hallii, Eubacterium rectale, Porphyromonas asaccharolytica, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Eubacterium hallii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N417	CDAD,T2D	7	9	77.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N418	CDAD,T2D	8	9	66.7	77.8	(clade_262 or clade_262i), clade_281, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or clade_444i), clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Eubacterium hallii, Eubacterium rectale, Porphyromonas asaccharolytica, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Eubacterium hallii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N419	CDAD,T2D	6	9	44.4	77.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_494, clade_500, (clade_98 or	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Streptococcus salivarius, Streptococcus vestibularis	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis

						clade_98i)		
N420	CDAD,T2D	8	9	66.7	88.9	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_478 or clade_478i), clade_485, clade_494	Bifidobacterium catenulatum, Bifidobacterium longum, Desulfovibrio desulfuricans, Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Bifidobacterium longum, Desulfovibrio desulfuricans, Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N421	CDAD,T2D	8	9	66.7	88.9	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_419, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Bacteroides salanitronis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans, Subdoligranulum variabile

N422	CDAD,T2D	7	9	66.7	100	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Eubacterium rectale, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Eubacterium rectale, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N423	CDAD,T2D	7	9	77.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_538	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N424	CDAD,T2D	6	9	44.4	77.8	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_494, clade_500, (clade_98 or	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis, Streptococcus salivarius, Streptococcus vestibularis	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis

						clade_98i)		
N425	CDAD,T2D	8	9	66.7	88.9	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_478 or clade_478i), clade_485, clade_494	Bifidobacterium catenulatum, Bifidobacterium longum, Desulfovibrio desulfuricans, Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Bifidobacterium longum, Desulfovibrio desulfuricans, Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N426	CDAD,T2D	7	9	66.7	100	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Eubacterium rectale, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Eubacterium rectale, Parabacteroides johnsonii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

N427	CDAD,T2D	7	9	77.8	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_538	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N428	CDAD,T2D	8	9	66.7	88.9	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_419, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Bacteroides salanitronis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans, Subdoligranulum variabile
N429	CDAD,T2D	7	8	75	87.5	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_504, clade_519, (clade_522 or clade_522i)	Bacteroides xylanisolvens, Campylobacter hominis, Dorea formicigenerans, Eubacterium eligens, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile	Bacteroides xylanisolvens, Dorea formicigenerans, Eubacterium eligens, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile

N430	CDAD	7	8	100	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_519, (clade_522 or clade_522i)	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia faecalis, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N431	CDAD	7	8	87.5	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_519	Bacteroides ovatus, Dorea longicatena, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N432	CDAD,T2D	6	8	87.5	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or	Clostridium leptum, Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Streptococcus sanguinis	Clostridium leptum, Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

						clade_444i), (clade_478 or clade_478i), clade_537, (clade_98 or clade_98i)		
N433	CDAD	6	8	87.5	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_65 or clade_65e)	Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides thetaiotaomicron, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N434	CDAD	7	8	87.5	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_522 or clade_522i)	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

N435	CDAD	6	8	87.5	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques
N436	CDAD	5	8	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i)	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N437	CDAD	5	8	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or	Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						clade_478i)		
N438	CDAD,T2D	7	8	50	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_494, clade_500, clade_504	Alistipes putredinis, Alistipes shahii, Campylobacter hominis, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N439	CDAD,T2D	8	8	75	87.5	(clade_262 or clade_262i), clade_293, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes shahii, Bifidobacterium bifidum, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N440	CDAD,T2D	8	8	87.5	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_537	Clostridium leptum, Clostridium symbiosum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Clostridium leptum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N441	CDAD	7	8	100	25	clade_252, clade_253, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_351 or clade_351e), (clade_354 or clade_354e), (clade_360 or clade_360c or clade_360g or clade_360h clade_360i)	Blautia producta, Clostridium butyricum, Clostridium celatum, Clostridium glycolicum, Clostridium innocuum, Dorea formicigenerans, Eubacterium tenue, Ruminococcus torques	Dorea formicigenerans, Ruminococcus torques
N442	CDAD,T2D	6	8	87.5	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i),	Clostridium leptum, Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Streptococcus sanguinis	Clostridium leptum, Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

						(clade_478 or clade_478i), clade_537, (clade_98 or clade_98i)		
N443	CDAD,T2D	7	8	75	87.5	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_504, clade_519, (clade_522 or clade_522i)	Bacteroides xylanisolvens, Campylobacter hominis, Dorea formicigenerans, Eubacterium eligens, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile	Bacteroides xylanisolvens, Dorea formicigenerans, Eubacterium eligens, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile
N444	CDAD,T2D	7	8	50	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_494, clade_500, clade_504	Alistipes putredinis, Alistipes shahii, Campylobacter hominis, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N445	CDAD,T2D	8	8	75	87.5	(clade_262 or clade_262i), clade_293, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or	Alistipes shahii, Bifidobacterium bifidum, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

						clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500		
N446	CDAD,T2D	7	8	50	87.5	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_396, (clade_444 or clade_444i), clade_466, clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides coprocola, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans
N447	CDAD,T2D	7	8	87.5	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_506	Dialister invisus, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

N448	CDAD,T2D	7	8	75	100	(clade_262 or clade_262i), clade_286, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides johnsonii, Roseburia inulinivorans, Subdoligranulum variabile	Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides johnsonii, Roseburia inulinivorans, Subdoligranulum variabile
N449	CDAD,T2D	8	8	87.5	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_537	Clostridium leptum, Clostridium symbiosum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Clostridium leptum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N450	CDAD,T2D	7	8	75	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i),	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

						clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)		
N451	CDAD,T2D	6	8	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N452	CDAD,T2D	7	8	50	87.5	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), clade_396, (clade_444 or clade_444i), clade_466, clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides coprocola, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans

N453	CDAD,T2D	7	8	87.5	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_506	Dialister invisus, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N454	CDAD,T2D	7	8	75	87.5	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Anaerotruncus colihominis, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N455	CDAD,T2D	6	8	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

						clade_360g or clade_360h or clade_360i), clade_396, (clade_478 or clade_478i), clade_500		
N456	CDAD,T2D	7	8	75	100	(clade_262 or clade_262i), clade_286, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides johnsonii, Roseburia inulinivorans, Subdoligranulum variabile	Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Parabacteroides johnsonii, Roseburia inulinivorans, Subdoligranulum variabile
N457	CDAD	6	7	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_522 or clade_522i)	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

N458	CDAD	7	7	85.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_522 or clade_522i)	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N459	CDAD	6	7	85.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bacteroides ovatus, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

N460	CDAD	6	7	85.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), clade_466, (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques
N461	CDAD	5	7	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N462	CDAD,T2D	5	7	71.4	85.7	(clade_262 or clade_262i), clade_281, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Faecalibacterium prausnitzii, Porphyromonas asaccharolytica, Subdoligranulum variabile	Bacteroides xylanisolvens, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Faecalibacterium prausnitzii, Subdoligranulum variabile

N463	CDAD	6	7	85.7	85.7	clade_170, clade_246, (clade_262 or clade_262i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_576	Bacteroides finegoldii, Clostridiales sp. SS3/4, Clostridium lactatifermentans, Faecalibacterium prausnitzii, Gemmiger formicilis, Roseburia inulinivorans, Ruminococcus torques	Bacteroides finegoldii, Clostridium lactatifermentans, Faecalibacterium prausnitzii, Gemmiger formicilis, Roseburia inulinivorans, Ruminococcus torques
N464	CDAD	7	7	100	28.6	clade_252, clade_253, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_351 or clade_351e), (clade_354 or clade_354e), (clade_360 or clade_360c or clade_360g or clade_360h clade_360i)	Blautia producta, Clostridium butyricum, Clostridium celatum, Clostridium glycolicum, Clostridium innocuum, Dorea formicigenerans, Ruminococcus torques	Dorea formicigenerans, Ruminococcus torques
N465	CDAD,T2D	6	7	71.4	85.7	clade_168, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Prevotella copri, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N466	CDAD,T2D	6	7	71.4	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i),	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Solobacterium moorei	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

						(clade_38 or clade_38e or clade_38i)8, (clade_444 or clade_444i), clade_494, clade_500		
N467	CDAD,T2D	5	7	71.4	85.7	(clade_262 or clade_262i), clade_281, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Faecalibacterium prausnitzii, Porphyromonas asaccharolytica, Subdoligranulum variabile	Bacteroides xylanisolvens, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Faecalibacterium prausnitzii, Subdoligranulum variabile
N468	CDAD,T2D	6	7	42.9	85.7	(clade_262 or clade_262i), clade_293, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_466, clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium bifidum, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus
N469	CDAD,T2D	6	7	42.9	85.7	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_466, clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium catenulatum, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus

N470	CDAD,T2D	6	7	71.4	85.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_485, (clade_98 or clade_98i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Ruminococcus lactaris, Streptococcus vestibularis, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Ruminococcus lactaris, Subdoligranulum variabile
N471	CDAD,T2D	6	7	57.1	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus mitis	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N472	CDAD,T2D	6	7	71.4	85.7	clade_168, (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_500	Alistipes putredinis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Prevotella copri, Roseburia inulinivorans	Alistipes putredinis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Roseburia inulinivorans
N473	CDAD,T2D	7	7	57.1	85.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_432, (clade_444 or clade_444i),	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans, Sutterella wadsworthensis	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans

						clade_466, clade_500		
N474	CDAD,T2D	7	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_566 or clade_566f)	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N475	CDAD,T2D	6	7	85.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_85	Bacteroides cellulosilyticus, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile	Bacteroides cellulosilyticus, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile
N476	CDAD,T2D	6	7	42.9	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides cellulosilyticus, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides cellulosilyticus, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques

N477	CDAD,T2D	6	7	57.1	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus thermophilus	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N478	CDAD,T2D	6	7	57.1	100	clade_170, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides caccae, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides caccae, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N479	CDAD,T2D	6	7	57.1	100	clade_110, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides uniformis, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides uniformis, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N480	CDAD,T2D	6	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500, clade_537	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques
N481	CDAD,T2D	6	7	71.4	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N482	CDAD,T2D	6	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i),	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

						clade_500, (clade_522 or clade_522i)		
N483	CDAD,T2D	6	7	57.1	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques
N484	CDAD,T2D	6	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N485	CDAD,T2D	6	7	71.4	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i)8, (clade_444 or clade_444i), clade_494, clade_500	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Solobacterium moorei	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N486	CDAD,T2D	6	7	42.9	85.7	(clade_262 or clade_262i), clade_293, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_466, clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium bifidum, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus
N487	CDAD,T2D	6	7	42.9	85.7	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_466, clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium catenulatum, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus
N488	CDAD	6	7	85.7	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or clade_444i), (clade_478 or	Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Streptococcus infantis	Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

						clade_478i), (clade_98 or clade_98i)		
N489	CDAD,T2D	6	7	57.1	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus mitis	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N490	CDAD	6	7	85.7	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Streptococcus australis	Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N491	CDAD,T2D	7	7	57.1	85.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_432, (clade_444 or clade_444i), clade_466, clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans, Sutterella wadsworthensis	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Odoribacter splanchnicus, Roseburia inulinivorans

N492	CDAD,T2D	6	7	71.4	85.7	clade_168, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Prevotella copri, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N493	CDAD	7	7	100	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_354 or clade_354e), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494	Clostridium bartlettii, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N494	CDAD,T2D	7	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_566 or clade_566f)	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N495	CDAD,T2D	6	7	42.9	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides cellulosilyticus, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides cellulosilyticus, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N496	CDAD,T2D	6	7	57.1	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques, Streptococcus thermophilus	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N497	CDAD	6	7	42.9	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Bacteroides dorei, Bacteroides ovatus, Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Bacteroides dorei, Bacteroides ovatus, Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum

N498	CDAD,T2D	6	7	57.1	100	clade_170, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides caccae, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides caccae, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N499	CDAD,T2D	6	7	57.1	100	clade_110, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides uniformis, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Bacteroides uniformis, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N500	CDAD,T2D	6	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500,	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus obeum, Ruminococcus torques

						clade_537		
N501	CDAD,T2D	6	7	71.4	85.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i)	Alistipes putredinis, Alistipes shahii, Collinsella aerofaciens, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N502	CDAD,T2D	6	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N503	CDAD,T2D	6	7	57.1	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus obeum, Ruminococcus torques
N504	CDAD,T2D	6	7	71.4	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N505	CDAD,T2D	6	7	71.4	85.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_485, (clade_98 or clade_98i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Ruminococcus lactaris, Streptococcus vestibularis, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Odoribacter splanchnicus, Ruminococcus lactaris, Subdoligranulum variabile

N506	CDAD,T2D	6	7	71.4	85.7	clade_168, (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_500	Alistipes putredinis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Prevotella copri, Roseburia inulinivorans	Alistipes putredinis, Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Roseburia inulinivorans
N507	CDAD,T2D	6	7	85.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_85	Bacteroides cellulosilyticus, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile	Bacteroides cellulosilyticus, Coprococcus comes, Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile
N508	CDAD	5	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

N509	CDAD	6	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_519	Dorea longicatena, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N510	CDAD	6	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_522 or clade_522i)	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N511	CDAD	4	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques

N512	CDAD	4	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i)	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N513	CDAD	5	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N514	CDAD	6	6	50	50	(clade_378 or clade_378e), clade_393, (clade_420 or clade_420f), (clade_444 or clade_444i), (clade_522 or clade_522i), clade_558	Bacteroides sp. 3_1_40A, Clostridiales bacterium oral clone P4PA, Coprococcus catus, Eubacterium eligens, Eubacterium rectale, Tannerella sp. 6_1_58FAA_CT1	Coprococcus catus, Eubacterium eligens, Eubacterium rectale
N515	CDAD	6	6	50	66.7	(clade_378 or clade_378e), clade_393, (clade_444 or clade_444i), clade_521, (clade_522 or clade_522i),	Bacteroides sp. 3_1_40A, Bilophila wadsworthia, Citrobacter sp. 30_2, Coprococcus catus, Eubacterium eligens, Eubacterium rectale	Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

						(clade_92 or clade_92e or clade_92i)		
N516	CDAD	6	6	50	83.3	(clade_378 or clade_378e), clade_393, (clade_444 or clade_444i), clade_445, clade_521, (clade_522 or clade_522i)	Bacteroides sp. 3_1_40A, Bilophila wadsworthia, Coprococcus catus, Desulfovibrio desulfuricans, Eubacterium eligens, Eubacterium rectale	Bilophila wadsworthia, Coprococcus catus, Desulfovibrio desulfuricans, Eubacterium eligens, Eubacterium rectale
N517	CDAD	6	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes shahii, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N518	CDAD,T2D	6	6	100	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_354 or clade_354e), clade_396, (clade_444 or clade_444i), clade_494	Clostridium bartlettii, Eubacterium hallii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium hallii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N519	CDAD,T2D	6	6	83.3	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_401, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494	Eubacterium rectale, Faecalibacterium prausnitzii, Lactococcus lactis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N520	CDAD,T2D	6	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_494, clade_537, (clade_566 or clade_566f)	Clostridium leptum, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Clostridium leptum, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N521	CDAD,T2D	6	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_393, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Coprococcus catus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Coprococcus catus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N522	T2D	6	6	66.7	50	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_466, clade_494,	Clostridium symbiosum, Clostridium thermocellum, Eubacterium siraeum, Heliobacterium modesticaldum, Odoribacter splanchnicus,	Eubacterium siraeum, Odoribacter splanchnicus, Pseudoflavonifractor capillosus

						clade_495, clade_538, clade_560	Pseudoflavonifractor capillosus	
N523	CDAD,T2D	5	6	83.3	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_432, (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Sutterella wadsworthensis	Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N524	CDAD,T2D	5	6	83.3	83.3	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i)8, clade_466, (clade_478 or clade_478i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus lactaris, Solobacterium moorei, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus lactaris, Subdoligranulum variabile
N525	CDAD,T2D	5	6	83.3	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_445, clade_494	Anaerostipes caccae, Clostridium bolteae, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N526	CDAD,T2D	5	6	83.3	83.3	(clade_262 or clade_262i), clade_271, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i)	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans, Rothia mucilaginosa	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans
N527	CDAD,T2D	5	6	50	83.3	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium pseudocatenulatum, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii
N528	CDAD,T2D	6	6	66.7	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_445, clade_494, clade_500	Alistipes shahii, Clostridium symbiosum, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N529	CDAD,T2D	6	6	83.3	83.3	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or	Bifidobacterium pseudocatenulatum, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

						clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_494		
N530	CDAD,T2D	5	6	50	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus australis	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N531	CDAD,T2D	6	6	83.3	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_401, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494	Eubacterium rectale, Faecalibacterium prausnitzii, Lactococcus lactis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N532	CDAD,T2D	6	6	100	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_354 or clade_354e), clade_396, (clade_444 or	Clostridium bartlettii, Eubacterium hallii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium hallii, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

						clade_444i), clade_494		
N533	CDAD,T2D	5	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_494	Clostridium nexile, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Clostridium nexile, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N534	CDAD,T2D	6	6	66.7	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_65 or clade_65e)	Alistipes shahii, Bacteroides fragilis, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N535	CDAD,T2D	5	6	83.3	100	clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella atypica	Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella atypica

N536	CDAD,T2D	5	6	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i), clade_500	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Veillonella atypica	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Veillonella atypica
N537	CDAD,T2D	6	6	83.3	83.3	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_494	Clostridium scindens, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N538	CDAD,T2D	6	6	66.7	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_445, clade_494, clade_500	Alistipes shahii, Clostridium hathewayi, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N539	CDAD,T2D	5	6	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i), clade_500	Alistipes shahii, Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Veillonella parvula	Alistipes shahii, Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Veillonella parvula
N540	CDAD,T2D	6	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_494, clade_537, (clade_566 or clade_566f)	Clostridium leptum, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Clostridium leptum, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N541	CDAD,T2D	6	6	100	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_494	Clostridium asparagiforme, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N542	CDAD,T2D	5	6	50	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500, clade_88	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Haemophilus parainfluenzae, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N543	CDAD,T2D	6	6	100	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_354 or clade_354e), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_494	Clostridium bartlettii, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N544	CDAD,T2D	6	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_393, (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Coprococcus catus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques	Coprococcus catus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus obeum, Ruminococcus torques
N545	CDAD,T2D	5	6	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium siraeum, Roseburia intestinalis, Roseburia inulinivorans	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium siraeum, Roseburia intestinalis, Roseburia

						clade_360i), clade_396, (clade_444 or clade_444i), clade_538		inulinivorans
N546	CDAD,T2D	5	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques
N547	CDAD,T2D	5	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile
N548	CDAD	6	6	66.7	83.3	(clade_262 or clade_262i), clade_299, (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494	Bacteroides xylanisolvens, Eubacterium rectale, Faecalibacterium prausnitzii, Propionibacterium acnes, Pseudoflavonifractor capillosus, Ruminococcus torques	Bacteroides xylanisolvens, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques

