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MICROBIAL SURFACTANTS: AN OVERVIEW

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ABSTRACT

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. Currently, biosurfactants are not widely utilized in the petroleum industry due to high production costs associated with use of expensive substrates and inefficient product recovery methods. The economics of biosurfactant production could be significantly impacted through use of media optimization and application of inexpensive carbon substrates such as agricultural process residuals. Utilization of biosurfactants produced from agricultural residuals may result in an economic advantage for surfactant production and technology application, and convert a substantial agricultural waste stream to a value added product. 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 and different microorganisms produce different biosurfactants. 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: biosurfactants, classification, renewable-resources, application

INTRODCUTION

Microbial surfactants or biosurfactants are the surface-active molecules derived from
a large number of microorganisms. These microbially produced surface-active compounds possess the ability to reduce the surface and interfacial tension between two immiscible fluid phases. It is only in the past few decades that surface active molecules of microbial origin, referred to as biosurfactants, have gained considerable interest. Biosurfactants have advantages over their chemicals counterparts because they are biodegradable [1], have low toxicity [2], are effective at extreme temperatures or pH values [3] and show better environmental compatibility [4].

BIOSURFACTANT PRODUCING MICROORGANISMS

Various types of biosurfactants are synthesized by a number of microbes particularly during their growth on water-immiscible substrates. A majority of biosurfactants are produced by bacteria. *Bacillus, Clostridium* and *Pseudomonas* species have often been used in the production of biosurfactant.

Classification of Biosurfactant

The microbial surfactants are complex molecules covering a wide range of chemical types including peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, etc. Rosenberg and Ron [5] 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. A brief discussion about each class of biosurfactant is given below.

Glycolipids:

Glycolipids are the most common types of biosurfcatnt. They are carbohydrates in combination with long-chain aliphatic acids or hydroxyaliphatic acids. Among the glycolipids, the best known are rhamnolipids, trehalolipids, and sophorolipids.

Rhamnolipids

Rhamnolipids, in which one or two molecules of rhamnose are linked to one or two molecules of β-hydroxydecanoic acid. These are the best-studied glycolipids. Production of rhamnose-containing glycolipids was first described in *Pseudomonas aeruginosa* by Jarvis and Johnson [6]. Rhamnoipids produced by *Pseudomonas aeruginosa* strains are among the most effective surfactants when applied for the removal of hydrophobic compounds from contaminated soils [7].

Trehalolipids

The production of trehalose lipids seen in many members of the genus *Mycobacterium.* The typical structure is due to the presence of trehalose esters on the cell surface [8] from different species of *Mycobacteria* [8], *Corynebacteria, Nocardia,* and *Brevibacteria* differ in size and structure of the mycolic acid esters. Trehalose lipids from *Rhodococcus erythropolis* and *Arthrobacter* sp. lowered the surface and interfacial tension in culture broth from 25 to 40 and 1 to 5 mN/m respectively [9].

Sophorolipids

These glycolipids are produced mainly by yeast such as *Torulopsis bombicola* [10]. Sophrolipids (SLs) consist of a dimeric sugar (sophorose) and a hydroxyl fatty acid, linked by a β-glycisidic bond [11] . According to Hu and Ju, [12] there are two types of SLs namely, the acidic (non-lactonic) SLs and the lactonic SLs. The hydroxyl fattyacids moity of the acidic SLe s has a free carboxylic acid functional group whilst that of the lactonic SLs forms a macrocyclic lactone ring with 4”- hydroxyl group of sophorose by intramolecular esterification.

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 [13].

Surfactin

Surfactin just as any other biosurfcatant reduces surface tension from 72-27 mN m^{-1} with concentration as low as 0.005%, making surfcatn one of the most powerful biosurfctants [14]. The cyclic lipopeptides surfactin produced by *Bacillus subtilis* ATCC 21332 is an example of 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.

Lichenysin

Lichenysin is formed during growth of *Bacillus licheniformis* JF2 under both aerobic and anaerobic conditions [15]. It lowers the surface tension of water from 72 mN m-1 to 28 mN m-1.

The detailed characterization of lichenysin A showed that isoleucine was the C-terminal amino acid instead of leucine and an asparagine residue was present instead of aspartic acid as in the surfactin peptide.

Fatty Acids, Phospholipids, and Neutral Lipids

Fatty acids produced from alkanes as a result of microbial oxidations have been considered as surfactants [16]. Several bacteria and yeasts produce large quantities of fatty acid and phospholipid surfactants during growth on *n*-alkanes [17]. Phospholipids are known to form major components of microbial membranes. When certains hydrocarbon-degrading bacteria or yeast are grown on alkane substrates, the level of phospholipids increases greatly [18].

