

## MINIREVIEW

### Microbial Surfactant

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**Abstract.** Microbial surface active agents (biosurfactant) have recently been recognized as important microbial products with properties applicable in a number of industries and processes. Being capable of lowering surface- and interfacial-tension, biosurfactants are today thought to be efficient replacers and possible enhancer of chemically synthesized surface-active agents. Some of their superior, such as absence of toxicity, biodegrade ability, and their specificity, make these microbial products both attractive for specific industries and environmentally acceptable. Most of the emphasis to date has been on the application of biosurfactants in petroleum-related activities and industries. They offer attractive products for use in enhanced oil recovery, in cleaning oil spills, in oil emulsification, and in breaking industrially derived oil-in-oil emulsions. Their in situ and ex situ utilization in enhanced oil recovery represent attractive alternatives. More recently, other applications of biosurfactants have also been under development. These include applications in the food industry, pharmaceuticals, and cosmetics, this article emphasizes the effect of nutritional and environmental factors on the production of biosurfactants.

**Keywords.** Biosurfactant, Classification, Carbon sources, Nitrogen sources, Production

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#### SURFACTANT

Surfactants are **SUR**Face **ACT**ive Age**NTS** with wide ranging properties including the lowering of surface and interfacial tensions of liquids. Surface tension is defined as the free surface enthalpy per unit area (**OECD** 1995) and is the force acting on the surface of a liquid leading to minimization of the area of that surface. Both synthetic and natural surfactants exist capable of reducing the surface tension of water from 72 mN m<sup>-1</sup> to around 27 mN m<sup>-1</sup> (Christofi and Ivshina 2002). Biosurfactants are biological compounds that exhibit high surface-active properties (Georgiou *et al.*, 1992). Microbial-derived surfactants or biosurfactants are produced by a wide variety of microbes and are amphipathic molecules with a hydrophilic and a hydrophobic domain, seem to facilitate the uptake of hydrocarbons into cells. Because of these traits, biosurfactants accumulate at interfaces, can form micelles, lower the surface tension and thereby enhance the solubility of poorly soluble compounds in water (Kuiper *et al.*, 2004). Wide spectra of microbial compounds, including glycolipids,

lipopeptides, fatty acids, and polymeric biosurfactants, have been found to have surface activity (Morikawa *et al.*, 2000). Biosurfactants have important advantages, such as biodegradability, low toxicity, and various possible structures, relative to chemically synthesized surfactants (Benincasa *et al.*, 2002). With environmental compatibility becoming an increasingly important factor in the selection of industrial chemicals, the use of biosurfactants in environmental applications, such as in bioremediation and the dispersion of oil spills, is increasing (Banat 1995). In addition, biosurfactants have other uses in the petroleum industry, such as in enhanced oil recovery (Kim *et al.*, 2000) and the transportation of crude oil. Other possible application fields are in the food, cosmetics, and pharmaceutical industries. In these indus-

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tries, most biosurfactants are used as emulsifiers (Desai and Banat, 1997). However, biosurfactants have not yet been employed extensively in industry because of the relatively high production and recovery costs involved. Considerable attention has been given in the past to the production of the surface-active molecules of biological origin because of their potential utilization in food processing (Mata-Sandoval *et al.*, 1999) pharmacology, and oil industry. Although the type and amount of the microbial surfactants produced depend primarily on the producer organism, factors like carbon and nitrogen, trace elements, temperature, and aeration also affected their production by the organism. Hydrophobic pollutants present in petroleum hydrocarbons and soil and water environment require solubilization before being degraded by microbial cells.

Mineralization is governed by adsorptions of hydrocarbons from soil. Surfactants can increase the surface area of hydrophobic materials, such as pesticides in soil and water environment, thereby increasing their water solubility. Hence, the presence of surfactants may increase microbial degradation of pollutants. Use of biosurfactants for degradation of pesticides in soil and water environment has become important recently (Jennings and Tanner 2000). The worldwide surfactant market totals approximately 9.4 billion US\$ per annum, and the demand for surfactants is expected to increase at a rate of 35% per annum (Desai and Banat, 1997). According to Karanth *et al.* (1999), the type, quality and quantity of biosurfactant production is dependent on the culture conditions such as pH, temperature, agitation, dilution rate in continuous culture, the concentration of metal ions and the nature of the carbon source and nitrogen source in the medium. Moreover, the efforts were based on conventional optimization methods where only one parameter is varied at any one time with the others being kept constant. As such, the interactions amongst these parameters are neglected, resulting in only an 'apparent' set of optimal conditions.