N549	CDAD,T2D	5	6	83.3	83.3	(clade_262 or clade_262i), clade_271, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i)	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans, Rothia mucilaginosa	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans
N550	CDAD,T2D	5	6	50	83.3	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_500	Alistipes putredinis, Alistipes shahii, Bifidobacterium pseudocatenulatum, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Alistipes putredinis, Alistipes shahii, Coprococcus comes, Dorea longicatena, Eubacterium hallii
N551	CDAD,T2D	6	6	83.3	83.3	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_494	Bifidobacterium pseudocatenulatum, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N552	CDAD,T2D	5	6	50	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i),	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus australis	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques

						clade_500, (clade_98 or clade_98i)		
N553	CDAD,T2D	5	6	83.3	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_432, (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Sutterella wadsworthensis	Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N554	CDAD,T2D	5	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_494	Clostridium nexile, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Clostridium nexile, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N555	CDAD,T2D	6	6	66.7	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_65 or clade_65e)	Alistipes shahii, Bacteroides fragilis, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N556	CDAD,T2D	5	6	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i), clade_500	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Veillonella atypica	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Veillonella atypica
N557	CDAD,T2D	6	6	83.3	83.3	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, clade_494	Clostridium scindens, Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N558	CDAD,T2D	5	6	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i), clade_500	Alistipes shahii, Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Veillonella parvula	Alistipes shahii, Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques, Veillonella parvula

N559	CDAD,T2D	6	6	100	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_494	Clostridium asparagiforme, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N560	CDAD,T2D	5	6	50	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500, clade_88	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Haemophilus parainfluenzae, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N561	CDAD,T2D	6	6	100	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_354 or clade_354e), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or	Clostridium bartlettii, Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

						clade_478i), clade_494		
N562	CDAD,T2D	5	6	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), clade_538	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium siraeum, Roseburia intestinalis, Roseburia inulinivorans	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium siraeum, Roseburia intestinalis, Roseburia inulinivorans
N563	CDAD,T2D	5	6	83.3	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus obeum, Ruminococcus torques
N564	CDAD,T2D	5	6	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i),	Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile	Dorea formicigenerans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Subdoligranulum variabile

						(clade_478 or clade_478i)		
N565	CDAD,T2D	5	6	83.3	83.3	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i)8, clade_466, (clade_478 or clade_478i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus lactaris, Solobacterium moorei, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Ruminococcus lactaris, Subdoligranulum variabile
N566	CDAD,T2D	5	6	83.3	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_445, clade_494	Anaerostipes caccae, Clostridium bolteae, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N567	CDAD,T2D	6	6	66.7	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or	Alistipes shahii, Clostridium symbiosum, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

						clade_408f or clade_408g or clade_408h), clade_445, clade_494, clade_500		
N568	CDAD,T2D	5	6	83.3	100	clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella atypica	Dorea formicigenerans, Eubacterium hallii, Faecalibacterium prausnitzii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella atypica
N569	CDAD,T2D	6	6	66.7	83.3	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_445, clade_494, clade_500	Alistipes shahii, Clostridium hathewayi, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N570	CDAD,T2D	5	5	80	80	(clade_172 or clade_172i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i),	Bifidobacterium adolescentis, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum

						(clade_444 or clade_444i), (clade_478 or clade_478i)		
N571	CDAD	5	5	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N572	CDAD,T2D	5	5	40	100	clade_286, (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Parabacteroides merdae	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Parabacteroides merdae
N573	CDAD	5	5	60	80	(clade_378 or clade_378e), clade_393, (clade_444 or clade_444i), clade_521, (clade_522 or clade_522i)	Bacteroides sp. 3_1_40A, Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale	Bilophila wadsworthia, Coprococcus catus, Eubacterium eligens, Eubacterium rectale

N574	CDAD,T2D	5	5	80	80	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_478 or clade_478i), clade_537, (clade_98 or clade_98i)	Clostridium leptum, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Streptococcus thermophilus	Clostridium leptum, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum
N575	CDAD,T2D	5	5	60	100	clade_170, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Bacteroides caccae, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Bacteroides caccae, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N576	CDAD,T2D	5	5	40	100	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i), clade_445, (clade_478 or clade_478i), clade_85	Bacteroides eggerthii, Bacteroides ovatus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides eggerthii, Bacteroides ovatus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques
N577	CDAD,T2D	4	5	80	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_537, clade_567	Clostridium leptum, Coprococcus comes, Dorea longicatena, Ruminococcus torques, Victivallis vadensis	Clostridium leptum, Coprococcus comes, Dorea longicatena, Ruminococcus torques, Victivallis vadensis
N578	CDAD,T2D	5	5	60	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i),	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Sutterella wadsworthensis	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

						clade_432, clade_445, (clade_478 or clade_478i)		
N579	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus infantis	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N580	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus australis	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N581	CDAD,T2D	5	5	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_494, clade_538	Eubacterium siraeum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium siraeum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N582	CDAD	5	5	100	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), (clade_478 or clade_478i)	Clostridium symbiosum, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N583	CDAD	5	5	100	0	clade_252, clade_253, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_351 or clade_351e), (clade_354 or clade_354e)	Blautia producta, Clostridium butyricum, Clostridium celatum, Clostridium glycolicum, Clostridium innocuum	
N584	CDAD	5	5	100	20	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_553 or clade_553i)	Blautia producta, Clostridium symbiosum, Collinsella aerofaciens, Coprococcus comes, Lachnospiraceae bacterium 5_1_57FAA	Coprococcus comes

N585	T2D	3	5	80	80	(clade_444 or clade_444i), (clade_478 or clade_478i), clade_88	Eubacterium rectale, Faecalibacterium prausnitzii, Haemophilus parainfluenzae, Roseburia intestinalis, Roseburia inulinivorans	Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans
N586	CDAD,T2D	3	5	100	80	clade_368, (clade_444 or clade_444i), (clade_478 or clade_478i)	Blautia hydrogenotrophica, Eubacterium rectale, Roseburia intestinalis, Roseburia inulinivorans, Subdoligranulum variabile	Eubacterium rectale, Roseburia intestinalis, Roseburia inulinivorans, Subdoligranulum variabile
N587	CDAD,T2D	5	5	60	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Clostridium methylpentosum, Dorea formicigenerans, Odoribacter splanchnicus, Subdoligranulum variabile	Alistipes putredinis, Dorea formicigenerans, Odoribacter splanchnicus, Subdoligranulum variabile
N588	CDAD,T2D	5	5	80	60	clade_396, (clade_516 or clade_516c or clade_516g or clade_516h), clade_519, clade_521, clade_538	Bacteroides pectinophilus, Bilophila wadsworthia, Eubacterium siraeum, Eubacterium ventriosum, Ruminococcus albus	Bilophila wadsworthia, Eubacterium siraeum, Eubacterium ventriosum
N589	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), clade_293, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or	Bifidobacterium bifidum, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

						clade_478i)		
N590	CDAD,T2D	4	5	80	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_401, (clade_478 or clade_478i), clade_485	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Lactococcus lactis, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Subdoligranulum variabile
N591	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_401, (clade_444 or clade_444i), clade_494	Eubacterium rectale, Lactococcus lactis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N592	CDAD,T2D	4	5	80	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_537, clade_567	Clostridium leptum, Coprococcus comes, Dorea longicatena, Ruminococcus torques, Victivallis vadensis	Clostridium leptum, Coprococcus comes, Dorea longicatena, Ruminococcus torques, Victivallis vadensis
N593	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus infantis	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques

						clade_444i), (clade_98 or clade_98i)		
N594	CDAD,T2D	4	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, clade_494	Blautia hansenii, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N595	CDAD,T2D	4	5	60	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), (clade_98 or clade_98i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Streptococcus mitis, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Subdoligranulum variabile
N596	CDAD,T2D	5	5	60	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_432, clade_445, (clade_478 or clade_478i)	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques, Sutterella wadsworthensis	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N597	CDAD,T2D	4	5	80	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), (clade_522 or clade_522i), (clade_98 or clade_98i)	Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Streptococcus australis, Subdoligranulum variabile	Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Subdoligranulum variabile

N598	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus australis	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N599	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, clade_494, (clade_566 or clade_566f)	Desulfovibrio desulfuricans, Eggerthella lenta, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N600	CDAD,T2D	5	5	80	80	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_478 or clade_478i), clade_537, (clade_98 or clade_98i)	Clostridium leptum, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum, Streptococcus thermophilus	Clostridium leptum, Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus obeum
N601	CDAD,T2D	5	5	40	100	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i), clade_445, (clade_478 or clade_478i), clade_85	Bacteroides eggerthii, Bacteroides ovatus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides eggerthii, Bacteroides ovatus, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques

N602	CDAD,T2D	5	5	80	100	clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea formicigenerans, Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella parvula	Dorea formicigenerans, Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella parvula
N603	CDAD,T2D	5	5	80	80	(clade_172 or clade_172i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bifidobacterium adolescentis, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N604	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_85	Bacteroides stercoris, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides stercoris, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N605	CDAD,T2D	5	5	60	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_500, clade_85	Alistipes shahii, Bacteroides eggerthii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides eggerthii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N606	CDAD,T2D	5	5	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_494, clade_538	Eubacterium siraeum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium siraeum, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N607	CDAD,T2D	5	5	60	100	clade_170, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Bacteroides caccae, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Bacteroides caccae, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N608	CDAD,T2D	5	5	40	100	clade_286, (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i)	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Parabacteroides merdae	Bacteroides xylanisolvens, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Parabacteroides merdae

N609	CDAD,T2D	5	5	60	100	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500	Alistipes shahii, Eubacterium rectale, Parabacteroides merdae, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Parabacteroides merdae, Ruminococcus obeum, Ruminococcus torques
N610	CDAD,T2D	5	5	60	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500, (clade_98 or clade_98i)	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis
N611	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), (clade_478 or clade_478i)	Bacteroides dorei, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides dorei, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N612	CDAD,T2D	5	5	60	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i),	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus salivarius	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques

						(clade_444 or clade_444i), clade_500, (clade_98 or clade_98i)		
N613	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), (clade_478 or clade_478i)	Bacteroides vulgatus, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N614	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_500, clade_537	Alistipes shahii, Clostridium leptum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Clostridium leptum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N615	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or	Bacteroides xylanisolvens, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides xylanisolvens, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

						clade_38e or clade_38i), (clade_478 or clade_478i)		
N616	CDAD,T2D	4	5	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Faecalibacterium prausnitzii	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Faecalibacterium prausnitzii
N617	CDAD,T2D	4	5	60	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Ruminococcus obeum, Ruminococcus torques
N618	CDAD	5	5	60	80	clade_281, (clade_444 or clade_444i), clade_445, clade_519, clade_537	Clostridium leptum, Desulfovibrio desulfuricans, Eubacterium ventriosum, Porphyromonas asaccharolytica, Roseburia inulinivorans	Clostridium leptum, Desulfovibrio desulfuricans, Eubacterium ventriosum, Roseburia inulinivorans
N619	CDAD,T2D	3	5	100	80	clade_368, (clade_444 or clade_444i), (clade_478 or clade_478i)	Blautia hydrogenotrophica, Eubacterium rectale, Roseburia intestinalis, Roseburia inulinivorans, Subdoligranulum variabile	Eubacterium rectale, Roseburia intestinalis, Roseburia inulinivorans, Subdoligranulum variabile

N620	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), clade_293, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Bifidobacterium bifidum, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N621	CDAD	5	5	100	80	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), (clade_478 or clade_478i)	Anaerostipes caccae, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N622	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_401, (clade_444 or clade_444i), clade_494	Eubacterium rectale, Lactococcus lactis, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N623	CDAD	5	5	60	80	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Veillonella dispar	Bacteroides xylanisolvens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N624	CDAD	5	5	100	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_494	Clostridium hathewayi, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N625	CDAD	5	5	60	80	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Bacteroides xylanisolvens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Streptococcus salivarius	Bacteroides xylanisolvens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum

N626	CDAD	5	5	100	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_494, (clade_566 or clade_566f)	Eggerthella lenta, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N627	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_85	Bacteroides stercoris, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides stercoris, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N628	CDAD,T2D	5	5	60	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_500, clade_85	Alistipes shahii, Bacteroides eggerthii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides eggerthii, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N629	CDAD	5	5	100	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_494	Clostridium asparagiforme, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N630	CDAD,T2D	5	5	60	100	(clade_262 or clade_262i), clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500	Alistipes shahii, Eubacterium rectale, Parabacteroides merdae, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Eubacterium rectale, Parabacteroides merdae, Ruminococcus obeum, Ruminococcus torques
N631	CDAD,T2D	5	5	60	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500, (clade_98 or clade_98i)	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus parasanguinis

N632	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), (clade_478 or clade_478i)	Bacteroides dorei, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides dorei, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N633	CDAD,T2D	5	5	60	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), clade_500, (clade_98 or clade_98i)	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques, Streptococcus salivarius	Alistipes shahii, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N634	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), (clade_478 or clade_478i)	Bacteroides vulgatus, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N635	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_500, clade_537	Alistipes shahii, Clostridium leptum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Clostridium leptum, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N636	CDAD,T2D	5	5	80	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38c or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides xylanisolvens, Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N637	CDAD,T2D	4	5	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_478 or clade_478i)	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Faecalibacterium prausnitzii	Coprococcus comes, Dorea formicigenerans, Dorea longicatena, Eubacterium hallii, Faecalibacterium prausnitzii
N638	CDAD,T2D	4	5	60	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Dorea longicatena, Ruminococcus obeum, Ruminococcus torques

						clade_360g or clade_360h or clade_360i), clade_500		
N639	CDAD,T2D	5	5	60	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Clostridium methylpentosum, Dorea formicigenerans, Odoribacter splanchnicus, Subdoligranulum variabile	Alistipes putredinis, Dorea formicigenerans, Odoribacter splanchnicus, Subdoligranulum variabile
N640	CDAD,T2D	5	5	80	60	clade_396, (clade_516 or clade_516c or clade_516g or clade_516h), clade_519, clade_521, clade_538	Bacteroides pectinophilus, Bilophila wadsworthia, Eubacterium siraeum, Eubacterium ventriosum, Ruminococcus albus	Bilophila wadsworthia, Eubacterium siraeum, Eubacterium ventriosum
N641	CDAD,T2D	4	5	80	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_401, (clade_478 or clade_478i), clade_485	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Lactococcus lactis, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis, Subdoligranulum variabile
N642	CDAD,T2D	4	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, clade_494	Blautia hansenii, Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques

N643	CDAD,T2D	4	5	60	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, (clade_478 or clade_478i), (clade_98 or clade_98i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Streptococcus mitis, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Odoribacter splanchnicus, Subdoligranulum variabile
N644	CDAD,T2D	4	5	80	80	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), (clade_522 or clade_522i), (clade_98 or clade_98i)	Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Streptococcus australis, Subdoligranulum variabile	Dorea formicigenerans, Eubacterium eligens, Faecalibacterium prausnitzii, Subdoligranulum variabile
N645	CDAD,T2D	5	5	80	80	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, clade_494, (clade_566 or clade_566f)	Desulfovibrio desulfuricans, Eggerthella lenta, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N646	CDAD,T2D	5	5	80	100	clade_358, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea formicigenerans, Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella parvula	Dorea formicigenerans, Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile, Veillonella parvula

N647	CDAD	4	4	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N648	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Dorea longicatena, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N649	CDAD,T2D	4	4	50	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_378 or clade_378e), (clade_444 or clade_444i), clade_445	Bacteroides dorei, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum	Bacteroides dorei, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum
N650	CDAD	4	4	75	50	clade_368, (clade_378 or clade_378e), (clade_444 or clade_444i), (clade_522 or clade_522i)	Bacteroides sp. 3_1_40A, Blautia hydrogenotrophica, Eubacterium eligens, Eubacterium rectale	Eubacterium eligens, Eubacterium rectale

N651	CDAD,T2D	4	4	75	50	(clade_172 or clade_172i), (clade_444 or clade_444i), (clade_516 or clade_516c or clade_516g or clade_516h), (clade_522 or clade_522i)	Anaerotruncus colihominis, Bifidobacterium catenulatum, Eubacterium eligens, Roseburia inulinivorans	Eubacterium eligens, Roseburia inulinivorans
N652	CDAD	4	4	75	75	(clade_378 or clade_378e), clade_393, (clade_444 or clade_444i), (clade_522 or clade_522i)	Bacteroides sp. 3_1_40A, Coprococcus catus, Eubacterium eligens, Eubacterium rectale	Coprococcus catus, Eubacterium eligens, Eubacterium rectale
N653	CDAD,T2D	4	4	100	75	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i)	Anaerostipes caccae, Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum
N654	CDAD,T2D	4	4	100	75	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_516 or clade_516c or clade_516g or clade_516h)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus albus, Ruminococcus obeum	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum

N655	CDAD,T2D	3	4	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Subdoligranulum variabile	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Subdoligranulum variabile
N656	CDAD	4	4	75	75	clade_170, (clade_262 or clade_262i), clade_396, clade_537	Bacteroides caccae, Bacteroides pectinophilus, Clostridium leptum, Ruminococcus lactaris	Bacteroides caccae, Clostridium leptum, Ruminococcus lactaris
N657	CDAD,T2D	3	4	25	100	(clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides dorei, Bacteroides ovatus, Bacteroides xylanisolvens, Faecalibacterium prausnitzii	Bacteroides dorei, Bacteroides ovatus, Bacteroides xylanisolvens, Faecalibacterium prausnitzii
N658	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), clade_445, (clade_478 or clade_478i), clade_85	Bacteroides stercoris, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides stercoris, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques
N659	CDAD,T2D	3	4	25	100	clade_171, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Faecalibacterium prausnitzii	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Faecalibacterium prausnitzii
N660	CDAD	3	4	25	75	(clade_172 or clade_172i), clade_466, clade_485	Bifidobacterium longum, Bifidobacterium pseudocatenulatum, Holdemania filiformis, Odoribacter splanchnicus	Bifidobacterium longum, Holdemania filiformis, Odoribacter splanchnicus
N661	CDAD,T2D,UC	4	4	0	100	clade_110, clade_170, clade_567, clade_85	Bacteroides finegoldii, Bacteroides stercoris, Bacteroides uniformis, Victivallis vadensis	Bacteroides finegoldii, Bacteroides stercoris, Bacteroides uniformis, Victivallis vadensis
N662	CDAD,T2D	3	4	50	75	clade_110, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i),	Bacteroides uniformis, Dorea formicigenerans, Dorea longicatena, Sutterella wadsworthensis	Bacteroides uniformis, Dorea formicigenerans, Dorea longicatena

						clade_432		
N663	CDAD,T2D	4	4	50	75	clade_110, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_98 or clade_98i)	Bacteroides uniformis, Dorea longicatena, Roseburia inulinivorans, Streptococcus sanguinis	Bacteroides uniformis, Dorea longicatena, Roseburia inulinivorans
N664	CDAD	4	4	75	75	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e), (clade_516 or clade_516c or clade_516g or clade_516h), clade_519	Bacteroides dorei, Clostridium methylpentosum, Dorea longicatena, Eubacterium ventriosum	Bacteroides dorei, Dorea longicatena, Eubacterium ventriosum
N665	CDAD,T2D	3	4	50	100	clade_286, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i)	Dorea formicigenerans, Parabacteroides johnsonii, Parabacteroides merdae, Roseburia intestinalis	Dorea formicigenerans, Parabacteroides johnsonii, Parabacteroides merdae, Roseburia intestinalis
N666	CDAD	4	4	75	75	(clade_444 or clade_444i), clade_476, clade_521, (clade_522 or clade_522i)	Bilophila wadsworthia, Eubacterium eligens, Eubacterium nodatum, Eubacterium rectale	Bilophila wadsworthia, Eubacterium eligens, Eubacterium rectale
N667	CDAD,T2D	3	4	25	100	clade_500, (clade_522 or clade_522i), clade_567	Alistipes putredinis, Alistipes shahii, Eubacterium eligens, Victivallis vadensis	Alistipes putredinis, Alistipes shahii, Eubacterium eligens, Victivallis vadensis
N668	CDAD,T2D	3	4	0	100	clade_286, (clade_378 or clade_378e), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides dorei, Parabacteroides johnsonii	Alistipes putredinis, Alistipes shahii, Bacteroides dorei, Parabacteroides johnsonii

