Chapter 6

Biomedical and Therapeutic Applications of Biosurfactants

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Abstract

During the last years, several applications of biosurfactants with medical purposes have been reported. Biosurfactants are considered relevant molecules for applications in combating many diseases and as therapeutic agents due to their antibacterial, antifungal and antiviral activities. Furthermore, their role as anti-adhesive agents against several pathogens illustrate their utility as suitable anti-adhesive coating agents for medical insertional materials leading to a reduction of a large number of hospital infections without the use of synthetic drugs and chemicals. Biomedical and therapeutic perspectives of biosurfactants applications are presented and discussed in this chapter.

Introduction

Biosurfactants are microbial compounds that exhibit pronounced surface and emulsifying activities. These compounds comprise a wide range of chemical structures, such as glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids and neutral lipids.[1-7] Therefore, it is reasonable to expect diverse properties and physiological functions for different groups of biosurfactants. Comparing with chemical surfactants, these compounds have several advantages such as lower toxicity, higher biodegradability and effectiveness at extreme temperatures or pH values.[6-10] Although these compounds present interesting features as compared with their chemical counterparts, many of the envisaged applications depend considerably on whether they can be produced economically. Hence, much effort in process optimization and at the engineering and biological levels has been carried out. Biosurfactants production from inexpensive waste substrates and low cost raw materials, thereby decreasing their production cost,[11-18] has been reported. Furthermore, these molecules can be tailor-made to suit different applications by changing the growth substrate or growth conditions.[19-20] Most biosurfactants are considered secondary metabolites, though, some may play essential roles for the survival of the producing-microorganisms either through facilitating nutrient transport, microbe-host interactions or as biocide agents.[6] Biosurfactant roles include increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, quorum sensing and biofilm formation.[21] An interface is any boundary between two different phases and microbial life may be more common at interfaces as evidenced by microbial biofilms, surface films and aggregates. Given that, all microbial life is impacted by interfacial phenomena and biosurfactants are a common mechanism by which microorganisms deal with interfacial challenges.[6] Biosurfactants are amphipatic molecules with both hydrophilic and hydrophobic moieties that partition preferentially at the interface between fluid phases that have different degrees of polarity and hydrogen bonding, such as oil and water, or air and water interfaces. In addition to this behaviour,

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Biosurfactants, edited by Ramkrishna Sen.

their diversity, environmentally friendly nature, suitability for large-scale production and selectivity, has driven most of the research in biosurfactants field for environmental applications.[7,22-25] Legal aspects such as stricter regulations concerning environmental pollution by industrial activities and health regulations will also strongly influence the chances of biodegradable biosurfactants replacing their chemical counterparts.[7,10,19,22-25]

Regardless of the potential and biological origin of biosurfactants few studies were carried out on applications related to biomedical applications.[11,26-30] Nevertheless, some biosurfactants have proven to be suitable alternatives to synthetic medicines and antimicrobial agents and may therefore be used as safe and effective therapeutic agents (Table 1).

The biosurfactants potential applications in the medical field, as well as their main mechanisms of interaction are discussed in this chapter.

Biomedical and Therapeutic Applications of Biosurfactants

As discussed above a broad range of chemical structures have been attributed to biosurfactants.[1-2,4-5] Some of these biosurfactants were described for their potential as biological active compounds and applicability in the medical field. Therefore, they are a suitable alternative to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents.[21,25] Recently, there has been an increasing interest in the effect of biosurfactants on human and animal cells and cell lines.[28,42,83] Lipopeptides produced by *Bacillus subtilis*[36] and *Bacillus licheniformis*,[19,49-51] mannosylerythritol lipids produced by *Candida antartica*[53] and rhamnolipids produced by *Pseudomonas aeruginosa,*[33-34] have been shown to have antimicrobial activities.

