REVIEW ARTICLE

Biosurfactants: Properties, commercial production and application

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Biosurfactants or microbial surfactants are surface-active biomolecules that are produced by a variety of microorganisms. Biosurfactants have gained importance in the fields of enhanced oil recovery, environmental bioremediation, food processing and pharmaceuticals owing to their unique properties such as higher biodegradability and lower toxicity. Interest in the production of biosurfactants has steadily increased during the past decade. However, large-scale production of these molecules has not been realized because of low yields in production processes and high recovery and purification costs. This article describes some practical approaches that have been adopted to make the biosurfactant production process economically attractive. These include the use of cheaper raw materials, optimized and efficient bioprocesses and overproducing mutant and recombinant strains for obtaining maximum productivity. Here, we discuss the role and applications of biosurfactants focusing mainly on medicinal and therapeutic perspectives. With these specialized and cost-effective applications in biomedicine, we can look forward to biosurfactants as the molecules of the future.

Keywords: Biodegradability, biosurfactants, critical micelle concentration, cytotoxic, production process.

BIOSURFACTANTS are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or excreted extracellularly and contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively[1]. They are a structurally diverse group of surface-active molecules synthesized by microorganisms[2]. Rhamnolipids from *Pseudomonas aeruginosa*, surfactin from *Bacillus subtilis*, emulsan from *Acinetobacter calcoaceticus* and sophorolipids from *Candida bombicola* are some examples of microbial-derived surfactants. Originally, biosurfactants attracted attention as hydrocarbon dissolution agents in the late 1960s, and their applications have been greatly extended in the past five decades as an improved alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters), especially in food, pharmaceutical and oil industry[3,4]. The reason for their popularity as high-value microbial products is primarily because of their specific action, low toxicity, higher biodegradability, effectiveness at extremes of temperature, pH, salinity and widespread applicability, and their unique structures which provide new properties that classical surfactants may lack[4,5]. Biosurfactants possess the characteristic property of reducing the surface and interfacial tension using the same mechanisms as chemical surfactants. Unlike chemical surfactants, which are mostly derived from petroleum feedstock, these molecules can be produced by microbial fermentation processes using cheaper agro-based substrates and waste materials. During the past few years, biosurfactant production by various microorganisms has been studied extensively. Also various aspects of biosurfactants, such as their biomedical and therapeutic properties[6–8], natural roles[9], production on cheap alternative substrates[10–12] and commercial potential[4,13], have been recently reviewed. No attempt has been made, to the best of our knowledge, to describe the research and development strategies of making the biosurfactant production process cheaper and commercially attractive. The principle aim of the present article is to focus on such studies, with special emphasis on the development and use of mutant and recombinant hyperproducers of biosurfactants, and indication of direction towards their commercial production. Most of the work on biosurfactant applications has been focusing on bioremediation of pollutants[14] and microbial enhanced oil recovery[15]. However, these microbial compounds exhibit a variety of useful properties and applications in various fields. In this review, we discuss the potential roles and applications of biosurfactants mainly focusing on areas such as food and food-related industries (as emulsifiers, foaming, wetting, solubilizers, antiadhesive agents), biomedicine and therapeutics (as antimicrobial agents, immunoregulators and immunomodulators, their possible role in signalling and cytotoxic activity). With these specialized and cost-effective applications in biomedicine, we can look forward to biosurfactants as the molecules of the future.

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Classification of biosurfactants

Unlike chemically synthesized surfactants, which are usually classified according to the nature of their polar grouping, biosurfactants are generally categorized mainly by their chemical composition and microbial origin. Rosenberg and Ron[16] suggested that biosurfactants can be divided into low-molecular-mass molecules, which efficiently lower surface and interfacial tension, and high-molecular-mass polymers, which are more effective as emulsion-stabilizing agents. The major classes of low-mass surfactants include glycolipids, lipopeptides and phospholipids, whereas high-mass surfactants include polymeric and particulate surfactants. Most biosurfactants are either anionic or neutral and the hydrophobic moiety is based on long-chain fatty acids or fatty acid derivatives, whereas the hydrophilic portion can be a carbohydrate, amino acid, phosphate or cyclic peptide[17] (Table 1). A brief discussion about each class of biosurfactant is given below.

Glycolipids

Most known biosurfactants are glycolipds. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids. The linkage is by means of either ether or an ester group. Among the glycolipids, the best known are rhamnolipids, trehalolipids and sophorolipids.

Table 1. Major biosurfactant classes and microorganisms involved[1,4,16]

Surfactant class	Microorganism
Glycolipids	
Rhamnolipids	*Pseudomonas aeruginosa*
Trehalose lipids	*Rhodococcus erithropolis*
	Arthobacter sp.
Sophorolipids	*Candida bombicola, C. apicola*
Mannosylerythritol lipids	*C. antartica*
Lipopeptides	
Surfactin/iturin/fengycin	*Bacillus subtilis*
Viscosin	*P. fluorescens*
Lichenysin	*B. licheniformis*
Serrawettin	*Serratia marcescens*
Phospholipids	*Acinetobacter* sp.
	Corynebacterium lepus
Surface-active antibiotics	
Gramicidin	*Brevibacterium brevis*
Polymixin	*B. polymyxa*
Antibiotic TA	*Myxococcus xanthus*
Fatty acids/neutral lipids	
Corynomicolic acids	*Corynebacterium insidibasseosum*
Polymeric surfactants	
Emulsan	*Acinetobacter calcoaceticus*
Alasan	*A. radioresistens*
Liposan	*C. lipolytica*
Lipomanan	*C. tropicalis*
Particulate biosurfactants	
Vesicles	*A. calcoaceticus*
Whole microbial cells	*Cyanobacteria*