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 called emulsan [19]. It is an effective emisifying agent for hydrocarbons in water, even at a concentration as low as 0.001 to 0.01%. Additionally, it is noted as one of the most powerful emulsion stabilizers known with the ability to resist inversion even at water to oil ratio of 1:4 [20]. Liposan is an extracellular water-souble emulsifier synthesized by *Candida lipolytica* and is composed of 83% carbohydrate and 17% protein [21].

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 [22].

PRODUCTION OF BIOSURFACTANT FROM RENEWABLE SOURCE

Though biosurfactant have advantage over synthetic surfactants, it can only replace the synthetic if the cost of the raw material and the process is minimal. So far, several renewable substrates form various sources; especially from industrial wastes have been intensively studied for microorganisms' cultivation and surfactant production at an experimental scale. 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.

Plant derived oils

Several studies with plant-derived oils have shown that they can act as effective and cheap raw materials for biosurfactant production. Vegetable oils are a lipidic carbon source and are mostly comprised of saturated or unsaturated fatty acids with 16-18 carbon atoms chain. Researchers have used variety of vegetable oils from canola, corn, sunflower, safflower, olive, rapeseed, grape seed, palm, coconut, fish and soybean oil.

Haba *et al.* [23] used olive or sunflower cooking oil as carbon source for biosurfactant production by 36 isolated bacteria. Kitamoto *et al*. [24] studied the interfacial and antimicrobial properties of two kinds of mannosylerythritol lipids (MEL-A and B), biosurfactants, produced by *Candida antarctica T-34*, when grown on soybean oil as substrate. In a study of Sim *et al*. [25] they tested mixture of vegetable oils (canola oil, soy bean and glucose), for rhamnolipid production by *P. aeruginosa* UW-1 and reported 10-12 fold increase in rhamnolipid production on vegetable oils in comparison to glucose. Camargo-de-Morais *et al*. [26] studied the production of a glycolipid with emulsifier properties during cultivation of *Penicillium citrinum* on mineral medium with 1% olive oil as carbon source.

Thaniyavarn *et al*. [27] studied the biosurfactant production by marine isolates Pseudomonas aeruginosa A41 by using the defined medium containing 2% vegetable oil or fatty acid as a carbon source. Oliveira *et al*. [28] used palm oil, a low-cost agricultural byproduct which is used in as raw material for soap and food industries, for biosurfactant production using *Pseudomonas alcaligenes* (a strain isolated from crude oil contaminated soil).

Oil wastes

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 [23]. Soapstock is a gummy, ambercolored byproduct of oilseed processing. It is produced when hexane and other chemicals are used to extract and refine edible oil from the seeds. Shabtai [29] reported the production of two extracellular capsular heteropolysaccharides, emulsan and biodispersan by *Acinetobacter calcoaceticus* RAG-1 and *A. calcoaceticus* A2, respectively using soap stock as a carbon source. Mercade *et al*. [30] were the first group to show the production of rhamnolipids by P. *aeruginosa* 47T2 when grown on olive oil mill effluent (OOME) as the sole carbon source (a major waste problem in Spain). Soy molasses is a byproduct of soybean oil processing industry, is a best substrate for biosurfactant production. Soy molasses were used to produce sophorolipids by *Candida bombicola* [31]. The yield of pure biosurfactant was 21 g/l. In an effort to economize biosurfactant production Thavasi *et al*. [32] used a mixture of peanut oil cake and waste motor lubricant oil as a substrate for the biosurfactant production.

Another raw materials associated with vegetable industry is residual cooking or frying oil which is a major source of nutrient rich low cost fermentative waste. Sadouk *et al*. [33] in an approach for reducing the cost of production of glycolipids by Rhodococcus erythropolis 16 LM.USTHB converted residual sunflower frying oil, a cheap renewable substrate, into extracellular glycolipids.

Starchy substances

Starch is a major agricultural product of corn, tapioca, wheat and potatoes which are major crops. Sugar and starch processing industries also produce large amount of solid residues of starch containing wastewater. The processing of agroindustrial raw materials such as cassava or potato produces the large amount of waste, whose accumulation leads to environmental pollution. Due to the high amounts of starch or reducing sugar, those wastes has been recognized as a suitable feedstock for industrial fermentations such as production of pullulan [34] and volatile compounds [35].

Das and Mukherjee [36] studied the efficiency of two *Bacillus subtilis* strains for the production of biosurfactants in two fermentation systems using powdered potato peels as substrate. Wang *et al*. [37] applied a *Bacillus subtilis* strain B6-1, for production of biosurfactant using soybean and sweet potato residues in solid-state fermentation. In addition, Nitschke and Pastore [38] used a cassava flour-processing effluent as a substrate for surfactant production by *Bacillus subtilis* LB5a and *Bacillus subtilis* ATCC 21332.