Biological Activity

Glycolipids

Glycolipids are the most common group of biosurfactants of which the most effective regarding surface active properties are the trehalose lipids obtained from *Mycobacterium* and related bacteria, the rhamnolipids obtained from *Pseudomonas* sp. and the sophorolipids obtained from yeasts. Otto and coworkers[12] described the production of sophorose lipids using deproteinized whey concentrate as substrate by a two-stage process. Several antimicrobial, immunological and neurological properties have been attributed to mannosylerythritol lipid (MEL), a yeast glycolipid biosurfactant, produced from vegetable oils by *Candida* strains.[62,84] Kitamoto et al[53] showed that MEL exhibits antimicrobial activity particularly against Gram-positive bacteria. Isoda et al[54] investigated the biological activities of seven extracellular microbial glycolipids including MEL-A, MEL-B, polyol lipid, rhamnolipid, sophorose lipid and succinoyl trehalose lipid STL-1 and STL-3. Except for rhamnolipid, all the other tested glycolipids induced cell differentiation instead of cell proliferation in the human promyelocytic leukaemia cell line HL60. These glycolipids induced the human myelogenous leukaemia cell line K562 and the human basophilic leukaemia cell line Ku812 to differentiate into monocytes, granulocytes and megakaryocytes. STL and MEL differentiation-inducing activity was attributed to a specific interaction with the plasma membrane instead of a simple detergent-like effect.

In addition, the effects of several kinds of microbial extracellular glycolipids on neutrite initiation in PC12 cells were investigated.[55] The PC12 cell line derived from a rat pheochromocytoma, provides a relatively simple and homogeneous system for studying various aspects of neuronal differentiation, because PC12 cells can survive and proliferate without requiring the presence of neutrotrophic factors. A significant neutrite outgrowth was observed as a consequence of the addition of MEL-A, MEL-B and sophorose lipid (SL) to PC12 cells. MEL-A increased acetylcholinesterase activity to an extent similar to nerve growth factor (NGF). MEL-A induced neutrite outgrowth after treatment of PC12 cells with an anti-NGF receptor antibody that obstructed the NGF action. It was shown that MEL-A and NGF induce differentitation of PC12 cells through different mechanisms. Moreover, MEL was found to induce the outgrowth of neutrites, enhance the activity of acetylcholinesterase and increase the levels of galactosylceramide from PC12 pheochromocytoma cells.[56]

Table 1. Examples of biosurfactant applications in the medical field (adapted from Rodrigues et al[28])

Microorganism	Biosurfactant Type	Activity/Application	Reference
Pseudomonas aeruginosa	Rhamnolipid	• Antimicrobial activity against *Mycobacterium tuberculosis*	
		• Anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses	31-35
		• Induced dose-dependent hemolysis and coagulation of platelet-poor plasma but is not detrimental to chicken lung, liver, heart and kidney tissues	
Bacillus subtilis	Surfactin	• Antimicrobial and antifungal activities	
		• Inhibition of fibrin clot formation	36-43
		• Hemolysis and formation of ion channels in lipid membranes	
		• Antitumor activity against Ehrlich's ascite carcinoma cells	
		• Antiviral activity against human immunodeficiency virus 1 (HIV-1)	
		• Induction of apoptosis in human leukemia K562	
Bacillus subtilis	Pumilacidin	• Antiviral activity against herpes simplex virus 1 (HSV-1)	
	(surfactin analog)	• Inhibitory activity against H^+, K^+-ATPase and protection against gastric ulcers in vivo	44
Bacillus subtilis	Iturin	• Antimicrobial activity and antifungal activity against profound mycosis	
		• Effect on the morphology and membrane structure of yeast cells	5,45-48
		• Increase in the electrical conductance of biomolecular lipid membranes	