Rhamnolipids: These glycolipids, in which one or two molecules of rhamnose are linked to one or two molecules of β-hydroxydecanoic acid, are the best studied. While the OH group of one of the acids is involved in glycosidic linkage with the reducing end of the rhamnose disaccharide, the OH group of the second acid is involved in ester formation[1]. Production of rhamnose containing glycolipids was first described in *Pseudomonas aeruginosa* by Jarvis and Johnson[18]. L-Rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (Figure 1) and L-rhamnosyl-β-hydroxydecanoyl-β-hydrocydecanoate, referred to as rhamnolipids 1 and 2 respectively, are the principal glycolipids produced by *P. aeruginosa*[19].

Trehalolipids: Several structural types of microbial trehalolipid biosurfactants have been reported (Figure 2). Disaccharide trehalose linked at C-6 and C-6′ to mycolic acid is associated with most species of *Mycobacterium*, *Nocardia* and *Corynebacterium*. Mycolic acids are long-chain, α-branched-β-hydroxy fatty acids. Trehalolipids from different organisms differ in the size and structure of mycolic acid, the number of carbon atoms and the degree of unsaturation[20]. Trehalose lipids from *Rhodococcus erythropolis* and *Arthrobacter* sp. lowered the surface and

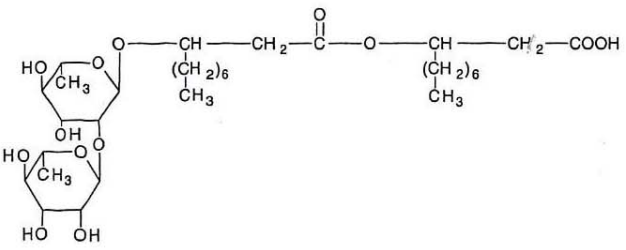

Figure 1. Structure of rhamnolipid.

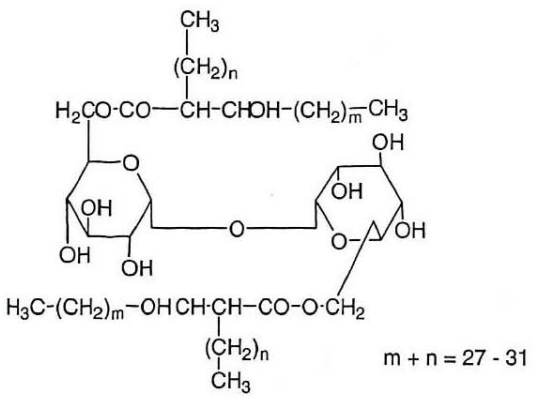

Figure 2. Structure of trehalose lipids.

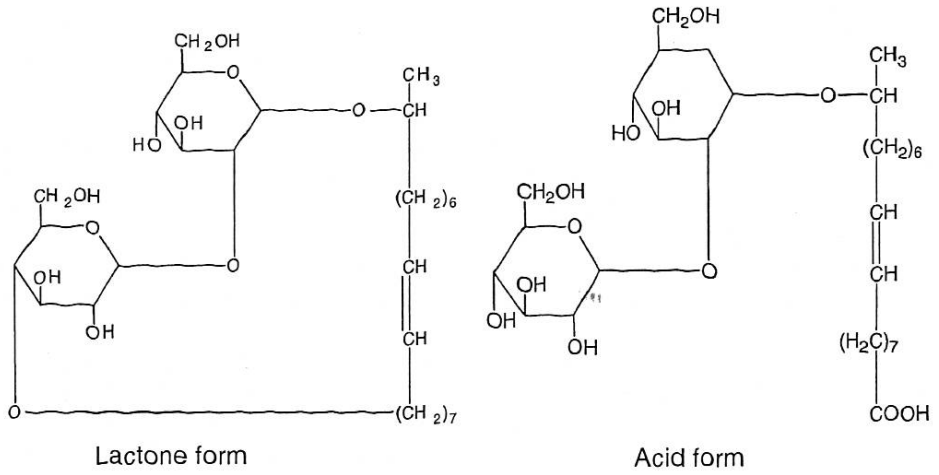

Figure 3. Structure of lactonized and free-acid forms of sophorolipids.

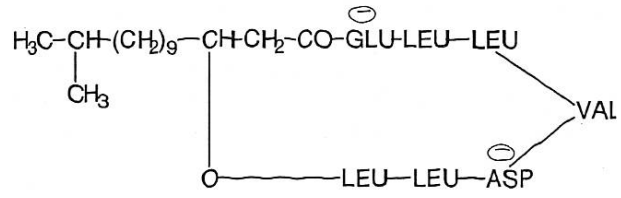

Figure 4. Structure of surfactin.

interfacial tension in culture broth from 25 to 40 and 1 to 5 mN/m respectively[21].

