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Biosurfactants: potential applications in medicine

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The use and potential commercial application of biosurfactants in the medical field has increased during the past decade. Their antibacterial, antifungal and antiviral activities make them relevant molecules for applications in combating many diseases and as therapeutic agents. In addition, their role as anti-adhesive agents against several pathogens indicates their utility as suitable anti-adhesive coating agents for medical insertional materials leading to a reduction in a large number of hospital infections without the use of synthetic drugs and chemicals. This review looks at medicinal and therapeutic perspectives on biosurfactant applications.

Keywords: antimicrobial activities, antiviral activities, anti-adhesive coatings, therapeutic agents, anti-carcinogenic agents

Introduction

Microbial compounds that exhibit pronounced surface and emulsifying activities are classified as biosurfactants. Biosurfactants comprise a wide range of chemical structures, such as glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids and neutral lipids.[1–5] For instance, Cooper and Goldenberg[6] described different bioemulsifiers produced by two *Bacillus* species in water-soluble substrates with distinct emulsifying and surface activities. It is, therefore, reasonable to expect diverse properties and physiological functions for different groups of biosurfactants. Moreover, these molecules can be tailor-made to suit different applications by changing the growth substrate or growth conditions.[7] Although most biosurfactants are considered to be secondary metabolites, some may play essential roles for the survival of biosurfactant-producing microorganisms through facilitating nutrient transport or microbe–host interactions or by acting as biocide agents. Biosurfactant roles include increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, quorum sensing and biofilm formation.[8] Biosurfactants are amphipathic 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. This property explains their broad use in environmental applications.[9–11] Most work on biosurfactant applications has been focused on their use in environmental applications owing to their diversity, environmentally friendly nature, suitability for large-scale production and selectivity.[12] Despite their potential and biological origin only a few studies have been carried out on applications related to the biomedical field.[13–15] Some biosurfactants are suitable alternatives to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents (Table 1).

Microbial surfactants have several advantages over chemical surfactants such as lower toxicity, higher biodegradability and effectiveness at extreme temperatures or pH values.[16,17] Many of the potential applications that have been considered for biosurfactants depend on whether they can be produced economically; however, much effort in process optimization and at the engineering and biological levels has been carried out. Biosurfactant production from inexpensive waste substrates, which decreases their production cost,[15,18] has been reported. In addition, 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]

This review aims to cover the applications of various biosurfactants in the medical field and also to provide an overview of biosurfactant activities and mechanisms of interaction that could be exploited further in developing alternative drugs, lines of therapy or biomaterials.

Biosurfactants: mechanisms of interaction

Biosurfactants are microbial amphiphilic polymers and polyphilic polymers that tend to interact with the phase boundary between

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Table 1. Examples of biosurfactant applications in the medical field

Microorganism	Biosurfactant type	Activity/application	Reference(s)
Pseudomonas aeruginosa	rhamnolipid	• antimicrobial activity against *Mycobacterium tuberculosis* • anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses	51, 63, 76, 77
Bacillus subtilis	surfactin	• antimicrobial and antifungal activities • inhibition of fibrin clot formation • haemolysis and formation of ion channels in lipid membranes • antitumour activity against Ehrlich's ascite carcinoma cells • antiviral activity against human immunodeficiency virus 1 (HIV-1)	31, 33, 83–86
Bacillus pumilus	pumilacidin (surfactin analogue)	• antiviral activity against herpes simplex virus 1 (HSV-1) • inhibitory activity against H^+, K^+-ATPase and protection against gastric ulcers *in vivo*	53
Bacillus subtilis	iturin	• antimicrobial activity and antifungal activity against profound mycosis • effect on the morphology and membrane structure of yeast cells • increase in the electrical conductance of biomolecular lipid membranes • non-toxic and non-pyrogenic immunological adjuvant	5, 27–29, 88
Bacillus licheniformis	lichenysin	• antibacterial activity • chelating properties that might explain the membrane-disrupting effect of lipopeptides	78–81
Candida antartica	mannosylerythritol lipids	• antimicrobial, immunological and neurological properties • induction of cell differentiation in the human promyelocytic leukemia cell line HL60 • induction of neuronal differentiation in PC12 cells	37–43
Rhodococcus erythropolis	treahalose lipid	• antiviral activity against HSV and influenza virus	89, 90
Streptococcus thermophilus	glycolipid	• anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses	26, 55, 74, 95, 96
Streptococcus mitis	not identified	• anti-adhesive activity against ***S**treptococcus mutans*	24, 25
Lactobacillus	surlactin	• anti-adhesive activity against several pathogens including enteric bacteria	62, 97–100
Lactococcus lactis	not identified	• anti-adhesive activity against several bacterial and yeast strains isolated from voice prostheses	55, 73

