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9. Advances in the research of new biosurfactants and their potential use in the biomedical and pharmaceutical industry

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Abstract. Biosurfactants (BS) are a structurally diverse group of surface-active substances produced by microorganisms. Interest in BS production has markedly increased during the past decade, although large-scale production has not been possible because of low production yields and high total costs. At present, BS have gained importance in environmental applications, while new applications in the pharmaceutical, biomedical, cosmetic and food industry, with a high added value, are still developing. This article describes classical and new BS producer bacteria together with their new BS applications. With these specialized and cost-effective applications, BS can be considered as an interesting option for the near future.

1. Introduction

Surfactants are amphipathic molecules that accumulate at interfaces, decrease interfacial tensions and form aggregate structures such as micelles [1]. Surface active agents or surfactants are an integral part of our everyday life and are products with a worldwide production. The enormous market

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demand for surfactants is currently met by numerous synthetic, mainly petroleum-based, chemical surfactants. About 54% of the total surfactant output is utilized in household/laundry detergents, with only 32% destined for industrial use [2]. Chemical surfactants are available in many forms, and are generally classified based on charge as anionic, non-ionic, cationic and amphoteric. Cationic surfactants are the most toxic and have historically been used as antimicrobials, while anionics are less toxic and are more active against Gram positive than Gram negative bacteria, and non-ionics are often considered non-toxic. Important surfactants include linear alkylbenzene sulfonates (LAS), fatty alcohol ethoxylates (FAEO) and lauryl ether sulfate (LES) [3].

The growing awareness towards the use of renewable-based products and "green products" has stimulated the development of alternatives to these chemical surfactants. Biosurfactants (BS) are an example of such environmental friendly options [4]. BS can be obtained either by chemical synthesis from renewable resources, by microbial fermentation processes or by enzymatic syntheses [5].

The amphiphilic nature of BS allows the partition at water/air, oil/air or the oil/water interfaces where it lowers surface and/or interfacial tension. Such characteristics enable emulsifying, foaming, detergency and dispersing properties [6]. One important feature of BS is that they have very low critical micelle concentrations (CMC). This means that BS are effective at low concentrations, lower than many chemically made surfactants. The fact that only small amounts of BS are needed to reduce surface tension coupled with their known biodegradability makes them excellent candidates for "green" detergents and surfactants.

Comparing with chemical surfactants, BS have several advantages such as lower toxicity, higher biodegradability and diversity, effectiveness at extreme temperatures or pH values and widespread applicability. Additionally, their unique structures provide new properties that classical surfactants may lack. BS occur naturally in soil, which makes them acceptable from a social and ecological point of view [2].

The most remarkable difficulty for BS commercial application is the BS production at low concentrations, which makes product recovery difficult and expensive. In order to reduce their production costs and increase their competitiveness, various researches have been working in the development of new and cheaper processes and/or in the use of low-cost raw materials [4].

2. Natural Roles of Biosurfactant

Recent research indicates that there are a number of reasons why microorganisms make BS; mainly these reasons relate to the need to change surface or interfacial properties of the cell or local environment. Prokaryotic cells communicate with the environment through their envelopes. Smaller cells have a higher surface/volume ratio, and thus can have a more efficient exchange of nutrients with their surrounding than larger cells. To have an effective relationship with the environment, microorganisms need surfactants [7]. When considering the natural roles of BS, it is important to emphasize that they are produced by a wide variety of diverse microorganisms and have very different chemical structures and surface properties. This diversity makes difficult to generalize about the natural role of BS [8]. Nevertheless, BS appear to play a role whenever a microbe encounters an interface.

BS regulate the attachment-detachment of microorganisms to and from surfaces. If a BS is excreted, it can modify the interface and enable or inhibit bacterial attachment. BS that low interfacial tension are particularly effective in mobilizing bound hydrophobic molecules and making them available for biodegradation [1,8]. BS may be used as carbon and energy storage molecules, as a protective mechanism against high ionic strength, and may simply be byproducts released in response to environmental change such as extracellular coverings [1]. Surface or interfacial tension changes are needed in the complex social responses that control cell development.

