ides from four separate regions of the poliovirus type 1 id protein VP1 induce neutralising antibodies. *Proceedings e National Academy of Sciences USA* 1985; **82:** 910–14.
on M, Evans DMA, Magrath DI, Minor PD, Almond JW, ld GC. Induction of broadly reactive, type specific ralising antibody to poliovirus type 3 by synthetic peptides. *logy* 1985; **143:** 505–15.
nd DC, Jameson BA, Brown J, Kohara M, Abe S, Itoh H, natsu T, Arita M, Kuge S, Osterhaus ADME, Crainic R, noto A, Wimmer E. Antigenic variation and resistance to tralization in poliovirus type 1. *Science* 1983; **229:** 1090–3.
PD, Evans DMA, Ferguson M, Schild GC, Westrop G, Almond JW. Principal and subsidiary antigenic sites VP1 involved in the neutralization of poliovirus type 3. *Journal of General Virology* 1985; **65:** 1159–65.
11 Blondel B, Crainic R, Fichot O, Dufraisse G, Cardrea A, Girard M, Horaud F. Mutations conferring resistance to neutralization with monoclonal antibodies in type 1 poliovirus can be located outside or inside the antibody binding site. *Virology* 1986, (in press).
12 Wychowski C, van der Werf S, Siffert O, Crainic R, Bruneau P, Girard M. A poliovirus type 1 neutralization epitope is located within amino acid residues 93–104 of viral capsid polypeptide VP1. *EMBO Journal* 1983; **2:** 2019–24.

Biosurfactants

DG Cooper*

Many different types of biosurfactants are synthesized by microorganisms. As the structures and properties are elucidated, yields increased and costs of recovery from the fermentation media reduced, biosurfactants will become important industrial chemicals.

Introduction

Conventional surfactants are currently used for a broad range of purposes in a large variety of different applications.[1] Most requirements for a conventional surfactant could be met by a biosurfactant. To justify replacement of a synthetic surfactant with a biological compound, it is necessary to find a more effective agent for a given application, and/or one that can be produced more cheaply.

This article discusses the structures and properties of biosurfactants, their production and isolation from fermentation broths, and their potential for commercial exploitation (particularly the more unusual compounds produced by microbes). These have unique properties because of their structures and thus have the best potential for application to a specific niche.

A surfactant is a molecule which has both water-soluble and water-insoluble (usually hydrocarbon) portions.[2] This balance of hydrophilic and hydrophobic moieties in the same molecule imparts unusual properties, including an ability to lower the surface tension of water. Unfortunately, the term biosurfactant has generally been used very loosely to refer to any compound which has some influence on interfaces. For example, it is often applied to biopolymers which have emulsifying properties but do not lower the surface tension of water appreciably or demonstrate other characteristics of a classical surfactant. This article considers both the biological compounds which fit the classical definition of a surfactant as well as the larger, poorly defined polymers or cell fragments which have some form of surface activity. Several longer review articles have been published on various aspects of biosurfactants.[3–5]

Structures

Biosurfactants have many different structures. Most are lipids, which have the typical amphiphilic structure of a surfactant. The lipophilic portion of lipids is almost always the hydrocarbon tail of one or more fatty acids (Figure 1) which may be saturated or unsaturated and may contain cyclic structures or hydroxyl functions. The polar, water-soluble part of a biosurfactant may be as simple as a carboxylate or hydroxyl function or a complex mixture of phosphate, carbohydrate, amino acids, etc.

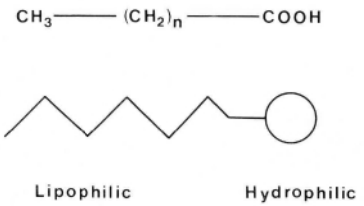

Figure 1. *Carboxylic acids and other lipids have the amphipathic structure of a surfactant.*

Most biosurfactants are either neutral or negatively charged. In anionic biosurfactants the charge is due to a carboxylate and/or phosphate or, occasionally, to a sulphate group. A small number of cationic biosurfactants contain amine functions.