two phases in a heterogeneous system, defined as the interface. For all interfacial systems, it is known that organic molecules from the aqueous phase tend to immobilize at the solid interface. There they eventually form a film known as a conditioning film, which will change the properties (wettability and surface energy) of the original surface.[19] In an analogy to organic conditioning films, biosurfactants may interact with the interfaces and affect the adhesion and detachment of bacteria. In addition, the substratum surface properties determine the composition and orientation of the molecules conditioning the surface during the first hour of exposure. After about 4 h, a certain degree of uniformity is reached and the composition of the adsorbed material becomes substratum independent.[20]

Owing to the amphiphilic nature of biosurfactants, not only hydrophobic but a range of interactions are involved in the possible adsorption of charged biosurfactants to interfaces. Most natural interfaces have an overall negative or, rarely, positive charge. Thus, the ionic conditions and the pH are important parameters if interactions of ionic biosurfactants with interfaces are to be investigated.[21] Gottenbos *et al.*[22] demonstrated that positively charged biomaterial surfaces exert an antimicrobial effect on adhering Gram-negative bacteria, but not on Gram-positive bacteria. In addition, the molecular structure of a surfactant will influence its behaviour at interfaces. In describing the surface-active approach, an effort is made to elaborate on the possible theoretical locations and orientations of the biosurfactants. Nevertheless, it must be kept in mind that the situation in natural systems is far more complex and requires the consideration of many additional parameters.

Biological activity of biosurfactants

As described above, a broad range of chemical structures, such as glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids and neutral lipids, have been attributed to biosurfactants.[1,2,4,5] Some of these biosurfactants were described for their potential to act as biologically active compounds and applicability in the medical field.

Lipopeptides

Among the several categories of biosurfactants, lipopeptides are particularly interesting because of their high surface activities and antibiotic potential. Lipopeptides can act as antibiotics, antiviral and antitumour agents, immunomodulators or specific toxins and enzyme inhibitors. Ahimou *et al*.[5] reported that lipopeptide profile and bacterial hydrophobicity vary greatly with the strains, iturin A being the only lipopeptide type produced by all *Bacillus subtilis* strains. Surfactin was found to be more efficient than iturin A in modifying the *B. subtilis* surface hydrophobic character. This aspect appears essential, in association with the antifungal properties of lipopeptides involved, in the biological control of plant diseases. 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.

Iturin biosurfactants. Produced by the strains of *B. subtilis*, iturin A is a potent antifungal lipopeptide with many properties, of which antimicrobial activity was the first reported.[5,27] Iturin A's mechanism of action is related to the disruption of the plasma membrane by the formation of small vesicles and the aggregation of intramembranous particles in yeast cells. Moreover, it also significantly increases the electrical conductance of biomolecular lipid membranes.[28] Iturin A has been proposed as an effective antifungal agent for profound mycosis.[29] 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.[30] Thus, the members of the iturin-like biosurfactant group have the potential to be used as alternative potent antifungal agents.

Surfactin biosurfactants. Surfactin, a cyclic lipopeptide, is also produced by *B. subtilis* strains and has well-known antimicrobial properties.[5] It has been reported to interact with artificial and biomembrane systems, for example bacterial protoplasts and enveloped viruses.[31] There are three different types of surfactins, A, B and C, which are classified according to the differences in their amino acid sequences.