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Table 1. Continued

Microorganism	Biosurfactant Type	Activity/Application	Reference
		• Nontoxic and nonpyrogenic immunological adjuvant	
Bacillus licheniformis	Lichenysin	• Antibacterial activity	
		• Chelating properties that might explain the membrane disrupting effect of lipopeptides	49-52
Candida antartica	Mannosylerythritol lipids	• Antimicrobial, immunological and neurological properties	
		• Induction of cell differentiation in the human promyelocytic leukemia cell line HL60	53-66
		• Induction of neuronal differentiation in PC12 cells	
		• Enhancement of gene transfection efficiency	
Rodococcus erythropolis	Treahalose lipid	• Antiviral activity against HSV and influenza virus	67-68
Streptococcus thermophilus	Glycolipid	• Anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses	69-73
Streptococcus mitis	Not identified	• Anti-adhesive activity against *Streptococcus mutans*	74-75
Lactobacillus	Surlactin	• Anti-adhesive activity against several pathogens including enteric bacteria	76-81
Lactococcus lactis	Not identified	• Anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses	70, 82

Glycolipids have also been implicated with growth arrest, apoptosis and the differentiation of mouse malignant melanoma cells.[57-58] Exposure of B16 cells to MEL resulted in the condensation of the chromatin, DNA fragmentation and sub-G1 arrest (the sequence of events of apoptosis). Furthermore, MEL was reported to markedly inhibit the growth of mouse melanoma B16 cells in a dose-dependent manner. Moreover, MEL exposure stimulated the expression of differentiation markers of melanoma cells, such as tyrosinase activity and the enhanced production of melanin, which is an indication that MEL triggered both apoptotic and cell differentiation programs. In addition, exposure of PC12 cells to MEL enhanced the activity of acetylcholinesterase and interrupted the cell cycle at the G1 phase, with resulting outgrowth of neutrites and partial cellular differentiation.[59] MEL has been implicated in the induction of neuronal differentiation in PC12 cells and therefore provides the basis for the use of glycolipids as therapeutical agents for cancer treatment. Nevertheless, further studies of the molecular basis of the signalling cascade that follows exposure of PC12 cells to MEL may ultimately lead to a better understanding of the processes that result in the outgrowth of neutrites and the commitment to differentiation of PC12 cells.

In other studies, four analogs of STL-3 at their critical micelle concentration were evaluated for their ability to inhibit growth and induce differentiation of HL60 human promyelocytic leukaemia cells.[85] It was found that the effect of STL-3 and its analogs on HL60 cells was dependent on the hydrophobic moiety of STL-3. Furthermore, a high binding-affinity of MEL towards human immunoglobulin G (HIgG) was shown by Im et al.[86] They suggested the possibility of using MEL-A as an alternative ligand for immunoglobulins. In subsequent studies they evaluated the potential of MEL (-A, -B and -C) attached to PHEMA beads (poly(2-hydroxyethyl methacrylate)), for binding, affinity to HIgG.[87] Of these three composite compounds, those bearing MEL-A exhibited the highest binding capacity to HIgG. More significantly, the bound HIgG was efficiently recovered (approximately 90%) under significantly mild elution conditions, with phosphate buffer at pH 7, indicating a great potential of the glycolipids as an affinity ligand material. Other researchers also demonstrated that MEL-A assembled monolayers would be useful as noble affinity ligand system for various immunoglobulins.[64-65] Inoh et al[88-89] reported that MEL-A significantly increased the efficiency of gene transfection mediated by cationic liposomes with a cationic cholesterol derivative. Among the cationic liposomes tested, the liposome bearing cholesteryl-3β-carboxyamindoethylene-*N*-hydroxyethylamine and MEL-A showed the best efficiency for delivery of plasmids encoding luciferase (ρGL3) into the target cells (NIH3T3, COS-7 and HeLa). The properties, production and applications of MEL were widely studied by Kitamoto and coworkers[90] and by Ueno et al,[60-61] particularly the exceptional interfacial properties and differentiation-inducing activities of MEL. They also focused on the excellent biological and self-assembling actions of MEL and examined the effect of MEL-A on the gene transfection using cationic liposomes. These results were also demonstrated by other researchers that studied the transfection efficiency in human cervix carcinoma HeLa cells[66] and the potential of these liposomes as vectors for herpes simplex virus thymidine kinase gene therapy.[63]

The succinoyl-trehalose lipid produced by *Rhodococcus erythropolis* has also been reported to inhibit HSV and influenza virus.[67-68] The deficiency of pulmonary surfactant which is responsible for respiration failure in premature infants[91] may be corrected through the isolation of genes for protein molecules of this surfactant and cloning in bacteria for possible fermentative production and use in medical application.[33] Sano et al[92] demonstrated the different actions of pulmonary surfactant protein A upon distinct serotypes of LPS which is the major constituent of the outer membrane of Gram-negative bacteria.

