Biosurfactants Properties

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Biosurfactants (BSs) are emerging surface-active molecules with high potential for a wide range of applications in the biomedical and pharmaceutical fields. BSs are extremely attractive due to their significant antimicrobial (against bacteria, fungi and viruses), antiadhesive and biofilm disruptive properties. Their use, either on their own or in combination with other antimicrobial or chemotherapeutic drugs, might pave the way for a future strategy of prevention and counteraction of microbial infections, biofilm formation and proliferation. In addition, BSs have recently attracted the attention of the scientific community as a new potential generation of pharmaceutics to be included in anticancer, immunomodulatory, wound healing, cosmetic and drug delivery agents.

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1. Introduction

Biosurfactants (BSs) are a structurally heterogeneous group of biomolecules that share pronounced surface and emulsifying activities. They can be either located on microbial cell surfaces or released in the extracellular space by different bacteria (*Bacillus, Lactobacillus, Pseudomonas, Burkholderia, Mycobacterium, Rhodococcus, Arthrobacter, Nocardia, Gordonia* and *Acinetobacter*), yeast and filamentous fungi (*Candida, Saccharomyces, Starmerella, Trichosporon, Pseudozyma* and *Ustilago*) ^{[1][2]}. They are, therefore, mostly classified by their structural features, the producing microorganisms and their molecular mass. BSs have a hydrophilic region (carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol) and a hydrophobic region (saturated, unsaturated, linear, or branched long-chain fatty acids or hydrocarbon acids). This amphipathic structure allows a reduction in surface tension at the interfaces of phases with dissimilar polarities (liquid–air, liquid–liquid or liquid–solid) ^{[3][4]}. They have the ability to form molecular aggregates, including micelles. The micellar aggregation of BSs is originated at the critical micelle concentration (CMC) typically from 1 to 200 mg/L and, interestingly, about 10- to 40-fold lower than that of chemical surfactants ^[5].

Based on their molecular weight, BSs are commonly divided into two main classes: the low molecular weight compounds efficiently lower surface tension and interfacial tension and are appropriately called "biosurfactants"; conversely, the high molecular weight polymers are more effective as emulsion-stabilizing agents and are usually called "bioemulsifiers". According to the chemical composition, BSs can be classified into five major groups: glycolipids, lipopeptides, phospholipids, polymeric compounds and neutral lipids ^[6].

The most widely studied groups of BSs are lipopeptides, such as surfactin, fengycin and iturin, and glycolipids, such as rhamnolipids, sophorolipids, mannosylerythritol lipids and trehalose lipids ^[7]. Since the 1980s, these

amphipathic molecules have been extensively applied in the biodegradation and detoxification of industrial effluents, bioremediation, industrial emulsions and enhanced oil recovery due to their emulsification, wetting, foaming, cleansing, phase separation, surface activity and reduction in heavy liquid viscosity ^{[8][9][10]}.

BSs might present valuable alternatives to petroleum-based surfactants. Additional advantageous properties, emphasizing the uniqueness of these natural molecules, include the possibility to modify their chemical composition through genetic engineering or the use of biological and biochemical techniques to alter the metabolic end products, thus tailoring them to meet specific functional requirements ^{[11][12]}. In addition, they are claimed to be more biodegradable and eco-friendly than synthetic surfactants ^{[13][14][15][16]}, less toxic and effective even at extremes temperatures, pH conditions, and salinity ^{[6][13][17][18][19]}.

Despite having a large number of advantages, some disadvantages are also linked to biosurfactants, such as high production cost and the need for purification for some specific applications (e.g., pharmaceutical). Biotechnological processes involved in the synthesis of biosurfactants are rather expensive, and the purification of surfactants is problematic. Several research groups are engaged in finding a solution for cost reductions in biosurfactant production by using easily available and renewable bioresources as cheap raw materials, industrial wastes or by-products ^[15].