Biosurfactants may be classified on the basis of their lipid types.[3] The simple neutral lipid surfactants include esters, alcohols and mono-, di- and triglycerides. The phospholipids contain diglyceride structures, phosphate and a wide range of polar groups. Glycolipids vary from the ubiquitous glycosyl glycerides to the many complex compounds produced by microbes. Finally, there are several examples of lipopeptide biosurfactants.

Carboxylic acids, neutral lipids and phospholipids are well known constituents of all cells and the usual types will not be considered here. A more unusual group of hydroxycarboxylic acids are common in microbial biosurfactants. They have useful surfactant properties on their own and are common constituents of complex biosurfactants.

Figure 2 illustrates one common type of hydroxy-acid. These α-branced, β-hydroxy acids are highly variable.[3] The shorter, corynomycolic acids with 20 to 40 carbon atoms are particularly common in biosurfactants; unbranched, hydroxy acids are also found. The hydroxyl group can be either adjacent to the carboxylic group or at the opposite end of the hydrocarbon chain. A more complex carboxylic acid surfactant is 4-hydroxy-4, 5-dicarboxypentadecanoic acid, with three carboxyl groups and a hydroxyl group in the polar portion.[6]

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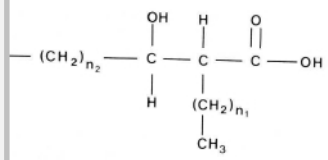
Received 13 December, 1985.

pids with the most unusual structures, and hence test potential for unique properties, are the l glycolipids and lipopeptides. In general, these e synthesized as mixtures and in most cases there ciable variation in the types of fatty acids incor- and even in the polar groups or the points of ent between the two portions.

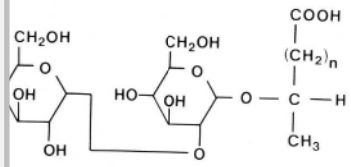
rst of these glycolipid surfactants to be studied e sophorose lipids produced by *Torulopsis* (Figure 3).[3–5] The sophorose is attached to an xy fatty acid through the hydroxyl moiety, g in a surfactant structure with a single hydro- portion between two polar structures. Several have been issued for this biosurfactant.[7,8] The lipids from *Pseudomonas aeruginosa* and the e lipids isolated from a variety of bacteria also various hydroxy fatty acids and carbohydrates e surfactant properties.[3–5,9]

ost thoroughly studied lipopeptide surfactant is d by *Bacillus subtilis* and given the trivial name n (Figure 4).[3–5] The seven amino acids in this nd form a ring and are bonded to both the l and hydroxyl groups of the acid. Other ide surfactants have been reported but only a e been characterized completely.

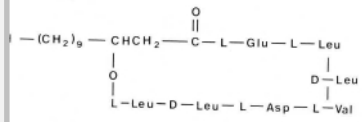
are also examples of one or two amino acids



An α-branched, β-hydroxy carboxylic acid. For cory- acids n_1 plus n_2 varies between 20 and 40.



One type of glycolipid from Torulopsis *species g sophorose and an ω-hydroxy carboxylic acid.*



Structure of surfactin from Bacillus subtilis.

attached to fatty acids. These biosurfactants are zwitterionic (they can carry both positive and negative charges). The most interesting example is cerilipin from *Gluconobacter cerinus*, which contains the unusual amino acid taurine: this makes it one of the few biosurfactants with a sulphate group.

The remaining types of surface-active compounds are polymeric. These are often referred to as biosurfactants but a more appropriate word would be bioemulsifiers. In general, these are poorly defined polysaccharides and often contain some protein or carboxylic acids.[3–5,10,11] The most thoroughly studied bioemulsifier is emulsan, produced by *Acinetobacter calcoaceticus* and composed mainly of amino sugars and fatty acids.[12] For the polymeric emulsifiers which have been reported, the emulsifying properties have been characterized much more thoroughly than the structures.

Properties

The simplest test for surface activity is the measurement of the surface tension of an aqueous system. In most cases, the preliminary testing is done with the whole microbial growth medium. An organism can be considered promising if it produces compounds which reduce the surface tension to below 40 mN m^{-1}. A good biosurfactant will produce values below 35 mN m^{-1}, but the most effective biosurfactant reported is surfactin from *Bacillus subtilis* (producing a surface tension of 27 mN m^{-1}).