Sophorolipids: These glycolipids, which are produced mainly by yeast such as *Torulopsis bombicola*[22,23] (Figure 3), *T. petrophilum* and *T. apicola* consist of a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage. Generally, sophorolipids occur as a mixture of macrolactones and free acid form. It has been shown that the lactone form of the sophorolipid is necessary, or at least preferable, for many applications[24]. These biosurfactants are a mixture of at least six to nine different hydrophobic sophorolipids.

Lipopeptides and lipoproteins

A large number of cyclic lipopetides, including decapeptide antibiotics (gramicidins) and lipopeptide antibiotics (polymyxins) are produced. These consist of a lipid attached to a polypeptide chain.

Surfactin: The cyclic lipopeptide surfactin (Figure 4), produced by *Bacillus subtilis* ATCC 21332, is one of the most powerful biosurfactants. It is composed of a seven amino-acid ring structure coupled to a fatty-acid chain via lactone linkage. It lowers the surface tension from 72 to 27.9 mN/m at concentrations as low as 0.005% (ref. 25).

Lichenysin: Bacillus licheniformis produces several biosurfacants which act synergistically and exhibit excellent temperature, pH and salt stability. These are also similar in structural and physio-chemical properties to the surfactin[26]. The surfactants produced by *B. licheniformis* are capable of lowering the surface tension of water to 27 mN/m and the interfacial tension between water and *n*-hexadecane to 0.36 mN/m.

Fatty acids, phospholipids, and neutral lipids

Several bacteria and yeast produce large quantities of fatty acids and phospholipid surfactants during growth on *n*-alkanes[27]. The hydrophilic and lipophilic balance (HLB) is directly related to the length of the hydrocarbon chain in their structures. In *Acinetobacter* sp. strain HO1-N, phosphatidylethanolamine-rich vesicles are produced[28], which form optically clear microemulsions of alkanes in water. Phosphatidylethanolamine produced by *R. erythropolis* grown on *n*-alkane causes a lowering of interfacial tension between water and hexadecane to less than 1 mN/m and a critical micelle concentration (CMC) of 30 mg/l (ref. 21).

Polymeric biosurfactants

The best-studied polymeric biosurfactants are emulsan, liposan, alasan, lipomanan and other polysaccharide–protein complexes. *Acinetobacter calcoaceticus* RAG-1 produces an extracellular potent polyanionic amphipathics heteropolysaccharide bioemulsifier[29]. Emulsan is an effective emisifying agent for hydrocarbons in water[30], even at a concentration as low as 0.001 to 0.01%. Liposan is an extracellular water-souble emulsifier synthesized by *Candida lipolytica* and is composed of 83% carbohydrate and 17% protein[31].

Particulate biosurfactants

Extracellular membrane vesicles partition hydrocarbons to from a microemulsion, which plays an important role in alkane uptake by microbial cells. Vesicles of *Acinetobacter* sp. strain HO1-N with a diameter of 20–50 nm and a buoyant density of 1.158 cubic g/cm are composed of protein, phospholipids and lipopolysaccharide[28].

Properties of biosurfactants

Biosurfactants are of increasing interest for commercial use because of the continually growing spectrum of available substances. There are many advantages of biosurfactants compared to their chemically synthesized counterpart. The main distinctive features of biosurfactants and a brief description of each property are given below.

Surface and interface activity

A good surfactant can lower surface tension of water from 72 to 35 mN/m and the interfacial tension of water/hexadecane from 40 to 1 mN/m (ref. 14). Surfactin from *B. subtilis* can reduce the surface tension of water to 25 mN/m and interfacial tension of water/hexadecane to <1 mN/m (ref. 32). Rhamnolipids from *P. aeruginosa* decrease the surface tension of water to 26 mN/m and the interfacial tension of water/hexadecane to <1 mN/m (ref. 33). The sophorolipids from *T. bombicola* have been reported to reduce the surface tension to 33 mN/m and the interfacial tension to 5 mN/m (ref. 34). In general, biosurfactants are more effective and efficient and their CMC is about 10–40 times lower than that of chemical surfactants, i.e. less surfactant is necessary to get a maximum decrease in surface tension[4].

Temperature, pH and ionic strength tolerance

Many biosurfactants and their surface activities are not affected by environmental conditions such as temperature and pH. McInerney *et al.*[26] reported that lichenysin from *B. licheniformis* JF-2 was not affected by temperature (up to 50°C), pH (4.5–9.0) and by NaCl and Ca concentrations up to 50 and 25 g/l respectively. A lipopeptide from *B. subtilis* LB5a was stable after autoclaving (121°C/20 min) and after 6 months at –18°C; the surface activity did not change from pH 5 to 11 and NaCl concentrations up to 20% (ref. 35).

Biodegradability

Unlike synthetic surfactants, microbial-produced compounds are easily degraded[36] and particularly suited for environmental applications such as bioremediation[14] and dispersion of oil spills.