BS are important for motility (gliding and swarming motility as well as de-adhesion from surfaces) and cell-cell interactions (biofilm formation, maintenance and maturation, quorum sensing, amensalism and pathogenicity). In response to certain environmental signals, bacteria will differentiate from an independent free-living or to an interdependent surface attached mode of live. Surfactants such as rhamnolipids may be able to maintain open channels in biofilm formations by affecting cell-cell interactions and the attachment of bacterial cells to surfaces. Rhamnolipid synthesis is an active mechanism whereby the bacteria exploit intracellular interaction and communication to actively maintain these channels [9]. Rhamnolipid production not only guarantees the structure, and therefore the nutritional balance of the biofilm, but these surfactants also prevent outside invading bacteria from colonizing the open spaces in the resident biofilm [10].

Much research has been directed towards determining how microorganisms can enhance acces to poorly soluble substrates prior to either passive or active uptake into the cell. BS may play a role mediating interactions between the hydrophobicity of the cell surface and the substrate surface. It is known that exogenously added BS can increase the apparent water solubility of organic compounds and alter its bioavailability. BS are used to complex with heavy metals. Rhamnolipid eliminated cadmium toxicity when added to the medium by a combination of metal complexation and rhamnolipd interaction with the cell surface to alter cadmium uptake [8,11,12]. It has been described that di-rhamnolipid removed from artificial contaminated soil, not only the leachable or available fraction of Cd and Pb but also the bound metals. Additionally, the microbial population of the contaminated soil was increased after removal of metals, indicating the decrease of toxicity of heavy metals to soil microflora. Heavy metals removal from sediments in a continuous flow configuration also was possible whith rhamnolipids [13].

3. Biosurfactant Classification

BS are classified based on their chemical structure. A typical BS is composed of a hydrophilic component (mainly amino acids, peptides or proteins, mono/disaccharides, polysaccharides) and a hydrophobic component (mainly saturated or unsaturated fatty acids, hydroxyl fatty acids or fatty alcohols) [6]. BS molecular masses generally range from 500 to 1500 Da and their CMC values range from 1 to 200 mg/L [1].

BS are structurally diverse and can have various chemical structures mainly consisting of glycolipids, lipopeptides, phospholipids, neutral lipids, polymeric surfactants and particulate BS, depending on the producing microorganism, raw matter and process conditions [5,6]. Low molecular weight BS are able to reduce the surface tension of water to 25-30 mN/m. The main property of high molecular weight BS (polymeric and particulate) is their ability to stabilize oil/water emulsions and they are therefore called bioemulsifiers [8,14]. A brief description about each class of BS is given below.

Glycolipids: Most known BS are glycolipids. They are commonly mono or disaccharides acylated with long chain fatty acids or hydroxyl fatty acids. Among them, rhamnolipids, trehalose lipids and sophorolipids are the best studied. Cellobioselipids and mannosyl erythritol lipids are also included in this group.

- Rhamnolipids are exoproducts of the opportunistic pathogen *Pseudomonas aeruginosa* and were described as a mixture of four congeners. Naturally produced rhamnolipids are always found as mixtures of different congeners, as observed with various strains of *P. aeruginosa* [15,16,17]. The development of more sensitive analytical techniques has lead to the further discovery of a wide diversity of rhamnolopid congeners and homologues that are produced at different concentrations by various *Pseudomonas* species. Recently, bacteria belonging to other families, classes or even phyla have been isolated and described as rhamnolipid producers [18].
- Different trehalose containing glycolipids are known to be produced by several microorganisms belonging to mycolates group, such as *Mycobacteria, Corynebacteria*, *Arthrobacter, Nocardia*, *Gordonia*

and specially *Rhodococcus*. Most of the trehalose lipids synthesized by this group are bound to the cell envelope and are produced mainly when microorganisms are grown on hydrocarbons probably as strategy to overcome the low solubility of hydrocarbons and enhance their transport. Microbial commercial success of trehalose lipids is scarce because its high cost of production related to the fact that are bound to the cellular envelope and requires non-renewable *n*-alkane carbon sources [14].

- Sophorolipids are produced by various yeast species, the most intensively studied being *Candida bombicola*. There are generally considered as the most promising glycolipids, because the producing microorganism is non-pathogenic and because of the high substrate conversion, high productivity and product recovery [3].

Lipopeptides: Several types of cyclic lipopeptides with surface active properties are produced mainly by members of the *Bacillus* sp. Up to now, lipopeptides are the most effective surfactants with high efficency, that is, low BS concentration produces significant reduction of surface tension. Antimicrobial activity is a frequent property present in this group.