Lipopeptides

Several features and biological activities have been reported for lipopeptides, mainly for iturin A and surfactin. They have been described as antibiotics, antiviral and antitumor agents, immunomodulators or specific toxins and enzyme inhibitors. Ahimou et al[5] reported that lipopeptide profile and bacterial hydrophobicity vary greatly with the producing strains, iturin A being the

only lipopeptide type produced by all *B. subtilis* strains. Surfactin was found to be more efficient than iturin A in modifying the *B. subtilis* surface hydrophobic character. Morikawa et al[1] identified and characterized a biosurfactant, arthrofactin, produced by *Arthrobacter* species, which was found to be seven times more effective than surfactin. Jenny and coworkers[49] determined the structural analysis and characterized surface activities of biosurfactants produced by *B. licheniformis*, while several researchers described their continuous production.[50,93] Yakimov and coworkers[51] demonstrated the antibacterial activity of lichenysin A, a biosurfactant produced by *B. licheniformis* that favourably compares to others surfactants. More recently Grangemard et al[52] reported the chelating properties of lichenysin, which might explain the membrane disrupting effect of lipopeptides.

In another study, Carrillo and its collaborators[94] proposed a molecular mechanism of membrane permeabilization by surfactin, which may explain surfactin induced pore formation underlying the antibiotic and haemolytic action of these lipopeptides. This study also suggested that the membrane barrier properties are likely to be damaged in the areas where surfactin oligomers interact with the phospholipids, at concentrations much below the onset for solubilisation. Such properties can cause structural fluctuations that may well be the primary mode of the antibiotic action of this lipopeptide. Surfactin type peptides that can rapidly act on membrane integrity rather than other vital cellular processes may perhaps constitute the next generation of antibiotics. Lipopeptide surfactin has been found to interact with artificial and biomembrane systems, for example bacterial protoplasts or enveloped viruses.[36] Several biological activities have been attributed to surfactin including the induction of ion channels formation in lipid bilayer membranes,[40] the inhibition of fibrin clot formation and haemolysis,[39] the inhibition of cyclic adenosine monophosphate (cAMP), the inhibition of platelet and spleen cytosolic phospholipase A2 (PLA2)[95] and antimicrobial, antiviral and antitumor activity against Ehrlich's ascite carcinoma cells.[38,95] According to the differences in their amino acid sequences, different types of surfactins (A, B and C) have been identified. Surfactin C was found to enhance the activation of prourokinase (plasminogen activator) and the conformational change in plasminogen, leading to increased fibrinolysis in vitro and in vivo.[96] The plasminogen-plasmin system is involved in blood clot dissolution, as well as in a variety of physiological and pathological processes requiring localized proteolysis. In a rat pulmonary embolism model, surfactin C increases plasma clot lyses when injected in combination with prourokinase.[83] The results gathered in this study point to the possible use of surfactin in thrombolytic therapy related to pulmonary, myocardial and cerebral disorders.

Vollenbroich and coworkers[36] showed that a surfactin treatment improved proliferation rates and lead to changes in the morphology of mammalian cells that had been contaminated with mycoplasma. Furthermore, the low cytotoxicity of surfactin to mammalian cells allowed specific inactivation of mycoplasmas without significant damaging effects on cell metabolism.[42,43] Additionally, surfactin and surfactin analogs have been reported as antiviral agents, namely it was demonstrated a significant inhibitory effect of pumilacidin on herpes simplex virus 1 (HSV-1)[44] and an inhibitory activity against H^+, K^+-ATPase and protection against gastric ulcers in vivo. The potential of surfactin against human immunodeficiency virus 1 (HIV-1) was reported by Itokawa et al.[41] The antiviral action of surfactin was suggested to be due to physicochemical interactions between the membrane-active surfactant and the virus lipid membrane, which causes permeability changes and at higher concentrations leads finally to the disintegration of the mycoplasma membrane system by a detergent effect.[37] Furthermore, surfactin was found to be active against Semliki Forest virus, herpes simplex virus, suid herpes virus, vesicular stomatitis virus, simian immunodeficiency virus, feline calicivirus and murine encephalomyocarditis virus.[37]

