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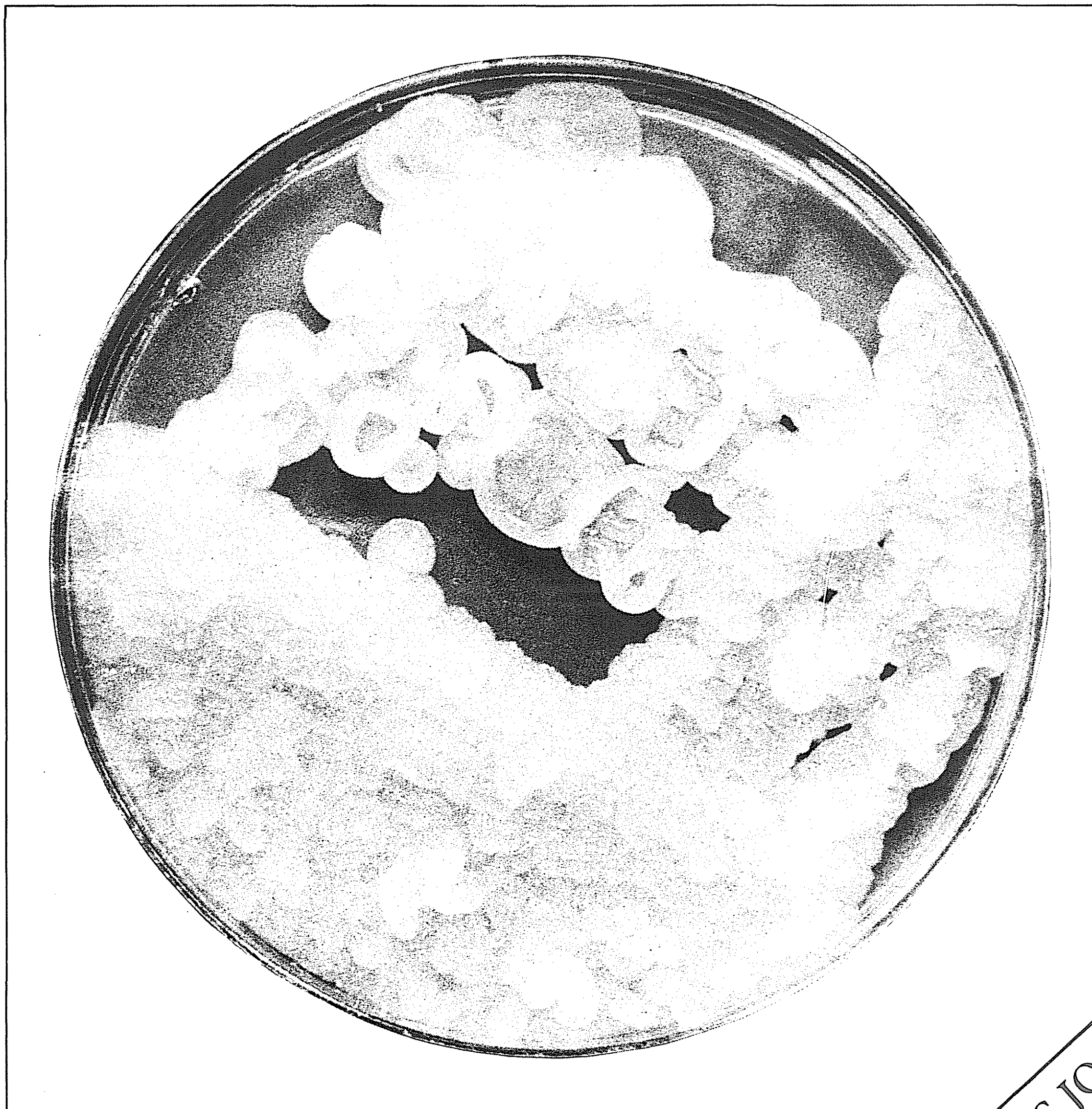

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Biosurfactants

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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.

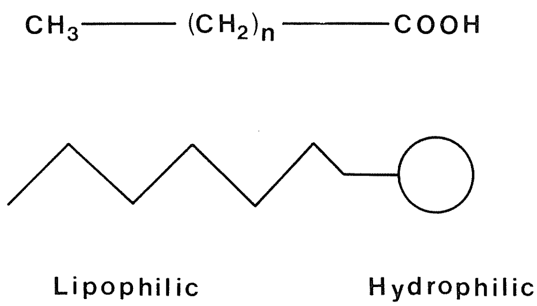


Figure 1. *Carboxylic acids and other lipids have the amphipathic structure of a surfactant.*

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

The lipids with the most unusual structures, and hence the greatest potential for unique properties, are the microbial glycolipids and lipopeptides. In general, these lipids are synthesized as mixtures and in most cases there is appreciable variation in the types of fatty acids incorporated and even in the polar groups or the points of attachment between the two portions.