In addition to antifungal and antibacterial properties, surfactin has also been related to several biological activities, namely the inhibition of fibrin clot formation, the induction of ion channel formation in lipid bilayer membranes, the inhibition of cyclic adenosine monophosphate, the inhibition of platelet and spleen cytosolic phospholipase A2 (PLA2), and antiviral and antitumour activities.[32] Kim *et al*.[32] 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. Vollenbroich *et al*.[31] showed that surfactin treatment improved proliferation rates and led to changes in the morphology of mammalian cells that had been contaminated with mycoplasma. In addition, the low cytotoxicity of surfactin to mammalian cells permitted specific inactivation of mycoplasmas without significant damaging effects on cell metabolism and the proliferation rate of cells in culture. In another study, the same authors[33] showed that surfactin is active against several viruses, including Semliki Forest virus, herpes simplex virus (HSV), suid herpes virus, vesicular stomatitis virus, simian immunodeficiency virus, feline calicivirus and murine encephalomyocarditis virus. The inactivation of enveloped viruses, especially herpesviruses and retroviruses, was significantly more efficient than that of non-enveloped viruses, suggesting that the antiviral action of surfactin is primarily due to a physicochemical interaction between the membrane-active surfactant and the outer part of the virus lipid membrane bilayer, which causes permeability changes and at higher concentrations leads finally to the disintegration of the mycoplasma membrane system by a detergent effect.

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*.[34] 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 increased plasma clot lysis when injected in combination with prourokinase.[35] These results point to the potential use of surfactin in thrombolytic therapy related to pulmonary, myocardial and cerebral disorders.

Various nosocomial infections such as those related to the use of central venous catheters, urinary catheters, prosthetic heart valves, voice prostheses and orthopaedic devices are clearly associated with biofilms that adhere to the biomaterial surface. These infections share common characteristics even though the microbial causes and host sites vary greatly. The most important of these characteristics is that bacteria in biofilms evade host defences and withstand antimicrobial chemotherapy. As antimicrobial resistance is nowadays a growing source of concern in modern medicine, genetic engineering of the known biosurfactant molecules is a key factor for the development of alternative prophylactic and therapeutic agents. Symmank *et al*.[36] produced a novel lipohexapeptide with altered antimicrobial activities by genetic engineering of the surfactin biosynthesis mechanism. Reduced detectable haemolytic activity concomitant with an increase in growth inhibition of bacterial cells, including *Bacillus licheniformis*, was observed. Thus, similar surfactin derivatives may exhibit reduced toxicity against eukaryotic cells, which could improve their therapeutic applications.

Glycolipids

Glycolipids are the most common class of biosurfactants, of which the most effective from the point of view of surface-active properties are the trehalose lipids obtained from *Mycobacterium* and related bacteria, the rhamnolipids obtained from *Pseudomonas* species and the sophorolipids obtained from yeasts. Otto *et al*.[18] described the production of sophorose lipids (SLs) using deproteinized whey concentrate as the 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. Kitamoto *et al*.[37] showed that MEL exhibits antimicrobial activity, particularly against Gram-positive bacteria. Isoda *et al*.[38] investigated the biological activities of seven extracellular microbial glycolipids, including MEL-A, MEL-B, polyol lipid, rhamnolipid, SL and succinoyltrehalose lipids STL-1 and STL-3. Except for rhamnolipid, all the other glycolipids tested induced cell differentiation instead of cell proliferation in the human promyelocytic leukaemia cell line HL60. 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 neurite initiation in PC12 cells were investigated.[39] 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 neurite outgrowth was observed as a consequence of the addition of MEL-A, MEL-B and SL to PC12 cells. MEL-A increased acetylcholinesterase activity to an extent similar to nerve growth factor (NGF). MEL-A induced neurite 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 differentiation of PC12 cells through different mechanisms. Moreover, MEL was found to induce the outgrowth of neurites, enhance the activity of acetylcholinesterase and increase the levels of galactosylceramide from PC12 pheochromocytoma cells.[40]

Glycolipids have also been implicated in growth arrest, apoptosis and the differentiation of mouse malignant melanoma cells.[41,42] 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). In addition MEL was also 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 mechanisms. In addition, exposure of PC12 cells to MEL enhanced the activity of acetylcholinesterase and interrupted the cell cycle at the G_1 phase, with resulting outgrowth of neurites and partial cellular differentiation.[43] MEL has been implicated in the induction of neuronal differentiation in PC12 cells and therefore provides the basis for the use of glycolipids as therapeutic agents for treatment of cancer cells. Nevertheless, further studies on 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 neurites and the commitment to differentiation of PC12 cells.