Relatively little work has been done with purified biosurfactants.[9] The presence of other materials in the whole broth samples causes some deviation from the true value for the active compounds but this is insignificant when compared to the large change from the surface tension of pure water (72 mN m^{-1}). In fact, most applications of biosurfactants will be with the whole broth or only partially purified mixtures because of economic considerations. Thus, the surface tensions and other properties of these crude systems are the relevant data for these uses. These properties have been used to screen for suitable biosurfactants.

Many studies have been carried out to select microorganisms for use in the petroleum industry and especially those suitable for enhanced oil recovery.[4,13–15]

It is possible to select for biosurfactants which can wet solid surfaces. It has been known for some time that *Thiobacillus thiooxidans* produces mixtures of phospholipids which wet sulphur particles.[3] A recent study to select biosurfactants to enhance peat de-watering, demonstrated a correlation between the ability to wet wax, extracted from the peat, and the ability to improve water loss.[16]

The ability of surfactin to lyse red blood cells is of limited use, but this discovery has led to a quick method for screening microbes growing on solid media for their ability to produce biosurfactants.[4,17]

Many studies have been published on the testing of microbes for their ability to produce emulsifiers.[3,4,18,19]

By altering the aqueous phase it was possible to test for stability of the emulsions to pH changes, salt additions, heat, etc. A wide range of different oil phases were used in the tests including pure hydrocarbons, crude oils and vegetable oils. Many of the emulsifiers that were characterized were found to be polymeric, with minimal ability to lower surface tension.

Production

Biosurfactants are produced by a wide range of microbes. While all microbes produce lipids which have surface activity, most interest lies with those capable of producing good yields of extracellular products. The highest yield reported is for the sophorose lipids from *Torulopsis bombicola* (35 g g^{-1} of substrate).[20] Unfortunately, most yields are much less than this and are not high enough to be economic. Work has begun to develop strain-selection techniques and this will allow the development of overproducing mutants.[17]

It has been suggested that microbes release biosurfactants as a mechanism for obtaining water-insoluble substrates.[3,5,13] This cannot be the only biological function as there are many examples of microbes producing surface-active agents when growing on soluble substrates.[3,10,21] Some microbes can produce surfactants when grown on many different substrates ranging from carbohydrates to hydrocarbons. Changing the substrate often alters the structure of the product which, in turn, alters its surfactant properties; this can be useful when designing a product with the appropriate properties for a given application.

There are many examples in the literature of changes in the carboxylic acids incorporated into lipids achieved by manipulating the substrate.[3,22] It has been shown that small variations in carboxylic acids can have dramatic effects on surfactant properties. There are also examples of more substantial changes, such as modification of the polar group in a biosurfactant by changing the substrate or growth conditions.

Relatively few of the microbes known to have some surface activity associated with them have been grown and monitored in fermenters. There is no standard pattern for all those that have been studied, but many synthesize a biosurfactant throughout the exponential growth phase. This has been observed for the production of both biosurfactants and bioemulsifiers.[3,11,22,23]

Very distinct maxima are observed during the exponential growth of *Corynebacterium lepus* producing an unidentified surfactant.[22] In the *C. lepus* fermentation the free corynomycolic acid disappears because it is incorporated into a lipopeptide surfactant which appears late in the fermentation. Similar behaviour has been reported for the production of a bioemulsifier by *Candida lipolytica*.[24]

Rhodococcus erythropolis produces glycolipids in two stages:[25] after an early plateau in biosurfactant concentration, there is a second burst of production. *Bacillus subtilis* can be induced to yield a second, large amount of the lipopeptide surfactin by the addition of iron or manganese salts to the fermenter after active growth is over.[21,22]

Late production of glycolipid by *Torulopsis bombicola* can be induced by substrate manipulation.[20] This yeast requires both carbohydrate and vegetable oil to yield biosurfactant; if it is grown on only one substrate and the second is added after growth is finished there is an immediate burst of product formation.

The many different modes of biosynthesis of surfactants support the contention that cells are producing them for a variety of purposes. In many cases there are opportunities to influence the fermentation to increase yields and decrease costs.