N669	CDAD	4	4	50	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, clade_85	Bacteroides eggerthii, Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus	Bacteroides eggerthii, Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus
N670	CDAD	4	4	50	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466, clade_85	Bacteroides stercoris, Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus	Bacteroides stercoris, Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus
N671	CDAD	4	4	50	100	clade_170, (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466	Bacteroides finegoldii, Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus	Bacteroides finegoldii, Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus
N672	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_299, (clade_444 or clade_444i), clade_494	Eubacterium rectale, Propionibacterium acnes, Pseudoflavonifractor capillosus, Ruminococcus torques	Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus torques
N673	CDAD,T2D	3	4	100	100	(clade_262 or clade_262i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus torques	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus torques
N674	CDAD,T2D	4	4	75	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, (clade_478 or clade_478i), clade_519	Desulfovibrio desulfuricans, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus obeum	Desulfovibrio desulfuricans, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus obeum

N675	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i), (clade_65 or clade_65e)	Bacteroides ovatus, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides ovatus, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus torques
N676	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_494, (clade_566 or clade_566f)	Eggerthella lenta, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N677	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Clostridium hathewayi, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N678	CDAD,T2D	4	4	50	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, (clade_478 or clade_478i), clade_583	Akkermansia muciniphila, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum	Akkermansia muciniphila, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum

N679	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Clostridium asparagiforme, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N680	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_368, (clade_478 or clade_478i)	Blautia hydrogenotrophica, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N681	CDAD,T2D	3	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i)	Coprococcus comes, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N682	CDAD	4	4	75	100	clade_170, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bacteroides caccae, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Bacteroides caccae, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum

N683	CDAD	4	4	50	100	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i), clade_85	Bacteroides eggerthii, Bacteroides ovatus, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides eggerthii, Bacteroides ovatus, Faecalibacterium prausnitzii, Ruminococcus torques
N684	CDAD,T2D,UC	4	4	0	100	clade_110, clade_170, clade_567, clade_85	Bacteroides finegoldii, Bacteroides stercoris, Bacteroides uniformis, Victivallis vadensis	Bacteroides finegoldii, Bacteroides stercoris, Bacteroides uniformis, Victivallis vadensis
N685	Obesity	4	4	25	100	clade_170, clade_286, clade_469, (clade_65 or clade_65e)	Bacteroides caccae, Bacteroides thetaiotaomicron, Catenibacterium mitsuokai, Parabacteroides merdae	Bacteroides caccae, Bacteroides thetaiotaomicron, Catenibacterium mitsuokai, Parabacteroides merdae
N686	Obesity	4	4	25	100	(clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), clade_445, clade_485	Bacteroides dorei, Bacteroides ovatus, Desulfovibrio piger, Holdemania filiformis	Bacteroides dorei, Bacteroides ovatus, Desulfovibrio piger, Holdemania filiformis
N687	T2D	4	4	100	50	clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_543	Bacteroides pectinophilus, Coprococcus eutactus, Faecalibacterium prausnitzii, Roseburia intestinalis	Faecalibacterium prausnitzii, Roseburia intestinalis
N688	CDAD,T2D	4	4	75	50	(clade_172 or clade_172i), (clade_444 or clade_444i), (clade_516 or clade_516c or clade_516g or clade_516h), (clade_522 or clade_522i)	Anaerotruncus colihominis, Bifidobacterium catenulatum, Eubacterium eligens, Roseburia inulinivorans	Eubacterium eligens, Roseburia inulinivorans
N689	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_516 or clade_516c or clade_516g or clade_516h)	Clostridium methylpentosum, Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans

N690	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i)8, clade_396, (clade_444 or clade_444i)	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans, Solobacterium moorei	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N691	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_299, (clade_444 or clade_444i), clade_494	Eubacterium rectale, Propionibacterium acnes, Pseudoflavonifractor capillosus, Ruminococcus torques	Eubacterium rectale, Pseudoflavonifractor capillosus, Ruminococcus torques
N692	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_556	Acidaminococcus fermentans, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Coprococcus comes, Dorea longicatena, Eubacterium hallii
N693	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_396, clade_401, (clade_444 or clade_444i)	Coprococcus comes, Eubacterium hallii, Lactococcus lactis, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N694	CDAD,T2D	4	4	100	75	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i)	Anaerostipes caccae, Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum	Eubacterium hallii, Eubacterium rectale, Ruminococcus obeum
N695	CDAD,T2D	4	4	50	25	(clade_378 or clade_378e), clade_396, (clade_516 or clade_516c or clade_516g or	Bacteroides vulgatus, Eubacterium hallii, Methanobrevibacter smithii, Ruminococcus albus	Eubacterium hallii

						clade_516h), clade_588		
N696	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans, Streptococcus vestibularis	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N697	CDAD,T2D,UC	4	4	0	100	clade_110, clade_170, clade_567, clade_85	Bacteroides finegoldii, Bacteroides stercoris, Bacteroides uniformis, Victivallis vadensis	Bacteroides finegoldii, Bacteroides stercoris, Bacteroides uniformis, Victivallis vadensis
N698	CDAD,T2D	3	4	75	75	(clade_172 or clade_172i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bifidobacterium catenulatum, Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile	Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile
N699	CDAD,T2D	3	4	25	100	clade_500, (clade_522 or clade_522i), clade_567	Alistipes putredinis, Alistipes shahii, Eubacterium eligens, Victivallis vadensis	Alistipes putredinis, Alistipes shahii, Eubacterium eligens, Victivallis vadensis
N700	CDAD,T2D	4	4	50	75	clade_110, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_98 or clade_98i)	Bacteroides uniformis, Dorea longicatena, Roseburia inulinivorans, Streptococcus sanguinis	Bacteroides uniformis, Dorea longicatena, Roseburia inulinivorans
N701	CDAD,T2D	3	4	75	75	(clade_172 or clade_172i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bifidobacterium pseudocatenulatum, Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile	Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile
N702	CDAD,T2D	3	4	0	100	clade_286, (clade_378 or clade_378e), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides dorei, Parabacteroides johnsonii	Alistipes putredinis, Alistipes shahii, Bacteroides dorei, Parabacteroides johnsonii

N703	CDAD,T2D	4	4	100	75	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_485	Blautia hansenii, Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis
N704	CDAD,T2D	3	4	50	100	clade_286, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i)	Dorea formicigenerans, Parabacteroides johnsonii, Parabacteroides merdae, Roseburia intestinalis	Dorea formicigenerans, Parabacteroides johnsonii, Parabacteroides merdae, Roseburia intestinalis
N705	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans, Streptococcus mitis	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N706	CDAD,T2D	3	4	50	75	clade_110, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_432	Bacteroides uniformis, Dorea formicigenerans, Dorea longicatena, Sutterella wadsworthensis	Bacteroides uniformis, Dorea formicigenerans, Dorea longicatena
N707	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_368, (clade_478 or clade_478i)	Blautia hydrogenotrophica, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N708	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Clostridium hathewayi, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N709	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_566 or clade_566f)	Coprococcus comes, Eubacterium hallii, Gordonibacter pamelaeae, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Gordonibacter pamelaeae, Roseburia inulinivorans
N710	CDAD,T2D	4	4	50	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500, (clade_566 or clade_566f)	Alistipes putredinis, Dorea formicigenerans, Gordonibacter pamelaeae, Subdoligranulum variabile	Alistipes putredinis, Dorea formicigenerans, Gordonibacter pamelaeae, Subdoligranulum variabile
N711	CDAD,T2D	3	4	25	100	clade_171, (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Faecalibacterium prausnitzii	Alistipes putredinis, Alistipes shahii, Bacteroides intestinalis, Faecalibacterium prausnitzii
N712	CDAD,T2D	3	4	100	75	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Clostridium scindens, Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile

N713	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_494, (clade_566 or clade_566f)	Eggerthella lenta, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N714	CDAD,T2D	3	4	75	75	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Streptococcus thermophilus, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile
N715	CDAD,T2D	4	4	50	100	clade_170, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500	Alistipes shahii, Bacteroides finegoldii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides finegoldii, Ruminococcus obeum, Ruminococcus torques
N716	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Clostridium bolteae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N717	CDAD,T2D	3	4	75	75	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i),	Dorea formicigenerans, Faecalibacterium prausnitzii, Haemophilus parainfluenzae,	Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile

						(clade_478 or clade_478i), clade_88	Subdoligranulum variabile	
N718	CDAD,T2D	4	4	50	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, (clade_478 or clade_478i), clade_583	Akkermansia muciniphila, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum	Akkermansia muciniphila, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus obeum
N719	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Clostridium asparagiforme, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N720	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), clade_445, (clade_478 or clade_478i), clade_85	Bacteroides stercoris, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides stercoris, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Ruminococcus torques
N721	CDAD,T2D	3	4	25	100	(clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides dorei, Bacteroides ovatus, Bacteroides xylanisolvens, Faecalibacterium prausnitzii	Bacteroides dorei, Bacteroides ovatus, Bacteroides xylanisolvens, Faecalibacterium prausnitzii

N722	CDAD,T2D	4	4	100	75	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_516 or clade_516c or clade_516g or clade_516h)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus albus, Ruminococcus obeum	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N723	CDAD,T2D	4	4	50	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_378 or clade_378e), (clade_444 or clade_444i), clade_445	Bacteroides dorei, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum	Bacteroides dorei, Desulfovibrio desulfuricans, Eubacterium rectale, Ruminococcus obeum
N724	CDAD,T2D	3	4	100	100	(clade_262 or clade_262i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus torques	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus lactaris, Ruminococcus torques
N725	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_65 or clade_65e)	Bacteroides thetaiotaomicron, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Bacteroides thetaiotaomicron, Coprococcus comes, Dorea longicatena, Eubacterium hallii
N726	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i), (clade_65 or clade_65e)	Bacteroides ovatus, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides ovatus, Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus torques

N727	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), (clade_65 or clade_65e)	Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N728	CDAD,T2D	4	4	75	75	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396	Bifidobacterium adolescentis, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Coprococcus comes, Dorea longicatena, Eubacterium hallii
N729	CDAD,T2D	4	4	75	75	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i)	Bifidobacterium adolescentis, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N730	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), clade_286, clade_396, (clade_444 or clade_444i)	Coprococcus comes, Eubacterium hallii, Parabacteroides merdae, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Parabacteroides merdae, Roseburia inulinivorans
N731	CDAD,T2D	4	4	75	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, (clade_478 or clade_478i), clade_519	Desulfovibrio desulfuricans, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus obeum	Desulfovibrio desulfuricans, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus obeum

N732	CDAD,T2D	3	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i)	Coprococcus comes, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Coprococcus comes, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N733	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), clade_500	Alistipes shahii, Bacteroides ovatus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides ovatus, Ruminococcus obeum, Ruminococcus torques
N734	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500, clade_521	Alistipes shahii, Bilophila wadsworthia, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bilophila wadsworthia, Ruminococcus obeum, Ruminococcus torques
N735	CDAD,T2D	3	4	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Subdoligranulum variabile	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Subdoligranulum variabile

N736	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Dorea longicatena, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea longicatena, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N737	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_393, (clade_444 or clade_444i)	Coprococcus catus, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques	Coprococcus catus, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N738	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_485	Faecalibacterium prausnitzii, Holdemania filiformis, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Holdemania filiformis, Ruminococcus obeum, Ruminococcus torques
N739	CDAD,T2D	3	4	50	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii

N740	CDAD,T2D	3	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i)	Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N741	CDAD,T2D	3	4	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500	Alistipes putredinis, Alistipes shahii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Ruminococcus obeum, Ruminococcus torques
N742	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N743	CDAD,T2D	4	4	50	100	clade_170, (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500	Alistipes shahii, Bacteroides finegoldii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides finegoldii, Ruminococcus obeum, Ruminococcus torques

N744	CDAD	4	4	75	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Veillonella parvula	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum, Veillonella parvula
N745	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_65 or clade_65e)	Bacteroides thetaiotaomicron, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Bacteroides thetaiotaomicron, Coprococcus comes, Dorea longicatena, Eubacterium hallii
N746	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), (clade_65 or clade_65e)	Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Bacteroides thetaiotaomicron, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N747	CDAD,T2D	4	4	75	75	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i)	Bifidobacterium adolescentis, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques

N748	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_38 or clade_38e or clade_38i), clade_500	Alistipes shahii, Bacteroides ovatus, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bacteroides ovatus, Ruminococcus obeum, Ruminococcus torques
N749	CDAD,T2D	4	4	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500, clade_521	Alistipes shahii, Bilophila wadsworthia, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Bilophila wadsworthia, Ruminococcus obeum, Ruminococcus torques
N750	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_393, (clade_444 or clade_444i)	Coprococcus catus, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques	Coprococcus catus, Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N751	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_485	Faecalibacterium prausnitzii, Holdemania filiformis, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Holdemania filiformis, Ruminococcus obeum, Ruminococcus torques
N752	CDAD,T2D	3	4	50	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii	Alistipes putredinis, Alistipes shahii, Dorea formicigenerans, Faecalibacterium prausnitzii

						clade_478i), clade_500		
N753	CDAD,T2D	3	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i)	Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Roseburia intestinalis, Ruminococcus obeum, Ruminococcus torques
N754	CDAD,T2D	3	4	50	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500	Alistipes putredinis, Alistipes shahii, Ruminococcus obeum, Ruminococcus torques	Alistipes putredinis, Alistipes shahii, Ruminococcus obeum, Ruminococcus torques
N755	CDAD,T2D	4	4	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N756	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_516 or clade_516c or clade_516g or clade_516h)	Clostridium methylpentosum, Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans

N757	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i)8, clade_396, (clade_444 or clade_444i)	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans, Solobacterium moorei	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N758	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_556	Acidaminococcus fermentans, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Coprococcus comes, Dorea longicatena, Eubacterium hallii
N759	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_396, clade_401, (clade_444 or clade_444i)	Coprococcus comes, Eubacterium hallii, Lactococcus lactis, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N760	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans, Streptococcus vestibularis	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N761	CDAD,T2D	3	4	75	75	(clade_172 or clade_172i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bifidobacterium catenulatum, Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile	Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile
N762	CDAD,T2D	3	4	75	75	(clade_172 or clade_172i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Bifidobacterium pseudocatenulatum, Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile	Eubacterium rectale, Roseburia intestinalis, Subdoligranulum variabile

N763	CDAD,T2D	4	4	100	75	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_485	Blautia hansenii, Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis	Dorea formicigenerans, Faecalibacterium prausnitzii, Holdemania filiformis
N764	CDAD,T2D	4	4	75	75	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans, Streptococcus mitis	Coprococcus comes, Eubacterium hallii, Roseburia inulinivorans
N765	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_566 or clade_566f)	Coprococcus comes, Eubacterium hallii, Gordonibacter pamelaeae, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Gordonibacter pamelaeae, Roseburia inulinivorans
N766	CDAD,T2D	4	4	50	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_500, (clade_566 or clade_566f)	Alistipes putredinis, Dorea formicigenerans, Gordonibacter pamelaeae, Subdoligranulum variabile	Alistipes putredinis, Dorea formicigenerans, Gordonibacter pamelaeae, Subdoligranulum variabile
N767	CDAD,T2D	3	4	100	75	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Clostridium scindens, Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile

N768	CDAD,T2D	3	4	75	75	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Streptococcus thermophilus, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile
N769	CDAD	4	4	100	75	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), (clade_478 or clade_478i), clade_494	Clostridium scindens, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques	Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques
N770	CDAD,T2D	4	4	100	75	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Clostridium bolteae, Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N771	CDAD,T2D	3	4	75	75	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_88	Dorea formicigenerans, Faecalibacterium prausnitzii, Haemophilus parainfluenzae, Subdoligranulum variabile	Dorea formicigenerans, Faecalibacterium prausnitzii, Subdoligranulum variabile
N772	CDAD,T2D	4	4	75	75	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i),	Bifidobacterium adolescentis, Coprococcus comes, Dorea longicatena, Eubacterium hallii	Coprococcus comes, Dorea longicatena, Eubacterium hallii

						clade_396		
N773	CDAD,T2D	4	4	75	100	(clade_262 or clade_262i), clade_286, clade_396, (clade_444 or clade_444i)	Coprococcus comes, Eubacterium hallii, Parabacteroides merdae, Roseburia inulinivorans	Coprococcus comes, Eubacterium hallii, Parabacteroides merdae, Roseburia inulinivorans
N774	CDAD,T2D	3	3	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum
N775	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), clade_286, clade_396	Coprococcus comes, Eubacterium hallii, Parabacteroides johnsonii	Coprococcus comes, Eubacterium hallii, Parabacteroides johnsonii
N776	CDAD,T2D	3	3	66.7	33.3	(clade_172 or clade_172i), (clade_516 or clade_516c or clade_516g or clade_516h), clade_538	Bifidobacterium catenulatum, Eubacterium siraeum, Ruminococcus albus	Eubacterium siraeum
N777	CDAD,T2D	3	3	66.7	66.7	clade_358, (clade_444 or clade_444i), clade_519	Eubacterium ventriosum, Roseburia inulinivorans, Veillonella dispar	Eubacterium ventriosum, Roseburia inulinivorans
N778	CDAD,T2D	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i)	Eubacterium rectale, Ruminococcus obeum, Veillonella dispar	Eubacterium rectale, Ruminococcus obeum

