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*Review*

# Microbial Biosurfactants: Properties, Types, and Production

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## Abstract

Surfactants are surface active agents that reduce the surface tension between immiscible phases. They are amphiphilic molecules which can be produced by chemical and biological methods. Compared with chemical surfactants with the same functionality, biosurfactants have advantages such as being able to operate under extreme temperature, pH and salinity conditions, being non-toxic or very low toxic and biodegradable. In addition, since they are of biological origin, they can be produced from renewable substrates and structurally modified by genetic engineering and biochemical methods, and they can reach different markets with innovative formulations. Recently, interest in biological surfactants has increased considerably. With their unique physicochemical properties and various biological activities, they have application areas in detergent, cosmetics, medicine, food, bioremediation and agriculture sectors. However, despite the huge market demand, the production of biosurfactants is not as competitive as their synthetic counterparts. In order to improve biosurfactant production, different parameters should be considered. In this review, the types of biosurfactants and the factors affecting microbial biosurfactant production are discussed.

*Keywords:* Biosurfactants; Rhamnolipid; fermentation; sophorolipid; surfactin.

## 1. Introduction

Surfactants (SURFace ACTive AgeNTS) are compounds that can reduce the surface tension between immiscible phases due to their hydrophilic (polar) and hydrophobic (non-polar) parts (Fig. 1). While the hydrophilic head usually contains polar functional groups such as sugar, amino acid, phosphate, carboxylic acid or alcohol, the hydrophobic tail usually contains the hydrocarbon chain of β-hydroxy fatty acids [1]. Due to their amphiphilic nature, they can accumulate between phases such as liquid/solid, liquid/gas, liquid/liquid (oil/water), reducing the surface tension between these two phases and forming an emulsion. With these properties, surfactants have great effects on all of our lives, as they form the main component of most of the products we use in our daily life, and they are versatile chemical groups used in various industrial processes. Detergents, cleaning agents, cosmetics and personal care products constitute a large part of the global demand in the growth of biosurfactants market.

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**Figure 1**. Structure of bio/surfactants

Biosurfactants are biological surfactants synthesized as a component of cell membrane or extracellularly by some yeasts, fungi and especially certain bacteria [2,3]. Compared to synthetic surfactants with the same functionality, biosurfactants have advantages such as being able to work in extreme temperature, pH and salinity conditions, being non-toxic or very low toxicity, and being biodegradable. In addition, since they are of biological origin, they can be produced from renewable substrates and modified structurally by genetic engineering and biochemical methods, and they can reach different markets with innovative formulations [1,4].

The benefits of biosurfactants to humanity in many areas of life have been studied and reported [5,6]. Biosurfactants that can accumulate at different interfaces such as liquid/solid, liquid/gas, liquid/liquid and reduce surface and internal surface tension have stabilizing, emulsifying (liquid-liquid mixture), wetting, spreading, foaming and cleaning properties. With these properties, they are frequently used in detergent, food, agriculture, petroleum, cosmetics and pharmaceutical industries [7–10]. Biosurfactants have also wide applications in bioremediation, medicine, food and agriculture due to its stabilizing, emulsifying (liquid-liquid mixture), wetting, spreading, foaming and cleaning properties and antimicrobial, antifungal and antiviral activities [11–13].

## 2. Types of Biosurfactants

Surfactants are classified as anionic, cationic, amphoteric and uncharged surfactants according to the charge of the hydrophilic heads involved in solubility. Anionic surfactants include linear alkylbenzene sulfonate (LAS) and sodium dodecyl sulfate (SDS), which are frequently used in laundry detergents, household and personal cleaning products, as well as examples of anionic surfactants that are frequently used in the cosmetic industry, pharmaceutical industry, and soil hydrocarbon removal [14,15]. Cationic surfactants are used in detergents, softeners and conditioners. In addition, due to their bacteriostatic effects, they prevent the growth of bacteria and can be used to develop cleaning agents and antibiotic products [16]. Amphoteric surfactants such as lauryl betaine can show both cationic and anionic properties depending on the environment and pH. Uncharged surfactants are widely used as emulsifiers, wetting agents and foam stabilizers.

Microorganisms can produce biosurfactants with unique molecular compositions. It is generally divided into two large groups as high and low molecular weight biosurfactants. Lipoprotein, lipopolysaccharide, protein, polysaccharide and biopolymer complexes are grouped as high molecular weight biosurfactants. Lipopeptide, glycolipid and phospholipid-based ones have been characterized as low molecular weight biosurfactants [5,17]. Biosurfactants obtained from microorganisms can be glycolipids, lipopeptides and lipoproteins, phospholipids and polymeric surfactants according to their chemical compositions. Apart from these, neutral lipids, fatty acids and particulate compounds can also be considered as biosurfactants (Table).