All of the above fermentations were aerobic. There has been very little investigation of the production of these compounds by anaerobic culture. A recent study of *Bacillus licheniformis* characterized a very effective biosurfactant isolated after strict anaerobic growth.[26] This compound, which may have an application in *in situ* enhanced oil recovery, was a lipopeptide and appears to be very similar to surfactin from *Bacillus subtilis*.

A major problem with the economics of biosurfactants is that the fermentations result in dilute aqueous solutions of the desired products. Often solvent extractions are used to recover these compounds.[3,11,18] Some can be recovered by altering the pH and collecting a precipitate,[21,26] but crude sophorolipid is unusually easy to obtain, separating as a denser phase from the fermentation broth of *T. bombicola*.[20]

Applications

Surface-active agents are needed for a very large number of diverse applications[1] and there is no industry which does not have some use for these compounds. Emulsion stabilization is a very common requirement for food products, cosmetics, cutting oils, etc. Demulsifiers are required, for example in de-watering of crude oil. Surfactants are also useful as soaps and detergents, both for simple cleaning applications and for more exotic purposes, such as enhanced oil recovery and oil spill clean-up.

Surface-wetting and solid dispersal are important properties for froth-flotation separation of ores or preparation of coal slurries for pipelining. Colloid preparations are necessary for paints and related products. Penetration rates of inks, dyes, etc. are important for the pulp and paper and textile industries.

There are many instances where excessive foaming must be regulated with surfactants, and the property of foam stabilization is also necessary for fire extinguishers and in the food industry. Other desirable properties are lubrication, corrosion inhibition and static inhibition.

The above cursory list gives some overall indications of the breadth of applications of these compounds in industry. The total use of all of these products in the United States alone in 1982 was 2.5 million metric tons.[1] Virtually all of these compounds were synthesized

lly. Large amounts of natural products, especi-
n and triglycerides, were used as feedstocks but
cant portion were prepared from petroleum.
many surfactants already available it is reason-
question whether biosurfactants from microbes
uture in industry. A strong argument in their
s that any new surfactant is potentially useful.
e so many different applications, each requiring
y different mix of properties, that it is always
have new products of this type. Many of the
es of the biosurfactants are so different from the
compounds that they will have novel combina-
properties. Ideally, one is looking for a biosur-
which has unique characteristics suitable for an
ion with a high enough value to justify the cost of
entation and product isolation.

ny of the applications of surfactants there is a
nent for a number of compounds with a grada-
some property. For example, in applications
g crude oil there is a broad range of types of oils
rvoir conditions and each requires slightly dif-
urfactant properties. The ability to make small
ns in biosurfactant structure by altering growing
ns or the substrate is a simple method of gener-
amily of related compounds with a spectrum of
es.

rfactants can be synthesized from renewable
es. Inevitably, petroleum resources are being
l and, at some time in the future, the only source
ctants will be from renewable feedstocks. In
, synthetic surfactants usually require pure
ls, or at least classes of chemicals (i.e. alkanes)
ce the desired product. This is not crucial for a
ation: in fact, it is possible to produce biosurfac-
fermenting waste streams containing carbo-
s, fatty acids or similar compounds.

y, biosurfactants are all biodegradable and do
ent the same pollution problems as some surfac-
here is less likelihood of toxicity problems
the intrinsic surfactant properties could be
ous regardless of the source of the compound.

trial application of biosurfactants remains at the
ment stage. The oil industry is a clear target for
mpounds since it uses large amounts of surface-
mpounds and the application is such that crude
or even a whole fermentation broth could be
addition, there is less rigorous testing than there
od and cosmetic formulations, and many of the
ctant-producing microorganisms can use pet-
products as substrates.

cations are also being considered for biosurfac-
industries involving food, cosmetics, pulp and
coal beneficiation, textiles and ore-processing.
ommercially viable, these compounds must be
d cheaply and easily; the surfactant properties
e uniquely suited to the application.

otential use of biosurfactants to de-water fuel-
at is an example of an application where biosur-
have unique advantages over conventional agents.[16] Biosurfactants are added to the process before mechanical pressing to improve water loss significantly. The wastewater stream contains dissolved organics and is a potential pollutant: some of the biosurfactant-producing microbes, notably *Bacillus subtilis*, can use this waste stream as a substrate.[27] This lowers the biological oxygen demand of the effluent and produces the de-watering additive. As the peat bogs are often in remote locations, there is a distinct advantage in producing the de-watering agent on site from a readily available feedstock.