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

The most thoroughly studied lipopeptide surfactant is produced by *Bacillus subtilis* and given the trivial name surfactin (Figure 4).[3–5] The seven amino acids in this compound form a ring and are bonded to both the carboxyl and hydroxyl groups of the acid. Other lipopeptide surfactants have been reported but only a few have been characterized completely.

There are also examples of one or two amino acids 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.

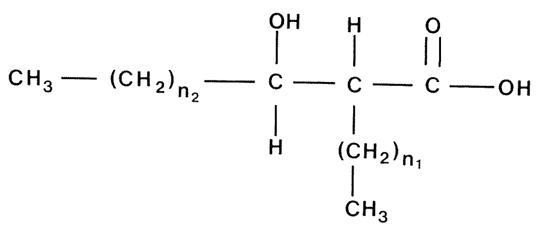


Figure 2. *An α-branched, β-hydroxy carboxylic acid. For corynomycolic acids n_1 plus n_2 varies between 20 and 40.*

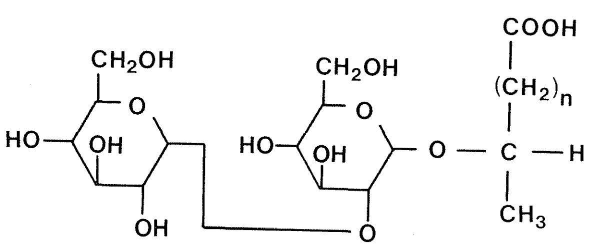
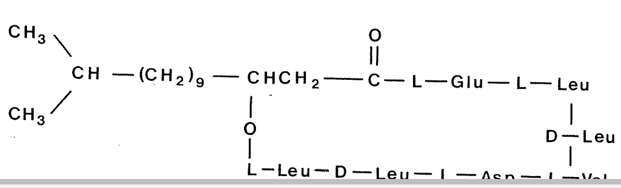


Figure 3. *One type of glycolipid from* Torulopsis *species containing sophorose and an ω-hydroxy carboxylic acid.*



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

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 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 chemically. Large amounts of natural products, especially lignin and triglycerides, were used as feedstocks but a significant portion were prepared from petroleum. With so many surfactants already available it is reasonable to question whether biosurfactants from microbes have a future in industry. A strong argument in their favour is that any new surfactant is potentially useful. There are so many different applications, each requiring a slightly different mix of properties, that it is always useful to have new products of this type. Many of the structures of the biosurfactants are so different from the synthetic compounds that they will have novel combinations of properties. Ideally, one is looking for a biosurfactant which has unique characteristics suitable for an application with a high enough value to justify the cost of the fermentation and product isolation.

In many of the applications of surfactants there is a requirement for a number of compounds with a gradation of some property. For example, in applications involving crude oil there is a broad range of types of oils and reservoir conditions and each requires slightly different surfactant properties. The ability to make small alterations in biosurfactant structure by altering growing conditions or the substrate is a simple method of generating a family of related compounds with a spectrum of properties.

Biosurfactants can be synthesized from renewable substrates. Inevitably, petroleum resources are being depleted and, at some time in the future, the only source of surfactants will be from renewable feedstocks. In addition, synthetic surfactants usually require pure chemicals, or at least classes of chemicals (i.e. alkanes) to produce the desired product. This is not crucial for a fermentation: in fact, it is possible to produce biosurfactants by fermenting waste streams containing carbohydrates, fatty acids or similar compounds.

Finally, biosurfactants are all biodegradable and do not present the same pollution problems as some surfactants. There is less likelihood of toxicity problems although the intrinsic surfactant properties could be deleterious regardless of the source of the compound.

Industrial application of biosurfactants remains at the development stage. The oil industry is a clear target for these compounds since it uses large amounts of surface-active compounds and the application is such that crude extracts or even a whole fermentation broth could be used. In addition, there is less rigorous testing than there is for food and cosmetic formulations, and many of the biosurfactant-producing microorganisms can use petroleum products as substrates.

Applications are also being considered for biosurfactants in industries involving food, cosmetics, pulp and paper, coal beneficiation, textiles and ore-processing. To be commercially viable, these compounds must be produced cheaply and easily; the surfactant properties should be uniquely suited to the application.

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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.

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