- *Bacillus subtilis* produces a cyclic lipopeptid called surfactin, which has been reported to be one of the most active BS that has been discovered to date [8].
- *Bacillus licheniformis* produced lichenysin, a lipopeptide (1006 to 1034 Da) with a lipid moiety mixture of 14 linear and branched beta-hydroxy fatty acids (C_{12}-C_{17}) and aminoacids such as glutamic acid, aparagine, valine, leucine and isoleucine. This BS is a powerful surface active agent (surface tension: 28 mN/m; CMC: 12 mg/L) and presents good antibacterial activity [19].
- The polymyxins are a group of lipopeptides produced by *Bacillus polymixa* and related bacilli. Polimixin B is a decapeptide in which amino acids 3-10 form a cyclic octapeptide with a branched-chain fatty acid connected to the terminal 2,4-diaminobutyric acid (DAB). The hydrophobic side-chain of the fatty acid together with the cationic γ-amino groups of the DAB residues give these antibiotics the surface-active properties of a catinoc detergent [8].

Phospholipids: Kretschmer *et al.* [20] described in 1982 the lipophilic compounds with surfactant properties of the culture suspension containing *Rhodococcus erythropolis*. Thirteen major lipids from organic crude extract were isolated. Non polar and polar lipids from which nonionic triglycerides and α,α-trehalose corynomycolates were the most abundant lipids. From components described, phosphatidylethanolamines (PE) acted as the most powerful surfactants reducing the interfacial tension below 1mN/m.

Neutral lipids: The report of the production of surface active bile acids by *Myroides* SM1 bacterial strains was performed by Maneerat *et al* (2005) [21]. These compounds are composed of cholic acid, deoxycholic acid and their glycine conjugates.

The biosphere offers a vast natural resource of BS. Sampling and isolation of bacteria are the basis for screening of BS producing microbe. Hydrocarbon-contaminated sites are promising for the isolation of BS producing microbes. Mercadé *et al.* (1996) [22] isolated 5 BS producing strains from petroleum-contaminated soil samples by using waste lubricating oil as the sole carbon source being *Pseudomonas* sp. the most frequent isolate, followed by *Rhodococcus* sp. Espuny *et al.* (1995) [23] and Abalos *et al.* (2001) [15] also described the isolation BS producing strains from vegetal oil-contaminated soil sample. Nevertheless, undisturbed soils also can have BS producing bacteria. Strain 6.2S, isolated from volcanic soil and identified as *Sphingobacterium* sp., was recently reported as a new BS producer [7].

The exploration of the marine habitat which has been underexplored has brought about new microorganisms. This represents a great opportunity to discover new bioactive compounds (new functional products). Up to now, more than 10,000 metabolites with biological activities have been isolated from marine microorganisms, meeting the needs of the industry in the new era of the white biotechnology, the "green chemistry". Beside the well known soil microorganisms, BS-yielding marine microbes emerge as a wide source of BS producers.

A number of new BS has been recently described and accelerated advances in molecular and cellular biology are expected to expand our insight into the diversity of structures and applications of BS. Inside of glycolipid class, of special interest are the first *Ochrobactrum* sp. (*α-Proteobacteria*) and *Brevibacterium* sp. (*Actinobacteria*) isolates which produce a glycolipid with surface activity [24]. Some marine γ-proteobacteria as *Pseudomonas fluorescens,* produced a complex BS composed of trehaloselipids link to diacyl-monoglyceride-protein [25]. This complex renders the cell capable of degrading hydrocarbons. New *Halomonas* sp. glycoprotein has been described with a high emulsifying activity. Other strain of *Halomonas* sp. accumulated glycolipids (oligosaccharide-lipids) and a noteworthy sulfated heteropolysaccharie was produced by *Halomonas eurithalina* [26]. *Alcanivorax borkumensis*, besides to be a good polyhydroxy-alkanoate producer, produces an anionic glycolipid containing glycine for the uptake of hydrophobic substrates [27]. Other bacteria belonging to the β-proteobacteria such as *Alcaligenes* sp. [28] produces glycolipids, or *Arthrobacter* sp. which produces a trehalose-lipid, very effective BS [29]. Recently, a marine *Azotobacter chroochocum* has been described as a BS producer [30].