Conclusions

Biosurfactants are diverse and ubiquitous and there is a high probability of finding a compound with the appropriate combination of properties for a specific application. They can be biosynthesized from inexpensive, renewable substrates and they are biodegradable. Before most of these compounds can be successfully commercialized, it will be necessary to improve yields and lower product-separation costs.

References

1 Layman PL. Industrial surfactants set for strong growth. *Chemical and Engineering News* 1985; **63 (3):** 23–48.
2 Tadros TF, ed. *Surfactants*. London: Academic Press, 1984.
3 Cooper DG, Zajic JE. Surface-active compounds from microorganisms. *Advances in Applied Microbiology* 1980; **26:** 229–53.
4 Singer ME. Microbial biosurfactants. *Microbes and Oil Recovery* 1985; **1:** 19–38.
5 Zajic JE, Seffens W. Biosurfactants. *Critical Reviews in Biotechnology* 1984; **1:** 87–107.
6 Zajic JE, Ban T. Spiculisporic acid. *Microbes and Oil Recovery* 1985; **1:** 310–20.
7 Inoue S, Kimura Y, Kinta M. Process for producing a glycolipid ester. *US Patent 4,215,213. Issued July 29, 1980.*
8 Inoue S, Kimura Y, Kinta M. Dehydrating purification process for a fermentation product. *US Patent 4,197,166. Issued April 8, 1980.*
9 Krestschmer A, Bock H, Wagner F. Chemical and physical characterization of interfacial-active lipids from *Rhodococcus erythropolis* grown on *n*-alkanes. *Applied and Environmental Microbiology* 1982; **44:** 864–70.
10 Cooper DG, Paddock DA. *Torulopsis petrophilum* and surface activity. *Applied and Environmental Microbiology* 1983; **46:** 1426–9.
11 Cirigliano MC, Carman GM. Purification and characterization of liposan, a bioemulsifier from *Candida lipolytica*. *Applied and Environmental Microbiology* 1985; **50:** 846–50.
12 Gutnick DL, Rosenberg E, Belsky I, Zosim Z. Emulsans. *US Patent. Issued July 26, 1983.*
13 Janshekar H. Microbial enhanced oil recovery processes. *Microbes and Oil Recovery* 1985; **1:** 54–84.
14 Jack TR, Thompson BG. Patents employing microorganisms in oil production. In: Zajic JE, Cooper DG, Jack TR, Kosaric N, eds. *Microbial Enhanced Oil Recovery*, Tulsa, Oklahoma: Penn Well Books 1983; 14–25.
15 Zajic JE, Gerson DF. Microbial extraction of bitumen from Athabasca oil sand. In: Strausz O, ed. *Oil Sands and Oil Shale*, New York: Verlag Chemie, 1978, 145–61.
16 Cooper DG, Pillon DW, Mulligan CN, Sheppard JD. Biological additives for improved mechanical de-watering of fuel-grade peat. *Fuel* 1986; **65:** 255–9.