Low toxicity

Very little data are available in the literature regarding the toxicity of microbial surfactants. They are generally considered as low or non-toxic products and therefore, appropriate for pharmaceutical, cosmetic and food uses. A report suggested that a synthetic anionic surfactant (Corexit) displayed an LC50 (concentration lethal to 50% of test species) against *Photobacterium phosphoreum* ten times lower than rhamnolipids, demonstrating the higher toxicity of the chemical-derived surfactant. When comparing the toxicity of six biosurfactants, four synthetic surfactants and two commercial dispersants, it was found that most biosurfactants degraded faster, except for a synthetic sucrose-stearate that showed structure homology to glycolipids and was degraded more rapidly than the biogenic glycolipids. It was also reported that biosurfactants showed higher EC50 (effective concentration to decrease 50% of test population) values than synthetic dispersants[37]. A biosurfactant from *P. aeruginosa* was compared with a synthetic surfactant (Marlon A-350) widely used in the industry, in terms of toxicity and mutagenic properties. Both assays indicated higher toxicity and mutagenic effect of the chemical-derived surfactant, whereas the biosurfactant was considered slightly non-toxic and non-mutagenic[38].

Emulsion forming and emulsion breaking

Stable emulsions can be produced with a lifespan of months and years[39]. Biosurfactants may stabilize (emulsifiers) or destabilize (de-emulsifiers) the emulsion. High-molecular-mass biosurfactants are in general better emulsifiers than low-molecular-mass biosurfactants. Sophorolipids from *T. bombicola* have been shown to reduce surface and interfacial tension, but are not good emulsifiers[34]. By contrast, liposan does not reduce surface tension, but has been used successfully to emulsify edible oils[27]. Polymeric surfactants offer additional advantages because they coat droplets of oil, thereby forming stable emulsions. This property is especially useful for making oil/water emulsions for cosmetics and food.

Chemical diversity

The chemical diversity of naturally produced biosurfactants offers a wide selection of surface-active agents with properties closely related to specific applications.

Medium cheap substrates: Economical and promising alternatives

Production economy is the major bottleneck in biosurfactant production, as in the case with most biotechnological

Table 2. Use of inexpensive raw materials for the production of biosurfactants by various microbial strains

Low cost or waste raw material	Biosurfactant type	Producer microbial strain	Maximum yield (g/l)	Reference
Rapeseed oil	Rhamnolipids	*Pseudomonas* sp. DSM 2874	45	41
Babassu oil	Sophorolipids	*Candida lipolytica* IA 1055	11.72	42
Turkish corn oil	Sophorolipids	*Candida bombicola* ATCC 22214	400	43
Sunflower and soybean oil	Rhamnolipid	*Pseudomonas aeruginosa* DS10–129	4.31	44
Sunflower oil	Lipopeptide	*Serratia marcescens*	2.98	44
Soybean oil	Mannosylerythritol lipid	*Candida* sp. SY16	95	45
Oil refinery waste	Glycolipids	*Candida antarctica*, *Candida apicola*	10.5	53
Curd whey and distillery waste	Rhamnolipid	*Pseudomonas aeruginosa* strain BS2	0.92	48
Potato process effluents	Lipopeptide	*Bacillus subtilis*	2.7	51
Cassava flour wastewater	Lipopeptide	*B. subtilis* ATCC 21332, *B. subtilis* LB5a	2.2	35

processes. Often the amount and type of a raw material can contribute considerably to the production cost; it is estimated that raw materials account for 10–30% of the total production cost in most biotechnological processes. Thus to reduce this cost it is desirable to use low-cost raw materials (Table 2) for the production of biosurfactants[10,40]. One possibility explored extensively is the use of cheap and agro-based raw materials as substrates for biosurfactant production. A variety of cheap raw materials, including plant-derived oils, oil wastes, starchy substances, lactic whey and distillery wastes have been reported to support biosurfactant production.

Vegetable oils and oil wastes

Several studies with plant-derived oils have shown that they can act as effective and cheap raw materials for biosurfactant production; for example, rapeseed oil[41], Babassu oil and corn oil[42,43]. Similarly, vegetable oils such as sunflower and soybean oil were used for the production of rhamnolipid, sophorolipid and mannosylerythritol lipid biosurfactants by various microorganisms[44,45]. Apart from various vegetable oils, oil wastes from vegetable-oil refineries and the food industry were also reported as good substrates for biosurfactant production. Furthermore, various waste oils with their origins at the domestic level, in vegetable-oil refineries or soap industries were found to be suitable for microbial growth and biosurfactant production[46,47].

Lactic whey and distillery wastes

The effluent from the dairy industry, known as dairy wastewater, supports good microbial growth and is used as a cheap raw material for biosurfactant production[48]. Dubey and Juwarkar[49] cultivated *P. aeruginosa* BS2 on whey waste; within 48 h of incubation the yield of biosurfactant obtained was 0.92 g/l. Strain BS2 produced a crystalline biosurfactant as the secondary metabolites and its maximal production occurred after the onset of nitrogen-limiting conditions. The isolated biosurfactant possessed the potent surface-active properties, as it effectively reduced the surface tension of water from 72 to 27 mN/m and formed 100% stable emulsion of a variety of water-insoluble compounds.

Starchy substrates

Potato process effluents (waste from potato-processing industries) were used to produce biosurfactant by *B. subtilis*[50,51]. Cassava wastewater, another carbohydrate-rich residue, which is generated in large amounts during the preparation of cassava flour, is also an attractive substrate and has been used for surfactin production by *B. subtilis*[35]. Several other starchy waste substrates, such as rice water (effluent from rice processing industry and domestic cooking), cornsteep liquor and wastewater from the processing of cereals, pulses and molasses, have tremendous potential to support microbial growth and biosurfactant production.