In terms of biodegradability, as water-soluble molecules, BSs may be susceptible to fast biodegradation by other microorganisms, thus limiting hydrocarbon degradation during bioremediation ^[20]. Additionally, it is also important to remark that for many applications, especially in biomedical and pharmaceutical processes, it would be interesting if biosurfactants were not biodegraded immediately to develop their function in the formulations where they have been included. However, from an environmental point of view, it could represent a problem, not only because of the changes in microbiota caused by the antimicrobial effect of biosurfactants but also due to the costs that could imply their exclusion ^[21]. Consequently, it is necessary to study the biodegradation process of biosurfactants to establish not only their environmental impact but also to determine their optimal formulation conditions and stability when applied in different industrial sectors ^[22].

In addition, critical "Life Cycle Assessment", which typically considers industrial processes from the basic acquisition of raw materials, to the manufacturing of products, consumer use and, finally, the disposal of waste materials. Such approach does not fundamentally show that a biosurfactant has a much lower environmental impact, in terms of greenhouse gas emissions, than petrochemically derived surfactant processes ^[11].

Studies for potential applications of biosurfactants in the medical field have increased during the past decade; the pertinence in these fields is mostly related to their biological properties, such as their ability to affect cell membrane permeability, emulsification and adhesion to biotic and abiotic surfaces.

This review focuses on recent advances in the understanding of BSs' antimicrobial, antiviral, antiadhesive, antibiofilm, wound healing, anticancer and immune-modulatory activities and their promising application in the field

of human health [18][23][24][25] (Figure 1). Some critical issues related to the production and application of these molecules will also be presented.



Figure 1. Biomedical, therapeutic and pharmaceutical applications of biosurfactants.

2. Biosurfactant Properties and Biological Activities Useful for Biomedical and Pharmaceutical Applications

In nature, BSs modulate various biological activities, including microbial metabolism, motility and survival. These molecules increase the surface areas and bioavailability of hydrophobic water-insoluble substrates and are responsible for the removal of heavy metals from the surrounding environment. They also regulate the attachment/detachment of microorganisms to and from surfaces, mobilization, cell surface conditioning, aggregation at interfaces and surfaces on which the interaction takes place. In addition, cellular differentiation, substrate accession and resistance to toxic compounds are all roles attributed to microbial surface-active compounds ^[26]. Rhamnolipids, for example, play multiple roles in the survival of microorganisms. They are crucial for the preservation of biofilm architecture and are considered as one of the virulence factors in *Pseudomonas* sp. ^{[27][28]} and as part of a natural mechanism evolved to improve the uptake of hydrophobic substrates by bacterial cells. However, current evidence confirms that rhamnolipids are part of a mechanism which controls the fundamental elements of microbial existence, such as the stimulation of bacterial motility, formation and disruption of biofilms, virulence and antimicrobial activity ^[29].

Overall, BSs confer a selective advantage to the producer microorganism; consequently, they exert antimicrobial activity against other microorganisms that do not produce BSs. BSs can act as virulence factors and as quorum-sensing molecules, regulating the expression of other virulence factors, such as those promoting biofilm formation, maintenance and, ultimately, biofilm dispersal. In addition, they are crucial in maintaining channels for gas and nutrient exchange across, and diffusion into, the biofilm surface and structure ^{[26][27][30][31][32]}.

In recent years, a growing number of studies have pointed out that BSs harbor many biological properties exploitable by biomedical and pharmaceutical fields. BSs mechanism of action on microbial cell surfaces involves binding/attachments to membranes, causing changes in wettability and surface energy, leading to a reduction in

hydrophobicity and an increase in permeability through the release of LPS and the formation of transmembrane pores. They, therefore, disrupt membrane integrity, leading to cell lysis and metabolite leakage; loss of membrane functions, such as transport and energy generation processes; and disruption of protein structures (Figure 2) ^{[Z][33]} ^[34]. Several reports have suggested that, in addition to their direct action against pathogens, biosurfactants are able to interfere with biofilm formation, modulating microbial interaction with interfaces ^[26] due to changes in surface tension and bacterial cell-wall charge ^[35].



Figure 2. Mechanisms of action of biosurfactants against microbial cell membranes and biofilms.