From the phylum of the *Bacteroidetes,* the genus *Myriodes* (formerly *Flavobacterium*), commonly found in marine habitat especially after oil-spills, produced ornithine-lipids often cell associated, which allow the cell to degrade hydrocarbons, however, surface active properties diminished in extreme conditions [31]. In the genus *Myroides*, recently *Myroides odoratus* has been reported to produce bile acids [21]. *Corynebacterium kutscher*, a glicopeptide-lipid producer, is a new BS producer from sea belonging to phylum *Actinobacteria* [32].

The family of the lipopeptides, a highly effective BS group, has been expanded with the description of *Bacillus circulans* as a new species producing a lipopeptide with low toxicity [33] and by a new type of lichenysin, produced by *B. licheniformis,* whose most novel characteristic is that the alkyl-chain size ranges between C_{12} to C_{17} [33]. Another lipopeptide type surfactant is produced by *Azotobacter chroococum* growing in motor lubricant oil. A sponge-associate *Actinomyces* has been recenly reported. It is noteworthly to mention that a glycopeptide surfactant has been detected in a strain of *Bacillus megaterium* [34].

The diversity of BS is limited only by biological evolution and suggests that only a tiny fraction of BS has been characterized to date. New BS discoveries have relied on the employment of advanced analytical methods and, often, significant screening efforts [1]. Although the improvements obtained from these strategies to successfully compete with synthetic surfactants, novel microorganisms must be designed. Data on the genes involved on the production of BS are critical to designing organisms with improved features and new properties [36].

4. *Sphingobacterium* sp., a New Biosurfactant Producer Bacterium

Recently, the isolation of a new BS-producing bacterium was carried out on a soil sample of the Azores islands. From 10 volcanic earth samples 54 different isolate colonies were obtained and strain 6.2S was selected on the basis of its capacity to decrease surface and interfacial tension. Strain 6.2S was identified as a *Sphingobacterium* sp. and is the first strain in this genus to be reported as a BS producer.

After 48 h culture of *Sphingobacterium* sp. 6.2S in G-Mineral Salts Medium (G-MSM), of 3.8 mg/mL of protein and 190 mg/L crude extract were obtained. Compositional analysis revealed that the crude extract consisted primarily of lipids (71.6%) and a minor fraction of carbohydrate (5.6%) and protein (4.4%). When *Sphingobacterium* sp. grew on G-MSM with n-alkenes the surface tension was reduced from 55 mN/m to 32mN/m. The crude extract (BS mixture) strongly reduced surface tension (22 mN/m at 10 g/L), producing one of the lowest values recorded for a microorganism-produced surfactant [7].

To purify crude extract, a modification of the method described by van Echten-Deckert (2000) [37] was used, and two fractions, fraction A and fraction B, were obtained (Figure 1), both with superficial activity [7].

Fraction A (28.7%, w/w of the total extract) decreased surface tension of distilled water to 33.0 mN/m, and presented a CMC of 180 mg/L. Three majority spots were visualized in thin layer chromatography with ninhydrin (amine group determination) and molybdenum blue (phospholipid determination) (Figure 1). These results indicated that the analyzed compounds belong to the family of phospholipids and some of them had in their structures nitrogen groups. An analysis of each spot with two dimensional-TLC using different standard phospholipids showed that fraction A was a mixture of phospholipids, being phosphatidyletanolamine the most abundant. Finally, these results were assessed