Olive oil mill effluent

Olive oil extraction involves an intensive consumption of water and produces large amounts of olive oil mill wastewater, thus causing deleterious environmental effects. Mercade *et al.*[52] found that *Pseudomonas* sp. could reduce the surface tension in culture medium comprising olive oil mill effluent (OOME; 100 g/l) and $NaNO_3$ (2.5 g/l). Surface-active compounds produced from *Pseudomonas* sp. cultured in OOME medium included rhamnolipids biosurfactant, a total conversion yield was estimated to be 14 g of rhamnolipids per kg of OOME after 150 h of cultivation time.

Animal fat

Animal fat and tallow can be obtained in large quantities from meat-processing industries and have been used as a cooking medium for food. Deshpande and Daniels[53] used

animal fat for the production of sophorolipid biosurfactant by yeast, *C. bombicola*. When only fat was provided as a sole carbon source, the growth was poor. A mixture of 10% glucose and 10% fat gave the highest level of growth. Sophorolipid was produced at levels of 97 and 12 g/l without and with pH control respectively.

Soapstock

Soapstock is a gummy, amber-coloured by-product of oilseed processing. It is produced when hexane and other chemicals are used to extract and refine edible oil from the seeds. Shabtai[54] reported the production of two extracellular capsular heteropolysaccharides, emulsan and biodispersan by *A. calcoaceticus* RAG-1 and *A. calcoaceticus* A2 respectively, using soapstock as a carbon source. Emulsan forms and stabilizes the oil–water emulsion[55], whereas biodispersan disperses the large solid limestone granules, forming micrometre-sized water suspension[56].

Molasses

This is a co-product of sugar production, obtained from sugar cane as well as from sugar beet. Patel and Desai[57] used molasses and cornsteep liquor as the primary carbon and nitrogen source to produce rhamnolipid biosurfactant from *P. aeruginosa* GS3. The biosurfactant production reached a maximum when 7% (v/v) of molasses and 0.5% (v/v) of cornsteep liquor were used. Maximal surfactant production occurred after 96 h of incubation, when cells reached the stationary phase of growth. A rhamnose concentration of 0.25 g/l and a reduction of interfacial tension between surfactant and crude oil of up to 0.47 mN/m were obtained.

Bioprocess development: Optimum production and recovery

An efficient and economical bioprocess is the foundation for every profit-making biotechnology industry. Hence bioprocess development is the primary step towards commercialization of all biotechnological products, including biosurfactants. Any attempt to increase the yield of a biosurfactant demands optimal addition of media components and selection of the optimal culture conditions that will induce the maximum or optimum productivity. Similarly, efficient downstream processing techniques and methods are needed for maximum product recovery.

Process optimization: The best combination of essential factors

Several elements, media components and precursors are reported to affect the process of biosurfactant production and the final quantity and quality. Different elements, such as nitrogen, iron and manganese are reported to affect the yield of biosurfactants; for example, the limitation of nitrogen is reported to enhance biosurfactant production in *P. aeruginosa* BS-2 (ref. 49) and *Ustilago maydis*[58]. Similarly, addition of iron and manganese to the culture medium was reported to increase the production of biosurfactant by *B. subtilis*[59]. The ratios of different elements such as C : N, C : P, C : Fe or C : Mg affected biosurfactant production and their optimization enhanced it.

Downstream processing: Fast, efficient and cheap product recovery

Even if optimum production is obtained using optimal media and culture conditions, the production process is still incomplete without an efficient and economical means for recovery of the products. For many biotechnological products, the downstream processing costs account for ~60% of the total production costs. Several conventional methods for the recovery of biosurfactants, such as acid precipitation, solvent extraction, crystallization, ammonium sulphate precipitation and centrifugation, have been widely reported in the literature[4]. A few unconventional and interesting recovery methods have also been reported in recent years. Few examples of such biosurfactant recovery strategies (Table 3) include foam fractionation[60,61], ultrafiltration[62], adsorption–desorption on polystyrene resins and ion exchange chromatography[63], and adsorption–desorption on wood-based activated carbon[64]. One of the main advantages of these methods is their ability to operate in a continuous mode for recovering biosurfactants with high level of purity. However, the solvents that are generally used for biosurfactant recovery, for example, acetone, methanol and chloroform, are toxic in nature and harmful to the environment. Cheap and less toxic solvents such as methyl tertiary-butyl ether have been successfully used in recent years to recover biosurfactants produced by *Rhodococcus*[65]. These types of low cost, less toxic and readily available solvents can be used to cut the recovery expenses substantially and minimize environmental hazards. Often a single downstream processing technique is not enough for product recovery and purification. In such cases, a multi-step recovery strategy, using a sequence of concentration and purification steps, is more effective[63]. In such a multi-step recovery for biosurfactants, it will be possible to obtain the product at any required degree of purity.