Moreover, Kim and coworkers[95] demonstrated that surfactin is a selective inhibitor for cytosolic PLA2 and a putative anti-inflammatory agent through the inhibitory effect produced by direct interaction with cytosolic PLA2 and that inhibition of cytosolic PLA2 activity may suppress inflammatory responses.

Another lipopeptide, iturin A, produced by *B. subtilis* was reported to have effective antifungal properties[5,46] which affects the morphology and membrane structure of yeast cells. This lipopeptide

was shown to pass through the cell wall and disrupt the plasma membrane with the formation of small vesicles and the aggregation of intramembranous particles. Iturin also passes through the plasma membrane and interacts with the nuclear membrane and probably with membranes of other cytoplasmic organelles. This lipopeptide has been proposed as an effective antifungal agent for profound mycosis.[47] Other members of the iturin group, including bacillomycin D and bacillomycin Lc were also found to have antimicrobial activity against *Aspergillus flavus*, but the different lipid chain length apparently affected the activity of the lipopeptide against other fungi.[97] Thus, the members of the iturin-like biosurfactant group are considered alternative antifungal agents.

Possible applications of biosurfactants as emulsifying aids for drug transport to the infection site, for supplementing pulmonary surfactant and as adjuvants for vaccines were suggested by Kosaric.[98] Mittenbuhler et al[48] showed that bacterial lipopeptides constitute powerful nontoxic and nonpyrogenic immunological adjuvants when mixed with conventional antigens. A marked enhancement of the humoral immune response was obtained with the low molecular mass antigens iturin AL, herbicolin A and microcystin (MLR) coupled to poly-L-lysine (MLR-PLL) in rabbits and in chickens. Conjugates of lipopeptide—Th-cell epitopes also constituted effective adjuvants for the in vitro immunization of either human mononuclear cells or mouse B cells with MLR-PLL and result in a significantly increased yield of antibody-secreting hybridomas.

Other Biosurfactants

Nielsen and coworkers[99] reported viscosinamide, a cyclic depsipeptide, as a new antifungal surface active agent produced by *Pseudomonas fluorescens* and with different properties as compared to the biosurfactant viscosin, known to be produced from the same species and to have antibiotic activity.[100] Massetolides A-H, also cyclic depsipeptides, were isolated from *Pseudomonas* species, derived from a marine habitat and found to exhibit in vitro antimicrobial activity against *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare*.[31]

Precursors and degeneration products of sphingolipids biosurfactants were found to inhibit the interaction of *Streptococcus mitis* with buccal epithelial cells and of *Staphylococcus aureus* with nasal mucosal cells.[101] Gram-positive *Bacillus pumilis* cells were found to produce pumilacidin A, B, C, D, E, F and G which exhibited antiviral activity against herpes simplex virus 1 (HSV-1), inhibitory activity against H^+, K^+-ATPase and were found to be protective against gastric ulcers[44] probably through inhibiting microbial activity contributing to these ulcers.