It is envisaged that more in-depth studies of the natural role of BSs in microbial competitive interactions, cell-to-cell communication, pathogenesis, motility and biofilm formation and maintenance will improve and suggest many other interesting potential applications ^[5].

3. Antimicrobial Activity of BSs

The widespread use of antimicrobials has led to the rapid appearance of an increasing number of drug-resistant microbial strains generating many concerns for future healthcare systems worldwide. According to WHO, antibiotic resistance causes about 700,000 deaths/year, and in Europe alone, about 25,000 deaths/year with an impact cost of about EUR 1.5 billion ^[36]. In the United States alone, infections due to these types of microorganisms cause 23,000 deaths/year that result in an impact cost of USD 55–70 billion ^[37].

In this context, microbial metabolites are among the major sources of bioactive compounds. In particular, BSs are very attractive due to their potent antibacterial and antifungal properties for some of them, such as daptomycin ^[38], and the echinocandins caspofungin ^[39], micafungin ^[40] and anidulafungin ^[41], all of which have already reached a commercial antibiotic status.

3.1. Lipopeptides and Glycolipids as Antimicrobial Agents

Lipopeptides and glycolipids are the most commonly reported classes of BSs with antimicrobial activity ^[42]. In particular, Polymyxin A and Polymyxin B from *Bacillus polymyxa* ^[43]; surfactin, iturin, fengycin, mycosubtilins and bacillomycins produced by *Bacillus subtilis* ^[44]; pumilacidin produced by *Bacillus pumilus* ^[45]; lichenysin from *Bacillus licheniformis* ^[46]; and viscosin from *Pseudomonas fluorescens* ^[47] are well known as antimicrobial lipopeptides. Concerning glycolipids, rhamnolipids from *Pseudomonas aeruginosa* ^[48], sophorolipids from *Candida bombicola* ^[49] and mannosylerythritol lipids from *Candida antarctica* ^[50] are the best studied.

Yang et al. ^[51] discovered a new cationic lipopeptide produced by an environmental strain of *Brevibacillus laterosporus* with marked antimicrobial activities against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Lactobacillus plantarum* and *Enterococcus faecalis*, with Minimal Inhibitory Concentration (MIC) values comparable to that of vancomycin.

In 2017, the lipopeptide obtained from *B. subtilis* SPB1, already known for its antimicrobial activity against a wide range of bacteria ^[52] and phytopathogenic fungi ^[53], was used as an ingredient in a dentifrice formulation, and its antibacterial activity has been compared to that of a commercial toothpaste.

The BS-based formulation exhibited a remarkable inhibitory activity against *E. faecalis*, *Enterobacter* sp., *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Salmonella typhimurium* and *Micrococcus luteus* ^[54]. Cordeiro et al. ^[55] observed that the lipopeptide mixture TIM96 was able to kill *Trichosporon inkin* and *Trichosporon asahii* cells within 48 h of co-incubation, via a reduction in cellular ergosterol content and surface hydrophobicity as well as an increase in membrane permeability. Basit et al. ^[56] isolated 3 strains of *Bacillus cereus* from garden soil whose lipopeptide biosurfactants exhibited significant antibacterial and antifungal activity against *S. aureus*, *Escherichia coli*, *P. aeruginosa*, *K. pneumoniae*, *Aspergillus niger* and *Candida albicans*, with MIC values ranging from 0.52 to 7.6 mg/mL. More recently, Medeot et al. ^[57] demonstrated that fengycin form *Bacillus amyloliquefaciens* MEP218 was able to induce dramatic alterations in the surface topography of the opportunistic human pathogen *P. aeruginosa* PA01, leading to a decrease in cell height and loss in intracellular content. The surfactin and rhamnolipids mixtures produced by *B. amyloliquefaciens* ST34 and *P. aeruginosa* ST5, respectively, showed a pronounced antimicrobial activity against a broad spectrum of opportunistic and pathogenic microorganisms, including antibiotic-resistant bacterial strains, such as *S. aureus* and *E. coli* and the yeast *C. albicans* ^[58].