## MICROBIAL BIOSURFACTANTS

Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a hydrocarbon (C<sub>x</sub>H<sub>y</sub>) microorganisms facilitate their diffusion into the cell by producing a variety of substances, the biosurfactants. Some bacteria excrete ionic surfactant, which emulsify hydrocarbon substrates in the growth medium. Some examples of this group of biosurfactants are rhamnolipids which are produced by different *Pseudomonas* sp. (Guerra-Santos *et al.*, 1984; Guerra-Santos *et al.*, 1986), or the sophorolipids which are produced by several *Torulopsis* sp. (Cooper and Paddock, 1983).

Some other microorganisms are capable of changing the structure of their cell wall, by synthesising lipopolysaccha-

rides or non-ionic surfactants in their cell wall. Example of this group are: *Candida lipolytica* and *Candida tropicalis* which produce wall-bound lipopolysaccharides when growing on *n*-alkanes (Fukui and Tanaka, 1981), and *Rhodococcus erythropolis* and many *Mycobacterium* sp. which synthesise non-ionic trehalose corynomycolates (Rapp *et al.*, 1979; Ristau and Wagner 1983, Rubinovitz *et al.*, 1982). There are lipopolysaccharides, such as emulsan, synthesised by *Acinetobacter* sp. (Rosenberg *et al.*, 1979), and lipoproteins, such as surfactin and subtilisin, produced by *Bacillus subtilis* (Arima *et al.*, 1969, Cooper *et al.*, 1981). Other effective biosurfactants are:

1. Mycolates Corynomycolates which are produced by *Rhodococcus* sp., *Corynebacteria* sp., *Mycobacteria* sp., and *Nocardia* sp. (Cooper *et al.*, 1981, Kretschmer *et al.*, 1982, Macdonald *et al.*, 1981)
2. Ornithinlipides, which are produced by *Pseudomonas rubescens*, *Gluconobacter cerinus* and *Thiobacillus ferrooxidans* (Knoche and Shively, 1972, Tahara *et al.*, 1976).

The exact reason why some microorganisms produce surfactant is unclear (Deziel *et al.*, 1996), Biosurfactants produced by various microorganism together with their properties are listed in Table 1.

## CLASSIFICATION AND CHEMICAL NATURE OF BIOSURFACTANTS

Biosurfactants are categorised mainly by their chemical composition and their microbial origin. The microbial surfactants are complex molecules covering a wide range of chemical types including peptides, fatty acid, phospholipids, glycolipids, antibiotics and lipopeptides. Microorganisms also produce surfactants that are in some cases combination of many chemical types referred to as the polymeric microbial surfactants. Many microbial surfactants have been purified (Deziel *et al.*, 2000, Kim *et al.*, 2000). The high molecular weight microbial surfactants are generally polyanionic heteropolysaccharides containing both polysaccharides and proteins, the low molecular weight microbial surfactants are often glycolipids. The yield of microbial surfactants varies with the nutritional environment of the growing microorganism. Intact microbial cells that have high cell surface hydrophobicity are themselves surfactants. In some cases, surfactants themselves play a natural role in growth of microbial cells on water-insoluble substrates like hydrocarbon, sulphur. Exocellular surfactants are involved in cell adhesion, emulsification, dispersion, flocculation, cell aggregation and desorption phenomena (Karanth *et al.*, 1999). A broad classification of biosurfactants is given in Table 2.