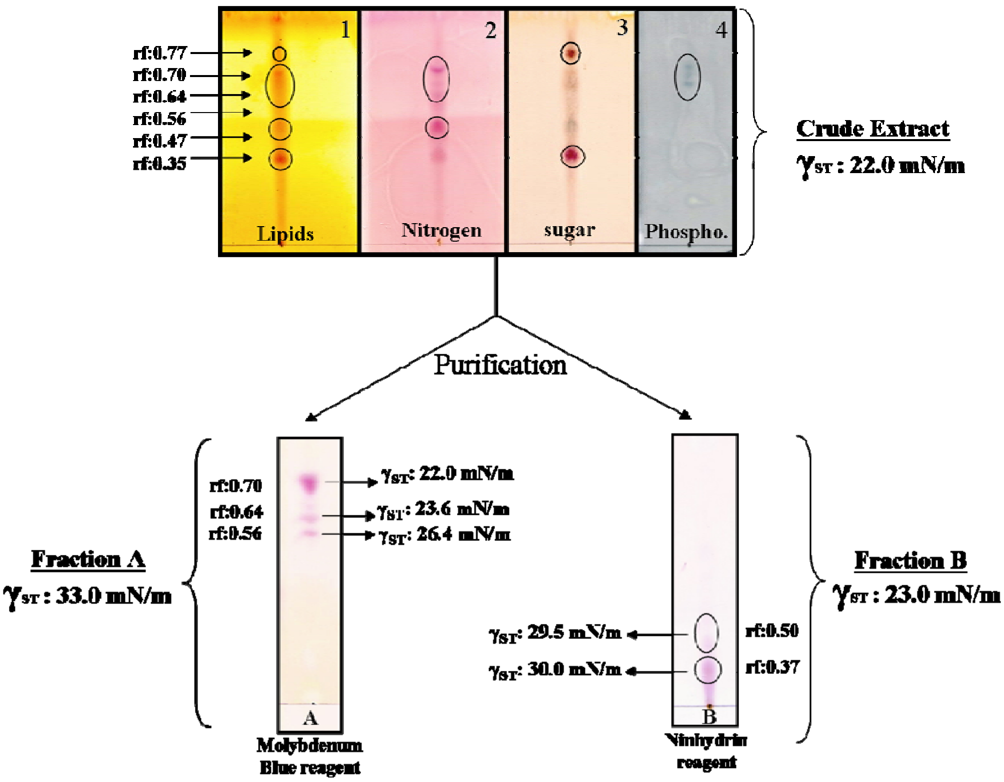

Figure 1. Analysis of the extract produced by *Sphingobacterium* sp. 6.2S. Crude extract developed by iodine vapours (lipids), ninhydrin (nitrogen), molish (sugar) and molybdenum blue (phospholipids). Fraction A: TLC obtained after purification and developed with Ninhydrin. Fraction B: TLC obtained after purification and developed with ninhydrin [7].

with a FTIR analysis, confirmating the presence of the functional groups characteristic of phospholipids.

Further analysis of fraction B (61.3%, w/w of the total extract) revealed that it was a mixture of lipopetides and at least one glycolipid. The surface tension-concentration curve showed two plateaux, the first of which can be attributed to a critical aggregation concentration of the BS with a protein (2.7 g/L) and the second to the true CMC in water (6.3 g/L) [7].

Emulsion capacity of supernatant produced by *Sphingobacterium* sp. was studied. At 25º C and pH 5, 70-65% of emulsion was measured and it was maintained during 5 days, and only a small decrease was observed (60-65% emulsion) after one month. A criterion used to identify bioemulsifiers is their ability to maintain at least 50% of the original emulsion volume after 24 h formation; therefore, 6.2S BS can be included in this family of compounds.

A thermal stability analysis was carried out between 0ºC and 100ºC and at pH 5, revealing that surfactant properties were maintained with the temperature increase and only a small decrease in interfacial and surface tension was observed after a thermal treatment of 121ºC. Nevertheless, it was found that after thermal treatment at 121ºC, the emulsification capacity of 6.2S BS decreased markedly from 65% to 25% (Figure 2). The surface activity was affected by pH. At acidic pH (1–5), surface and interfacial tension were lower (37 and 10 mN/m, respectively). At pH 7 an increase in surface tension and interface tension was observed. This change can be related to the pKa, indicating that the ionic form of the surfactant had lower surface activity. If developed to higher yields, BS from *Sphingobacterium* sp. 6.2S will lead to interesting applications.