The main glycolipid-type biosurfactants are rhamnolipids, trehalolipids and sophorolipids. Among these, rhamnolipids produced by *Pseudomonas aeruginosa* are the best known. It has the feature of emulsifying hydrocarbons, and its antibacterial and antifungal effects have also been reported [18,19].
Trehalolipids are mainly derived from *Rhodococcus sp.* and *Arthrobacter sp.* and are used for their immunomodulatory and antitumor activities in medicine, and used as hydrocarbon solubilizers and emulsifiers in bioremediation, microbial enhanced oil recovery, cosmetic and food applications [20,21].

**Table 1.** Main classes of biosurfactants and their producers

| Biosurfactants | | Microorganisms |
|---|---|---|
| **Grup** | **Biosurfactant types** | |
| **Glycolipids** | Rhamnolipids | *Pseudomonas aeruginosa* |
| | Trehalolipids | *Mycobacterium tuberculosis*<br>*Rhodococcus erythropolis*<br>*Arthrobacter sp.*<br>*Nocardia* sp.<br>*Corynebacterium* sp. |
| | Sophorolipids | *Torulopsis bombicola*<br>*Torulopsis petrophilum*<br>*Torulopsis apicola* |
| **Fatty acids, phospholipids and neutral fats** | Corinomycolic acid | *Corynebacterium lepus* |
| | Spiculisporic acid | *Penicillium spiculisporum* |
| | Phosphatidylethanolamine | Acinetobacter sp.<br>*Rhodococcus erythropolis* |
| **Lipopeptides** | Surfactin | *Bacillus subtilis* |
| | Lichenysins | *Bacillus licheniformis* |
| **Polymeric biosurfactants** | Emulsan | *Acinetobacter calcoaceticus* |
| | Alasan | *Acinetobacter radioresistens* |
| | Liposan | *Candida lipolytica* |

Sophorolipids are generally produced by yeast species such as *Torulopsis sp.* and *Candida sp.* and are used in stabilizing oil/water emulsions, in cosmetics with their wetting-moisturizing properties, and in medicine with their antimicrobial, anti-inflammatory and immune system regulatory effects [22,23].

The most known lipopeptide biosurfactants are surfactin and iturin from *Bacillus subtilis*, lichenisin from *B. licheniformis*, serravettin from *Serratia marcescens*, viscosine from *P. fluorescens*, gramicidin from *B. brevis*, polymyxin from *B. polymyxa*. Surfactant, one of the most important of this group, has been reported to have antibacterial, anti-inflammatory, antimycoplasma, antiviral, antitumor and thrombolytic activities [24,25].

Fatty acids and phospholipids are substances produced from alkanes as a result of microbial oxidation and considered as biosurfactants [26]. While fatty acids are frequently used in the food industry, phospholipids have found use in gene carrier systems due to their membrane component. Lecithin and lysolecithin can be given as examples of phospholipid biosurfactants [27].

The best known polymeric biosurfactants, emulsan, liposan and mannoprotein, have potent bioemulsifiers effects [28]. One of the strongest stabilizers known today is the emulsan extracted from *Acinetobacter calcoaceticus* [29].

## 3. Factors Affecting Biosurfactant Production

Although the amount and type of biosurfactants depend primarily on the producer microorganism, carbon (glycerol, oil, glucose, mannitol) and nitrogen source (ammonium salts, urea, peptone), fermentation time, ion concentration, pH, temperature and oxygen levels are the parameters affecting biosurfactant production (Fig. 2).

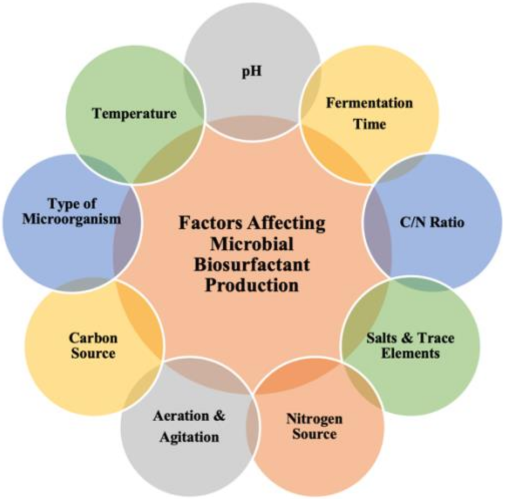


**Figure 2.** Factors affecting the production of biosurfactants

Both the type and amount of the carbon sources in the culture medium plays an important role in the biosurfactant production [30]. The carbon sources for biosurfactant production are divided into three groups as carbohydrates (glucose, sucrose, fructose, mannitol, lactose), hydrocarbons (n-hexadecane, n-hexane, octadecane) and vegetable oils (sunflower oil, soybean oil, olive oil) [31–33]. Waste frying oils, molasses, fruit and fruit residues, starch-rich agricultural products, renewable and inexpensive wastes for the production of biosurfactants have found wide scope in recent years [33–35].