In other studies four analogues 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.[44] It was found that the effect of STL-3 and its analogues 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*.[45] They suggested the possibility of using MEL-A as an alternative ligand for immunoglobulins. In subsequent studies they evaluated MEL-A, MEL-B and MEL-C attached to PHEMA beads [where PHEMA stands for poly(2-hydroxyethyl methacrylate)] for their binding affinity to HIgG.[46] Of these three composite compounds, those bearing MEL-A exhibited the highest binding capacity for HIgG. More significantly, the bound HIgG was efficiently recovered (~90%) under significantly mild elution conditions, with phosphate buffer at pH 7, indicating a great potential of the glycolipids as an affinity ligand material. Inoh *et al*.[47,48] 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 liposomes bearing cholesteryl-3β-carboxyamindoethylene-*N*-hydroxyethylamine and MEL-A showed the best efficiency for delivery of plasmids encoding luciferase (pGL3) into the target cells (NIH3T3, COS-7 and HeLa). The properties, production and applications of MEL were widely studied by Kitamoto *et al*.,[49] 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 gene transfection using cationic liposomes.

Other biosurfactants with biological activity

Nielsen *et al*.[50] reported viscosinamide, a cyclic depsipeptide, to be a new antifungal surface-active agent produced by *Pseudomonas fluorescens*, with different properties compared with the biosurfactant viscosin, known to be produced from the same species and shown to have antibiotic activity.[23] Massetolides A–H, also cyclic depsipeptides, were isolated from the *Pseudomonas* species, derived from a marine habitat, and found to exhibit *in vitro* antimicrobial activity against *Mycobacterium tuberculosis* and *Mycobacterium avium-intracellulare*.[51]

Precursors and degeneration products of sphingolipid biosurfactants were found to inhibit the interaction of *Streptococcus mitis* with buccal epithelial cells and of *Staphylococcus aureus* with nasal mucosal cells.[52] Gram-positive *Bacillus pumilis* cells were found to produce pumilacidin A, B, C, D, E, F and G, which exhibited antiviral activity against HSV-1 and inhibitory activity against H^+, K^+-ATPase, and were found to be protective against gastric ulcers,[53] probably through the inhibition of microbial activity contributing to these ulcers.

Antimicrobial activity of biosurfactants

The antimicrobial activity of several biosurfactants has been reported in the literature for many different applications.[54] For instance, the antimicrobial activity of two biosurfactants obtained from probiotic bacteria, *Lactococcus lactis* 53 and *Streptococcus thermophilus* A, against a variety of bacterial and yeast strains isolated from explanted voice prostheses was evaluated, as shown in Table 2.[55] We found that both biosurfactants have a high antimicrobial activity even at low concentrations against *Candida tropicalis* GB 9/9, one of the strains held responsible for prostheses failure. At the highest concentration tested both biosurfactants were active against all the bacterial and yeast strains studied. In another study, Reid *et al*.[56,57] 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 by Reid *et al*.[57] 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*.[58] determined that the probiotic strain, *Lactobacillus 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

Table 2. Antimicrobial activity of biosurfactants (at different concentrations) against several bacterial and yeast strains isolated from explanted voice prostheses

	Biosurfactant obtained from *L. lactis* 53				
Microorganism	5 mg/mL	10 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL
Staphylococcus epidermidis GB 9/6	±	±	+	+	+
Streptococcus salivarius GB 24/9	–	–	±	±	+
Staphylococcus aureus GB 2/1	±	±	±	+	+
Rothia dentocariosa GBJ 52/2B	–	–	±	±	±
Candida albicans GBJ 13/4A	–	±	±	+	+
Candida tropicalis GB 9/9	+	+	+	+	+
	Biosurfactant obtained from *S. thermophilus* A				
Microorganism	3 mg/mL	5 mg/mL	10 mg/mL	50 mg/mL	100 mg/mL
Staphylococcus epidermidis GB 9/6	±	±	±	+	+
Streptococcus salivarius GB 24/9	–	–	±	±	+
Staphylococcus aureus GB 2/1	–	–	±	±	+
Rothia dentocariosa GBJ 52/2B	–	–	±	±	±
Candida albicans GBJ 13/4A	–	–	±	+	+
Candida tropicalis GB 9/9	+	+	+	+	+

The experiments were scored as positive (+) when growth inhibition was observed (no colonies formed); a ± sign indicates that some colonies formed within the zones; and no growth inhibition was marked as negative (–). For details see Rodrigues *et al*.[55]

of adherence to surgical implants. A recent *in vitro* study of *Lactobacillus plantarum* 299v and *Lactobacillus rhamnosus* GG showed that these probiotic strains could inhibit the adhesion of *Escherichia coli* to intestinal epithelial cells by stimulating epithelial expression of mucins.[59] These strains, however, were also found to be biosurfactant producers.[60] These observations generally indicated that biosurfactants might also contain signalling factors that interact with the host and/or bacterial cells, leading to the inhibition of infections. Moreover, they support the assertion of a possible role in preventing microbial adhesion[61,62] and their potential in developing anti-adhesion biological coatings for implant materials.[63]