N779	CDAD,T2D	3	3	66.7	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Faecalibacterium prausnitzii, Roseburia intestinalis, Streptococcus australis	Faecalibacterium prausnitzii, Roseburia intestinalis
N780	CDAD,T2D	3	3	66.7	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Faecalibacterium prausnitzii, Roseburia intestinalis, Streptococcus infantis	Faecalibacterium prausnitzii, Roseburia intestinalis
N781	CDAD	3	3	66.7	66.7	clade_293, clade_393, (clade_444 or clade_444i)	Bifidobacterium bifidum, Coprococcus catus, Eubacterium rectale	Coprococcus catus, Eubacterium rectale
N782	CDAD,T2D	3	3	66.7	100	clade_171, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_522 or clade_522i)	Bacteroides intestinalis, Eubacterium eligens, Ruminococcus obeum	Bacteroides intestinalis, Eubacterium eligens, Ruminococcus obeum
N783	CDAD,T2D	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i)	Eubacterium rectale, Ruminococcus obeum, Veillonella parvula	Eubacterium rectale, Ruminococcus obeum, Veillonella parvula
N784	CDAD,T2D	3	3	66.7	100	clade_170, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i)	Bacteroides finegoldii, Eubacterium rectale, Ruminococcus obeum	Bacteroides finegoldii, Eubacterium rectale, Ruminococcus obeum
N785	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i),	Coprococcus comes, Dorea longicatena, Eubacterium ventriosum	Coprococcus comes, Dorea longicatena, Eubacterium ventriosum

						clade_519		
N786	CDAD	3	3	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus obeum
N787	CDAD	3	3	33.3	66.7	clade_110, (clade_378 or clade_378e), clade_537	Bacteroides coprocola, Bacteroides uniformis, Clostridium leptum	Bacteroides uniformis, Clostridium leptum
N788	CDAD	1	3	100	0	(clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Clostridium methylpentosum, Ruminococcus albus	
N789	CDAD	3	3	66.7	100	(clade_262 or clade_262i), clade_357, clade_537	Clostridium leptum, Oxalobacter formigenes, Ruminococcus lactaris	Clostridium leptum, Oxalobacter formigenes, Ruminococcus lactaris
N790	CDAD,T2D	3	3	33.3	66.7	clade_358, (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Faecalibacterium prausnitzii, Veillonella dispar	Bacteroides xylanisolvens, Faecalibacterium prausnitzii
N791	CDAD,T2D	3	3	33.3	66.7	clade_299, (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Faecalibacterium prausnitzii, Propionibacterium acnes	Bacteroides xylanisolvens, Faecalibacterium prausnitzii
N792	CDAD,T2D	3	3	100	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_516 or clade_516c or clade_516g or clade_516h)	Clostridium methylpentosum, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N793	CDAD,T2D	3	3	66.7	33.3	clade_504, (clade_516 or	Anaerotruncus colihominis,	Clostridium leptum

						clade_516c or clade_516g or clade_516h), clade_537	Campylobacter hominis, Clostridium leptum	
N794	CDAD,T2D	3	3	100	33.3	(clade_516 or clade_516c or clade_516g or clade_516h), clade_537, clade_543	Anaerotruncus colihominis, Clostridium leptum, Coprococcus eutactus	Clostridium leptum
N795	CDAD,T2D	2	3	33.3	66.7	clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Clostridium methylpentosum	Alistipes putredinis, Alistipes shahii
N796	CDAD,T2D	2	3	33.3	100	clade_500, clade_537	Alistipes putredinis, Alistipes shahii, Ruminococcus bromii	Alistipes putredinis, Alistipes shahii, Ruminococcus bromii
N797	CDAD,T2D	3	3	33.3	66.7	(clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Bacteroides xylanisolvens, Faecalibacterium prausnitzii, Streptococcus salivarius	Bacteroides xylanisolvens, Faecalibacterium prausnitzii
N798	CDAD,T2D	3	3	33.3	66.7	(clade_378 or clade_378e), clade_494, clade_588	Bacteroides dorei, Methanobrevibacter smithii, Pseudoflavonifractor capillosus	Bacteroides dorei, Pseudoflavonifractor capillosus
N799	CDAD	2	3	0	66.7	(clade_38 or clade_38e or clade_38i), clade_556	Acidaminococcus fermentans, Bacteroides ovatus, Bacteroides xylanisolvens	Bacteroides ovatus, Bacteroides xylanisolvens
N800	CDAD,T2D	3	3	33.3	66.7	clade_299, clade_445, clade_537	Clostridium leptum, Desulfovibrio desulfuricans, Propionibacterium acnes	Clostridium leptum, Desulfovibrio desulfuricans
N801	CDAD,T2D	3	3	33.3	66.7	clade_281, clade_445, clade_537	Clostridium leptum, Desulfovibrio desulfuricans, Porphyromonas asaccharolytica	Clostridium leptum, Desulfovibrio desulfuricans
N802	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_485,	Anaerostipes caccae, Clostridium leptum, Holdemania filiformis	Clostridium leptum, Holdemania filiformis

						clade_537		
N803	CDAD,T2D	3	3	0	66.7	(clade_38 or clade_38e or clade_38i), clade_432, clade_500	Alistipes shahii, Bacteroides xylanisolvens, Sutterella wadsworthensis	Alistipes shahii, Bacteroides xylanisolvens
N804	CDAD,T2D	3	3	100	66.7	clade_485, clade_494, clade_543	Coprococcus eutactus, Holdemania filiformis, Pseudoflavonifractor capillosus	Holdemania filiformis, Pseudoflavonifractor capillosus
N805	CDAD,T2D	3	3	33.3	66.7	clade_293, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445	Bifidobacterium bifidum, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans
N806	CDAD,T2D	3	3	33.3	66.7	(clade_172 or clade_172i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445	Bifidobacterium catenulatum, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans
N807	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i)	Anaerostipes caccae, Coprococcus comes, Subdoligranulum variabile	Coprococcus comes, Subdoligranulum variabile
N808	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_516 or clade_516c or clade_516g or clade_516h)	Clostridium methylpentosum, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans

N809	CDAD,T2D	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, (clade_478 or clade_478i)	Blautia hansenii, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii
N810	CDAD,T2D	3	3	33.3	66.7	(clade_172 or clade_172i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445	Bifidobacterium adolescentis, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans
N811	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_516 or clade_516c or clade_516g or clade_516h)	Desulfovibrio desulfuricans, Dorea formicigenerans, Ruminococcus albus	Desulfovibrio desulfuricans, Dorea formicigenerans
N812	CDAD,T2D	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides eggerthii, Dorea formicigenerans, Ruminococcus obeum	Bacteroides eggerthii, Dorea formicigenerans, Ruminococcus obeum
N813	CDAD,T2D	3	3	66.7	100	clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h	Dorea formicigenerans, Parabacteroides merdae, Ruminococcus obeum	Dorea formicigenerans, Parabacteroides merdae, Ruminococcus obeum

						clade_360i)		
N814	CDAD,T2D	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides stercoris, Dorea formicigenerans, Ruminococcus obeum	Bacteroides stercoris, Dorea formicigenerans, Ruminococcus obeum
N815	CDAD,T2D	3	3	66.7	33.3	(clade_172 or clade_172i), (clade_522 or clade_522i), (clade_553 or clade_553i)	Bifidobacterium catenulatum, Collinsella aerofaciens, Eubacterium eligens	Eubacterium eligens
N816	CDAD	3	3	33.3	66.7	(clade_38 or clade_38e or clade_38i), (clade_522 or clade_522i), (clade_98 or clade_98i)	Bacteroides ovatus, Eubacterium eligens, Streptococcus vestibularis	Bacteroides ovatus, Eubacterium eligens
N817	CDAD	3	3	66.7	66.7	clade_519, clade_537, (clade_98 or clade_98i)	Clostridium leptum, Eubacterium ventriosum, Streptococcus vestibularis	Clostridium leptum, Eubacterium ventriosum
N818	CDAD,T2D	3	3	33.3	100	clade_357, (clade_38 or clade_38e or clade_38i), clade_396	Bacteroides ovatus, Eubacterium hallii, Oxalobacter formigenes	Bacteroides ovatus, Eubacterium hallii, Oxalobacter formigenes
N819	CDAD,T2D	2	3	0	100	clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides eggerthii	Alistipes putredinis, Alistipes shahii, Bacteroides eggerthii
N820	CDAD,T2D	2	3	0	66.7	clade_168, clade_500	Alistipes putredinis, Alistipes shahii, Prevotella copri	Alistipes putredinis, Alistipes shahii
N821	CDAD,T2D	2	3	0	66.7	clade_500, (clade_98 or	Alistipes putredinis, Alistipes shahii,	Alistipes putredinis, Alistipes shahii

						clade_98i)	Streptococcus sanguinis	
N822	CDAD,T2D	2	3	0	66.7	clade_401, clade_500	Alistipes putredinis, Alistipes shahii, Lactococcus lactis	Alistipes putredinis, Alistipes shahii
N823	CDAD,T2D	2	3	0	66.7	clade_299, clade_500	Alistipes putredinis, Alistipes shahii, Propionibacterium acnes	Alistipes putredinis, Alistipes shahii
N824	CDAD,T2D	3	3	0	66.7	(clade_172 or clade_172i), (clade_38 or clade_38e or clade_38i), clade_521	Bacteroides xylanisolvens, Bifidobacterium catenulatum, Bilophila wadsworthia	Bacteroides xylanisolvens, Bilophila wadsworthia
N825	CDAD	3	3	66.7	100	(clade_444 or clade_444i), clade_521, (clade_522 or clade_522i)	Bilophila wadsworthia, Eubacterium eligens, Eubacterium rectale	Bilophila wadsworthia, Eubacterium eligens, Eubacterium rectale
N826	CDAD,T2D	2	3	33.3	66.7	clade_500, clade_543	Alistipes putredinis, Alistipes shahii, Coprococcus eutactus	Alistipes putredinis, Alistipes shahii
N827	CDAD	3	3	66.7	66.7	(clade_378 or clade_378e), (clade_444 or clade_444i), (clade_522 or clade_522i)	Bacteroides sp. 3_1_40A, Eubacterium eligens, Eubacterium rectale	Eubacterium eligens, Eubacterium rectale
N828	CDAD	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_98 or clade_98i)	Coprococcus comes, Dorea longicatena, Streptococcus australis	Coprococcus comes, Dorea longicatena
N829	CDAD,T2D	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_65 or clade_65e)	Bacteroides fragilis, Coprococcus comes, Dorea longicatena	Coprococcus comes, Dorea longicatena
N830	CDAD	3	3	33.3	66.7	(clade_262 or clade_262i), clade_466, (clade_98 or	Coprococcus comes, Odoribacter splanchnicus, Streptococcus	Coprococcus comes, Odoribacter splanchnicus

						clade_98i)	thermophilus	
N831	CDAD,T2D	3	3	33.3	66.7	(clade_172 or clade_172i), clade_396, clade_500	Alistipes shahii, Bifidobacterium pseudocatenulatum, Eubacterium hallii	Alistipes shahii, Eubacterium hallii
N832	CDAD,T2D	3	3	33.3	66.7	clade_293, (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Bifidobacterium bifidum, Subdoligranulum variabile	Bacteroides xylanisolvens, Subdoligranulum variabile
N833	CDAD	3	3	66.7	66.7	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), clade_466	Clostridium scindens, Coprococcus comes, Odoribacter splanchnicus	Coprococcus comes, Odoribacter splanchnicus
N834	CDAD,T2D	2	3	0	100	clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Streptococcus parasanguinis	Alistipes putredinis, Alistipes shahii, Streptococcus parasanguinis
N835	CDAD,T2D	2	3	0	100	clade_500, (clade_566 or clade_566i)	Alistipes putredinis, Alistipes shahii, Gordonibacter pamelaeae	Alistipes putredinis, Alistipes shahii, Gordonibacter pamelaeae
N836	CDAD	3	3	100	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i), clade_494	Pseudoflavonifractor capillosus, Ruminococcus gnavus, Subdoligranulum variabile	Pseudoflavonifractor capillosus, Subdoligranulum variabile
N837	CDAD	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_88	Coprococcus comes, Dorea longicatena, Haemophilus parainfluenzae	Coprococcus comes, Dorea longicatena
N838	CDAD,T2D	3	3	33.3	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or	Alistipes shahii, Bacteroides cellulosilyticus, Ruminococcus obeum	Alistipes shahii, Bacteroides cellulosilyticus, Ruminococcus obeum

						clade_309i), clade_500, clade_85		
N839	CDAD,T2D	3	3	33.3	66.7	(clade_335 or clade_335i), clade_445, (clade_478 or clade_478i)	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Parabacteroides distasonis	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii
N840	CDAD,T2D	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium rectale, Ruminococcus obeum, Streptococcus salivarius	Eubacterium rectale, Ruminococcus obeum
N841	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_478 or clade_478i), clade_537	Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus torques
N842	CDAD,T2D	2	3	33.3	100	clade_393, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus catus	Alistipes putredinis, Alistipes shahii, Coprococcus catus
N843	CDAD,T2D	2	3	33.3	100	(clade_262 or clade_262i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes	Alistipes putredinis, Alistipes shahii, Coprococcus comes
N844	CDAD	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_521	Bilophila wadsworthia, Coprococcus comes, Dorea longicatena	Bilophila wadsworthia, Coprococcus comes, Dorea longicatena
N845	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e)	Bacteroides dorei, Coprococcus comes, Dorea longicatena	Bacteroides dorei, Coprococcus comes, Dorea longicatena

N846	CDAD,T2D	3	3	66.7	100	clade_170, (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h clade_360i)	Bacteroides caccae, Coprococcus comes, Dorea longicatena	Bacteroides caccae, Coprococcus comes, Dorea longicatena
N847	CDAD,T2D	3	3	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum	Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum
N848	CDAD	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466	Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus	Coprococcus comes, Dorea longicatena, Odoribacter splanchnicus
N849	CDAD	3	3	100	66.7	(clade_444 or clade_444i), (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i), (clade_522 or clade_522i)	Clostridium cocleatum, Eubacterium eligens, Eubacterium rectale	Eubacterium eligens, Eubacterium rectale
N850	CDAD	3	3	100	0	(clade_351 or clade_351e), (clade_354 or clade_354e), (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i)	Clostridium bifermentans, Clostridium innocuum, Clostridium ramosum	

N851	CDAD	3	3	0	66.7	clade_445, clade_521, clade_556	Acidaminococcus fermentans, Bilophila wadsworthia, Desulfovibrio desulfuricans	Bilophila wadsworthia, Desulfovibrio desulfuricans
N852	CDAD,T2D	3	3	66.7	100	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_478 or clade_478i)	Bifidobacterium longum, Faecalibacterium prausnitzii, Ruminococcus torques	Bifidobacterium longum, Faecalibacterium prausnitzii, Ruminococcus torques
N853	CDAD	3	3	100	100	(clade_262 or clade_262i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus torques	Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus torques
N854	CDAD,T2D	3	3	66.7	100	clade_110, (clade_262 or clade_262i), (clade_478 or clade_478i)	Bacteroides uniformis, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides uniformis, Faecalibacterium prausnitzii, Ruminococcus torques
N855	CDAD,T2D	3	3	66.7	100	clade_170, (clade_262 or clade_262i), (clade_478 or clade_478i)	Bacteroides finegoldii, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides finegoldii, Faecalibacterium prausnitzii, Ruminococcus torques
N856	CDAD,T2D	2	3	100	100	(clade_262 or clade_262i), clade_494	Clostridium nexile, Pseudoflavonifractor capillosus, Ruminococcus torques	Clostridium nexile, Pseudoflavonifractor capillosus, Ruminococcus torques
N857	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_494	Blautia hansenii, Pseudoflavonifractor capillosus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques
N858	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Anaerostipes caccae, Pseudoflavonifractor capillosus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques

N859	CDAD,T2D	3	3	100	66.7	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), clade_494	Clostridium scindens, Pseudoflavonifractor capillosus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques
N860	CDAD,T2D	3	3	66.7	66.7	(clade_262 or clade_262i), clade_494, (clade_98 or clade_98i)	Pseudoflavonifractor capillosus, Ruminococcus torques, Streptococcus thermophilus	Pseudoflavonifractor capillosus, Ruminococcus torques
N861	CDAD	3	3	100	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h)	Clostridium hathewayi, Ruminococcus obeum, Ruminococcus torques	Ruminococcus obeum, Ruminococcus torques
N862	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_494	Pseudoflavonifractor capillosus, Ruminococcus gnavus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques
N863	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i)	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N864	CDAD	3	3	100	0	clade_253, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or	Blautia producta, Clostridium celatum, Clostridium innocuum	

						clade_309i), (clade_351 or clade_351e)		
N865	Obesity	2	3	0	0	(clade_335 or clade_335i), (clade_378 or clade_378e)	Bacteroides coprocola, Bacteroides plebeius, Parabacteroides distasonis	
N866	Obesity	3	3	33.3	66.7	clade_170, clade_286, clade_368	Bacteroides caccae, Blautia hydrogenotrophica, Parabacteroides merdae	Bacteroides caccae, Parabacteroides merdae
N867	Obesity	3	3	66.7	66.7	clade_396, clade_543, (clade_65 or clade_65e)	Bacteroides pectinophilus, Bacteroides thetaiotaomicron, Butyrivibrio crossotus	Bacteroides thetaiotaomicron, Butyrivibrio crossotus
N868	Obesity	3	3	66.7	33.3	clade_110, clade_368, (clade_566 or clade_566f)	Bacteroides uniformis, Blautia hydrogenotrophica, Eggerthella lenta	Bacteroides uniformis
N869	Obesity	3	3	33.3	100	(clade_38 or clade_38e or clade_38i), clade_537, (clade_566 or clade_566f)	Bacteroides xylanisolvens, Clostridium leptum, Gordonibacter pamelaeae	Bacteroides xylanisolvens, Clostridium leptum, Gordonibacter pamelaeae
N870	T2D	2	3	100	0	clade_252, clade_495	Clostridium beijerinckii, Clostridium botulinum, Clostridium thermocellum	
N871	CDAD,T2D	2	3	66.7	66.7	(clade_444 or clade_444i), clade_556	Acidaminococcus fermentans, Roseburia intestinalis, Roseburia inulinivorans	Roseburia intestinalis, Roseburia inulinivorans
N872	CDAD,T2D	3	3	66.7	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Faecalibacterium prausnitzii, Roseburia intestinalis, Streptococcus infantis	Faecalibacterium prausnitzii, Roseburia intestinalis
N873	CDAD,T2D	3	3	66.7	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Faecalibacterium prausnitzii, Roseburia intestinalis, Streptococcus australis	Faecalibacterium prausnitzii, Roseburia intestinalis

N874	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), clade_88	Eubacterium hallii, Haemophilus parainfluenzae, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans
N875	CDAD,T2D	2	3	100	66.7	(clade_444 or clade_444i), (clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Roseburia intestinalis, Roseburia inulinivorans	Roseburia intestinalis, Roseburia inulinivorans
N876	CDAD,T2D	2	3	66.7	100	clade_170, (clade_444 or clade_444i)	Bacteroides caccae, Roseburia intestinalis, Roseburia inulinivorans	Bacteroides caccae, Roseburia intestinalis, Roseburia inulinivorans
N877	CDAD,T2D	2	3	66.7	100	clade_110, (clade_444 or clade_444i)	Bacteroides uniformis, Roseburia intestinalis, Roseburia inulinivorans	Bacteroides uniformis, Roseburia intestinalis, Roseburia inulinivorans
N878	CDAD,T2D	2	3	100	100	(clade_444 or clade_444i), (clade_522 or clade_522i)	Eubacterium eligens, Roseburia intestinalis, Roseburia inulinivorans	Eubacterium eligens, Roseburia intestinalis, Roseburia inulinivorans
N879	CDAD,T2D	3	3	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum	Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus obeum
N880	CDAD,T2D	2	3	100	100	(clade_262 or clade_262i), (clade_444 or clade_444i)	Coprococcus comes, Eubacterium rectale, Roseburia inulinivorans	Coprococcus comes, Eubacterium rectale, Roseburia inulinivorans
N881	CDAD,T2D	2	3	100	100	clade_396, (clade_444 or clade_444i)	Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans	Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans
N882	CDAD,T2D	3	3	0	66.7	(clade_172 or clade_172i), (clade_38 or clade_38e or clade_38i), clade_521	Bacteroides xylanisolvens, Bifidobacterium catenulatum, Bilophila wadsworthia	Bacteroides xylanisolvens, Bilophila wadsworthia