Mutant and recombinant strains: The hyperproducers

The genetics of the producer organism is an important factor affecting the yield of all biotechnological products, because the capacity to produce a metabolite is bestowed

Table 3. Physico-chemical property-based biosurfactant recovery methods and their relative advantages[60–65]

Downstream recovery procedure	Biosurfactant property responsible for separation	Instrument/apparatus/ set-up required	Advantages
Acid precipitation	Biosurfactants become insoluble at low pH values	No set-up required	Low cost, efficient in crude biosurfactant recovery
Organic solvent extraction	Biosurfactants are soluble in organic solvents due to the presence of hydrophobic end	No set-up required	Efficient in crude biosurfactant recovery and partial purification, reusable nature
Ammonium sulphate precipitation	Salting-out of the polymeric or protein-rich biosurfactants	No set-up required	Effective in isolation of certain type of polymeric biosurfactants
Centrifugation	Insoluble biosurfactants get precipitated because of centrifugal force	Centrifuge required	Reusable, effective in crude biosurfactant recovery
Foam fractionation	Biosurfactants, due to surface activity, form and partition into foam	Specially designed bioreactors that facilitate foam recovery during fermentation	Useful in continuous recovery procedures, high purity of product
Membrane ultrafiltration	Biosurfactants form micelles above their critical micelle concentration, which are trapped by polymeric membranes	Ultrafiltration units with porous polymer membrane	Fast, one-step recovery, high level of purity
Adsorption on polystyrene resins	Biosurfactants are adsorbed on polymer resins and subsequently desorbed with organic solvents	Polystyrene resin packed in glass columns	Fast, one-step recovery, high level of purity, reusability
Adsorption on wood-activated carbon	Biosurfactants are adsorbed on activated carbon and can be desorbed using organic solvent	No set-up required. Can be added to culture broth. Can also be packed in glass columns	Highly pure biosurfactants, cheaper, reusability, recovery from continuous culture
Ion-exchange chromatography	Charged biosurfactants are attached to ion-exchange resins and can be eluted with proper buffer	Ion-exchange resins packed in columns	High purity, reusability, fast recovery
Solvent extraction (using methyl tertiary-butyl ether	Biosurfactants dissolve in organic solvents owing to the hydrophobic ends in the molecule	No set-up required	Less toxic than conventional solvents, reusability, cheap

by the genes of the organism. The bioindustrial production process is often dependent on the use of hyperproducing microbial strains, even with cheap raw materials, optimized medium and culture conditions, and efficient recovery processes. A production process cannot be made commercially viable and profitable until the yield of the final product by the producer organisms is naturally high. Moreover, the industrial production process is dependent on the availability of recombinant and mutant hyperproducers if good yields are lacking from the natural producer strains. Even if high-yielding natural strains are available, the recombinant hyperproducers are always required to economize further the production process and to obtain products with better commercially important properties. Besides the natural biosurfactant producer strains, a few mutant and recombinant varieties with enhanced biosurfactant production characteristics are reported in the literature (Table 4). These mutant varieties were produced using various agents, for example, transposons[66], chemical mutagens such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine[67–69], radiation[70] or by selection on the basis of resistance to ionic detergents such as CTAB[71]. In addition to these mutant-hyperproducing varieties, several recombinant strains producing biosurfactants in better yields and showing improved production properties have been developed in recent years.

Applications of biosurfactants

All surfactants are chemically synthesized. Nevertheless, in recent years, much attention has been directed towards biosurfactants due to their broad range of functional properties and diverse synthetic capabilities of microbes. Most important is their environmental acceptability, because they are readily biodegradable and have low toxicity than synthetic surfactants. These unique properties of biosurfactants allow their use and possible replacement of chemically synthesized surfactants in a great number of industrial operations. Moreover, they are ecologically safe and can be applied in bioremediation and wastewater treatment. Some of the potential applications of biosurfactants in pollution and environmental control are microbial enhanced oil recovery, hydrocarbon degradation

Table 4. Mutant and recombinant strains of microorganisms with enhanced biosurfactant yields and with improved product characteristics

Mutant and/or recombinant strain	Characteristic feature	Increased yield and/or improved production properties	Reference
P. aeruginosa 59C7	Transposon Tn5-GM-induced mutant of *P. aeruginosa* PG201	Two times more production	66
P. aeruginosa PTCC 1637	Random mutagenesis with *N*-methyl-*N'*-nitro-*N'*-nitrosoguanidine	Ten times more production	67
B. licheniformis KGL11	Random mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine	Twelve times more production	68
B. subtilis ATCC 55033	Random mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine	Approximately 4–6 times more production	69
P. aeruginosa EBN-8	Gamma ray-induced mutant of *P. aeruginosa* S8	2–3 times more production	70
A. calcoaceticus RAG-1	Mutant selection on basis of resistance to cationic detergent CTAB	2–3 times more production	71

Table 5. Industrial applications of biosurfactants[72]

Industry	Application	Role of biosurfactants
Petroleum	Enhanced oil recovery	Improving oil drainage into well bore, stimulating release of oil entrapped by capillaries, wetting of solid surfaces, reduction of oil viscosity and oil pour point, lowering of interfacial tension, dissolving of oil
	De-emulsification	De-emulsification of oil emulsions, oil solubilization, viscosity reduction, wetting agent
Environmental	Bioremediation	Emulsification of hydrocarbons, lowering of interfacial tension, metal sequestration
	Soil remediation and flushing	Emulsification through adherence to hydrocarbons, dispersion, foaming agent, detergent, soil flushing
Food	Emulsification and de-emulsification	Emulsifier, solubilizer, demulsifier, suspension, wetting, foaming, defoaming, thickener, lubricating agent
	Functional ingredient	Interaction with lipids, proteins and carbohydrates, protecting agent
Biological	Microbiological	Physiological behaviour such as cell mobility, cell communication, nutrient accession, cell–cell competition, plant and animal pathogenesis
	Pharmaceuticals and therapeutics	Antibacterial, antifungal, antiviral agents, adhesive agents, immunomodulatory molecules, vaccines, gene therapy
Agricultural	Biocontrol	Facilitation of biocontrol mechanisms of microbes such as parasitism, antibiosis, competition, induced systemic resistance and hypovirulence
Bioprocessing	Downstream processing	Biocatalysis in aqueous two-phase systems and microemulsions, biotransformations, recovery of intracellular products, enhanced production of extracellular enzymes and fermentation products
Cosmetic	Health and beauty products	Emulsifiers, foaming agents, solubilizers, wetting agents, cleansers, antimicrobial agents, mediators of enzyme action

