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Surfactants of microbial origin and its application in foods

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Surfactants are petroleum-derived compounds widely used globally in industrial areas such as pharmaceuticals, cosmetics, textile, among others. The synthesis and use of these compounds has generated environmental pollution, putting public health at risk. For several years, the synthesis of surfactants has been reported by various microorganisms called biosurfactants. Biosurfactants have been shown to reduce surface tension using emulsifying agents, in addition to presenting biodegradability and low toxicity. The objective of this mini review document is to deal with aspects related to the classification, producing microorganisms, physicochemical and biological properties of biosurfactants that have placed them as a potential biotechnological alternative regarding chemical counterparts in various industrial areas, including food production.

Key words: Amphiphilic, antimicrobials, biotechnology, biosurfactant, food safety, glycolipids.

INTRODUCTION

Surfactants are versatile chemical agents, which exert their effect on the contact surface between two immiscible phases (interface), through the reduction of surface tension (Cabanes, 2014). Because of their ability to reduce surface tension, these compounds are commonly used as detergents, foaming agents, emulsifiers, plasticizers, solubilizers, dispersants or humectants in various areas ranging from home to industrial areas such as textile, food, agriculture, mining, pharmaceutical, petroleum, cosmetics, paint, paper, among others, and its demand in recent years has had a considerable increase (Cruz and Del Valle, 1987; Cabanes, 2014; Martinez et al., 2015; Varjani and Upasani, 2017; Akbari et al., 2018).

Surfactants are amphiphilic molecules made up of hydrophilic and hydrophobic fractions that divide at the interface between fluid phases with different polarity and hydrogen bonds (oil / water or air / water interfaces); this allows reducing surface and interfacial tension and forming micro emulsions, favoring the solubility of hydrophobic compounds in water (Varjani and Upasani, 2017).

Most of the production and availability of surfactants are derived from the petrochemical industry, estimating that these compounds represent between 70% and 75% of active tensions used in industrialized nations (Campos et al., 2013; Akbari et al., 2018); this has contributed to greater environmental pollution with possible effects

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on public health, which is why there is currently the human and environmental challenge of developing strategies to replace petroleum, coal and natural gas-based products with renewable, biodegradable and sustainable energies (Akbari et al., 2018).

In the search for alternatives that favor the reduction of environmental pollution in the field of surfactants, the biosurfactants (BS) are found (Nitschke and Silva, 2018). BSs are compounds that have broad advantages compared to their chemical counterparts, such as: biodegradability, low toxicity, specificity, selectivity, stability under adverse conditions (pH and salinity), low critical micellar concentration (CMC), detergent capacity, emulsifier, solubilizer, foaming, biological activity, biocompatibility and digestibility, in addition to being synthesized from relatively inexpensive carbon sources or industrial waste (Cortés et al., 2013; Sobrinho et al., 2013; Nitschke and Costa, 2014; Chavez et al., 2016; Martinez et al., 2015; Santos et al., 2016; Varjani and Upasani, 2017; Bustos et al., 2018; Das et al., 2018). This has catapulted them as potential alternatives to surfactants of synthetic origin and its use in various industrial areas such as oil recovery, pharmaceutical, food, cosmetics, agricultural and livestock sectors (Martinez et al., 2015; Chavez et al., 2016; Bustos et al., 2018).

The objective of this mini review document is to address a brief and general description of surfactants of microbial origin covering aspects such as their classification, producing organisms, as well as physicochemical and biological properties that have made them to be considered for their potential biotechnological alternative application in the food industry.

Biosurfactants

Biosurfactants (BS) are molecules with surface activity capable of reducing the surface tension of water; it is considered a good biosurfactant when it is capable of reducing the surface tension of water from 72 mN / m to 35 mN / m and the interfacial tension of water / n-hexadecane from 40 mN / m to 1 mN / m (Sourav et al., 2015; Chavez et al., 2016).

BS are secondary metabolites synthesized, in the stationary phase of microbial growth, by a variety of bacteria, filamentous fungi and yeasts in various substrates, mainly in those immiscible in water such as oils, alkanes, sugars and waste, being secreted extracellularly or remain bound to cellular components (Cortés et al.,2013; Sourav et al., 2015; Chavez et al., 2016; Becerra and Horna, 2016; Varjani and Upasani, 2017). These biomolecules are compounds of amphiphilic nature because they are formed from a polar or hydrophilic part, consisting of mono-, oligo- or polysaccharides, amino acids, peptides or proteins, and another non-polar or hydrophobic, which are commonly saturated, unsaturated, hydroxylated fatty acids, or fatty alcohols (Chavez et al., 2016; Nitschke and Silva, 2018; Bustos et al., 2018; Kubicki et al.,2019).