The type of nitrogen source critically affects the production of biosurfactant by microorganisms. There are two types of nitrogen sources; organic (peptones, yeast and meat extract, urea) and inorganic nitrogen (ammonium sulfate, ammonium nitrate, sodium nitrate, potassium nitrate, ammonium chloride) sources. Organic nitrogen compounds with a complex structure are preferred as they do not lead to pH change drastically. Since the use of inorganic salts causes hydrolysis of cations or anions, it may affect the pH of the culture medium, thus reducing the fermentation efficiency [33,36]. However, nitrates, ammonia and amino acids are preferred nitrogen sources for *P. aeruginosa* [37,38]. Apart from these nitrogen sources, it is possible to use waste materials instead of commercial nitrogen sources in order to reduce the production cost. For this purpose, rhamnolipid production from *P. aeruginosa* was carried out using chicken feather peptone [34], ram horn peptone [39] and corn steep liquor [40].

In fermentation, the carbon/nitrogen (C/N) ratio also affects the production of biosurfactants [30]. Biosurfactant production usually occurs during the stationary phase of cell growth when the nitrogen source is depleted in the culture medium [33]. For this reason, a high C/N ratio (10-40) is required in the culture medium.

The addition of various metal salts to the fermentation medium greatly affects the biosurfactant production. Various metal supplements such as magnesium, calcium, iron and trace elements are often used for the production of biosurfactants. However, metal requirements vary depending on the microorganism [33,41].

Potassium dihydrogen phosphate ($KH_2PO_4$) and dipotassium hydrogen phosphate ($K_2HPO_4$) are often added to the production medium to maintain the desired pH during fermentation and encourage microbial growth [42]. Calcium (usually $CaCl_2$) works as a common mediator in transmitting signals from the cell surface to the intracellular processes of microorganisms. Both potassium and calcium ions play important roles in balancing the osmotic pressure and controlling the cell membrane potential, which can prevent the lysis of the cell in the environment [33]. Mostly, 0.1 g/L $Ca^{2+}$ ion is required for biosurfactant production from *P. aeruginosa* [43] and 0.02 g/L $Ca^{2+}$ ion is required for glycolipid production from *B. megaterium* [44]. In biosurfactant production, the magnesium ion ($Mg^{2+}$) is typically supplied in the form of magnesium sulfate ($MgSO_4$) and is approximately 50 times higher than the $Ca^{2+}$ concentration used in production medium [45].

Iron (Fe) is a very important cofactor in the metabolism of various microorganisms. The specific requirements of trace elements depend on the microorganism itself; however, the most important trace elements used in the production of biosurfactants are zinc (Zn), copper (Cu), boron (B), molybdenum (Mo) and cobalt (Co). Trace elements are chemical elements required for microorganisms in amounts less than 0.1% of the total working volume. In previous studies, it has been determined that Fe, Mg and manganese (Mn) are cofactors of enzymes involved in surfactin synthesis by *B. subtilis* [46].

Fermentation conditions (temperature, pH, agitation speed and oxygen level) are important parameters affecting biosurfactant production [30]. However, biosurfactant production can often vary depending on the optimum conditions for microorganism growth. In a study with *Acinetobacter* M6, the optimum temperature was determined as 37 °C, pH 7 and it was obtained at the end of the 7-day incubation [47]. Maximum rhamnolipid production in different *P. aeruginosa* species occurs at pH values ranging from pH 6 to 8, depending on the strain used [48], [49]. With the mutant *P. aeruginosa* 15GR obtained by gamma rays, rhamnolipid was produced in solid state fermentation at pH 8, 30 °C and 1% bacterial inoculum concentration [49]. In another study, optimum conditions for rhamnolipid production from *P. aeruginosa* TMN were reported as 37 ℃, pH 7 and 200 rpm shaking speed [50].

## 4. Conclusions

Although the production and characterization of specific biosurfactants from various microbial sources have been reported, they still have not become widespread enough to compete with synthetic surfactants in use. Both high production costs and low efficiency are the biggest obstacles to the expansion of the usage areas of biosurfactants. The majority of surfactants currently in use in daily life and in a wide variety of industries are synthetic and these chemical surfactants are used in large quantities. However, synthetic surfactants and their incomplete biodegradable products pose significant risks to the ecosystem. They are constantly mixed with surface and ground waters through wastewater. Biosurfactants, on the other hand, have the ability to maintain their stability and work under harsh conditions, and they do not cause the negative effects of synthetic surfactant accumulation due to their biodegradability when discharged.

The major change in attitude towards surfactants in recent years is due to the sustainability concerns. Companies that use surfactants in their products now want to replace some or all of their chemical surfactants with sustainable biological surfactants, that is, surfactants that are mainly produced using microorganisms from sustainable raw materials. As the importance of sustainable production processes and sustainability initiatives in general is appreciated and rises to the top of many companies' agendas, the interest and usage areas of biological surfactants will continue to increase.

## Conflict of Interest

The authors declare no conflict of interest.

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