(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 June 2008 (26.06.2008)

PCT

(10) International Publication Number
WO 2008/075949 A1

(51) International Patent Classification:
A61P 1/00 (2006.01) *A61P 3/10* (2006.01)
A61P 3/04 (2006.01) *A61P 1/14* (2006.01)
A61P 9/00 (2006.01) *A61P 37/00* (2006.01)
A61P 9/10 (2006.01) *A61K 35/74* (2006.01)
A61P 3/08 (2006.01)

(21) International Application Number:
PCT/NL2007/050667

(22) International Filing Date:
18 December 2007 (18.12.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
06126673.0 20 December 2006 (20.12.2006) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, 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, IS, JP, KE, KG, KM, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) **Title:** MODULATION OF HUMAN MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN (MTP OR MTTP) GENE EXPRESSION BY FOOD-GRADE/INGESTED DIETARY MICROORGANISMS

(57) **Abstract:** The present invention relates to the field of using microorganisms, especially food grade bacteria, to modulate intestinal MTP expression levels in order to treat and/or prevent weight gain, obesity, atherosclerosis, hyperglyceridaemia, hypercholesterolaemia, diabetes, dyslipidaemia and/or disorders associated with impaired intestinal immune response to antigens.

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Modulation of human microsomal triglyceride transfer protein (MTP or MTTP) gene expression by food-grade/ingested dietary microorganisms

FIELD OF THE INVENTION

The present invention relates to the field of food-grade microorganisms, especially bacteria and/or their components. Especially, the use of food-grade microorganisms, which are capable of modulating intestinal MTP gene expression, for the preparation of food or feed compositions, food or feed supplements or pharmaceutical compositions for the treatment and/or prevention of a sub-optimal (or non-healthy) intestinal microbiota, weight gain, obesity, atherosclerosis, hyperglyceridaemia, hypercholesterolaemia, diabetes, dyslipidaemia and/or disorders associated with impaired intestinal immune response to antigens is provided herein. Also provided are compositions comprising one or more microbial strains or components thereof (such as cell fractions) which are capable of modulating MTP gene expression in human intestinal cells or tissues *in vivo* and *in vitro*, as are methods for isolating such strains and for establishing administration/dosage regimes for such strains. Furthermore, the use of intestinal MTP gene expression as a biomarker for the health status of humans and their associated intestinal microbiota is provided herein.

BACKGROUND OF THE INVENTION

Microsomal triglyceride transfer protein (MTP), which catalyzes the transport of triglyceride, cholesterol ester and phospholipids between phospholipids surfaces, is a heterodimer, consisting of an 88 kDa catalytic domain which is non-covalently associated with a 58 kDa PDI (protein disulfide isomerase). The human cDNA and genomic DNA encoding the large subunit MTP have been cloned and characterized (Sharp et al. 1993, Nature 365: 65-69). The human MTP gene was found to be primarily expressed in liver and intestinal tissue, which is compatible with its proposed function in triglyceride transfer (Hagan et al. 1994, J Biol Chem 269: 28737-28744).

MTP plays a role in the assembly and secretion of apolipoprotein B (apoB) containing lipoproteins and high plasma levels of these lipoproteins may be associated with atherosclerosis and coronary heart diseases. Humans carrying non-functional MTP genes have a condition referred to as abetalipoproteinaemia and a defective production

of apoB-containing lipoproteins. Hepatic over-expression of MTP results in an increased *in vivo* secretion of VLDL (very low density lipoprotein) triglycerides and apoB (Tietge et al. 1999, J. Lipid Res. 40: 2134-2139). MTP liver-specific knock-out mice results in an abrogation of VLDL/LDL production (Chang et al. 1999, J Biol Chem 274: 6051-6055). Similarly, it was shown that the inhibition of MTP activity causes a decrease in the secretion rate of apoB-containing lipoproteins in human and intestinal cells in vitro (Jamil et al. 1996, PNAS 93: 11991-11995; Van Greevenbroek et al. 1998, J Lipid Res 39: 173-185). These findings, therefore, suggest that MTP plays a role in modulating lipoprotein production in the liver and intestine.

Recently, MTP was found to be also involved in modulating the intestinal immune response to antigens. Lipid antigens are presented to T cells by CD1 molecules, which are major histocampatibility complex (MHC) class I-homologues. CD1 is expressed on myeloid cells, hepatocytes and intestinal epithelial cells. The type I CD1 molecules (CD1a, CD1b and CD1c) are expressed on dendritic cells in the intestinal mucosa. It was shown previously that MTP regulates CD1d function and, hence, natural killer T (NKT) cell biogenesis (Brozovic et al. 2004, Nature Med 10: 5: 535-9). Recent observations that MTP is involved in antigen presentation, and is able to regulate CD1a, CD1b and CD1c production, suggest that MTP is important in the host response to microbial pathogens. The presence of type 1 CD1 molecules on dendritic cells indicates a putative role of MTP in the pathogenesis of mucosal inflammation-related disorders (Kaser A, Hava D, Yoshida M, Kuo T, Nagaishi T, Dougan S, Lugt Vander B, Haddad W, Brenner M, Blumberg R. Microsomal triglyceride transer protein regulates endogenous and exogenous antigen presentation by group 1 Cd1 molecules. Gastroenterology 2006;130: 4 suppl 2; 126). Furthermore, recent data suggest that MTP regulates an intestinal barrier-protective CD1d pathway that is mediated by NKT cells (Kaser A, Yoshida M, Furuta G, Zhu F, Davidson N, Colgan S, Blumberg R. Intestinal Microsomal Triglyceride Transfer Protein (MTP) regulates Cd1d function on the intestinal epithelium and protects from mortality in the oxazolone model. Gastroenterology 2006;130: 4 suppl 2; 126).