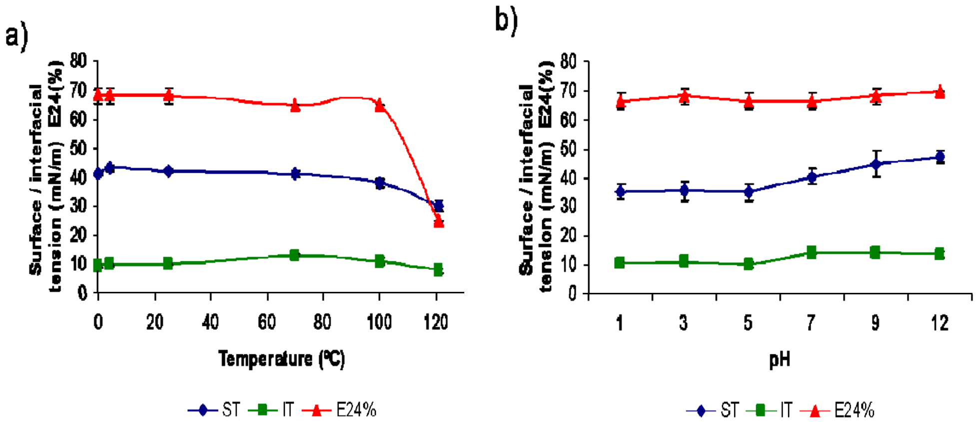

Figure 2. (a) Effect of temperature treatment on the stability of BS produced by *Sphingobacterium* sp., 6.2S grown on G-MSM. (b) Effect of pH on the stability of BS. Measurements were conducted on crude supernatant with unknown concentrations of BS at 25ºC [7].

5. Applications of Biosurfactants

Surfactants are an important class of chemical products with a high volume of use in a great variety of household and industrial applications. Most of these surfactants are petroleum based and are chemically synthesized [6]. BS are the natural choice because they are obtained by environmental friendly technologies and they possess many advantages over synthetic surfactants, such as lower toxicity, effectiveness at a wide range of physical conditions, but they are special as consequence of their biodegradability [6]. BS are more effective and efficient than many existing synthetic surfactants and they may provide new properties that the classical chemical surfactants lack [38,39].

BS find applications in an extremely wide variety of industrial processes that involve emulsification, foaming, detergency, wetting, dispersion and solubilization of hydrophobic compounds [19,40] and, in fact, the demand of BS is increasing worldwide in recent years [14,19,39,40,41,42]. At present, the main applications are the hydrocarbon bioremediation and oil and petroleum industry, in particular for microbial enhanced oil recovery (MEOR) due to their biodegradability avoiding environmental accumulation and toxicity [14]. In spite of their numerous advantages over the synthetic chemical surfactants the problem related with the large scale and cheap production still exits and is a major hurdle in economic competitiveness [6].

According to Syldatk and Hausmann (2010) [5] and Makkar *et al.* (2011) [6], the reasons for limited use of microbial surfactants in industry are the use of expensive substrates, limited product concentrations, low yields and formation of product mixtures rather than pure compounds. Nevertheless, their higher price is largely compensed by their environmental profile and performance benefits. In this regard, discovery of potent BS producing microorganisms would enhance their use and hence reduce total dependence towards the chemical synthetic surfactant industry [19].

The positive economical outlook can be enhanced by increasing their high throughput values or by harnessing other important properties such as pharmacological, antifungal and antiviral capabilities. Several BS have recently been used or anticipated to use in cost-effective applications in medicine, food and cosmetic industries [6]. BS have also potential applications as additives for agricultural use, food industry, mining and manufacturing processes, pulp and paper industries and as detergents or cosmetics [2,14,19,42,43].

6. Medical and Healthcare Applications

The biomedical and cosmetics area, in which low amount of high value BS is required in a high added value product, opens a large field where the research seems to be at its infancy for new applications. Next, possible applications of BS in these areas are described. The use of BS in medical applications has been proposed, due to several biological properties such as antimicrobial ones [14,42]. BS also have potential for use in vaccines and gene therapy [42,44]. A judicial and effective combination of these strategies might, in the future, lead the way towards large-scale profitable production of BS [42].

Due to their biological origin, BS are generally considered safer than synthetic pharmaceuticals, although, to date there have been very few studies carried out to confirm their lack of toxicity [14,36]. In a study of the skin potential irritation of trehalose lipids produced by *Rhodococcus erythropolis* 51T7 in mouse fibroblast and human keratinocyte lines, results indicated that the BS was less irritating than SDS, and could be therefore used in cosmetic preparations [39].

Trehalose-6,60-dimycolate (TDM), or cord factor, has exhibited a number of different biological activities, including antitumor and immunomodulating functions [14], although Mycobacterial TDM use is limited by the relatively high toxicity of the molecule and the potential pathogenicity of producer strains. TDM produced by *Rhodococcus* sp. 4306 was demonstrated to exhibit lower toxicity, both *in vivo* and *in vitro*, than mycobacterial TDM [14].

Sophorolipids are other promising modulators of the immune response, being able to decrease sepsis related mortality *in vivo*, adhesion molecules levels, cytokine production and IgE levels [42]. Sophorolipids in lactone form have also been proposed as anti-ageing agents due to their capacity to stimulate skin dermal fibroblast cell metabolism and, more particularly, collagen neosynthesis [42].