Antibacterial and antiphytoviral effects of various rhamnolipids have been described in the literature.[13,64] Seven different rhamnolipids were identified in cultures of *Pseudomonas aeruginosa* AT10 from soybean oil refinery wastes and these showed excellent antifungal properties against various fungi.[65] Golubev *et al*.[66] reported the production of an extracellular, low molecular weight, protease-resistant thermostable glycolipid fungicide from the yeast *Pseudozyma fusiformata* (*Ustilaginales*). This fungicide was active against >80% of the 280 yeast and yeast-like species tested under acidic conditions (pH 4.0) at 20–30°C.[67] The purified glycolipids enhanced non-specific permeability of the cytoplasmic membrane in sensitive cells, which resulted in ATP leakage.

Anti-adhesive activity of biosurfactants

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 might constitute a new and effective means of combating colonization by pathogenic microorganisms.[8] Pre-coating vinyl urethral catheters by running the surfactin solution through them before inoculation with media resulted in a decrease in the amount of biofilm formed by *Salmonella typhimurium*, *Salmonella enterica*, *E. coli* and *Proteus mirabilis*.[68] Given the importance of opportunistic infections with *Salmonella* species, including urinary tract infections of AIDS patients, these results have great potential for practical applications. In addition, the use of lactobacilli as a probiotic for the prevention of urogenital infections has been widely studied. The role of *Lactobacillus* species in the female urogenital tract as a barrier to infection is of considerable interest.[69] 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*.[70,71] Heinemann *et al*.[72] showed that *L. fermentum* RC-14 releases surface-active components that can inhibit adhesion of uropathogenic bacteria, including *Enterococcus faecalis*. Efforts in the development of strategies to prevent the microbial colonization of silicone rubber voice prostheses have been reported by Rodrigues *et al*.[73,74] 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 pre-coated 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. The biosurfactant

Table 3. Desorption percentages of microorganisms isolated from explanted voice prostheses adhered to silicone rubber as a result of rhamnolipid perfusion through the parallel-plate flow chamber with and without a following passage of a liquid–air interface

	Desorption percentages (%)	
Microorganism	rinsing with rhamnolipid solution	passage air–liquid interface
Staphylococcus epidermidis GB 9/6	80.2	89.5
Streptococcus salivarius GB 24/9	87.3	98.7
Staphylococcus aureus GB 2/1	21.0	67.4
Rothia dentocariosa GBJ 52/2B	63.3	98.9
Candida albicans GBJ 13/4A	81.8	95.5
Candida tropicalis GB 9/9	74.2	95.5

Results are averages of duplicate experiments varying within 10–15%. For details see Rodrigues *et al*.[63]

obtained from *S. thermophilus* A was more effective against *Rothia dentocariosa* GBJ 52/2B, which is one of the strains responsible for valve prosthesis failure. The initial deposition rates of the yeast strains were far less reduced in the presence of the biosurfactant than the other tested strains. Recently the authors also demonstrated that when rinsing flow chambers, designed to monitor microbial adhesion, with a rhamnolipid biosurfactant-containing solution the rate of deposition and adhesion was significantly reduced for a variety of bacterial and yeast strains isolated from explanted voice prostheses to silicone rubber, as shown in Table 3.[63] Therefore, we believe that this rhamnolipid may be useful as a biodetergent solution for prostheses cleaning, prolonging their lifetime and directly benefiting laryngectomized patients.

The role of surfactants in 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.[75]

Biomedical and therapeutic applications of biosurfactants

Some biosurfactants are a suitable alternative to synthetic medicines and antimicrobial agents and may be used as safe and effective therapeutic agents.[8,12] There has been increasing interest in the effect of biosurfactants on human and animal cells and cell lines.