The regulation of MTP gene expression seems to be complex. In hamsters, high fat and high cholesterol diets have been shown to up-regulate hepatic MTP mRNA levels. *In*

vitro studies on human hepatoma cells have shown that ethanol (Lin et al. 1997, FASEB J. 11, 1145-1152), fresh garlic (Lin et al. 2002, J Am Soc Nutr Sci, 132: 1165-1168) and insulin (Lin et al. 1995, J Lipid Res 36: 1073-1081) down-regulate hepatic MTP expression.

Some drugs, such as diaminoindanes and benimidazole-based compounds, with MTP-inhibitory activity are under investigation for the treatment of hyperlipidemia (Burnett JR 2006, IDrugs, Jul;9(7):495 -9; Chandler CE, Wilder DE, Pettini JL, Savoy YE, Petras SF, Chang G, Vincent J, Harwood HJ Jr, J Lipid Res. 2003 44(10):1887-901). These drugs mostly inhibit both liver and the intestinal MTP expression and may result in fatty livers. Intestinal-specific MTP inhibitors would be desirable, preferably ones which are based on natural food-grade products. Fresh garlic seems to contain MTP inhibiting substances which are relatively specific for inhibiting intestinal MTP expression in rats, 3 hours after oral administration (Lin MC, Wang EJ, Lee C, Chin KT, Liu D, Chiu JF, Kung HF, J Nutr. 2002 Jun;132(6):1165-8).
However, the components, which are responsible for this effect, have not yet been identified and it is not clear whether the effect and specificity would be the same in humans. Furthermore, the addition of large quantities of fresh garlic to food products would limit the applicability to certain types of food.

It is, therefore, an object of the invention to provide alternative, food grade MTP-expression modulators, especially MTP gene expression inhibitors and activators, methods for identifying and isolating these and compositions comprising these. It is a further object of the invention to provide a method for evaluating and monitoring the health status of the human intestinal microbiota, and means for distinguishing between a healthy intestinal microbiota and an unhealthy or sub-optimally healthy intestinal condition. In addition, means for treating and/or preventing a sub-optimal or abnormal intestinal microbiota are provided, whereby a sufficient amount of a MTP-gene expression modulating composition, comprising or consisting of at least one microorganism capable of modulating intestinal MTP-gene expression, is administered.

GENERAL DEFINITIONS

"Lactic acid bacteria" and "lactic acid producing bacteria", is used herein interchangeably and refers to bacteria, which produce lactic acid as an end product of fermentation, such as, but not limited to, bacteria of the genus *Lactobacillus*, *Streptococcus, Lactococcus, Oenococcus, Leuconostoc, Pediococcus, Carnobacterium, Enterococcus*. In addition, *Bifidobacterium* and *Propionibacterium* species are considered for this application to belong to lactic acid bacteria although they have a distinct phylogenetic position.

"Probiotics" or "probiotic strain(s)" refers to strains of live or viable micro-organisms, preferably bacteria, which when administered in adequate amounts provide a health benefit to the host subject, e.g. when ingested (e.g. orally, enterally or by inhalation) by a subject. Probiotics are defined as "viable microbial food supplements which, when taken in the right doses beneficially influence human health" (Salminen et al. 1998, WHO 2002).

"Micro-organisms" include bacteria and fungi, such as yeasts. When reference herein is made to bacteria, it is understood that the embodiments also apply to other microorganisms.

"Enteral" refers herein to the delivery directly into the gastrointestinal tract of a subject (e.g. orally or via a tube, catheter, capsules or stoma).

"Food-grade" micro-organisms are in particular organisms, which are considered as not harmful, when ingested by a human or animal subject.

"Components" of microorganisms or "inactivated" microorganisms refers to non-viable microorganisms, such as dead cells, cell fragments, and the like.

A "subject" refers herein to a human or animal, in particular a vertebrate, such as but not limited to domestic animals.

The term "comprising" is to be interpreted as specifying the presence of the stated parts, steps or components, but does not exclude the presence of one or more additional parts, steps or components. A composition comprising a lactic acid bacterium may thus comprise additional bacterial strains etc. However, a lactic acid bacterium or a mixture of several distinct lactic acid bacteria is preferably a main active component of a composition of the invention. More preferably, a lactic acid bacterium or a mixture of several distinct lactic acid bacteria is the sole active component of a composition of the invention.

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