N883	CDAD,T2D	3	3	33.3	66.7	(clade_172 or clade_172i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445	Bifidobacterium catenulatum, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans
N884	CDAD,T2D	2	3	0	66.7	clade_299, clade_500	Alistipes putredinis, Alistipes shahii, Propionibacterium acnes	Alistipes putredinis, Alistipes shahii
N885	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_485	Anaerostipes caccae, Faecalibacterium prausnitzii, Holdemania filiformis	Faecalibacterium prausnitzii, Holdemania filiformis
N886	CDAD,T2D	3	3	33.3	66.7	clade_299, (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Faecalibacterium prausnitzii, Propionibacterium acnes	Bacteroides xylanisolvens, Faecalibacterium prausnitzii
N887	CDAD,T2D	3	3	33.3	66.7	clade_299, clade_445, clade_537	Clostridium leptum, Desulfovibrio desulfuricans, Propionibacterium acnes	Clostridium leptum, Desulfovibrio desulfuricans
N888	CDAD,T2D	2	3	33.3	66.7	clade_500, clade_543	Alistipes putredinis, Alistipes shahii, Coprococcus eutactus	Alistipes putredinis, Alistipes shahii
N889	CDAD,T2D	3	3	100	33.3	(clade_516 or clade_516c or clade_516g or clade_516h), clade_537, clade_543	Anaerotruncus colihominis, Clostridium leptum, Coprococcus eutactus	Clostridium leptum
N890	CDAD,T2D	3	3	100	66.7	clade_485, clade_494, clade_543	Coprococcus eutactus, Holdemania filiformis, Pseudoflavonifractor capillosus	Holdemania filiformis, Pseudoflavonifractor capillosus
N891	CDAD,T2D	3	3	100	33.3	clade_368, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h),	Blautia hydrogenotrophica, Clostridium bolteae, Holdemania filiformis	Holdemania filiformis

						clade_485		
N892	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i)	Anaerostipes caccae, Coprococcus comes, Subdoligranulum variabile	Coprococcus comes, Subdoligranulum variabile
N893	CDAD,T2D	3	3	33.3	66.7	clade_281, clade_445, clade_537	Clostridium leptum, Desulfovibrio desulfuricans, Porphyromonas asaccharolytica	Clostridium leptum, Desulfovibrio desulfuricans
N894	CDAD,T2D	3	3	66.7	66.7	clade_293, (clade_444 or clade_444i), (clade_478 or clade_478i)	Bifidobacterium bifidum, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N895	CDAD,T2D	2	3	0	66.7	clade_168, clade_500	Alistipes putredinis, Alistipes shahii, Prevotella copri	Alistipes putredinis, Alistipes shahii
N896	CDAD,T2D	3	3	66.7	33.3	clade_504, (clade_516 or clade_516c or clade_516g or clade_516h), clade_537	Anaerotruncus colihominis, Campylobacter hominis, Clostridium leptum	Clostridium leptum
N897	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_485, clade_537	Anaerostipes caccae, Clostridium leptum, Holdemania filiformis	Clostridium leptum, Holdemania filiformis
N898	CDAD,T2D	3	3	66.7	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), clade_556	Acidaminococcus fermentans, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N899	CDAD,T2D	3	3	66.7	33.3	(clade_172 or clade_172i), (clade_516 or	Bifidobacterium catenulatum, Eubacterium siraeum,	Eubacterium siraeum

						clade_516c or clade_516g or clade_516h), clade_538	Ruminococcus albus	
N900	CDAD,T2D	3	3	33.3	66.7	(clade_378 or clade_378e), clade_494, clade_588	Bacteroides dorei, Methanobrevibacter smithii, Pseudoflavonifractor capillosus	Bacteroides dorei, Pseudoflavonifractor capillosus
N901	CDAD,T2D	3	3	33.3	66.7	(clade_262 or clade_262i), clade_401, clade_466	Lactococcus lactis, Odoribacter splanchnicus, Ruminococcus lactaris	Odoribacter splanchnicus, Ruminococcus lactaris
N902	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Anaerostipes caccae, Pseudoflavonifractor capillosus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques
N903	CDAD,T2D	3	3	33.3	66.7	clade_293, (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Bifidobacterium bifidum, Subdoligranulum variabile	Bacteroides xylanisolvens, Subdoligranulum variabile
N904	CDAD,T2D	2	3	0	66.7	clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Streptococcus sanguinis	Alistipes putredinis, Alistipes shahii
N905	CDAD,T2D	3	3	33.3	66.7	clade_293, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445	Bifidobacterium bifidum, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans
N906	CDAD,T2D	3	3	66.7	33.3	(clade_172 or clade_172i), (clade_522 or clade_522i), (clade_553 or clade_553i)	Bifidobacterium catenulatum, Collinsella aerofaciens, Eubacterium eligens	Eubacterium eligens
N907	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or	Dorea longicatena, Roseburia intestinalis, Streptococcus vestibularis	Dorea longicatena, Roseburia intestinalis

						clade_444i), (clade_98 or clade_98i)		
N908	CDAD,T2D	3	3	33.3	100	clade_357, (clade_38 or clade_38e or clade_38i), clade_396	Bacteroides ovatus, Eubacterium hallii, Oxalobacter formigenes	Bacteroides ovatus, Eubacterium hallii, Oxalobacter formigenes
N909	CDAD,T2D	2	3	33.3	66.7	clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Alistipes shahii, Clostridium methylpentosum	Alistipes putredinis, Alistipes shahii
N910	CDAD,T2D	3	3	33.3	66.7	(clade_172 or clade_172i), clade_396, clade_500	Alistipes shahii, Bifidobacterium pseudocatenulatum, Eubacterium hallii	Alistipes shahii, Eubacterium hallii
N911	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_516 or clade_516c or clade_516g or clade_516h)	Clostridium methylpentosum, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans
N912	CDAD,T2D	3	3	100	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_516 or clade_516c or clade_516g or clade_516h)	Clostridium methylpentosum, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N913	CDAD,T2D	3	3	100	33.3	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_516 or clade_516c or	Anaerotruncus colihominis, Clostridium symbiosum, Dorea formicigenerans	Dorea formicigenerans

						clade_516g or clade_516h)		
N914	CDAD,T2D	3	3	66.7	66.7	clade_358, (clade_444 or clade_444i), clade_519	Eubacterium ventriosum, Roseburia inulinivorans, Veillonella dispar	Eubacterium ventriosum, Roseburia inulinivorans
N915	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396	Blautia hansenii, Coprococcus comes, Eubacterium hallii	Coprococcus comes, Eubacterium hallii
N916	CDAD,T2D	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445, (clade_478 or clade_478i)	Blautia hansenii, Desulfovibrio desulfuricans, Faecalibacterium prausnitzii	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii
N917	CDAD,T2D	3	3	33.3	66.7	clade_358, (clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i)	Bacteroides xylanisolvens, Faecalibacterium prausnitzii, Veillonella dispar	Bacteroides xylanisolvens, Faecalibacterium prausnitzii
N918	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_494	Blautia hansenii, Pseudoflavonifractor capillosus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques

N919	CDAD,T2D	2	3	33.3	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500	Alistipes putredinis, Alistipes shahii, Blautia hansenii	Alistipes putredinis, Alistipes shahii
N920	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), clade_286, clade_396	Coprococcus comes, Eubacterium hallii, Parabacteroides johnsonii	Coprococcus comes, Eubacterium hallii, Parabacteroides johnsonii
N921	CDAD,T2D	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i)	Eubacterium rectale, Ruminococcus obeum, Veillonella dispar	Eubacterium rectale, Ruminococcus obeum
N922	CDAD,T2D	2	3	0	33.3	(clade_378 or clade_378e), (clade_38 or clade_38e or clade_38i)	Bacteroides ovatus, Bacteroides plebeius, Bacteroides vulgatus	Bacteroides ovatus
N923	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_98 or clade_98i)	Dorea longicatena, Roseburia intestinalis, Streptococcus mitis	Dorea longicatena, Roseburia intestinalis
N924	CDAD,T2D	3	3	0	66.7	(clade_38 or clade_38e or clade_38i), clade_432, clade_500	Alistipes shahii, Bacteroides xylanisolvens, Sutterella wadsworthensis	Alistipes shahii, Bacteroides xylanisolvens
N925	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus australis	Eubacterium hallii, Roseburia inulinivorans
N926	CDAD,T2D	2	3	0	66.7	clade_401, clade_500	Alistipes putredinis, Alistipes shahii, Lactococcus lactis	Alistipes putredinis, Alistipes shahii
N927	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i),	Dialister invisus, Eubacterium hallii, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans

						clade_506		
N928	CDAD,T2D	2	3	0	66.7	clade_500, clade_506	Alistipes putredinis, Alistipes shahii, Dialister invisus	Alistipes putredinis, Alistipes shahii
N929	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), clade_396, (clade_566 or clade_566f)	Coprococcus comes, Eggerthella lenta, Eubacterium hallii	Coprococcus comes, Eubacterium hallii
N930	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i)	Clostridium nexile, Eubacterium hallii, Roseburia inulinivorans	Clostridium nexile, Eubacterium hallii, Roseburia inulinivorans
N931	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)	Clostridium nexile, Dorea formicigenerans, Subdoligranulum variabile	Clostridium nexile, Dorea formicigenerans, Subdoligranulum variabile
N932	CDAD,T2D	2	3	100	100	(clade_262 or clade_262i), clade_494	Clostridium nexile, Pseudoflavonifractor capillosus, Ruminococcus torques	Clostridium nexile, Pseudoflavonifractor capillosus, Ruminococcus torques
N933	CDAD,T2D	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_65 or clade_65e)	Bacteroides fragilis, Coprococcus comes, Dorea longicatena	Coprococcus comes, Dorea longicatena
N934	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_478 or clade_478i), clade_485	Clostridium nexile, Faecalibacterium prausnitzii, Holdemania filiformis	Clostridium nexile, Faecalibacterium prausnitzii, Holdemania filiformis
N935	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), (clade_65 or clade_65e)	Bacteroides fragilis, Eubacterium hallii, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans

N936	CDAD,T2D	3	3	33.3	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500, (clade_65 or clade_65e)	Alistipes putredinis, Bacteroides fragilis, Dorea formicigenerans	Alistipes putredinis, Dorea formicigenerans
N937	CDAD,T2D	2	3	0	100	clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides eggerthii	Alistipes putredinis, Alistipes shahii, Bacteroides eggerthii
N938	CDAD,T2D	3	3	66.7	100	clade_171, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_522 or clade_522i)	Bacteroides intestinalis, Eubacterium eligens, Ruminococcus obeum	Bacteroides intestinalis, Eubacterium eligens, Ruminococcus obeum
N939	CDAD,T2D	3	3	66.7	66.7	(clade_262 or clade_262i), clade_494, (clade_98 or clade_98i)	Pseudoflavonifractor capillosus, Ruminococcus torques, Streptococcus thermophilus	Pseudoflavonifractor capillosus, Ruminococcus torques
N940	CDAD,T2D	3	3	33.3	66.7	(clade_38 or clade_38e or clade_38i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Bacteroides xylanisolvens, Faecalibacterium prausnitzii, Streptococcus salivarius	Bacteroides xylanisolvens, Faecalibacterium prausnitzii
N941	CDAD,T2D	3	3	33.3	66.7	(clade_335 or clade_335i), clade_445, (clade_478 or clade_478i)	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii, Parabacteroides distasonis	Desulfovibrio desulfuricans, Faecalibacterium prausnitzii
N942	CDAD,T2D	3	3	100	66.7	(clade_260 or clade_260c or clade_260g or clade_260h), clade_396, (clade_444 or clade_444i)	Clostridium scindens, Eubacterium hallii, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans

N943	CDAD,T2D	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides eggerthii, Dorea formicigenerans, Ruminococcus obeum	Bacteroides eggerthii, Dorea formicigenerans, Ruminococcus obeum
N944	CDAD,T2D	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium rectale, Ruminococcus obeum, Streptococcus salivarius	Eubacterium rectale, Ruminococcus obeum
N945	CDAD,T2D	2	3	33.3	66.7	(clade_260 or clade_260c or clade_260g or clade_260h), clade_500	Alistipes putredinis, Alistipes shahii, Clostridium scindens	Alistipes putredinis, Alistipes shahii
N946	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_485	Clostridium hathewayi, Faecalibacterium prausnitzii, Holdemania filiformis	Faecalibacterium prausnitzii, Holdemania filiformis
N947	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus thermophilus	Eubacterium hallii, Roseburia inulinivorans
N948	CDAD,T2D	2	3	0	100	clade_500, (clade_566 or clade_566f)	Alistipes putredinis, Alistipes shahii, Gordonibacter pamelaeae	Alistipes putredinis, Alistipes shahii, Gordonibacter pamelaeae
N949	CDAD,T2D	3	3	33.3	66.7	(clade_172 or clade_172i), (clade_360 or clade_360c or clade_360g or	Bifidobacterium adolescentis, Desulfovibrio desulfuricans, Dorea formicigenerans	Desulfovibrio desulfuricans, Dorea formicigenerans

						clade_360h or clade_360i), clade_445		
N950	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides stercoris, Coprococcus comes, Dorea longicatena	Bacteroides stercoris, Coprococcus comes, Dorea longicatena
N951	CDAD,T2D	3	3	100	66.7	(clade_260 or clade_260c or clade_260g or clade_260h), (clade_262 or clade_262i), clade_494	Clostridium scindens, Pseudoflavonifractor capillosus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques
N952	CDAD,T2D	2	3	0	100	clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides stercoris	Alistipes putredinis, Alistipes shahii, Bacteroides stercoris
N953	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides eggerthii, Coprococcus comes, Dorea longicatena	Bacteroides eggerthii, Coprococcus comes, Dorea longicatena
N954	CDAD,T2D	2	3	33.3	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_500	Alistipes putredinis, Alistipes shahii, Clostridium asparagiforme	Alistipes putredinis, Alistipes shahii
N955	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), (clade_478 or clade_478i)	Clostridium asparagiforme, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile

N956	CDAD,T2D	3	3	33.3	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500, clade_85	Alistipes putredinis, Bacteroides eggerthii, Dorea formicigenerans	Alistipes putredinis, Bacteroides eggerthii, Dorea formicigenerans
N957	CDAD,T2D	2	3	33.3	100	clade_500, clade_537	Alistipes putredinis, Alistipes shahii, Ruminococcus bromii	Alistipes putredinis, Alistipes shahii, Ruminococcus bromii
N958	CDAD,T2D	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_444 or clade_444i)	Eubacterium rectale, Ruminococcus obeum, Veillonella parvula	Eubacterium rectale, Ruminococcus obeum, Veillonella parvula
N959	CDAD,T2D	3	3	33.3	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500, clade_85	Alistipes shahii, Bacteroides cellulosilyticus, Ruminococcus obeum	Alistipes shahii, Bacteroides cellulosilyticus, Ruminococcus obeum
N960	CDAD,T2D	2	3	33.3	66.7	(clade_354 or clade_354e), clade_500	Alistipes putredinis, Alistipes shahii, Clostridium bartlettii	Alistipes putredinis, Alistipes shahii
N961	CDAD,T2D	3	3	100	66.7	(clade_354 or clade_354e), (clade_444 or clade_444i), (clade_478 or clade_478i)	Clostridium bartlettii, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N962	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_478 or clade_478i), clade_537	Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus bromii, Ruminococcus torques
N963	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or	Ruminococcus gnavus, Ruminococcus obeum, Ruminococcus torques	Ruminococcus obeum, Ruminococcus torques

						clade_360c or clade_360g or clade_360h clade_360i)		
N964	CDAD,T2D	3	3	66.7	100	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_478 or clade_478i)	Bifidobacterium longum, Faecalibacterium prausnitzii, Ruminococcus torques	Bifidobacterium longum, Faecalibacterium prausnitzii, Ruminococcus torques
N965	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_494	Clostridium bolteae, Pseudoflavonifractor capillosus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques
N966	CDAD,T2D	2	3	0	100	clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Alistipes shahii, Streptococcus parasanguinis	Alistipes putredinis, Alistipes shahii, Streptococcus parasanguinis
N967	CDAD,T2D	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides stercoris, Dorea formicigenerans, Ruminococcus obeum	Bacteroides stercoris, Dorea formicigenerans, Ruminococcus obeum
N968	CDAD,T2D	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_92 or clade_92e or clade_92i)	Escherichia coli, Ruminococcus obeum, Ruminococcus torques	Ruminococcus obeum, Ruminococcus torques

N969	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_516 or clade_516c or clade_516g or clade_516h)	Desulfovibrio desulfuricans, Dorea formicigenerans, Ruminococcus albus	Desulfovibrio desulfuricans, Dorea formicigenerans
N970	CDAD,T2D	3	3	66.7	100	clade_286, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h clade_360i)	Dorea formicigenerans, Parabacteroides merdae, Ruminococcus obeum	Dorea formicigenerans, Parabacteroides merdae, Ruminococcus obeum
N971	CDAD,T2D	3	3	66.7	100	clade_170, (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i)	Bacteroides finegoldii, Eubacterium rectale, Ruminococcus obeum	Bacteroides finegoldii, Eubacterium rectale, Ruminococcus obeum
N972	CDAD,T2D	3	3	100	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N973	CDAD,T2D	3	3	66.7	100	clade_170, (clade_262 or clade_262i), (clade_478 or clade_478i)	Bacteroides finegoldii, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides finegoldii, Faecalibacterium prausnitzii, Ruminococcus torques
N974	CDAD,T2D	3	3	0	66.7	(clade_172 or clade_172i), clade_466, clade_500	Alistipes putredinis, Bifidobacterium adolescentis, Odoribacter splanchnicus	Alistipes putredinis, Odoribacter splanchnicus

N975	CDAD,T2D	3	3	66.7	100	clade_170, (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h clade_360i)	Bacteroides caccae, Coprococcus comes, Dorea longicatena	Bacteroides caccae, Coprococcus comes, Dorea longicatena
N976	CDAD,T2D	3	3	33.3	100	clade_286, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466	Dorea formicigenerans, Odoribacter splanchnicus, Parabacteroides merdae	Dorea formicigenerans, Odoribacter splanchnicus, Parabacteroides merdae
N977	CDAD,T2D	3	3	66.7	100	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus parasanguinis	Eubacterium hallii, Roseburia inulinivorans, Streptococcus parasanguinis
N978	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e)	Bacteroides dorei, Coprococcus comes, Dorea longicatena	Bacteroides dorei, Coprococcus comes, Dorea longicatena
N979	CDAD,T2D	2	3	0	100	(clade_378 or clade_378e), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides dorei	Alistipes putredinis, Alistipes shahii, Bacteroides dorei
N980	CDAD,T2D	2	3	33.3	100	(clade_262 or clade_262i), clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus comes	Alistipes putredinis, Alistipes shahii, Coprococcus comes
N981	CDAD,T2D	3	3	66.7	100	clade_110, (clade_262 or clade_262i), (clade_478 or clade_478i)	Bacteroides uniformis, Faecalibacterium prausnitzii, Ruminococcus torques	Bacteroides uniformis, Faecalibacterium prausnitzii, Ruminococcus torques
N982	CDAD,T2D	3	3	66.7	100	(clade_172 or clade_172i), (clade_262 or clade_262i), clade_396	Bifidobacterium longum, Coprococcus comes, Eubacterium hallii	Bifidobacterium longum, Coprococcus comes, Eubacterium hallii
N983	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus salivarius	Eubacterium hallii, Roseburia inulinivorans

N984	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_494	Pseudoflavonifractor capillosus, Ruminococcus gnavus, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus torques
N985	CDAD,T2D	3	3	66.7	100	clade_110, (clade_262 or clade_262i), clade_396	Bacteroides uniformis, Coprococcus comes, Eubacterium hallii	Bacteroides uniformis, Coprococcus comes, Eubacterium hallii
N986	CDAD,T2D	3	3	66.7	100	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i)	Bifidobacterium longum, Ruminococcus obeum, Ruminococcus torques	Bifidobacterium longum, Ruminococcus obeum, Ruminococcus torques
N987	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), clade_396, clade_537	Coprococcus comes, Eubacterium hallii, Ruminococcus bromii	Coprococcus comes, Eubacterium hallii, Ruminococcus bromii
N988	CDAD,T2D	3	3	100	100	(clade_444 or clade_444i), (clade_478 or clade_478i), clade_537	Eubacterium rectale, Ruminococcus bromii, Subdoligranulum variabile	Eubacterium rectale, Ruminococcus bromii, Subdoligranulum variabile
N989	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_519	Coprococcus comes, Dorea longicatena, Eubacterium ventriosum	Coprococcus comes, Dorea longicatena, Eubacterium ventriosum
N990	CDAD,T2D	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_378 or clade_378e)	Bacteroides vulgatus, Coprococcus comes, Dorea longicatena	Coprococcus comes, Dorea longicatena