in soil environment and hexa-chloro cyclohexane degradation, heavy-metal removal from contaminated soil and hydrocarbon in aquatic environment (Table 5)[72]. In this review we discuss the potential roles and applications of biosurfactants, mainly focusing on areas such as food and food-related industries, biomedicine and therapeutics.

Potential food applications

Biosurfactants can be explored for several food-processing applications. In this section we emphasize their potential as food-formulation ingredients and antiadhesive agents.

Food-formulation ingredients: Apart from their obvious role as agents that decrease surface and interfacial tension, thus promoting the formation and stabilization of emulsions, surfactants can have several other functions in food. For example, to control the agglomeration of fat globules, stabilize aerated systems, improve texture and shelf-life of starch-containing products, modify rheological properties of wheat dough and improve consistency and texture of fat-based products[73]. In bakery and ice-cream formulations biosurfactants act by controlling consistency, retarding staling and solubilizing flavour oils; they are also utilized as fat stabilizers and antispattering

agents during cooking of oil and fats. Improvement in dough stability, texture, volume and conservation of bakery products is obtained by the addition of rhamnolipid surfactants[74]. The study also suggested the use of rhamnolipids to improve the properties of butter cream, croissants and frozen confectionery products. L-Rhamnose has considerable potential as a precursor for flavouring. It is already used industrially as a precursor of high-quality flavour components like furaneol.

Antiadhesive agents: A biofilm is described as a group of bacteria that have colonized a surface. The biofilm not only includes bacteria, but it also describes all the extracellular material produced at the surface and any material trapped within the resulting matrix. Bacterial biofilms present in the food industry surfaces are potential sources of contamination, which may lead to food spoilage and disease transmission[75]. Thus controlling the adherence of microorganisms to food-contact surfaces is an essential step in providing safe and quality products to consumers. The involvement of biosurfactants in microbial adhesion and detachment from surfaces has been investigated. A surfactant released by *Streptococcus thermophilus* has been used for fouling control of heat-exchanger plates in pasteurizers, as it retards the colonization of other thermophilic strains of *Streptococcus* responsible for fouling. The preconditioning of stainless steel surfaces with a biosurfactant obtained from *Pseudomonas fluorescens* inhibits the adhesion of *L. monocytogenes* L028 strain. The bioconditioning of surfaces through the use of microbial surfactants has been suggested as a new strategy to reduce adhesion.

Therapeutic and biomedical applications

Antimicrobial activity: Several biosurfactants have shown antimicrobial action against bacteria, fungi, algae and viruses. The lipopeptide iturin from *B. subtilis* showed potent antifungal activity[76]. Inactivation of enveloped virus such as herpes and retrovirus was observed with 80 mM of surfactin[77]. Rhamnolipids inhibited the growth of harmful bloom algae species, *Heterosigma akashivo* and *Protocentrum dentatum* at concentrations ranging from 0.4 to 10.0 mg/l. A rhamnolipid mixture obtained from *P. aeruginosa* AT10 showed inhibitory activity against the bacteria *Escherichia coli*, *Micrococcus luteus*, *Alcaligenes faecalis* (32 mg/ml), *Serratia arcescens*, *Mycobacterium phlei* (16 mg/ml) and *Staphylococcus epidermidis* (8 mg/ml) and excellent antifungal properties against *Aspergillus niger* (16 mg/ml), *Chaetonium globosum*, *Enicillium crysogenum*, *Aureobasidium pullulans* (32 mg/ml) and the phytopathogenic *Botrytis cinerea* and *Rhizoctonia solani* (18 mg/ml)[78]. Sophorolipids and rhamnolipids were found to be effective antifungal agents against plant and seed pathogenic fungi. The mannosylerythritol lipid (MEL), a glycolipid surfactant from *Candida antartica*, has demonstrated antimicrobial activity particularly against Gram-positive bacteria[79].