Several BS exhibit antibacterial, antifungal and antiviral activities, which make them relevant molecules for applications in combating many diseases and infections. BS with known antimicrobial activity include surfactin and iturin produced by *Bacillus subtilis* strains, mannosylerythritol lipids from *Candida antarctica*, and BS isolated from *Streptococcus thermophilus* A and *Lactococcus lactis* [45]. Cyclic lipopeptides are often found to be antimicrobial. For example, the cyclic lipopeptide antibiotic surfactin has antibiotic, anticlotting, haemolytic and antiviral properties. In vesicle studies, surfactin was found to incorporate into membranes at low concentrations and induce slow leakage due to changes in membrane ultrastructure [1,46,47]. Two cyclic lipodepsipeptides isolated from *Pseudomonas fluorescens* have also shown antifungal and anti-weed activities [1]. A rhamnolipid mixture obtained from *P. aeruginosa* AT10 has exhibited inhibitory activity against the bacteria *Escherichia coli*, *Micrococcus luteus*, *Alcaligenes faecalis*, *Serratia marcescens*, *Mycobacterium phlei* and *Staphylococcus epidermidis* and excellent antifungal properties against *Aspergillus niger*, *Chaetonium globosum*, *Penicillium chrysogenum*, *Aureobasidium pullulans* and the phytopathogenic *Botrytis cinerea* and *Rhizoctonia solani* [15]. *Pseudomonas aeruginosa* 47T2, growing with waste frying oil as a carbon source produces a mixture of ramnolipids with wide antimicrobial activity [48]. Rhamnolipids are also able to inhibit the growth of harmful bloom algae species, *Heterosigma akashivo* and *Protocentrum dentatum.* The mannosylerythritol lipid (MEL), a glycolipid surfactant from *Candida antarctica*, has demonstrated antimicrobial activity particularly against Gram-positive bacteria [42].

Infections resulting from microbial adhesion to biomaterial surfaces have been observed on nearly al medical devices with severe economic and medical consequences. Biofilm infections pose a number of challenges. BS have been studied as antiadhesive agents in surgicals, representing an interesting approach because it may be possible to modify the surface properties to make it simultaneously anti-adhesive and give it antimicrobial activity [36, 42]. *Lactobacillus paracasei* sp. *paracasei* BS showed anti-adhesive activity against *Streptococcus sanguis* (72.9%), *Staphylococcus aureus* (76.8%), *Staphylococcus epidermidis* (72.9%) and *Streptococcus agalactiae* (66.6%). This finding is promising to control infections in the urinary, vaginal and gastrointestinal tracts, and in the skin [45].

Concerning the antiviral properties of BS, inhibition of growth of HIV in leukocytes by BS has been cited in the literature [49]. The sophorolipid produced by *Candida bombicola* and its structural analogues have been studied for their spermicidal, anti-HIV and cytotoxic activities [42].

The succinoyl-trehalose lipid of *Rhodococcus erythropolis* has been reported to inhibit herpes simplex virus and influenza virus and a 1% emulsion of rhamnolipids has been shown to be effective for the treatment of leaves of *Nicotiana glutinosa* infected with tobacco mosaic virus and for the control of potato virus-x disease [14,40,49].

Moreover, a trehalose lipid BS produced by *Rhodococcus* spp. has been shown to cause hemolysis of human erythrocytes through a colloidosmotic mechanism. This pore-type behavior can be explained by the formation of enhanced permeability domains in the erythrocyte membrane, as observed in model membranes [50,51].

Concerning the potential antitumoural activity of BS, first evidences of growth arrest, apoptosis and differentiation induced by glycolipids in different cancer cell line have been reported [14,42].

Another interesting application of BS could be as pulmonary surfactant in cases of deficiency. The isolation of genes for protein molecules of this surfactant and cloning in bacteria has made possible its fermentative production for medical applications [40,42,49].

BS are also very attractive in the health care and cosmetic industries. A large number of compounds for cosmetic applications are prepared by enzymatic conversion of hydrophobic molecules by various lipases and whole cells. The cosmetic industry demands surfactants with a minimum shelf life of three years. Therefore, saturated acyl groups are preferred over the unsaturated compounds. Monoglycerides, one of the widely used surfactant in the cosmetic industry, has been reported to be produced from glycerol-tallow by using *Pseudomonas fluorescens* lipase treatment [40,49].