N991	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i)	Eubacterium hallii, Roseburia inulinivorans, Ruminococcus lactaris	Eubacterium hallii, Roseburia inulinivorans, Ruminococcus lactaris
N992	CDAD,T2D	2	3	0	66.7	(clade_378 or clade_378e), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides vulgatus	Alistipes putredinis, Alistipes shahii
N993	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500	Alistipes putredinis, Dorea formicigenerans, Ruminococcus lactaris	Alistipes putredinis, Dorea formicigenerans, Ruminococcus lactaris
N994	CDAD,T2D	2	3	0	100	(clade_38 or clade_38e or clade_38i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens
N995	CDAD,T2D	3	3	66.7	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500, clade_537	Alistipes putredinis, Clostridium leptum, Dorea formicigenerans	Alistipes putredinis, Clostridium leptum, Dorea formicigenerans
N996	CDAD,T2D	3	3	100	100	clade_393, clade_396, (clade_444 or clade_444i)	Coprococcus catus, Eubacterium hallii, Roseburia inulinivorans	Coprococcus catus, Eubacterium hallii, Roseburia inulinivorans
N997	CDAD,T2D	2	3	33.3	100	clade_393, clade_500	Alistipes putredinis, Alistipes shahii, Coprococcus catus	Alistipes putredinis, Alistipes shahii, Coprococcus catus
N998	CDAD,T2D	3	3	100	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, (clade_478 or clade_478i)	Coprococcus catus, Dorea formicigenerans, Faecalibacterium prausnitzii	Coprococcus catus, Dorea formicigenerans, Faecalibacterium prausnitzii
N999	CDAD,T2D	3	3	100	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum	Dorea formicigenerans, Faecalibacterium prausnitzii, Ruminococcus obeum

						clade_360g or clade_360h or clade_360i), (clade_478 or clade_478i)		
N1000	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), clade_396, clade_466	Coprococcus comes, Eubacterium hallii, Odoribacter splanchnicus	Coprococcus comes, Eubacterium hallii, Odoribacter splanchnicus
N1001	CDAD,T2D	3	3	100	100	clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile	Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile
N1002	CDAD,T2D	2	3	33.3	100	(clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Subdoligranulum variabile
N1003	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_494	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N1004	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445	Desulfovibrio desulfuricans, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Ruminococcus obeum, Ruminococcus torques
N1005	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500	Alistipes shahii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Ruminococcus obeum, Ruminococcus torques

N1006	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i)	Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N1007	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i)	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus obeum, Ruminococcus torques
N1008	CDAD	3	3	66.7	100	(clade_262 or clade_262i), (clade_478 or clade_478i), clade_521	Bilophila wadsworthia, Faecalibacterium prausnitzii, Ruminococcus torques	Bilophila wadsworthia, Faecalibacterium prausnitzii, Ruminococcus torques
N1009	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i)	Eubacterium hallii, Roseburia inulinivorans, Ruminococcus lactaris	Eubacterium hallii, Roseburia inulinivorans, Ruminococcus lactaris
N1010	CDAD	3	3	100	100	(clade_262 or clade_262i), (clade_478 or clade_478i), clade_537	Clostridium leptum, Faecalibacterium prausnitzii, Ruminococcus torques	Clostridium leptum, Faecalibacterium prausnitzii, Ruminococcus torques
N1011	CDAD,T2D	2	3	66.7	66.7	(clade_444 or clade_444i), clade_556	Acidaminococcus fermentans, Roseburia intestinalis, Roseburia inulinivorans	Roseburia intestinalis, Roseburia inulinivorans
N1012	CDAD,T2D	3	3	66.7	66.7	clade_293, (clade_444 or clade_444i), (clade_478 or clade_478i)	Bifidobacterium bifidum, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N1013	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or	Dorea longicatena, Roseburia intestinalis, Streptococcus vestibularis	Dorea longicatena, Roseburia intestinalis

						clade_444i), (clade_98 or clade_98i)		
N1014	CDAD,T2D	2	3	33.3	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_500	Alistipes putredinis, Alistipes shahii, Blautia hansenii	Alistipes putredinis, Alistipes shahii
N1015	CDAD,T2D	3	3	66.7	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_98 or clade_98i)	Dorea longicatena, Roseburia intestinalis, Streptococcus mitis	Dorea longicatena, Roseburia intestinalis
N1016	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus australis	Eubacterium hallii, Roseburia inulinivorans
N1017	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), clade_506	Dialister invisus, Eubacterium hallii, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans
N1018	CDAD,T2D	2	3	0	66.7	clade_500, clade_506	Alistipes putredinis, Alistipes shahii, Dialister invisus	Alistipes putredinis, Alistipes shahii
N1019	CDAD	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_358, (clade_478 or clade_478i)	Faecalibacterium prausnitzii, Ruminococcus obeum, Veillonella atypica	Faecalibacterium prausnitzii, Ruminococcus obeum, Veillonella atypica
N1020	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i)	Clostridium nexile, Eubacterium hallii, Roseburia inulinivorans	Clostridium nexile, Eubacterium hallii, Roseburia inulinivorans
N1021	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i),	Bacteroides fragilis, Eubacterium hallii, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans

						(clade_65 or clade_65e)		
N1022	CDAD,T2D	3	3	33.3	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500, (clade_65 or clade_65e)	Alistipes putredinis, Bacteroides fragilis, Dorea formicigenerans	Alistipes putredinis, Dorea formicigenerans
N1023	CDAD	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_98 or clade_98i)	Dorea longicatena, Ruminococcus torques, Streptococcus salivarius	Dorea longicatena, Ruminococcus torques
N1024	CDAD,T2D	3	3	100	66.7	(clade_260 or clade_260c or clade_260g or clade_260h), clade_396, (clade_444 or clade_444i)	Clostridium scindens, Eubacterium hallii, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans
N1025	CDAD,T2D	2	3	33.3	66.7	(clade_260 or clade_260c or clade_260g or clade_260h), clade_500	Alistipes putredinis, Alistipes shahii, Clostridium scindens	Alistipes putredinis, Alistipes shahii
N1026	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus thermophilus	Eubacterium hallii, Roseburia inulinivorans
N1027	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides stercoris, Coprococcus comes, Dorea longicatena	Bacteroides stercoris, Coprococcus comes, Dorea longicatena
N1028	CDAD,T2D	2	3	0	100	clade_500, clade_85	Alistipes putredinis, Alistipes shahii, Bacteroides stercoris	Alistipes putredinis, Alistipes shahii, Bacteroides stercoris

N1029	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_85	Bacteroides eggerthii, Coprococcus comes, Dorea longicatena	Bacteroides eggerthii, Coprococcus comes, Dorea longicatena
N1030	CDAD,T2D	2	3	33.3	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_500	Alistipes putredinis, Alistipes shahii, Clostridium asparagiforme	Alistipes putredinis, Alistipes shahii
N1031	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), clade_88	Eubacterium hallii, Haemophilus parainfluenzae, Roseburia inulinivorans	Eubacterium hallii, Roseburia inulinivorans
N1032	CDAD,T2D	2	3	33.3	66.7	(clade_354 or clade_354e), clade_500	Alistipes putredinis, Alistipes shahii, Clostridium bartlettii	Alistipes putredinis, Alistipes shahii
N1033	CDAD,T2D	3	3	100	66.7	(clade_354 or clade_354e), (clade_444 or clade_444i), (clade_478 or clade_478i)	Clostridium bartlettii, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N1034	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_360 or clade_360c or clade_360g or clade_360h clade_360i)	Ruminococcus gnavus, Ruminococcus obeum, Ruminococcus torques	Ruminococcus obeum, Ruminococcus torques
N1035	CDAD	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), (clade_98 or	Faecalibacterium prausnitzii, Ruminococcus obeum, Streptococcus mitis	Faecalibacterium prausnitzii, Ruminococcus obeum

						clade_98i)		
N1036	CDAD,T2D	3	3	66.7	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_92 or clade_92e or clade_92i)	Escherichia coli, Ruminococcus obeum, Ruminococcus torques	Ruminococcus obeum, Ruminococcus torques
N1037	CDAD	3	3	66.7	66.7	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), clade_88	Faecalibacterium prausnitzii, Haemophilus parainfluenzae, Ruminococcus obeum	Faecalibacterium prausnitzii, Ruminococcus obeum
N1038	CDAD	3	3	66.7	100	(clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_478 or clade_478i), (clade_98 or clade_98i)	Faecalibacterium prausnitzii, Ruminococcus obeum, Streptococcus parasanguinis	Faecalibacterium prausnitzii, Ruminococcus obeum, Streptococcus parasanguinis
N1039	CDAD,T2D	2	3	100	66.7	(clade_444 or clade_444i), (clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Roseburia intestinalis, Roseburia inulinivorans	Roseburia intestinalis, Roseburia inulinivorans
N1040	CDAD,T2D	3	3	100	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), (clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile

N1041	CDAD,T2D	3	3	0	66.7	(clade_172 or clade_172i), clade_466, clade_500	Alistipes putredinis, Bifidobacterium adolescentis, Odoribacter splanchnicus	Alistipes putredinis, Odoribacter splanchnicus
N1042	CDAD,T2D	2	3	66.7	100	clade_170, (clade_444 or clade_444i)	Bacteroides caccae, Roseburia intestinalis, Roseburia inulinivorans	Bacteroides caccae, Roseburia intestinalis, Roseburia inulinivorans
N1043	CDAD,T2D	3	3	66.7	100	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus parasanguinis	Eubacterium hallii, Roseburia inulinivorans, Streptococcus parasanguinis
N1044	CDAD,T2D	2	3	0	100	(clade_378 or clade_378e), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides dorei	Alistipes putredinis, Alistipes shahii, Bacteroides dorei
N1045	CDAD,T2D	2	3	66.7	100	clade_110, (clade_444 or clade_444i)	Bacteroides uniformis, Roseburia intestinalis, Roseburia inulinivorans	Bacteroides uniformis, Roseburia intestinalis, Roseburia inulinivorans
N1046	CDAD,T2D	3	3	66.7	66.7	clade_396, (clade_444 or clade_444i), (clade_98 or clade_98i)	Eubacterium hallii, Roseburia inulinivorans, Streptococcus salivarius	Eubacterium hallii, Roseburia inulinivorans
N1047	CDAD	3	3	100	100	(clade_262 or clade_262i), (clade_478 or clade_478i), clade_485	Faecalibacterium prausnitzii, Holdemania filiformis, Ruminococcus torques	Faecalibacterium prausnitzii, Holdemania filiformis, Ruminococcus torques
N1048	CDAD,T2D	3	3	66.7	100	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i)	Bifidobacterium longum, Ruminococcus obeum, Ruminococcus torques	Bifidobacterium longum, Ruminococcus obeum, Ruminococcus torques
N1049	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), clade_396, clade_537	Coprococcus comes, Eubacterium hallii, Ruminococcus bromii	Coprococcus comes, Eubacterium hallii, Ruminococcus bromii
N1050	CDAD,T2D	3	3	100	100	(clade_444 or clade_444i), (clade_478 or clade_478i), clade_537	Eubacterium rectale, Ruminococcus bromii, Subdoligranulum variabile	Eubacterium rectale, Ruminococcus bromii, Subdoligranulum variabile

N1051	CDAD	3	3	100	66.7	(clade_262 or clade_262i), (clade_478 or clade_478i), (clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Faecalibacterium prausnitzii, Ruminococcus torques	Faecalibacterium prausnitzii, Ruminococcus torques
N1052	CDAD,T2D	2	3	0	66.7	(clade_378 or clade_378e), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides vulgatus	Alistipes putredinis, Alistipes shahii
N1053	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500	Alistipes putredinis, Dorea formicigenerans, Ruminococcus lactaris	Alistipes putredinis, Dorea formicigenerans, Ruminococcus lactaris
N1054	CDAD,T2D	2	3	0	100	(clade_38 or clade_38e or clade_38i), clade_500	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens	Alistipes putredinis, Alistipes shahii, Bacteroides xylanisolvens
N1055	CDAD,T2D	3	3	66.7	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500, clade_537	Alistipes putredinis, Clostridium leptum, Dorea formicigenerans	Alistipes putredinis, Clostridium leptum, Dorea formicigenerans
N1056	CDAD	2	3	33.3	100	(clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Faecalibacterium prausnitzii	Alistipes putredinis, Alistipes shahii, Faecalibacterium prausnitzii
N1057	CDAD,T2D	3	3	100	100	clade_393, clade_396, (clade_444 or clade_444i)	Coprococcus catus, Eubacterium hallii, Roseburia inulinivorans	Coprococcus catus, Eubacterium hallii, Roseburia inulinivorans
N1058	CDAD,T2D	3	3	100	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, (clade_478 or clade_478i)	Coprococcus catus, Dorea formicigenerans, Faecalibacterium prausnitzii	Coprococcus catus, Dorea formicigenerans, Faecalibacterium prausnitzii
N1059	CDAD,T2D	2	3	100	100	(clade_444 or clade_444i), (clade_522 or clade_522i)	Eubacterium eligens, Roseburia intestinalis, Roseburia inulinivorans	Eubacterium eligens, Roseburia intestinalis, Roseburia inulinivorans

N1060	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), clade_396, clade_466	Coprococcus comes, Eubacterium hallii, Odoribacter splanchnicus	Coprococcus comes, Eubacterium hallii, Odoribacter splanchnicus
N1061	CDAD	3	3	100	100	(clade_262 or clade_262i), clade_396, (clade_478 or clade_478i)	Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus torques	Eubacterium hallii, Faecalibacterium prausnitzii, Ruminococcus torques
N1062	CDAD,T2D	2	3	100	100	(clade_262 or clade_262i), (clade_444 or clade_444i)	Coprococcus comes, Eubacterium rectale, Roseburia inulinivorans	Coprococcus comes, Eubacterium rectale, Roseburia inulinivorans
N1063	CDAD,T2D	2	3	100	100	clade_396, (clade_444 or clade_444i)	Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans	Eubacterium hallii, Roseburia intestinalis, Roseburia inulinivorans
N1064	CDAD,T2D	3	3	100	100	clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i)	Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile	Eubacterium hallii, Roseburia inulinivorans, Subdoligranulum variabile
N1065	CDAD,T2D	2	3	33.3	100	(clade_478 or clade_478i), clade_500	Alistipes putredinis, Alistipes shahii, Subdoligranulum variabile	Alistipes putredinis, Alistipes shahii, Subdoligranulum variabile
N1066	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_494	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques	Pseudoflavonifractor capillosus, Ruminococcus obeum, Ruminococcus torques
N1067	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_445	Desulfovibrio desulfuricans, Ruminococcus obeum, Ruminococcus torques	Desulfovibrio desulfuricans, Ruminococcus obeum, Ruminococcus torques
N1068	CDAD,T2D	3	3	66.7	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or	Alistipes shahii, Ruminococcus obeum, Ruminococcus torques	Alistipes shahii, Ruminococcus obeum, Ruminococcus torques

						clade_309h or clade_309i), clade_500		
N1069	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), (clade_444 or clade_444i)	Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques	Eubacterium rectale, Ruminococcus obeum, Ruminococcus torques
N1070	CDAD	3	3	66.7	33.3	(clade_262 or clade_262i), clade_299, (clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Coprococcus comes, Propionibacterium acnes	Coprococcus comes
N1071	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_485	Anaerostipes caccae, Faecalibacterium prausnitzii, Holdemania filiformis	Faecalibacterium prausnitzii, Holdemania filiformis
N1072	CDAD,T2D	3	3	100	33.3	clade_368, (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), clade_485	Blautia hydrogenotrophica, Clostridium bolteae, Holdemania filiformis	Holdemania filiformis
N1073	CDAD,T2D	3	3	66.7	66.7	(clade_444 or clade_444i), (clade_478 or clade_478i), clade_556	Acidaminococcus fermentans, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N1074	CDAD,T2D	3	3	33.3	66.7	(clade_262 or clade_262i), clade_401, clade_466	Lactococcus lactis, Odoribacter splanchnicus, Ruminococcus lactaris	Odoribacter splanchnicus, Ruminococcus lactaris

N1075	CDAD	3	3	33.3	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_98 or clade_98i)	Desulfovibrio desulfuricans, Dorea formicigenerans, Streptococcus infantis	Desulfovibrio desulfuricans, Dorea formicigenerans
N1076	CDAD,T2D	3	3	100	33.3	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_516 or clade_516c or clade_516g or clade_516h)	Anaerotruncus colihominis, Clostridium symbiosum, Dorea formicigenerans	Dorea formicigenerans
N1077	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), (clade_309 or clade_309c or clade_309e or clade_309g or clade_309h or clade_309i), clade_396	Blautia hansenii, Coprococcus comes, Eubacterium hallii	Coprococcus comes, Eubacterium hallii
N1078	CDAD	3	3	33.3	66.7	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_445, (clade_98 or clade_98i)	Desulfovibrio desulfuricans, Dorea formicigenerans, Streptococcus australis	Desulfovibrio desulfuricans, Dorea formicigenerans
N1079	CDAD,T2D	3	3	100	66.7	(clade_262 or clade_262i), clade_396, (clade_566 or clade_566f)	Coprococcus comes, Eggerthella lenta, Eubacterium hallii	Coprococcus comes, Eubacterium hallii
N1080	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or	Clostridium nexile, Dorea formicigenerans, Subdoligranulum variabile	Clostridium nexile, Dorea formicigenerans, Subdoligranulum variabile

						clade_360h or clade_360i), (clade_478 or clade_478i)		
N1081	CDAD,T2D	3	3	100	100	(clade_262 or clade_262i), (clade_478 or clade_478i), clade_485	Clostridium nexile, Faecalibacterium prausnitzii, Holdemania filiformis	Clostridium nexile, Faecalibacterium prausnitzii, Holdemania filiformis
N1082	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_478 or clade_478i), clade_485	Clostridium hathewayi, Faecalibacterium prausnitzii, Holdemania filiformis	Faecalibacterium prausnitzii, Holdemania filiformis
N1083	CDAD,T2D	3	3	100	66.7	(clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), (clade_478 or clade_478i)	Clostridium asparagiforme, Eubacterium rectale, Subdoligranulum variabile	Eubacterium rectale, Subdoligranulum variabile
N1084	CDAD,T2D	3	3	33.3	100	(clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_500, clade_85	Alistipes putredinis, Bacteroides eggerthii, Dorea formicigenerans	Alistipes putredinis, Bacteroides eggerthii, Dorea formicigenerans
N1085	CDAD,T2D	3	3	33.3	100	clade_286, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_466	Dorea formicigenerans, Odoribacter splanchnicus, Parabacteroides merdae	Dorea formicigenerans, Odoribacter splanchnicus, Parabacteroides merdae
N1086	CDAD,T2D	3	3	66.7	100	(clade_172 or clade_172i), (clade_262 or clade_262i), clade_396	Bifidobacterium longum, Coprococcus comes, Eubacterium hallii	Bifidobacterium longum, Coprococcus comes, Eubacterium hallii
N1087	CDAD,T2D	3	3	66.7	100	clade_110, (clade_262 or	Bacteroides uniformis, Coprococcus comes,	Bacteroides uniformis, Coprococcus comes,

						clade_262i), clade_396	Eubacterium hallii	Eubacterium hallii
N1088	CDAD	2	2	100	100	clade_393, (clade_444 or clade_444i)	Coprococcus catus, Eubacterium rectale	Coprococcus catus, Eubacterium rectale
N1089	CDAD	2	2	100	100	(clade_478 or clade_478i), clade_576	Clostridium lactatifermentans, Faecalibacterium prausnitzii	Clostridium lactatifermentans, Faecalibacterium prausnitzii
N1090	CDAD	2	2	0	0	clade_293, (clade_378 or clade_378e)	Bacteroides sp. 3_1_40A, Bifidobacterium bifidum	
N1091	CDAD	2	2	50	50	(clade_444 or clade_444i), clade_561	Alistipes indistinctus, Eubacterium rectale	Eubacterium rectale
N1092	CDAD	2	2	100	50	clade_393, (clade_481 or clade_481a or clade_481b or clade_481e or clade_481g or clade_481h or clade_481i)	Clostridium cocleatum, Coprococcus catus	Coprococcus catus
N1093	CDAD	2	2	50	100	(clade_522 or clade_522i), clade_583	Akkermansia muciniphila, Eubacterium eligens	Akkermansia muciniphila, Eubacterium eligens
N1094	CDAD	2	2	0	0	(clade_497 or clade_497e or clade_497f), clade_89	Granulicatella adiacens, Proteus penneri	
N1095	CDAD	2	2	100	100	(clade_444 or clade_444i), (clade_522 or clade_522i)	Eubacterium eligens, Eubacterium rectale	Eubacterium eligens, Eubacterium rectale
N1096	T2D	2	2	50	50	(clade_378 or clade_378e), (clade_522 or clade_522i)	Bacteroides coprocola, Eubacterium eligens	Eubacterium eligens
N1097	T2D	2	2	50	50	clade_543, clade_88	Butyrivibrio crossotus, Haemophilus parainfluenzae	Butyrivibrio crossotus
N1098	T2D	2	2	100	100	(clade_444 or clade_444i), (clade_478 or clade_478i)	Faecalibacterium prausnitzii, Roseburia intestinalis	Faecalibacterium prausnitzii, Roseburia intestinalis

Table 9.