Although there is an increasing potential for the application of biosurfactants in the biomedical field, some of these molecules may constitute a risk for humans. For instance, *P. aeruginosa* is a bacterium responsible for severe nosocomial infections, life-threatening infections in immunocompromised persons and chronic infections in cystic fibrosis patients; thus rhamnolipids have to be well-investigated prior to such uses. *P. aeruginosa* strain's virulence depends on a large number of cell-associated and extracellular factors.[102-104] Cell-to-cell signalling systems control the expression and allow a coordinated, cell-density-dependent production of many extracellular virulence factors. The possible role of cell-to-cell signalling in the pathogenesis of *P. aeruginosa* infections and a rationale for targeting cell-to-cell signalling systems in the development of new therapeutic approaches was discussed by Van Delden and Iglewski.[102] Synthesis of rhamnolipids is regulated by a very complex genetic regulatory system that also controls different *P. aeruginosa* virulence-associated traits.[34] The possible application of rhamnolipids in the pharmaceutical industry is still being studied by some researchers.[35,105] The cosmetic and health care industries use large amounts of surfactants for a wide variety of products including insect repellents, antacids, acne pads, contact lens solutions, hair colour and care products, deodorants, nail care products, lipstick, eye shadow, mascara, toothpaste, denture cleaners, lubricated condoms, baby products, foot care products, antiseptics, shaving and depilatory products.[8] Biosurfactants are known to have advantages over synthetic surfactants such as low irritancy or anti-irritating effects and compatibility with skin. Rhamnolipids in particular are being used as cosmetic additives and have been patented to make some liposomes and emulsions,[103-104] both of which are important in the cosmetic industry.

Anti-Adhesive Activity

Biosurfactants have been found to inhibit the adhesion of pathogenic organisms to solid surfaces or to infection sites, thus prior adhesion of biosurfactants to solid surfaces of implant materials might constitute a new and effective means of combating colonization by pathogenic microorganisms.[21] Precoating vinyl urethral catheters by running the surfactin solution through them before inoculation with media resulted in a decrease of the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enterica*, *Escherichia coli* and *Proteus mirabilis*.[106] Given the importance of opportunistic infections with *Salmonella* species, including urinary tract infections of AIDS patients, these results have great potential for practical applications.

A role for biosurfactants as defence weapons in post adhesion competition with other strains or species has to date been suggested only for biosurfactants released by *S. mitis* strains against *Streptococcus mutans* adhesion[74-75] and for biosurfactants released by lactobacilli against adhesion of uropathogens.[77-78] The biosurfactant surlactin,[79] produced by several *Lactobacillus* isolates, was suggested as a suitable anti-adhesive coating for catheter materials. The role of *Lactobacillus* species in the female urogenital tract as a barrier to infection is of considerable interest.[107] These organisms are believed to contribute to the control of vaginal microbiota by competing with other microorganisms for adherence to epithelial cells and by producing biosurfactants. There are reports of inhibition of biofilm formation by uropathogens and yeast on silicone rubber with biosurfactants produced by *Lactobacillus acidophilus*.[108-109] Heinemann and coworkers showed that *Lactobacillus fermentum* RC-14 releases surface-active components that can inhibit adhesion of uropathogenic bacteria, including *Enterococcus faecalis*.[110] Velraeds et al[80] also reported on the inhibition of adhesion of pathogenic enteric bacteria by a biosurfactant produced by a *Lactobacillus* strain and later showed that the biosurfactant caused an important, dose-related inhibition of the initial deposition rate of *E. coli* and other bacteria adherent on both hydrophobic and hydrophilic substrata.[76]

Dairy *S. thermophilus* strains were found to be biosurfactant-producers and Busscher et al[72-73] showed that this biosurfactant inhibited adhesion onto silicone rubber and growth of several bacterial and yeast strains isolated from explanted voice prostheses. Efforts in the development of strategies to prevent the microbial colonization of silicone rubber voice prostheses have been reported by Rodrigues et al.[71,82] The ability of biosurfactants obtained from the probiotic strains, *L. lactis* 53 and *S. thermophilus* A, to inhibit adhesion of four bacterial and two yeast strains isolated from explanted voice prostheses to precoated silicone rubber was evaluated. The results obtained showed that the biosurfactants were effective in decreasing the initial deposition rates, as well as the number of bacterial cells adhering after 4 h, for all microorganisms tested. Over 90% reductions in the initial deposition rates were achieved for most of the bacterial strains tested. Recently, the authors also demonstrated that a rhamnolipid biosurfactant containing solution may be useful for use as a biodetergent solution for prostheses cleaning, prolonging their lifetime and directly benefiting laryngectomized patients. Gotek et al[81] assessed the adhesive properties of several biosurfactant-producers *Lactobacillus* spp. strains to a monolayer of intestinal epithelium in vitro, represented by the Caco2 cell line. All tested *Lactobacillus* strains showed adhesion to Caco2 cells. A 50% reduction in the population of *Klebsiella pneumoniae* 2 cells adhering to the surface previously impregnated with a solution of biosurfactants synthesised by *Lactobacillus casei rhamnosus* CCM 1825, after the 3-hour contact with the tested surface was also observed.