Finally, BS have several promising applications in the food industry as food additives. Bioemulsifiers may stabilize oil-in-water (O/W) and water-in-oil (W/O) emulsions. The type of emulsion formed by the water and oil depends primarily on the nature of the emulsifying agent and, to a certain extent, on the process used in preparing the emulsion and the relative proportions of oil and water present. Lecithin and its derivatives, fatty acid esters containing glycerol, sorbitano, or ethylene glycol, and ethyoxylated derivatives of monoglycerides are currently in use as emulsifiers in the food industries worldwide [40, 49]. A novel bioemulsifier from *Candida utilis* has shown potential use in salad dressing [49]. Haba et al. [17] tested the ability of rhamnolipids to emulsify different oils used in a number of industries forming a stable emulsion with liseed oil. Trealose tetraester (THL) produced by *Rhodococcus erythropolis* 51T7, revealed effective emulsification with water and paraffin or isopropyl myristate. The composition of 11.3-7.5-81.8 (isopropyl myristate-THL-W) was stable for at least 3 months. To examine the solubilization behaviour of isopropyl myristate and paraffin oil in the multicomponent BS solutions, two-phase diagrams of the ternary system water–surfactant–oil were developed. Figure 3 shows the zone of 100% emulsion phase (shaded area) obtained after mixing different quantities of components. When paraffin was used as the oil phase, the diagram revealed a wide zone of total emulsion. Out of the shaded area, partial emulsion zones with different texture and aspect were observed.

Due to its more hydrophilic character, when isopropyl myristate was used as the hydrophobic component, the total emulsion zone moved to the left vertex, which is the area of higher water proportion. THL proved to be an effective emulsifier when there was a low amount of myristate oil in the mixture [52]. Apart from their obvious role as emulsifying agents, BS can have several other functions in food. For example, to control the agglomeration of fat globules, stabilize aerated systems, improve texture and

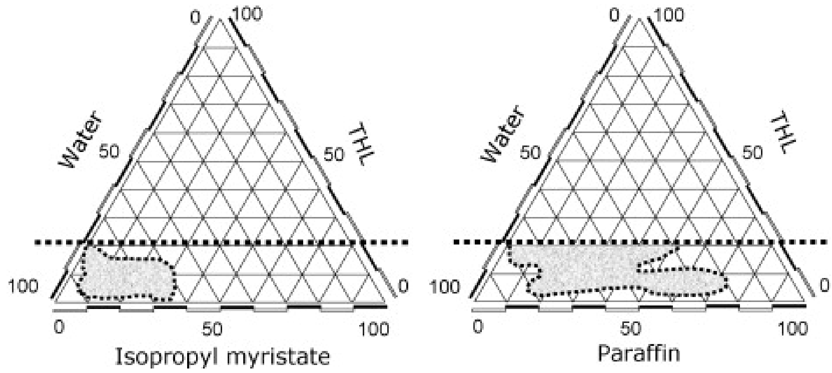

Figure 3. Phase diagram illustrating the emulsion area with isopropyl myristate (left) and paraffin (right) [39].

shelf-life of starch-containing products, modify rheological properties of wheat dough and improve consistency and texture of fat-based products. 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 [42].

The involvement of BS in microbial adhesion and detachment from surfaces has also been investigated for food industry application. 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 BS obtained from *Pseudomonas fluorescens* is able to inhibit the adhesion of *Listeria monocytogenes* L028 strain [42]. It is evident that the anti-adherent property of BS is very interesting in medicine. The use of plastic material (catether, sondes, etc) is a challenge consequence of bacterial adhesion. A BS pre-treatement on this material can confer anti-adherent and antimicrobial properties.

7. Conclusions

Surfactants are an important class of chemical products in view of the volumes sold and their great variety of applications. During the last decades, a wide variety of microorganisms have been reported to produce numerous types of BS. Their biodegradability and lower toxicity give them an advantage over their chemical counterparts. The most important factor limiting BS use is the production cost, however, the small amounts of product required and the special properties described makes BS high valuable molecules. Application of BS as food additives, specialty chemicals, biocontrol agents and as a new generation molecules for health and beauty care permits to think in these compunds as the molecules of the future.

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