MELs produced by *Candida antartica*,[37] rhamnolipids produced by *P. aeruginosa*[76,77] and lipopeptides produced by *B. subtilis*[31] and *B. licheniformis*[7,78–80] have been shown to have antimicrobial activities. Jenny *et al*.[78] determined the structure and characterized surface activities of biosurfactants produced by *B. licheniformis*, while Lin *et al*.[79] described their continuous production. Yakimov *et al*.[80] demonstrated the antibacterial activity of lichenysin A, a biosurfactant produced by *B. licheniformis* that compares favourably with other surfactants. More recently, Grangemard *et al*.[81] reported the chelating properties of lichenysin, which might explain the membrane-disrupting effect of lipopeptides. In another study, Carrillo *et al*.[82] noted 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 solubilization. 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 was also reported to have an antitumour activity against Ehrlich's ascite carcinoma cells[83] and an antifungal activity as well as various pharmacological applications such as inhibiting fibrin clot formation and haemolysis[84] and formation of membrane ion channels.[85] In addition, surfactin and surfactin analogues have been reported as antiviral agents: a significant inhibitory effect of pumilacidin on HSV-1 was demonstrated[53] as well as 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*.[86] The antiviral action of surfactin was suggested to be due to physicochemical interactions between the membrane-active surfactant and the virus lipid membrane.[33]

Another lipopeptide, iturin, produced by *B. subtilis* was reported to have antifungal properties,[28] affecting the morphology and membrane structure of yeast cells. Iturin 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.

Possible applications of biosurfactants as emulsifying agents for drug transport to the infection site, as agents supplementing the pulmonary surfactant and as adjuvants for vaccines were suggested by Kosaric.[87]

Mittenbuhler *et al*.[88] showed that bacterial lipopeptides constitute potent non-toxic and non-pyrogenic 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 resulted in a significantly increased yield of antibody-secreting hybridomas.

The biological activities of MELs obtained from *C. antartica* were investigated by Isoda *et al*.[38] and an induction of cell differentiation in the human promyelocytic leukaemia cell line HL60 was reported. 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. The succinoyl-trehalose lipid produced by *Rhodococcus erythropolis* has also been reported to inhibit HSV and influenza virus.[89,90] The deficiency of pulmonary surfactant described earlier which is responsible for

respiration failure in premature infants[75] 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.[76] Sano *et al*.[91] demonstrated the different actions of the pulmonary surfactant protein A upon distinct serotypes of lipopolysaccharide, which is the major constituent of the outer membrane of Gram-negative bacteria.

Although there is an increasing potential for the application of biosurfactants in the biomedical field, some of these molecules may pose 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. The virulence of a *P. aeruginosa* strain depends on a large number of cell-associated and extracellular factors.[92–94] 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 were discussed by Van Delden and Iglewski.[92] Synthesis of rhamnolipids is regulated by a very complex genetic regulatory system that also controls different *P. aeruginosa* virulence-associated traits.[77] The cosmetic and healthcare 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 and shaving and depilatory products.[16] 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,[93,94] both of which are important in the cosmetic industry.

Another approach to the use of biosurfactants in biomedical applications is the development of suitable anti-adhesion biological coatings for implant materials. Dairy *S. thermophilus* strains produced a biosurfactant which caused its own desorption from glass, leaving a completely non-adhesive coating.[26] Busscher *et al*.[95,96] also showed that biosurfactant release by *S. thermophilus* inhibited adhesion on to silicone rubber and growth of several bacterial and yeast strains isolated from explanted voice prostheses. Rodrigues *et al*.,[73] using an artificial throat model, showed that biosurfactants obtained from probiotic strains greatly reduced microbial numbers on voice prostheses and also induced a decrease in the airflow resistance of voice prostheses after biofilm formation, which may constitute a mechanism by which the lifetime of indwelling silicone rubber voice prostheses can be prolonged. 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[24,25] and for biosurfactants released by lactobacilli against adhesion of uropathogens.[97,98] The biosurfactant surlactin,[99] produced by several *Lactobacillus* isolates, was suggested as a suitable anti-adhesive coating for catheter materials. Velraeds *et al*.[100] 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.[62]

Conclusions

A host of interesting features of biosurfactants have led to a wide range of potential applications in the medical field. They are useful as antibacterial, antifungal and antiviral agents, and they also have the potential for use as major immunomodulatory molecules and adhesive agents and in vaccines and gene therapy. Biosurfactants have been used for gene transfection, as ligands for binding immunoglobulins, as adjuvants for antigens and also as inhibitors for fibrin clot formation and activators of fibrin clot lysis. 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 medical insertional materials, 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.

In spite of the immense 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 investigations on human cells and natural microbiota are needed 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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