Anticancer activity: The biological activities of seven microbial extracellular glycolipids, including mannosylerythritol lipids-A, mannosylerythritol lipids-B, polyol lipid, rhamnolipid, sophorose lipid, succinoyl trehalose lipid (STL)-1 and succinoyl trehalose lipid-3 have been investigated[80]. All these glycolipids, except rhamnolipid, were found to induce cell differentiation instead of cell proliferation in the human promyelocytic leukaemia cell line HL60. STL and MEL markedly increased common differentiation characteristics in monocytes and granulocytes respectively. Exposure of B16 cells to MEL resulted in the condensation of chromatin, DNA fragmentation and sub-G1 arrest (the sequence of events of apoptosis). This is the first evidence that growth arrest, apoptosis and differentiation of mouse malignant melanoma cells can be induced by glycolipids[81]. In addition, exposure of PC12 cells to MEL enhanced the activity of acetylcholine esterase and interrupted the cell cycle at the G1 phase, with resulting outgrowth of neurites and partial cellular differentiation[82]. This suggests that MEL induces neuronal differentiation in PC12 cells and provides the groundwork for the use of microbial extracellular glycolipids as novel reagents for the treatment of cancer cells. Another report suggested that the cytotoxic effects of sophorolipid on cancer cells of H7402, A549, HL60 and K562 were investigated by MTT assay. The results showed a dose-dependent inhibition ratio on cell viability according to the drug concentration <62.5 g/ml. These findings suggested that the sophorolipid produced by *W. domercqiae* have anticancer activity[83].

Immuno modulatory action: Sophorolipids are promising modulators of the immune response. It has been previously demonstrated that sophorolipids, (1) decreased sepsis related mortality at 36 h *in vivo* in a rat model of septic peritonitis by modulation of nitric oxide, adhesion molecules and cytokine production and (2) decreased IgE production *in vitro* in U266 cells possibly by affecting plasma cell activity. The results show that sophorolipids decrease IgE production in U266 cells by downregulating important genes involved in IgE pathobiology in a synergistic manner. These data continue to support the utility of sophorolipids as an anti-inflammatory agent and a novel potential therapy in diseases of altered IgE regulation[84].

Anti-human immunodeficiency virus and sperm-immobilizing activity: The increased incidence of human immunodeficiency virus (HIV)/AIDS in women aged 15–49 years has identified the urgent need for a female-controlled, efficacious and safe vaginal topical microbicide. To meet this challenge, sophorolipid produced by *C. bombicola*

and its structural analogues have been studied for their spermicidal, anti-HIV and cytotoxic activities[85]. The sophorolipid diacetate ethyl ester derivative is the most potent spermicidal and virucidal agent of the series of sophorolipids studied. Its virucidal activity against HIV and sperm-immobilizing activity against human semen are similar to those of nonoxynol-9. However, it also induced enough vaginal cell toxicity to raise concerns about its applicability for long-term microbicidal contraception.

Agents for respiratory failure: A deficiency of pulmonary surfactant, a phospholipid protein complex is responsible for the failure of respiration in prematurely born infants. Isolation of genes for protein molecules of this surfactant and cloning in bacteria have made possible its fermentative production for medical applications[12].

Agents for stimulating skin fibroblast metabolism: The use of sophorolipids in lactone form comprises a major part of diacetyl lactones as agents for stimulating skin dermal fibroblast cell metabolism and more particularly, as agents for stimulating collagen neosynthesis, at a concentration of 0.01 ppm at 5% (p/p) of dry matter in formulation. This is applicable in cosmetology and dermatology. The purified lactone sophorolipid product is of importance in the formulation of dermis anti-ageing, repair and restructuring products because of its effect on the stimulation of dermis cells. By encouraging the synthesis of new collagen fibres, purified lactone sophorolipids can be used both as a preventive measure against ageing of the skin and used in creams for the body, and in body milks, lotions and gels for the skin[86].

Antiadhesive agents in surgicals: Pre-treatment of silicone rubber with *S. thermophilus* surfactant inhibited by 85% adhesion of *C. albicans*[87], whereas surfactants from *L. fermentum* and *L. acidophilus* adsorbed on glass, reduced by 77% the number of adhering uropathogenic cells of *Enterococcus faecalis*. The biosurfactant from *L. fermentum* was reported to inhibit *S. aureus* infection and adherence to surgical implants[88]. Surfactin decreased the amount of biofilm formation by *Salmonella typhimurium*, *S. enterica*, *E. coli* and *Proteus mirabilis* in PVC plates and vinyl urethral catheters[89].

Future trends

Successful commercialization of every biotechnological product depends largely on its bioprocess economics. At present, the prices of microbial surfactants are not competitive with those of the chemical surfactants due to their high production costs and low yields. Hence, they have not been commercialized extensively. For the production of commercially viable biosurfactants, process optimization at the biological and engineering level needs to be improved. Improvement in the production technology of biosurfactants has already enabled a 10–20-fold increase in productivity, although further significant improvements are required. However, the use of cheaper substrates and optimal growth and production conditions coupled with novel and efficient multi-step downstream processing methods and the use of recombinant and mutant hyperproducing microbial strains can make biosurfactant production economically feasible. Novel recombinant varieties of these microorganisms, which can grow on a wide range of cheap substrates and produce biosurfactants at high yields, can potentially bring the required breakthrough in the biosurfactant production process. Although a large number of biosurfactant producers have been reported in the literature, biosurfactant research, particularly related to production enhancement and economics, has been confined mostly to a few genera of microorganisms such as *Bacillus*, *Pseudomonas* and *Candida*. As documented in this review, biosurfactants are not only useful as antibacterial, antifungal and antiviral agents, they also have the potential for use as major immunomodulatory molecules, adhesive agents and even in vaccines and gene therapy. A judicial and effective combination of these strategies might, in the future, lead the way towards large-scale profitable production of biosurfactants. This will make biosurfactants highly sought after biomolecules for present and future applications as fine specialty chemicals, biological control agents, and new generation molecules for pharmaceutical, cosmetic and health care industries.

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