BS are compounds of structural diversity classified according to: 1. Their chemical structure in: glycolipids, lipoproteins or lipopeptides, phospholipids, fatty acids or natural lipids, polymeric surfactants and surfactant particles (Santos et al., 2016; Varjani and Upasani, 2017), and 2. Based on its molecular weight: (a) low molecular weight compounds such as: glycolipids, lipopeptides, short chain grade acids and phospholipids, and (b) high molecular weight compounds such as: polymeric surfactants, lipopolysaccharide surfactant particles or lipoproteins considered bioemulsifiers (Sourav et al, 2015; Varjani and Upasani, 2017; Kubicki et al.,2019; Fenibo et al., 2019). Stressing that BS reduces surface and interfacial tension while bioemulsifiers are more effective in stabilizing oil-in-water emulsions (Varjani and Upasani, 2017).

Glycolipids are the largest class of biosurfactants widely known and studied; these compounds contain in their structure carbohydrates (hydrophilic fraction) linked to long chains of aliphatic or hydroxyaliphatic acids (hydrophobic fraction) by an ester group. Within the group of glycolipids there are rhamnolipids, trehalolipids and sophorolipids (Vijayakumar and Saravanan, 2015; Santos et al., 2016).

The physiological function of the BS in the producing cell is not fully understood yet, but it is believed that these compounds are synthesized and released to facilitate the transportation, absorption and metabolism of hydrophobic compounds by solubilization and emulsification processes, as well as acting as biocidal agents in the survival and competition in ecological niches (Dhanarajan and Sen, 2014; Chavez et al., 2016; Santos et al., 2016; Fenibo et al., 2019).

On the other hand, the synthesis of BS can be affected in quality and quantity by factors such as the nature of the carbon source (lipophilic or hydrophilic), nitrogen source, pH, temperature, aeration, agitation, metal ion concentration, incubation time, culture strategy (batch, fed-batch, continuous), dilution rate and cell stress conditions like low concentration of the nitrogen source (Saharan et al., 2011; Sourav et al., 2015; Usman et al., 2016; Santos et al., 2016; Fenibo et al., 2019).

Some methods have been developed for the search and detection of BS-producing microorganisms in nature such as phenotypic tests that is, hemolytic activity, emulsification index, blue agar plate method, hydrocarbon agar plate, drop collapse, or oil spreading; also, there are molecular techniques through the polymerase chain reaction (PCR); while, on the other hand, physicochemical and instrumental methods have been developed focused on the recovery, purification and characterization of these compounds that involve the use of organic solvents, acid precipitation, alkaline precipitation, crystallization, filtration, ultrafiltration, lyophilization, centrifugation, layer chromatography fine, ion exchange chromatography, high pressure liquid, gas chromatography coupled to mass spectrometry, infrared, nuclear magnetic resonance, matrix assisted laser desorption / ionization with ion detector, and mass spectrometry (MALDI-TOF MS) (Satpute et al., 2010; Saharan et al., 2011; Sachdev and Cameotra, 2013; Sourav et al., 2015; Mnif and Ghrib, 2016; Santos et al., 2016; Varjani and Upasani, 2017; Fenibo et al., 2019).

BIOSURFACTANTS IN FOOD

At the level of primary food production, the yield of agricultural land is negatively affected by the presence of organic and inorganic pollutants that give abiotic stress in crop plants. For this reason, and in order to increase the quality of soils contaminated by hydrocarbons and heavy metals, it is necessary to implement bioremediation processes to improve the soils dedicated to agriculture. Thus, microorganisms producing biosurfactants, or biosurfactants only, can be used to eliminate of hydrocarbons, pesticides and heavy metals in contaminated soils (Sachdev and Cameotra, 2013; Usman et al., 2016; Rangarajan and Narayanan, 2018).