**Table 1.** Structural Types of Microbial Surfactants

Biosurfactant	Source
<b>Glycolipids</b>	
Trehalolipids	<i>Rhodococcus erythropolis</i> <i>Nocardia erythropolis</i>
Trehalose Dimycolates	<i>Mycobacterium</i> sp. <i>Nocardia</i> sp.
Trehalose dicorynemycolates	<i>Arthrobacter</i> sp. <i>Corynebacterium</i> sp.
Rhamnolipids	<i>Pseudomonas aeruginosa</i> <i>Pseudomonas</i> sp.
Sophorolipids	<i>Torulopsis bombicola</i> <i>Torulopsis apicola</i> <i>Torulopsis petrophilum</i> <i>Torulopsis</i> sp.
Cellobiolipids	<i>Ustilago zaeae</i> <i>Ustilago maydis</i>
<b>Aminoacid-lipids</b>	
<b>Lipopeptides and lipoprotein</b>	
	<i>Streptomyces</i> sp. <i>Corynebacterium</i> sp. <i>Mycobacterium</i> sp.
Peptide-lipid	<i>Bacillus licheniformis</i>
Serrawettin	<i>Serratia marcescens</i>
Viscosin	<i>Pseudomonas fluorescens</i>
Surfactin	<i>Bacillus subtilis</i>
Subtilisin	<i>Bacillus subtilis</i>
Gramicidins	<i>Bacillus brevis</i>
Polymyxins	<i>Bacillus polymyxa</i>
<b>Ornithine-lipid</b>	
	<i>Pseudomonas</i> sp. <i>Thiobacillus</i> sp. <i>Agrobacterium</i> sp. <i>Gluconobacter</i> sp.
<b>Phospholipids</b>	
	<i>Candida</i> sp. <i>Corynebacterium</i> sp. <i>Micrococcus</i> sp. <i>Thiobacillus</i> sp.
<b>Fatty acids /Natural lipids</b>	
	<i>Acinetobacter</i> sp. <i>Pseudomonas</i> sp. <i>Micrococcus</i> sp. <i>Mycococcus</i> sp. <i>Candida</i> sp. <i>Penicillium</i> sp. <i>Aspergillus</i> sp.
<b>Polymeric surfactants</b>	
Emulsan	<i>Arthrobacter calcoaceticus</i>
Biodispersan	<i>Arthrobacter calcoaceticus</i>
Mannan-lipid-protein	<i>Candida tropicalis</i>
Liposan	<i>Candida lipolytica</i>
Carbohydrate-protein-lipid	<i>Pseudomonas fluorescens</i> <i>Debaryomyces polymorphis</i>
Protein PA	<i>Pseudomonas aeruginosa</i>
<b>Particulate biosurfactants</b>	
Vesicles and fimbriae	
Whole cells	<i>Arthrobacter calcoaceticus</i>

(Desai &amp; Banat 1997, Karanth et al. 1999)

**FACTORS AFFECTING BIOSURFACTANT PRODUCTION**

Biosurfactants are amphiphilic compounds. They contain a hydrophobic and hydrophilic moiety. The polar moiety can be a carbohydrate, an amino acid, a phosphate group, or some other compounds. The non polar moiety is mostly a long –carbon-chain fatty acid. Although the various biosurfactants possess different structures, these are some general phenomena concerning their biosynthesis. For example, hydrocarbons or other water-insoluble substrates can induce biosurfactants production (Radwan and Sorkhoh, 1993). An another striking phenomenon is the catabolic repression of biosurfactant synthesis by glucose and other primary metabolites. For example, in the case of *Arthrobacter paraffineus*, no surface-active agent could be isolated from the medium when glucose was used as the carbon source instead of hexadecane. Similarly a protein-like activator for *n*-alkane oxidation was formed by *Pseudomonas aeruginosa* S7B1 from hydrocarbon, but not from glucose, glycerol, or palmitic acid (Reddy et al., 1983). *Torulopsis petrophilum* did not produce any glycolipids when grown on a single-phase medium that contained water-soluble carbon source (Cooper and Paddock, 1983). When glycerol was used as substrate, rhamnolipid production by *Pseudomonas aeruginosa* was sharply reduced by adding glucose, acetate, succinate or citrate to the medium (Hauser and Karnovsky, 1958). Olive oil mill effluent, a major pollutant of the agricultural industry in Mediterranean countries, has been used as raw material for rhamnolipid biosurfactant production by *Pseudomonas* sp. JAMM. Many microorganisms are known to synthesise different types of biosurfactants when grown on several carbon sources. However, there have been examples of the use of a water-soluble substrate for biosurfactant production by microorganisms (Desai et al., 1988). The type, quality and quantity of biosurfactant produced are influenced by the nature of the carbon substrate, the concentration of nitrogen, phosphorus, magnesium, ferric, and manganese ions in the medium and the culture conditions, such as pH, temperature, agitation and dilution rate in continuous culture (Guerra-Santose et al., 1986).