The role for surfactants in the defence against infection and inflammation in the human body is a well-known phenomenon. The pulmonary surfactant is a lipoprotein complex synthesized and secreted by the epithelial lung cells into the extracellular space, where it lowers the surface tension at the air-liquid interface of the lung and represents a key factor against infections and inflammatory lung diseases.[91]

Antimicrobial Activity

The antimicrobial activity of several biosurfactants has been reported in the literature for many different applications.[111] For instance, the antimicrobial activity of two biosurfactants obtained from probiotic bacteria, *L. lactis* 53 and *S. thermophilus* A, against a variety of bacterial and yeast

strains isolated from explanted voice prostheses was evaluated.[70] In another study, Reid et al[112-113] emphasized a possible probiotic role for the biosurfactant-producing lactobacilli in the restoration and maintenance of healthy urogenital and intestinal tracts, conferring protection against pathogens and suggested a reliable alternative treatment and preventive regimen to antibiotics in the future. The first clinical evidence that probiotic lactobacilli can be delivered to the vagina following oral intake was provided[113] and although only a limited set of strains have any proven clinical effect or scientific basis, there are sufficient data to suggest that this approach could provide a valuable alternative to antibiotic prophylaxis and treatment of infection. By the use of a rat model of surgical implant infection, Gan et al[114] determined that the probiotic strain, *L. fermentum* RC-14 and its secreted biosurfactant reduced infections associated with surgical implants, which are mainly caused by *S. aureus* through inhibition of growth and reduction of adherence to surgical implants. A recent in vitro study of *Lactobacillus plantarum* 299v and *L. rhamnosus* GG showed that these probiotic strains could inhibit the adhesion of *E. coli* to intestinal epithelial cells by stimulating epithelial expression of mucins.[115] These strains however were also found to be biosurfactant producers.[14] These observations generally indicated that biosurfactants might also contain signalling factors that interact with host and/or bacterial cells leading to the inhibition of infections. Moreover they support the assertion of possible role in preventing microbial adhesion[76,116] and their potential in developing anti-adhesion biological coatings for implant materials.[32]

Conclusion

Interest in the use of biosurfactants in the medical field has been increasing in the last years as a result of many studies published on their unique features. Biosurfactants are not only useful as antibacterial, antifungal and antiviral agents, but also have potential for use as major immunomodulatory molecules, adhesive agents and even in vaccines and gene therapy. They have been used for gene tranfection, as ligands for binding immunoglobulins, as adjuvants for antigens and also as inhibitors for fibrin clot formation and activators of fibrin clot lyses. Promising alternatives to produce potent biosurfactants with altered antimicrobial profiles and decreased toxicity against mammalian cells may be exploited by genetic alteration of biosurfactants. Furthermore, biosurfactants have the potential to be used as anti-adhesive biological coatings for biomaterials, thus reducing hospital infections and use of synthetic drugs and chemicals. They may also be incorporated into probiotic preparations to combat urogenital tract infections and pulmonary immunotherapy.

Regardless of the enormous potential of biosurfactants in this field, their use still remains limited, possibly due to their high production and extraction cost and lack of information on their toxicity towards human systems. Further research on human cells and natural microbiota are required to validate the use of biosurfactants in several biomedical and health related areas. Nevertheless, there appears to be great potential for their use in the medical science arena waiting to be fully exploited.

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