Glycolipid-type BS such as sophorolipids, cellobiose lipids, mannosylerythritol lipids and rhamnolipids has reported agricultural utility through their growth-inhibiting effects of phytopathogenic fungi (*Sclerotinia sclerotiorum, Phomopsis helianthi, Botrytis cinerea, Alternaria tomatophilia, Alternaria solani,* among others). It has also been observed that they are involved in the stimulation of plant signaling and immunity processes, while rhamnolipids synthesized by *Pseudomonas* sp., in studies on insects have shown a pesticidal effect, being therefore considered as biocontrol agents for pests (Mnif and Ghribi, 2016). Meanwhile, lipopeptide type BS produced by species of the genus *Bacillus* presented growth inhibition of phytopathogens, such as *Fusarium* spp.*, Aspergillus* spp.*, Trichoderma* spp.*,* and *Biopolaris sorokiniana*, opening the alternative for using thus in biocontrol activities (Sachdev and Cameotra, 2013; Hafeez et al., 2019).

In food processing, one of the main goals is to provide safety and good sensory characteristics (Nitschke and Silva, 2018). Any substance that modifies the distinctive property of food when added is considered a food additive. The purpose of incorporating additives is to improve and maintain the nutritional value, texture, safety, taste and appearance of food. However, during some years there has been a change in the tendency of consumers towards natural additives over synthetics, along with the growing concerns about environmental and health contamination, it has created demand for natural or green additives in food, opening the door for BS due to their different shown properties (Nitschke and Silva, 2018).

Within the applications in the processing and food industry, the BS intervene as detergents, thickeners, foaming agents, humectants, and emulsifying agents, with the latter being possible by reducing the surface and interfacial tension, facilitating the formation and stabilization of emulsions (Nitschke and Costa, 2007; Shoeb et al., 2013; Sourav et al., 2015; Vijayakumar and Saravanan, 2015; Santos et al., 2016). Thus, in the food industry, BS is used in two main ways: (a) In the treatment/cleaning of surfaces in contact with food, and (b) food additives/ingredients (Nitschke and Silva, 2018).

BSs have been considered for potential uses as formulation ingredients to control the aggregation of fat globules, stabilization of aerated systems, improvement of the texture and shelf life of products containing starch, modification of the rheological properties of wheat mass and improving the consistency and texture of fat-based products; while in bakery and ice cream formulations, these compounds act by controlling consistency, slowing aging and solubilizing oil flavoring and flavoring (Nitschke and Costa, 2007; Vijayakumar and Saravanan, 2015). It has been reported that *Candida utilis* produces a bioemulsifier used in processed salad dressings and *Saccharomyces cerevisiae* produces a mannoprotein capable of stabilizing water / oil emulsions in biscuits, mayonnaise and ice cream (Santos et al., 2016). Other yeasts such as *Hansenula anomala, Rrhodotorula graminis, Rhodospiridium diobovatum,* red algae such as *Porphiridium cruentum*, and species of bacteria of the genus *Klebsiella* sp., and *Acinetobacter calcoaceticus* have been reported to produce extracellular bioemulsifiers with a better stabilizing activity than the Arabic gum and carboxycellulose (Campos et al., 2013).

BSs are also considered in the foods area as anti-adhesive and antimicrobial agents (Nitschke and Costa, 2007; Cortés et al., 2013; Akbari et al., 2018). Biofilms are heterogeneous communities of microorganisms that adhere and colonize a biotic or abiotic surface, where extracellular material produced is included. These biofilms are also considered as a form of resistance against antimicrobials and adverse conditions by the microorganisms that constitute them (Nitschke and Costa, 2007, 2014; Nitschke and Silva, 2018). The biofilms present in the food processing and production surfaces are of relevance, because when they are established they can cause the obstruction of pipes, corrosion of equipment, and the reduction of the efficiency of the temperature transfer systems; besides, they are also a source of contamination that can lead to food spoilage and disease transmission (Nitschke and Costa, 2007, 2014). Therefore, the BSs contribute to the breakdown and prevent the formation of biofilms, making it impossible for the microorganisms to adhere and colonize food contact surfaces, thus improving the quality and safety of food products (Nitschke and Costa, 2007; Cortés et al., 2013; Akbari et al., 2018).

Table 1. Types of biosurfactants and producing microorganisms.