Lactic whey and distillery wastes

The dairy industry has a considerable amount of byproducts such as buttermilk, whey, and their derivatives. Dubey and Juwarkar [39] cultivated *Pseudomonas aeruginosa* BS2 on whey waste for biosurfactant production. Daniel *et al*. [40] reported the high yields of sophorolipids production with whey concentrate and rapeseed oil as substrate. Molasses is also used as substrate for biosurfactant production [41- 43].

APPLICATION OF BIOSURFACTANTS

Biosurfactants are widely used in many industries such as agriculture, food production, chemistry, cosmetics and pharmaceutics. Some of the potential applications of biosurfactants in pollution and environmental control are microbial enhanced oil recovery, hydrocarbon degradation in soil environment and hexa-chloro cyclohexane degradation, heavy-metal removal from contaminated soil and hydrocarbon in aquatic environment.

Biosurfactant in food production

Biosurfactants can be explored for several food-processing applications. It has been applied in food industries usually as food additives (emulsifiers). For instance, lectin and its derivatives, fatty acids esters containing glycerol, sotbitan or ethylene glycol and ethoxylated derivatives of monoglycerides including recently synthesized oligopeptides [44]. These emulsifiers have a long way to improving the flavor, taste and quality of products with minimal health hazards.

It is also used 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 [45]. 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 [46]. Rhamnolipids was also used 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 [47]. Another application of biosurfactants in food industry is as antiadhesive agents. 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 [47].

Cosmetic application of Biosurfactant

Like synthetic surfactants, biosurfactant are excellent emulsifiers and maintain wetting and foaming properties, due to this characteristics of biosurfactant are valued in several applications in cosmetic industry. These compounds are as effective as chemical surfactants and have excellent skin compatibility that synthetic surfactants lack. Glycolipids surfactants have been most extensively analyzed, especially in the cosmetic industry due to exceptional skin compatibility [48]. Sophorolipids in particular are on the brink of being in-corporated into several cosmetic applications. Common types of glycolipids are sophorolipids, mannosylerythritol lipids and rhemnolipids. *C. bombicola* is yeast commonly used for the production of sophorolipids which exhibit moisturizing, antibacterial, and antioxidant properties [49].

Therapeutic and biomedical applications:

Biosurfactants have some therapeutic applications. The lipopeptide iturin from *B. subtilis* showed potent antifungal activity [50]. Rhamnolipids inhibited the growth of harmful bloom algae species, *Heterosigma akashivo* and *Protocentrum dentatum*. A rhamnolipid mixture obtained from *P. aeruginosa* AT10 showed inhibitory activity against the bacteria *Escherichia coli*, *Micrococcus luteus*, *Alcaligenes faecalis*, *Serratia arcescens*, *Mycobacterium phlei* and *Staphylococcus epidermidis* and excellent antifungal properties against *Aspergillus niger*, *Chaetonium globosum*, *Enicillium crysogenum*, *Aureobasidium pullulans* and the phytopathogenic *Botrytis cinerea* and *Rhizoctonia solani* [51]. The biosurfactant from *L. fermentum* was reported to inhibit *S. aureus* infection and adherence to surgical implants [52].

Agricultural application

Biosurfactant also have application in agriculturel industry. It is used as antifungal agent against the phytopathogen. Stanghellini and Miller [53] studied about the antifungal activity of rhamnolipids against three genera of zoosporic phytopathogen; *Pythium aphanidermatum, Phytophthora capsici* and *Plasmopara lactucea-radicis*. Biosurfactants are potentially used in various formulations of herbicides and pesticides [5]. An example is the use of glycolipoppetides produced by strains of *Bacillus* for emulsifying immiscible organophosphorus pesticides [54].

Biosurfactants in pollution and environmental control

A promising method that can improve bioremediation effectiveness of hydrocarbon contaminated environments is the use of biosurfactants. They can enhance hydrocarbon bioremediation by two mechanisms. [55]. By reducing surface and interfacial tensions, biosurfactants increase the surface areas of insoluble compounds leading to increased mobility and bioavailability of hydrocarbons. In consequence, biosurfactants enhance biodegradation and removal of hydrocarbons. Biosurfactant used degrade hydrocarbon in aquatic environment. Surfactants enhance degradation by dispersing and emulsifying hydrocarbons. Chakrabarty [56] reported that an emulsifier produced by *P. aeruginosa* SB30 was able to quickly disperse oil into fine droplets; therefore it may be useful in removing oil from contaminated beaches [57].

CONCLUSION

Biosurfactants have received more and more attention in recent years as surface-active compounds released by microorganisms that have some influence on interfaces, most notably on the surface tension of liquid – vapor interfaces. Over the years, biosurfactants are not widely utilized in the industries due to high production costs, if the scientific worlds overcome these problems by the use of inexpensive substrates and better biotechnological tools biosurfactant will shine as most valuable biological molecule in future.

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