Keystone OTUs that occur in Network Ecologies representing states of health for various disease indications (CDAD = Clostridium difficile associated diarrhea, T2D = Type 2 Diabetes, Obesity = clinical obesity, UC = ulcerative colitis)

OTU	Clade	Family	Genus	Disease Indication for which Health Keystone OTU
Akkermansia muciniphila	clade_583	Verrucomicrobiaceae	Akkermansia	CDAD
Alistipes putredinis	clade_500	Rikenellaceae	Alistipes	CDAD, T2D
Alistipes shahii	clade_500	Rikenellaceae	Alistipes	CDAD, T2D
Bacteroides caccae	clade_170	Bacteroidaceae	Bacteroides	CDAD, Obesity
Bacteroides cellulosilyticus	clade_85	Bacteroidaceae	Bacteroides	CDAD, T2D
Bacteroides dorei	clade_378	Bacteroidaceae	Bacteroides	CDAD, Obesity, T2D
Bacteroides eggerthii	clade_85	Bacteroidaceae	Bacteroides	T2D
Bacteroides finegoldii	clade_170	Bacteroidaceae	Bacteroides	CDAD, UC
Bacteroides intestinalis	clade_171	Bacteroidaceae	Bacteroides	T2D
Bacteroides ovatus	clade_38	Bacteroidaceae	Bacteroides	Obesity, T2D
Bacteroides sp. D20	clade_110	Bacteroidaceae	Bacteroides	CDAD
Bacteroides stercoris	clade_85	Bacteroidaceae	Bacteroides	CDAD, T2D, UC
Bacteroides thetaiotaomicron	clade_65	Bacteroidaceae	Bacteroides	CDAD, Obesity
Bacteroides uniformis	clade_110	Bacteroidaceae	Bacteroides	T2D, UC
Bacteroides xylanisolvens	clade_38	Bacteroidaceae	Bacteroides	Obesity
Bifidobacterium longum	clade_172	Bifidobacteriaceae	Bifidobacterium	CDAD
Bilophila wadsworthia	clade_521	Desulfovibrionaceae	Bilophila	CDAD
Blautia sp. M25	clade_309	Lachnospiraceae	Blautia	CDAD
Butyricicoccus pullicaecorum	clade_572	Clostridiaceae	Butyricicoccus	CDAD
Butyrivibrio crossotus	clade_543	Lachnospiraceae	Butyrivibrio	CDAD
Catenibacterium mitsuokai	clade_469	Erysipelotrichaceae	Catenibacterium	Obesity
Clostridium lactatifermentans	clade_576	Clostridiaceae	Clostridium	CDAD
Clostridium leptum	clade_537	Clostridiaceae	Clostridium	CDAD, Obesity, T2D
Clostridium nexile	clade_262	Clostridiaceae	Clostridium	CDAD
Clostridium sp. NML 04A032	clade_494	Clostridiaceae	Clostridium	CDAD
Coprococcus catus	clade_393	Lachnospiraceae	Coprococcus	CDAD, T2D
Coprococcus comes	clade_262	Lachnospiraceae	Coprococcus	CDAD, T2D
Desulfovibrio desulfuricans	clade_445	Desulfovibrionaceae	Desulfovibrio	T2D
Desulfovibrio piger	clade_445	Desulfovibrionaceae	Desulfovibrio	Obesity
Dorea formicigenerans	clade_360	Lachnospiraceae	Dorea	CDAD, T2D
Dorea longicatena	clade_360	Lachnospiraceae	Dorea	CDAD, T2D
Eubacterium coprostanoligenes	clade_537	Eubacteriaceae	Eubacterium	CDAD
Eubacterium eligens	clade_522	Eubacteriaceae	Eubacterium	T2D

OTU	Clade	Family	Genus	Disease Indication for which Health Keystone OTU
Eubacterium hadrum	clade_408	Lachnospiraceae	Anaerostipes	CDAD
Eubacterium hallii	clade_396	Eubacteriaceae	Eubacterium	CDAD, T2D
Eubacterium rectale	clade_444	Eubacteriaceae	Eubacterium	CDAD, T2D
Eubacterium siraeum	clade_538	Eubacteriaceae	Eubacterium	CDAD
Eubacterium ventriosum	clade_519	Eubacteriaceae	Eubacterium	T2D
Faecalibacterium prausnitzii	clade_478	Ruminococcaceae	Faecalibacterium	CDAD, T2D
Gemmiger formicilis	clade_478	Hyphomicrobiaceae	Gemmiger	CDAD
Gordonibacter pamelaeae	clade_566	Coriobacteriaceae	Gordonibacter	Obesity
Holdemania filiformis	clade_485	Erysipelotrichaceae	Holdemania	CDAD, Obesity, T2D
Lachnospiraceae bacterium 1_4_56FAA	clade_262	Lachnospiraceae		CDAD
Lachnospiraceae bacterium 3_1_57FAA_CT1	clade_408	Lachnospiraceae		CDAD
Odoribacter splanchnicus	clade_466	Porphyromonadaceae	Odoribacter	CDAD, T2D
Oscillibacter valericigenes	clade_540	Oscillospiraceae	Oscillibacter	CDAD
Oxalobacter formigenes	clade_357	Oxalobacteraceae	Oxalobacter	T2D
Parabacteroides johnsonii	clade_286	Porphyromonadaceae	Parabacteroides	CDAD, T2D
Parabacteroides merdae	clade_286	Porphyromonadaceae	Parabacteroides	CDAD, Obesity, T2D
Pseudoflavonifractor capillosus	clade_494		Pseudoflavonifractor	T2D
Roseburia hominis	clade_444	Lachnospiraceae	Roseburia	CDAD
Roseburia intestinalis	clade_444	Lachnospiraceae	Roseburia	CDAD, T2D
Roseburia inulinivorans	clade_444	Lachnospiraceae	Roseburia	CDAD, T2D
Ruminococcus bromii	clade_537	Ruminococcaceae	Ruminococcus	CDAD
Ruminococcus lactaris	clade_262	Ruminococcaceae	Ruminococcus	CDAD
Ruminococcus obeum	clade_309	Lachnospiraceae	Blautia	CDAD, T2D
Ruminococcus torques	clade_262	Lachnospiraceae	Blautia	CDAD, T2D
Sporobacter termitidis	clade_572	Ruminococcaceae	Sporobacter	CDAD
Streptococcus parasanguinis	clade_98	Streptococcaceae	Streptococcus	CDAD
Subdoligranulum variabile	clade_478	Ruminococcaceae	Subdoligranulum	CDAD
Veillonella atypica	clade_358	Veillonellaceae	Veillonella	CDAD
Veillonella parvula	clade_358	Veillonellaceae	Veillonella	CDAD
Victivallis vadensis	clade_567	Victivallaceae	Victivallis	UC

Table 10.

Non-Keystone OTUs that occur in Network Ecologies representing states of health

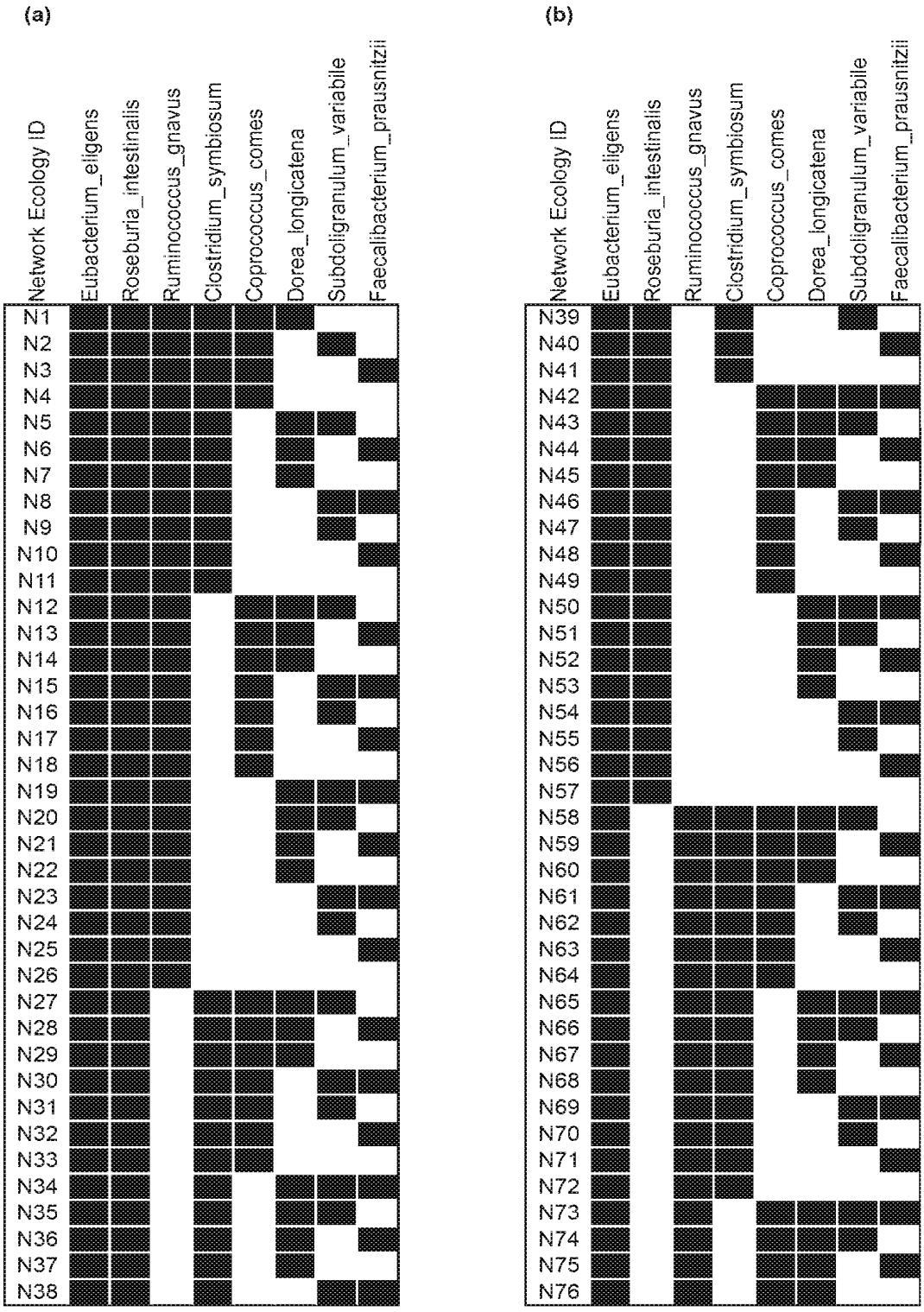
Non-Keystone OTUs
Acidaminococcus fermentans
Acinetobacter johnsonii
Alistipes indistinctus
Anaerostipes caccae
Anaerotruncus colihominis
Auritibacter ignavus
Bacteroides coprocola
Bacteroides coprophilus
Bacteroides fragilis
Bacteroides pectinophilus
Bacteroides plebeius
Bacteroides salanitronis
Bacteroides sp. 1_1_30
Bacteroides sp. 20_3
Bacteroides sp. 3_1_40A
Bacteroides sp. D2
Bacteroides vulgatus
Barnesiella viscericola
Bifidobacterium adolescentis
Bifidobacterium bifidum
Bifidobacterium catenulatum
Bifidobacterium dentium
Bifidobacterium pseudocatenulatum
Blautia hansenii
Blautia hydrogenotrophica
Blautia producta
Campylobacter hominis
Campylobacter upsaliensis
Capnocytophaga sp. oral clone ASCH05
Capnocytophaga sp. oral taxon 338
Chlamydiales bacterium NS13
Chromobacterium violaceum
Citrobacter sp. 30_2
Clostridiales bacterium oral clone P4PA
Clostridiales sp. SS3/4
Clostridium asparagiforme
Clostridium bartlettii
Clostridium beijerinckii
Clostridium bifermentans
Clostridium bolteae
Clostridium botulinum

[illegible]
Clostridium butyricum
Clostridium celatum
Clostridium cocleatum
Clostridium glycolicum
Clostridium hathewayi
Clostridium hylemonae
Clostridium innocuum
Clostridium methylpentosum
Clostridium orbiscindens
Clostridium ramosum
Clostridium scindens
Clostridium symbiosum
Clostridium tertium
Clostridium thermocellum
Collinsella aerofaciens
Collinsella tanakaei
Coprobacillus sp. D7
Coprococcus eutactus
Corynebacterium pseudogenitalium
Dialister invisus
Eggerthella lenta
Enhydrobacter aerosaccus
Enterococcus raffinosus
Enterococcus sp. CCRI 16620
Escherichia coli
Eubacterium desmolans
Eubacterium nodatum
Eubacterium sp. WAL 14571
Eubacterium tenue
Gardnerella vaginalis
Granulicatella adiacens
Haemophilus parainfluenzae
Halorubrum lipolyticum
Heliobacterium modesticaldum
Lachnobacterium bovis
Lachnospiraceae bacterium 1_1_57FAA
Lachnospiraceae bacterium 5_1_57FAA
Lactobacillus ruminis
Lactococcus lactis
Marvinbryantia formatexigens
Methanobrevibacter smithii
Mitsuokella multacida
Mycoplasmataceae genomosp P1 oral clone
Neisseria meningitidis

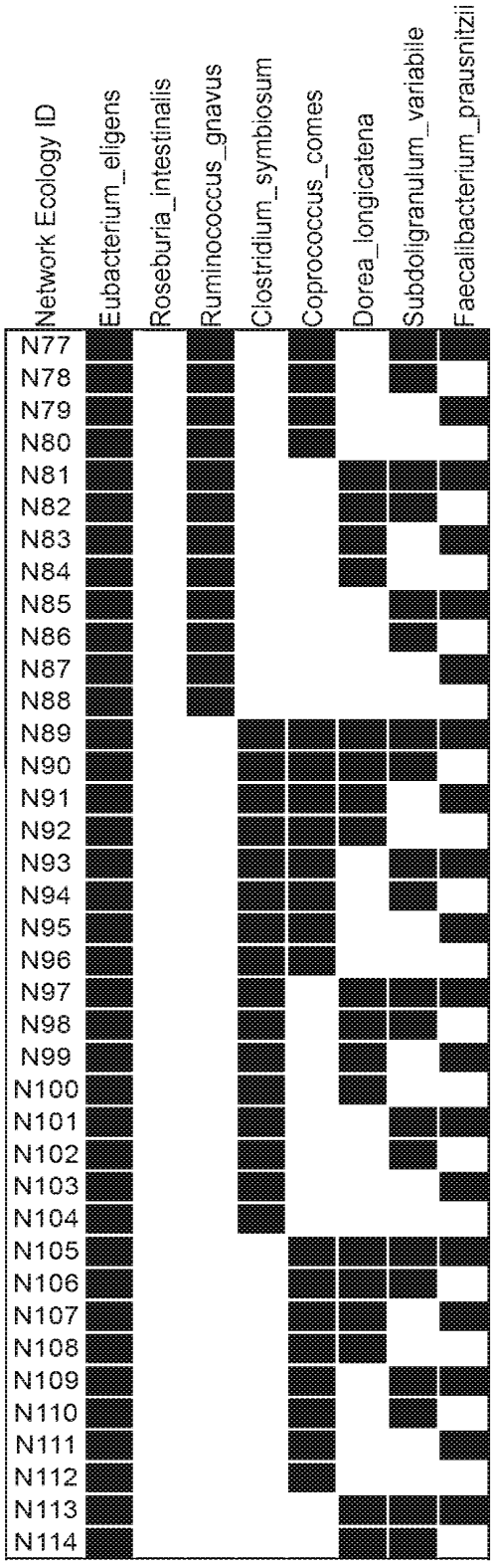
Non-Key Gene (NTT)
Parabacteroides distasonis
Peptoniphilus sp. gpac077
Phascolarctobacterium sp. YIT 12068
Porphyromonas asaccharolytica
Prevotella buccae
Prevotella buccalis
Prevotella copri
Propionibacterium acnes
Proteus penneri
Psychrobacter pulmonis
Roseburia faecalis
Rothia mucilaginosa
Ruminococcus albus
Ruminococcus gnavus
Ruminococcus sp. ID8
Scardovia inopinata
Solobacterium moorei
Staphylococcus warneri
Streptococcus australis
Streptococcus dysgalactiae
Streptococcus infantis
Streptococcus mitis
Streptococcus peroris
Streptococcus pyogenes
Streptococcus salivarius
Streptococcus sanguinis
Streptococcus sp. oral clone ASCF07
Streptococcus suis
Streptococcus thermophilus
Streptococcus vestibularis
Sutterella wadsworthensis
Synergistetes bacterium oral taxon D48
Tannerella sp. 6_1_58FAA_CT1
Tissierella praeacuta
Veillonella dispar

Table 11.