Type of biosurfactant	Producer microorganism
Glycolipids	
Rhamnolipids	*Pseudomonas* sp. *Acinetobacter calcoaceticus* *Serratia rubidea*
Trehalose lipids	*Rhodococcus erythropolis Arthrobacter* sp. *Nocardia* sp. *Corynebacterium* sp. *Tsukamurella spumae* *Tsukamurella pseudospumae*
Sophorolipids	*Candida apícola* *Candida bombicola* *Candida albicans O-13-1* *Candida antartica* *Aspergillus flavus* *Rhizopus oryzae*
Mannosylerythritol lipids	*Ustilago maydis* *Ustilago scitaminea* *Candida antárctica* *Pseudozyma aphidis*
Celobiose lipids	*Ustilago zeae* *Ustilago maydis*
Xylolipids	*Lactobacillus helveticus* *Lactococcus lactis* *Enterococcus faecium*
Lipopeptids and lipoproteins	
Subtilisin/surfactin/iturin/fengycin *Lichenysin* *Viscosin* *Serrawettin* *Polymyxin*	*Bacillus subtilis* *Bacillus licheniformis* *Pseudomonas fluorescens* *Serratia marcescens* *Bacillus polymyxa*
Fatty acids	*Nocardia erythropolis* *Arthrobacter paraffincus* *Corynebacterium lepus* *Penicillium spiculisporum*
Fosfolipids	*Acinetobacter* sp. *Corynebacterium lepus* *Thiobacillus thiooxidans*
Polimeric surfactants	
Emulsan/biodispersan *Liposan* *Yasan* *Alasan* *Mannoproteins* *Carbohydrate-lipid-protein*	*Acinetobacter calcoaceticus* *Candida lipolytica* *Yarrowia lipolytica* *Acinetobacter radioresistens* *Saccharomyces cerevisiae, Kluyveromyces marxianus* *Pseudomonas fluorescens*

Table 1. Contd.

Surfactant particles	
Vesicles	*Acinetobacter calcoaceticus*
Emulcyan	*Phormidium* J-1

Sources: Saravanakumari and Mani (2010), Saharan et al. (2011), Sobrinho et al. (2013), Dhanarajan and Sen (2014), Sharma et al. (2014, 2015), Santos et al. (2016), Mnif and Ghribi (2016), Nitschke and Silva (2018), Mnif et al. (2018) and Fenibo et al. (2019).

The antimicrobial activity of the BSs is potentially useful in food production as conservative agents. Studies show the utility in the control of microbial growth including that of human pathogens (Cortés et al., 2013; Shoeb et al., 2013; Nitschke and Costa, 2014; Nitschke and Silva, 2018).

The mechanism involved in antimicrobial activity by BSs is not well clarified yet; however, it is estimated that these compounds act by altering the permeability of the cytoplasmic membrane causing cellular damage, derived from their amphipathic nature that allows interaction with constituent phospholipids of microbial cell membrane (Shoeb et al., 2013; Cortés et al.,2013; Nitschke and Costa, 2014).

In related studies, Sambanthamoorthy et al. (2014) determined that BS produced by lactic bacteria, such as *Lactobacillus jensenii* and *Lactobacillus rhamnosus* presented anti-adhesive and antimicrobial effect at concentrations between 25 and 50 mg/ml against multi-resistant pathogenic strains such as *Acinetobacter baumannii, Escherichia coli,* and *Staphylococcus aureus*. Meanwhile, Mani et al. (2016) reported that the glycolipid-type BS produced *Staphylococcus saprophyticus* and showed antimicrobial activity against bacteria and pathogenic fungi in humans such as *Klebsiella pneumoniae* (4 µg/mL), *E. coli* (12 µg / mL), *Pseudomonas aeruginosa* (32 µg/mL), *Vibrio cholerae* (64 µg/mL), *Cryptococcus neoformans* (32 µg/mL) and *Candida albicans* (32 µg/mL); in addition, this BS was stable at a range of pH (3-9) and temperature (30 - 80°C), highlighting the promising use of these compounds in different industrial areas.

On the other hand, BSs favor the activity of probiotic strains (Cortés et al., 2013; Fariq and Saeed, 2016). Probiotics are defined as living microorganisms that when administered in adequate amount confer different benefits for the health of the consumer by preventing and treating different gastrointestinal disorders, allergies, reducing lactose intolerance, in addition to having modulation effects on the immune response and cholesterol (Cortés et al., 2013; Fariq and Saeed, 2016; Saha et al., 2018). Lactic acid bacteria (LAB) are widely distributed in nature and are an important part in the production of various foods in addition to being considered probiotics, some species of the genera that are part of this group are: *Lactobacillus, Bifidobacteria, Enterococcus, Pediococcus, Leuconostoc* and *Streptococcus* (Parra, 2010; Ramírez et al., 2011; Cortés et al., 2013; Sharma et al., 2015; Fariq and Saeed, 2016; Cortés and Barrón, 2017; Hajfarajollah et al., 2018). Probiotics can release various metabolites (free fatty acids, hydrogen peroxide, peptides, biosurfactants, diacetylated compounds, lactic acid, and bacteriocins) that may affect specific pathogenic microorganisms, thus benefiting the host and maintaining the balance of native microbial communities (Cortés et al., 2013; Sharma and Singh, 2014; Fariq and Saeed, 2016; Cortés and Barrón, 2017; Hajfarajollah et al., 2018).