An interesting antimicrobial activity against human pathogens was also reported for the glycolipid obtained by the marine strain *Staphylococcus saprophyticus* SBPS 15^[59]. The biosurfactant completely inhibited the growth of all the tested clinical isolates (e.g., *E. coli, K. pneumoniae, P. aeruginosa, Vibrio cholerae, S. aureus* and *C. albicans*) at concentrations of 4–64 µg/mL. More recently, Valotteau et al. ^[60] reported the biocidal activity of sophorolipids (SLs)-grafted gold monolayers against both Gram-positive (*E. faecalis, Staphylococcus epidermidis* and *Streptococcus pyogenes*) and Gram-negative (*E. coli, P. aeruginosa* and *S. typhimurium*) strains. The authors also reported that the exposure of all tested microorganisms to these surfaces caused a significant reduction in cell

viability resulting from cell membrane damage. In the same year, Elshikh et al. ^{[61][62]} demonstrated the efficacy of mixtures of rhamnolipids and lactonic sophorolipids of different origins in inhibiting the growth of oral bacterial pathogens, finding MIC values against *Streptococcus mutans*, *Streptococcus oralis*, *Actinomyces naeslundii*, *Neisseria mucosa* and *Streptococcus sanguinis* ranging from 0.1 to 0.4 mg/mL. More recently, Sen et al. ^[63] illustrated the antifungal activity of a rhamnolipid produced by *P. aeruginosa* SS14 against *Trichophyton rubrum*. This study also showed that purified biosurfactant (0.5 mg/mL) effectively induced a loss in cell membrane integrity, suppressed spore germination and hyphal proliferation, altered hyphal morphology in vitro and completely cured induced cutaneous dermatophytosis in 21 days when topically applied to infected mice.

In most studies, the antimicrobial mechanism of action of BS has been ascribed to the well-established disturbing activity on the cell membranes due to the amphiphilic nature of these compounds. However, evidence is emerging of the role of BSs in quorum sensing signaling ^{[29][64][65]}. Comparative studies regarding the biosynthesis of rhamnolipids by a strain of *P. aeruginosa* isolated from manure revealed that the cultivation in a selected mixed culture remarkably improved the production of rhamnolipids in terms of maximum yield compared to the axenic culture. This effect was suggested to be associated with interspecies communication via quorum sensing based on AI-2 signaling molecules, demonstrating the significance of interspecies communication for biosurfactant production ^[66]. This evidence suggests the need to explore the role of BS in microbial competitive interactions.

3.2. Biosurfactants from Lactic Acid Bacteria with Antimicrobial Activities

Lactic acid bacteria (LAB) are generally believed to positively influence human health and immune systems. Some of them have shown antimicrobial properties against a broad spectrum of microorganisms, including several pathogens in the intestinal tract and female genital tract due the production of heterogeneous structural biosurfactants ^{[57][58][59]}. The biosurfactants produced by *Lactobacillus jensenii* P6A and *Lactobacillus gasseri* P65 showed a marked antimicrobial activity against urogenital tract clinical isolates of *E. coli* (MIC = 16 μ g/mL), *Staphylococcus saprophyticus, Enterobacter aerogenes* and *K. pneumoniae* (MIC = 128 μ g/mL) ^[70]. In another study, Vecino et al. ^[71] suggested the use of a glycolipopeptides obtained from a *Lactobacillus pentosus* strain as a "natural" ingredient in cosmetic and personal care formulations due to their efficacy in inhibiting the growth of several microorganisms present in the skin microflora, such as *P. aeruginosa, Streptococcus agalactiae, S. aureus, E. coli, S. pyogenes* and *C. albicans*. Most recently, it has also been shown that the biosurfactant from *Pediococcus dextrinicus* SHU1593 is characterized by an interesting dose-dependent inhibitory activity against the planktonic cells of *E. coli, E. aerogenes* and *P. aeruginosa*, leading to a complete eradication at 25 mg/mL concentration ^[72].

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