The nitrogen source can be an important key to the regulation of biosurfactants synthesis. *Arthrobacter paraffineus* ATCC 19558 preferred ammonium to nitrate as inorganic nitrogen source for biosurfactants production. A change in growth rate of the concerned microorganisms is often sufficient to result in over production of biosurfactants (Kretschmer et al., 1982). In some cases, addition of multivalent cations to the culture medium can have a positive effect on biosurfactants production (Cooper et al., 1981). Besides the regulation of biosurfactants by chemicals indicated above, some compounds like ethambutol, penicillin (Horne and Tomasz, 1979), chloramphenicol (Rubinovitz et al., 1982), and EDTA (Reddy et al., 1982) influenced the formation of interfacially active compounds. The regulation of biosurfactants production by these compounds is either through their effect

**Table 2.** Classification of Biosurfactant

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1. Glycolipids
    - Trehalose lipids
    - Sophorolipids
    - Rhamnolipids
  2. Fatty acids
  3. Phospholipids
  4. Surface active antibiotics
    - Gramicidin
    - Polymixins
    - Surfactine
  5. Polymeric microbial surfactants
    - Emulsan from *Acinetobacter calcoaceticus* RAG-1 (ATCC 31012).
    - The polysaccharide protein complex of *Acinetobacter calcoaceticus* BD4.
    - Other *Acinetobacter* sp. emulsifiers.
    - Emulsifying protein from *Pseudomonas aeruginosa*.
    - Emulsifying and solubilizing factors from *Pseudomonas* sp. PG-1.
    - Biofloculant and emulcyan from the filamentous *Cyanobacterium phormidium* J-1.
  6. Particulate surfactant
    - Extracellular vesicles from *Acinetobacter* sp. HO1-N.
    - Microbial cell with high cell surface hydrophobicities.
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(Christofi and Ivshina, 2002, Karanth *et al.* 1999)

on solubilization of nonpolar hydrocarbon substrates or by increased production of water-soluble (polar) substrates. In some cases, pH and temperature regulate biosurfactants synthesis. For example in rhamnolipid production by *Pseudomonas* sp., in cellobioselipid formation by *Ustilago maydis* pH played an important role (Frautz *et al.*, 1986) and in the case of *Arthrobacter paraffineus* ATCC 19558 temperature was important (Duvnjak *et al.*, 1982).

**Carbon Source.** Water-soluble carbon sources such as glycerol, glucose, mannitol, and ethanol were all used for rhamnolipid production by *Pseudomonas* sp. Biosurfactant product, however, was inferior to that obtained with water-immiscible compounds such as *n*-alkanes and olive oil (Robert *et al.*, 1989). Syldatk *et al.*, (1985a) demonstrated that although different carbon sources in the medium affected the composition of biosurfactant production in *Pseudomonas* sp., substrates with different chain lengths exhibited no effect on the chain length of fatty acid moieties in glycolipids. On the other hand, Neidleman and Geigert (1984), showed evidence for qualitative variation, reflecting the carbon number of alkane for biosurfactant production in *Acinetobacter* sp. strains H13-A and HO1-N, respectively. When *Arthrobacter paraffineus* ATCC 19558 was grown on D-glucose, supplementation with hexadecane in the medium during the stationary growth phase resulted in a significant increase in biosurfactant yield (Duvnjak *et al.*, 1982). Duvnjak and Kosaric (1985), showed the presence of large amounts of biosurfactant bound to

*Corynebacterium lepus* cells when grown on glucose, and addition of hexadecane facilitated the release of surfactant from cells.

Others observed a little biosurfactant production, when cells were growing on a readily available carbon source, only when all the soluble carbon was consumed and when water-immiscible hydrocarbon was available was biosurfactant production triggered (Banat 1995, Banat *et al.*, 1991). Davila *et al.* (1992) demonstrated a high yield of sophorose lipids by overcoming product inhibition in *Candida bombicola* CBS6009 through the addition of ethyl esters of rape seed oil fatty acids in D-glucose medium. Using *Torulopsis apicola* IMET 43747, Stuwer *et al.* (1987) achieved a high glycolipid yield with a medium containing D-glucose and sunflower oil. Lee and Kim (1993) reported that in batch culture, 37% of the carbon input was channelled to produce sophorolipid by *Torulopsis bombicola*. However, in fed batch cultures, about 60% of the carbon inputs were incorporated into biosurfactant, increasing the yield. The availability of carbon source, particularly the carbohydrate used, has a great bearing on the type of biosurfactant produced (Li *et al.*, 1984).