Lipid oxidation tends to reduce the quality and safety of food through the development of toxic compounds resulting in rancidity and perception of unpleasant tastes and odors; therefore, antioxidants are relevant food additives focused on preventing lipid oxidation, and improving the shelf life of food (Nitschke and Silva, 2018). Some BS have exhibited antioxidant properties, as observed in the case of glycolipids called mannosylerythritol lipids synthesized by yeasts of the genus *Pseudozyma* and its free radical neutralizing effect analyzed through the method of 1,1-diphenyl-2-picrylhydrazine (DPPH) (Takahashi et al., 2012). On the other hand, an emulsifying polysaccharide of *Klebsiella* shows inhibition of the self-oxidation of soybean oil by encapsulation processes (Campos et al., 2013). *B. subtilis* produces a lipopeptide capable of neutralizing free radicals and that can be used as a natural antioxidant in foods by protecting cells from oxidative stress (Yalçın and Çavuşoğlu, 2010). Meanwhile, *Bacillus methylotrophicus* produces a lipopeptide with chelating activity for iron ions and antioxidant activity above commercial products such as Ethylene Diamine Tetra-Acetic Acid (EDTA), and hydroxybutylanisole (BHA) that makes it an alternative potential as an antioxidant agent (Jemil et al., 2017).

PRODUCTION OF BIOSURFACTANTS FROM AGROINDUSTRIAL WASTE

Despite the advantages of the BS with respect to their counterparts derived from the petrochemical industry, some drawbacks of these biomolecules are: 1) Acquisition of products with a high degree of purity is difficult due to the need for steps of consecutive purification on the culture; 2) Superproductive species are rare in such a way that the known producing species are not capable of producing high surfactant yields, and they require a complex culture medium; 3) Biosynthesis regulation is not fully understood as well; hence these biomolecules can be produced as secondary metabolites or in association with microbial growth; 4) Increased productivity is often affected by foaming, which requires the use of a diluted medium, and 5) Large-scale production is of high economic costs (Sobrinho et al., 2013; Fenibo et al., 2019).

Commonly, a large part of biotechnological processes requires high cost inputs, so it is necessary to ensure optimum product performance at the lowest cost through economical materials. On the other hand, microorganisms produce very low amounts of active tense and the subsequent processing of biotechnological products costs between 60 and 80% of the total production expenditure; therefore, in most cases, marketable BS products are economically high and it is important to reduce production costs through the use of economic and renewable substrates (Banat et al., 2014; Banat and Thavasi, 2018).

The relationship between BS and food is that different agroindustrial wastes, due to their high sugar and lipid content, can be used as a substrate by microorganisms producing these compounds. In this way, they are considered economic processes and cultivation conditions (impediment to the production of large scales), in addition to environmental care, recycling and recovery of this waste; being wastes from the oil, dairy, distiller, food-processing by-products, and carbohydrate-rich wastes mainly intended for the production of BS (Gautam and Tyagi, 2006; Nitschke and Costa, 2007; Saharan et al., 2011; Campos et al., 2013; Banat et al., 2014; Das et al., 2018; Singh et al., 2019).

Conclusion

The biosurfactants are compounds of biological origin synthesized by bacteria, fungi and yeasts during their growth mainly in hydrophobic carbon sources with the ability to reduce surface tension between immiscible liquids. The BS have shown a variety of physicochemical and biological properties (antimicrobial, anti-adhesive, biodegradability, low toxicity, among others) that make them potential alternatives for use regarding their chemical-type counterparts (toxic and contaminating) for using in various industrial areas including in food. The BS due to their different physicochemical and biological properties, are promising alternatives to synthetic surfactants. However, there are still disadvantages for its use at large scales, and it is such that the synthesis of BS is at low yields and high production costs, so research should focus on the search for producing and hyper-producing strains, improve and optimize conditions of microbial biosynthesis, as well as extraction and purification techniques through the best knowledge of biochemical and genetic processes (Chavez et al., 2016; Akbari et al., 2018). For the application of BS in the production, processing and preservation of food, further studies should be generated regarding the physicochemical and biological properties presented in addition to its safety, an essential quality that an additive must have for its use in food.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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