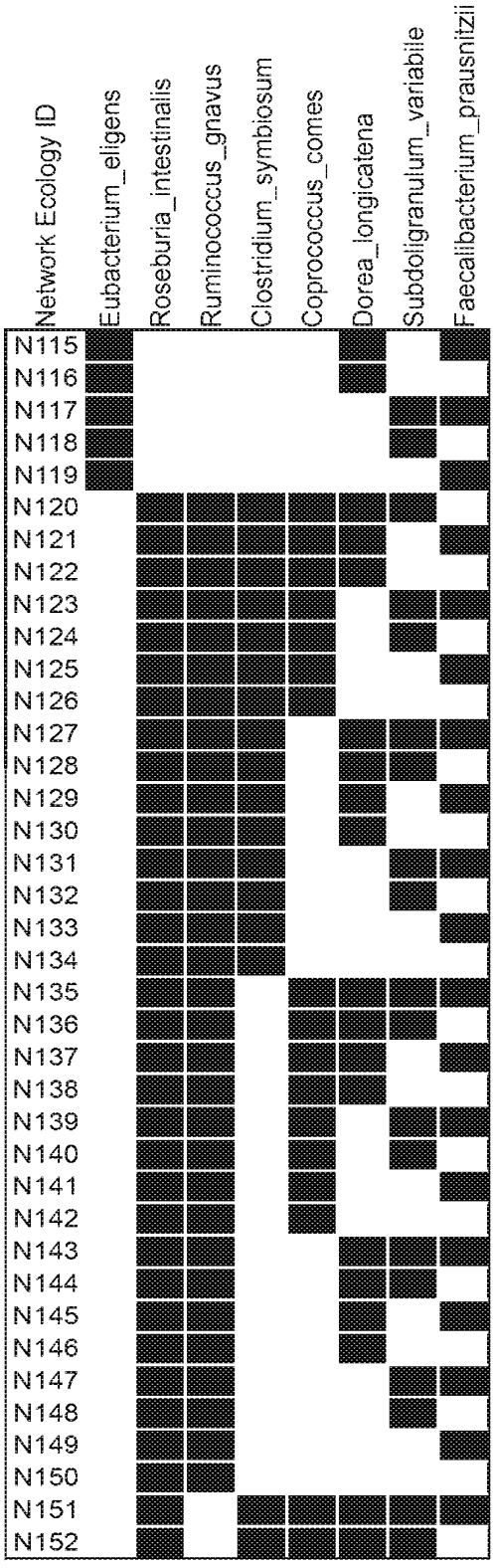
Exemplary Functional Network Ecologies that are found only in healthy individuals, that contain only Keystone OTUs, and that have OTUs with the functional capacity to form spores.



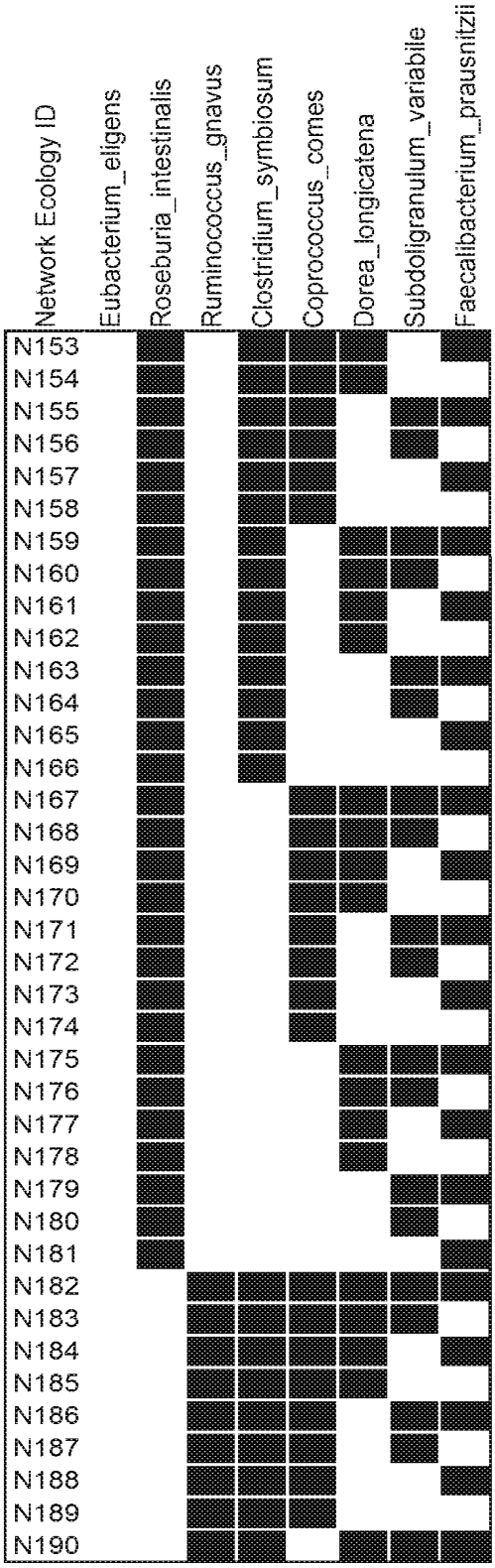
(c)



(d)



(e)


Network Ecology ID
Eubacterium_eligens
Roseburia_intestinalis
Ruminococcus_gnavus
Clostridium_symbiosum
Coprococcus_comes
Dorea_longicatena
Subdoligranulum_variabile
Faecalibacterium_prausnitzii
N153
N154
N155
N156
N157
N158
N159
N160
N161
N162
N163
N164
N165
N166
N167
N168
N169
N170
N171
N172
N173
N174
N175
N176
N177
N178
N179
N180
N181
N182
N183
N184
N185
N186
N187
N188
N189
N190

(f)

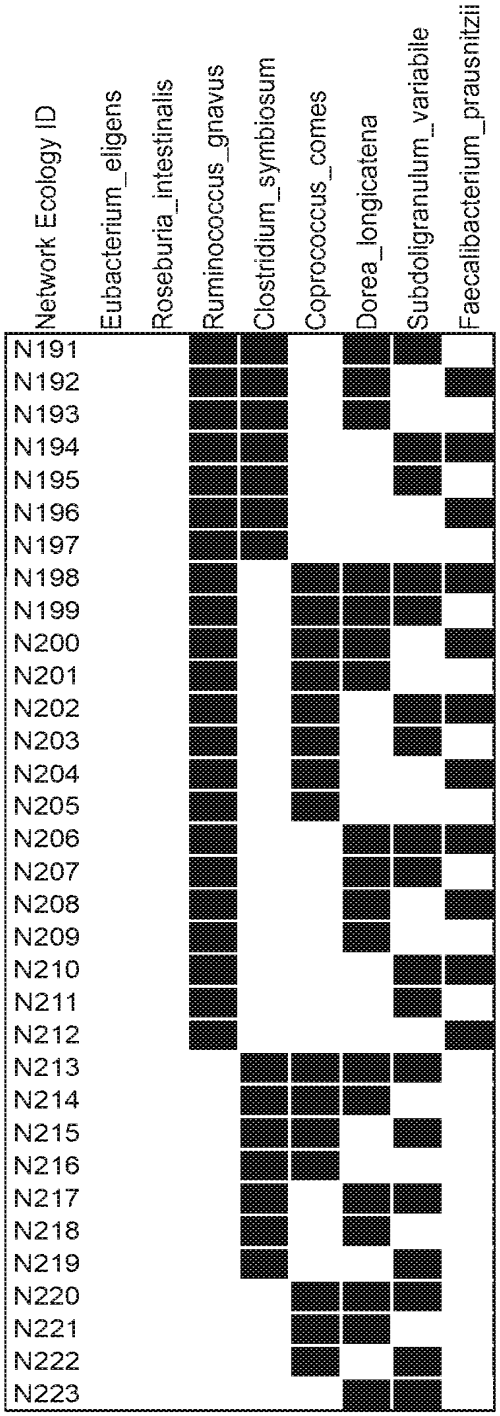

Network Ecology ID
Eubacterium_eligens
Roseburia_intestinalis
Ruminococcus_gnavus
Clostridium_symbiosum
Coprococcus_comes
Dorea_longicatena
Subdoligranulum_variabile
Faecalibacterium_prausnitzii
N191
N192
N193
N194
N195
N196
N197
N198
N199
N200
N201
N202
N203
N204
N205
N206
N207
N208
N209
N210
N211
N212
N213
N214
N215
N216
N217
N218
N219
N220
N221
N222
N223

Table 12.

Exemplary Network Ecologies Classes (a.k.a Core) defined from their occurrence in only healthy individuals, the size of the network, and the frequency of occurrence of the networks in individual subjects at or above the 90th percentile for the latter two metrics. OTUs represent diverse taxonomic families (see Table 1). Numbers in each cell represent the percentage of individual observed Network Ecologies within a given Network Class that contain the given OTU. **(a)** OTUs that are observed in at least 80% of a core's networks (dark grey) are primary signature OTUs of a given core. **(b-c)** OTUs observed in 11%-79% (mid grey) ≤10% (light grey) are secondary and tertiary signature OTUs respectively of a given Network Class. Primary signature OTUs are of greater significance than secondary signature OTUs which are of greater significance than tertiary keystone OTUs.

(a)

Core Network ID	Faecalibacterium_prausnitzii	Ruminococcus_obeum	Alistipes_putredinis	Alistipes_shahii	Ruminococcus_torques	Coprococcus_catus	Dorea_longicatena	Eubacterium_rectale	Bifidobacterium_adolescentis	Coprococcus_comes	Roseburia_inulinivorans	Clostridium_leptum	Gordonibacter_pamelaeae
Core1	100	100	15	15	99	99	100	100	99	13	99	13	
Core2	100	100	100	100	14	100	100	100	100	1	14	16	
Core3	100	100	100	100	8	5	100	100	100	100	8	8	
Core4	100	100	100	100	100	11	11	14		94	10	99	99
Core5	100	100	100	100	100	97	20	17		7	13	91	93
Core6	100	100	100	100	100	100	11	11	2	82	100	10	19

(b)

Core Network ID	Dorea_formicigenerans	Eubacterium_eligens	Eubacterium_hallii	Subdoligranulum_variabile	Bacteroides_ovatus	Eubacterium_ventriosum	Anaerotruncus_colihominis	Bacteroides_vulgatus	Bacteroides_xylanisolvens	Roseburia_intestinalis
Core1	13	12	11	13	11	12	9	9	9	11
Core2	16	15	14	16	10	14	10	11	10	9
Core3	8	8	8	8	8	8	8	7	8	8
Core4	13	11	11	11	11	10	10	15	7	6
Core5	19	20	19	12	17	13	16		7	7
Core6	11	9	10	9	9	9	10	10	5	5

(c)

Core Network ID	Bacteroides_uniformis	Holdemania_filiformis	Parabacteroides_merdae	Collinsella_aerofaciens	Bacteroides_dorei	Odoribacter_splanchnicus	Blautia_wadsworthia	Ruminococcus_lactaris	Bacteroides_finegoldii	Bacteroides_thetaiotaomicron	Ruminococcus_gnavus	Bacteroides_caccae	Bacteroides_stercoris	Akkermansia_muciniphila	Bifidobacterium_longum
Core1	7	7	8	7	8			3							
Core2	7	3	8	6	7										
Core3	7	7	7	7	7	7	6	7	6	6	4	6	4	5	4
Core4	5	6	6	5	3	7	5	7	5	4	5	1	1		
Core5	6	10				4	4		6						
Core6	4	4	3	4	3	6	4	3	6	6	3	4	3	3	3

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Table 13.

Network Ecologies Classes (a.k.a. Core) encompass specific phylogenetic signature families based on inclusion of OTUs that are observed in at least 80% of a Class' networks.

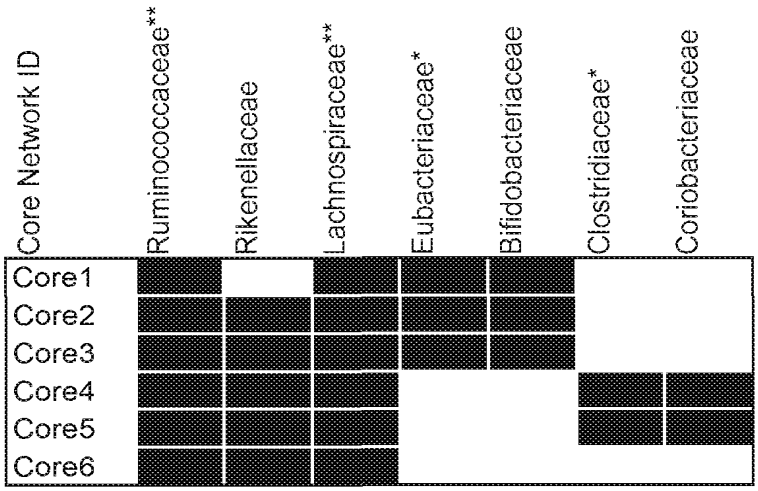

Table 14a.

Network Ecologies comprised of one or more clades, or two or more OTUs that are observed in the ethanol-treated spore preparation or the combined engrafted and augmented ecologies of at least one patient post-treatment with the bacterial composition. Network Ecologies are defined based on their clade or OTU content. The respective clades and 16S genetic sequences for each OTU are defined in Table 1. Network Ecology IDs with a ".s" indicates that the network is a subset of the computationally determined networks reported in Table 8 with the same Network Ecology ID.

Network Ecology ID	Num. of Clades in Network Ecology	Num. of OTUs in Network Ecology	Per. cent of OTUs that are Spore Forming	Per. cent of Net. mem. OTUs in Network Ecology	Exemplary Network clades	Exemplary Network OTUs	Exemplary keystone OTUs
N262.S	10	15	80	93.3	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Bifidobacterium dentium, Bifidobacterium longum, Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Bifidobacterium longum, Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N290.S	10	14	92.9	92.9	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_522 or clade_522i), clade_543	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Coprococcus eutactus, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans,	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques,

						Ruminococcus torques, Subdoligranulum variabile	Subdoligranulum variabile
N284.S	9	13	92.3	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_519, clade_538	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N271.S	9	13	84.6	92.3	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Streptococcus salivarius, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile

N282.S	9	13	84.6	92.3	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, clade_401, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Lactococcus lactis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N288.S	8	12	91.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N302.S	8	12	91.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_519	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile

N279.S	9	12	83.3	100	(clade_172 or clade_172i), (clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Bifidobacterium longum, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Bifidobacterium longum, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N310.S	7	11	90.9	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N323.S	7	11	90.9	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus catus, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile

N331.S	7	11	90.9	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_538	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Eubacterium siraeum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N332.S	8	11	81.8	90.9	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Streptococcus thermophilus, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N301.S	7	10	90	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N312.S	7	10	90	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques

N339.S	7	10	80	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_566 or clade_566f)	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Gordonibacter pamelaeae, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N325.S	8	10	90	90	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Anaerotruncus colihominis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques, Subdoligranulum variabile
N340.S	7	10	90	90	(clade_262 or clade_262i), (clade_354 or clade_354e), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Clostridium bartlettii, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N341.S	7	10	90	90	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Anaerotruncus colihominis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile

N346.S	7	10	90	90	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus albus, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N338.S	7	10	80	90	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i), (clade_98 or clade_98i)	Alistipes putredinis, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Streptococcus australis, Subdoligranulum variabile	Alistipes putredinis, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N336.S	6	9	88.9	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N345.S	7	9	88.9	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_494, clade_500	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile

N355.S	6	9	88.9	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_519	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N356.S	6	9	88.9	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_537	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus bromii, Ruminococcus torques, Subdoligranulum variabile
N343.S	7	9	77.8	100	(clade_262 or clade_262i), clade_286, (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Parabacteroides merdae, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Parabacteroides merdae, Roseburia inulinivorans, Ruminococcus torques
N329.S	6	9	88.9	88.9	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i)	Alistipes putredinis, Collinsella aerofaciens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N361.S	6	9	88.9	88.9	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_516 or clade_516c or clade_516g or clade_516h)	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus albus,	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques,

						Ruminococcus torques, Subdoligranulum variabile	Subdoligranulum variabile
N353.S	7	9	77.8	88.9	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500, clade_506	Alistipes putredinis, Dialister invisus, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N381.S	7	9	100	77.8	(clade_262 or clade_262i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, (clade_516 or clade_516c or clade_516g or clade_516h), (clade_522 or clade_522i), clade_537	Anaerotruncus colihominis, Clostridium leptum, Clostridium methylpentosum, Eubacterium eligens, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Clostridium leptum, Eubacterium eligens, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N344.S	6	8	87.5	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques
N352.S	5	8	87.5	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile

N357.S	6	8	87.5	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N358.S	5	8	87.5	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N369.S	6	8	87.5	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_522 or clade_522i)	Alistipes putredinis, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Eubacterium eligens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N372.S	6	8	87.5	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494, clade_500	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N375.S	6	8	87.5	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_537	Alistipes putredinis, Clostridium leptum, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Clostridium leptum, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile

N380.S	7	8	87.5	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_519, (clade_522 or clade_522i)	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques
N374.S	6	8	75	100	(clade_172 or clade_172i), (clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Bifidobacterium longum, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Bifidobacterium longum, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques
N377.S	6	8	75	100	clade_170, (clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Bacteroides caccae, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Bacteroides caccae, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N368.S	6	8	75	87.5	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_98 or clade_98i)	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques, Streptococcus salivarius	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N370.S	5	7	85.7	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus comes, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques

N373.S	6	7	85.7	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_485, clade_500	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Holdemania filiformis, Roseburia inulinivorans, Ruminococcus torques
N376.S	5	7	85.7	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Roseburia inulinivorans, Ruminococcus torques
N389.S	6	7	85.7	100	(clade_262 or clade_262i), clade_393, clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Coprococcus catus, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Coprococcus catus, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques
N394.S	5	7	85.7	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques, Subdoligranulum variabile
N431.S	6	7	85.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_519	Bacteroides ovatus, Dorea longicatena, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques
N434.S	6	7	85.7	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_38 or clade_38e or clade_38i), (clade_444 or clade_444i), (clade_478	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques	Bacteroides ovatus, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques

					or clade_478i), (clade_522 or clade_522i)		
N390.S	6	7	71.4	100	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, clade_521	Alistipes putredinis, Bilophila wadsworthia, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Bilophila wadsworthia, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques
N397.S	6	7	71.4	100	(clade_262 or clade_262i), (clade_38 or clade_38e or clade_38i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500	Alistipes putredinis, Bacteroides ovatus, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Bacteroides ovatus, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques
N387.S	6	7	85.7	85.7	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_553 or clade_553i)	Alistipes putredinis, Collinsella aerofaciens, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques
N440.S	7	7	85.7	85.7	(clade_262 or clade_262i), (clade_408 or clade_408b or clade_408d or clade_408f or clade_408g or clade_408h), (clade_444 or clade_444i), clade_445, (clade_478 or clade_478i), clade_494, clade_537	Clostridium leptum, Clostridium symbiosum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques	Clostridium leptum, Desulfovibrio desulfuricans, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques
N396.S	6	7	71.4	85.7	(clade_262 or clade_262i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_500, (clade_65 or clade_65e)	Alistipes putredinis, Bacteroides fragilis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques	Alistipes putredinis, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia inulinivorans, Ruminococcus torques

N399.S	6	6	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i), (clade_478 or clade_478i), clade_494	Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques	Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Faecalibacterium prausnitzii, Pseudoflavonifractor capillosus, Ruminococcus torques
N403.S	4	6	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i)	Coprococcus comes, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques	Coprococcus comes, Dorea longicatena, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques
N414.S	5	6	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), (clade_522 or clade_522i)	Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus torques	Coprococcus comes, Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Faecalibacterium prausnitzii, Ruminococcus torques
N430.S	6	6	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_519, (clade_522 or clade_522i)	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus torques	Dorea longicatena, Eubacterium eligens, Eubacterium rectale, Eubacterium ventriosum, Faecalibacterium prausnitzii, Ruminococcus torques
N432.S	4	6	100	100	(clade_262 or clade_262i), (clade_444 or clade_444i), (clade_478 or clade_478i), clade_537	Clostridium leptum, Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques	Clostridium leptum, Coprococcus comes, Eubacterium rectale, Faecalibacterium prausnitzii, Roseburia intestinalis, Ruminococcus torques
N436.S	4	6	100	100	(clade_262 or clade_262i), (clade_360 or clade_360c or clade_360g or clade_360h or clade_360i), clade_396, (clade_444 or clade_444i)	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Roseburia inulinivorans, Ruminococcus torques	Coprococcus comes, Dorea longicatena, Eubacterium hallii, Eubacterium rectale, Roseburia inulinivorans, Ruminococcus torques