**Nitrogen Source.** Medium constituents other than carbon source also affect the production of biosurfactants. Among the inorganic salts tested, ammonium salts and urea were preferred nitrogen sources for biosurfactant production by *Arthrobacter paraffineus*, whereas nitrate supported maximum surfactant production by *Pseudomonas aeruginosa* (Guerra-Santos *et al.*, 1986) and *Rhodococcus* sp. (Abu-Rawaida *et al.*, 1991a). Biosurfactant production by *Arthrobacter paraffineus* is increased by the addition of amino acid such as aspartic acid, glutamic acid, asparagine, and glycine to the medium. Robert *et al.* (1989) and Abu-Ruwaida *et al.* (1991a), observed nitrate to be the best source of nitrogen for biosurfactant production by *Pseudomonas* strain 44T1 and *Rhodococcus* strain ST-5 growing on olive oil and paraffin, respectively. Similarly, nitrogen limitation caused increased biosurfactant production in *Pseudomonas aeruginosa* (Ramana and Karanth, 1989), *Candida tropicalis* IIP-4 (Singh *et al.*, 1990), and *Nocardia* strain SFC-D (Kosaric *et al.*, 1990).

Syldatk *et al.* (1985b) showed that nitrogen limitation not only caused overproduction of biosurfactant but also changed the composition of the biosurfactant produced. Guerra-Santos *et al.* (1986), showed maximum rhamnolipid production after nitrogen limitation at a C:N ratio of 16:1 to 18:1 and no surfactant production below a C:N ratio of 11:1, where the culture was not nitrogen limited. According to Hommel *et al.* (1987) it was the absolute quantity of nitrogen and not its relative concentration that appeared to be important for optimum biomass yield, while concentration of hydrophobic carbon source determines the conversion of carbon available to the biosurfactant.

**Environmental Factors.** Environmental factors and growth conditions such as pH, temperature, agitation, and oxygen

availability also affect biosurfactant production through their effects on cellular growth or activity. The pH of the medium plays an important role in sophorolipid production by *Torulopsis bombicola* (Gobbert *et al.*, 1984). Rhamnolipid production in *Pseudomonas sp.* was at its maximum at a pH range from 6 to 6.5 and decrease sharply above pH 7 (Guerra-Santos *et al.*, 1984). In contrast, Powalla *et al.* (1989) showed that penta- and disaccharide lipid production in *Nocardia corynbacteroides* is unaffected in the pH range of 6.5 to 8. In addition, surface tension and critical micelle concentrations of a biosurfactant product remained stable over a wide range of pH values, whereas emulsification had a narrower pH range (Abu-Rawaida *et al.*, 1991b). In *Arthrobacter paraffineus* and *Pseudomonas sp.* strain DSM-2874 (Syldatk *et al.*, 1985b) temperature caused alteration in the composition of the biosurfactant produced. A thermophilic *Bacillus sp.* grew and produced biosurfactant at temperature above 40°C. Heat treatment of some biosurfactant caused no appreciable change in biosurfactant properties such as the lowering of surface tension and interfacial tension and the emulsification efficiency, all of which remained stable after autoclaving at 120°C for 15 min (Abu Rawaida *et al.*, 1991b).

An increase in agitation speed results in the reduction of biosurfactant yield due to the effect of shear in *Nocardia erythropolis* (Margaritis *et al.*, 1979). While studying the mechanism of biosurfactant production in *Acinetobacter calcoaceticus* RAG-1, Wang and Wang (1990), revealed that the cell-bound polymer/dry-cell ratio decrease as the shear stress increase. On the other hand, in yeast, biosurfactant production increases when the agitation and aeration rates increased. Sheppard and Cooper (1990) had concluded that oxygen transfer was one of the Key parameters for the process optimization and scale-up of surfactin production in *Bacillus subtilis*. Salt concentration also affected biosurfactant production depending on its effect on cellular activity. Some biosurfactant products, however, were not affected by salt concentrations up to 10% (w/v), although slight reduction in the critical micelle concentrations were detected (Abu Rawaida *et al.*, 1991b).

At present, the cost of production and insufficient experience in applications limit the use of bioemulsifiers. However, inasmuch as awareness of water quality and environmental conservation is increasing and demand for natural products is expanding, it appears inevitable that the high quality, microbially produced bioemulsifiers will replace the currently used chemical emulsifiers in many applications.

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