17 Mulligan CN, Cooper DG, Neufeld RJ. Selection of microbes producing biosurfactants in media without hydrocarbons. *Journal of Fermentation Technology* 1984; **62:** 311–14.
18 Akit J, Cooper DG, Manninen KI, Zajic JE. Investigation of potential biosurfactant production among phytopathogenic corynebacteria and related soil microbes. *Current Microbiology* 1981; **6:** 145–50.
19 Cooper DG, Liss SN, Longay R, Zajic JE. Surface activity of *Mycobacterium* and *Pseudomonas*. *Journal of Fermentation Technology* 1981; **59:** 97–101.
20 Cooper DG, Paddock DA. Production of a biosurfactant from *Torulopsis bombicola*. *Applied and Environmental Microbiology* 1984; **47:** 173–6.
21 Cooper DG, Macdonald CR, Duff SJB, Kosaric N. Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. *Applied and Environmental Microbiology* 1981; **42:** 408–12.
22 Cooper DG. Unusual aspects of biosurfactant production. In: Ratledge C, Dawson P, Rattray J, eds. *Biotechnology for the Oils and Fats Industry*. American Oil Chemists Society, 1984: 281–7.
23 Macdonald CR, Cooper DG, Zajic JE. Surface active lipids from *Nocardia erythropolis* grown on hydrocarbons. *Applied and Environmental Microbiology* 1981; **41:** 117–23.
24 Cirigliano MC, Carman GM. Isolation of a bioemulsifier from *Candida lipolytica*. *Applied and Environmental Microbiology* 1984; **48:** 747–50.
25 Rapp P, Bock H, Wray, Wagner F. Formation, isolation and characterization of trehalose dimycolates from *Rhodococcus erythropolis* grown on n-alkanes. *Journal of General Microbiology* 1979; **115:** 491–503.
26 Javaheri M, Jenneman GE, McInerney MJ, Knapp RJ. Anaerobic production of a biosurfactant by *Bacillus licheniformis* JF-2. *Applied and Environmental Microbiology* 1985; **50:** 698–700.
27 Mulligan CN, Cooper DG. Pressate from peat de-watering as a substrate for bacterial growth *Applied and Environmental Microbiology* 1985; **50:** 160–2.

Biotechnological applications of carboxydotrophic bacteria

E Williams* & J Colby

Carbon monoxide (CO) is a widespread pollutant and a hazard to man because of its extremely toxic nature. It is a major component of some industrial gas mixtures and may be derived from coal. The carboxydotrophic bacteria obtain energy and carbon from the oxidation of CO. These organisms may be used to produce new metabolites, and the oxidases from them may be used to produce fuel cells and biosensors for CO.

Introduction

Carbon monoxide is a major atmospheric pollutant occurring in rural areas at a concentration of 0.1 p.p.m. and in urban districts between 50–100 p.p.m.[1] It is a colourless, odourless, tasteless and explosive gas with flammable limits in air of 12–75% and an ignition point in air of 700°C. It is only sparingly soluble in water; 3.3 ml/100 ml H_2O at 0°C, 2.3 ml/100 ml H_2O at 20°C. Carbon monoxide is extremely toxic to aerobic organisms because of its affinity for the metal ions of respiratory chain components, and in man it binds easily to haemoglobin and can cause rapid and lethal toxaemia. Anthropogenic emissions of CO exceed all other pollutants and about 1.4×10^8 t are added annually from the incomplete combustion of fossil fuels. It is also a major component of volcanic gases (1 to 4%), resulting in a further annual addition of 1×10^8 t to the atmosphere and is released from the oceans (where supersaturation factors > 30 may be found in the surface layers) and from the earth's crust.[2,3]

Biogenic contributions to the global production of CO are extremely small in comparison to abiogenic sources, but a variety of living systems evolve CO in small amounts. Man produces small amounts of CO from the oxidation of haemoglobin by microsomal haem oxygenase and in enclosed conditions, such as in submarines, this may accumulate and may reach dangerous concentrations. However, man's contact with CO is usually from exposure to domestic and industrial combustion processes such as faulty domestic heating systems, blast furnace gas (25% CO), automobile exhaust gases (0.5 to 12% CO) and smoking tobacco (cigarette smoke contains 2 to 5% CO, cigar and pipe tobacco smoke contains 5 to 14% CO). Carbon monoxide may also accumulate in coal mines and during the treatment and transport of sewage.

Industrial gases containing carbon monoxide

Gas mixtures containing CO are widely used as cheap feedstocks in many chemical industries and there is increasing interest in producing these mixtures from coal, lignite and peat, which currently account for about 47% of the available energy content in recoverable reserves of known fossil fuels.[1] A coal-based synthesis gas industry existed in the United Kingdom until the 1960s converting Town gas into synthesis gas, consisting of carbon monoxide and hydrogen, for use in producing ammonia by the Haber process or for conversion to methanol. Cheaper naphtha and processes based on methane later replaced this coal-based technology in the

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Received 8 October, 1985.