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Bioengineering

Volume 2, Issue 3, 144-162.
DOI: 10.3934/bioeng.2015.3.144
Received date 24 June 2015,
Accepted date 03 August 2015,
Published date 14 August 2015

http://www.aimspress.com/

Review

Potential therapeutic applications of microbial surface-active compounds

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Abstract: Numerous investigations of microbial surface-active compounds or biosurfactants over the past two decades have led to the discovery of many interesting physicochemical and biological properties including antimicrobial, anti-biofilm and therapeutic among many other pharmaceutical and medical applications. Microbial control and inhibition strategies involving the use of antibiotics are becoming continually challenged due to the emergence of resistant strains mostly embedded within biofilm formations that are difficult to eradicate. Different aspects of antimicrobial and anti-biofilm control are becoming issues of increasing importance in clinical, hygiene, therapeutic and other applications. Biosurfactants research has resulted in increasing interest into their ability to inhibit microbial activity and disperse microbial biofilms in addition to being mostly nontoxic and stable at extremes conditions. Some biosurfactants are now in use in clinical, food and environmental fields, whilst others remain under investigation and development. The dispersal properties of biosurfactants have been shown to rival that of conventional inhibitory agents against bacterial, fungal and yeast biofilms as well as viral membrane structures. This presents them as potential candidates for future uses in new generations of antimicrobial agents or as adjuvants to other antibiotics and use as preservatives for microbial suppression and eradication strategies.

Keywords: biosurfactants; biofilm; anti-biofilm; anti-adhesive; antimicrobial; adjuvants

1. Introduction

Biosurfactants comprise a wide range of surface-active structurally different organic compounds produced by numerous prokaryotic and eukaryotic microorganisms. These compounds are generally extracellularly excreted or localized on microbial cell surfaces and are made of amphiphilic molecules in which the hydrophobic moiety may include an acid, mono-, di- or polysaccharides, peptide cations, or anions, while the hydrophobic moiety may be composed of saturated or unsaturated fatty acid or hydrocarbon chains [1]. Biosurfactants are generally grouped according to their chemical structure, molecular weight, and mode of action. The best studied biosurfactants are glycolipids such as rhamnolipids, trehalolipids, sophorolipids and mannosylerythritol lipids and lipopeptides such as surfactin and fengycin.

These compounds orientation and behaviour on surfaces and interphases confers to these compounds a range of properties, such as the ability to decrease surface and interfacial tension of liquids and the formation of microemulsions and micelles between different phases [2,3]. Biosurfactants tend to aggregate in heterogeneous systems and at interfaces or boundaries and to form molecular interfacial films that alters the original properties of these surfaces.

In the past twenty years, a large volume of research activity has been dedicated to biosurfactants as potential replacement for synthetic surfactants in many industrial and environmental applications such as detergent, textile, paint, cosmetic, bioremediation, enhanced oil recovery, food, agrochemical fields and several commercial products have already been manufactured [4].

More recently, numerous investigations have led to the discovery of several interesting biological and chemical properties of biosurfactants and several pharmaceutical and medical applications have been envisaged [5,6]. In particular, the ability to disturb membranes integrity destabilizing them and permeability leading to metabolite leakage and cell lysis [7–10], as well as their propensity to partition at the interfaces, modifying surface properties and thus affecting microorganisms adhesion, which are important functions for antimicrobial and anti-biofilm applications [11]. Additionally, some experimental results have suggested that they are non-toxic or less toxic when compared to synthetic surfactants [12,13], a valuable characteristic for biomedical applications.

In this review, we focus on recent advances on biosurfactants as antimicrobial and anti-adhesive compounds, with a brief overview on the latest outcome on innovative therapeutic and biotechnological applications.

2. Biosurfactants as Biological Control Agents

The urgent need for new antimicrobial compounds nowadays remains of major concern due to the newly emerging pathogens and other conventional ones the majority of which have become almost insensitive to existing antibiotics [14]. Microbial metabolites are also known as a major source of compounds categorized with potent biological activities and, among these, some biosurfactants have been described as adjuvants or potential alternatives to antimicrobial agents and synthetic medicines [11]. Moreover, in addition to their ability to modulate the interaction of cells with surfaces, biosurfactants are able to interfere with microbial adhesion and biofilm formation, an important and frequently hazardous manifestations on medical devices, especially as such biofilms contain bacterial strains that often become highly resistant to adverse environmental challenges and antibiotics [15,16]. It would be useful therefore to increase the efficacy of known biocides and

antibiotics with alternative strategies aimed at reducing the biofilm populations and decreasing bacterial adhesion to medical devices surfaces.

2.1. Mechanisms of action

Establishing the functional mechanisms of actions for biosurfactants is of immense importance to assist the discovery of interesting applications. Among biosurfactants, lipopeptides and glycolipids have the most potent antimicrobial activity and represent an important source for the identification of new antibiotics.

2.1.1. Lipopeptide type compounds

The antimicrobial activities of lipopeptides, such as surfactin [17] and fengycin [18], are due to their ability to self-associate and form micellular aggregates or pore-bearing channels inside the lipid membrane. Due to these properties, lipopeptides usually cause membrane disruption, increased membrane permeability, metabolite leakages and cell lysis. Furthermore, membrane structure changes and disruption of protein conformations alter vital membrane functions including energy generation and transport [19,20]. Studies carried out on daptomycin showed that the lipopeptide oligomer binding which can be Ca^{2+} dependent often leads to the formation of pores within the membranes [21]. These pores may lead to membrane disruption and cell death as a result of transmembrane ion influxes, including Na^{+} and K^{+} [22]. The bactericidal activity of lipopeptides increases with the presence of a lipid tail length of 10–12 carbons atoms whereas an enhanced antifungal activity is exhibited in lipopeptides with a lipid tail length of 14 or 16 carbon atoms [20]. In addition, due to the difficulty of the target cells to reorganize their membranes, the ability to develop resistant strains is significantly diminished [22].

Surfactin, which is often described as a powerful biosurfactant has the capability to disturb the integrity and permeability of membranes destabilizing them. In fact, surfactin generates physical structural changes in the membrane or disrupts protein conformations, which can change some central membrane functions such as the generation of energy and transport [7–9,23]. One of the crucial steps for cellular membrane leakage and destabilization is the dimerization of surfactin into its bilayer [17]. Surfactin incorporation into membranes, *in vitro,* leads to the dehydration of the head groups of the phospholipid and bilayer instability due to the perturbation of lipid packing which ultimately leads to the alteration and distortion of the membrane barrier properties [17]. For antiviral activity, surfactin acts directly on the mainly lipidic viral envelope causing leakages or complete disintegration of the envelope exposing the capsid of the virus particles, which leads to loss infectivity.

Mechanisms of action and activity of other lipopeptides have recently been reviewed by Cochrane and Vederas [24]. Polymyxins primarily exert their strong bactericidal effect against Gram-negative bacteria through the binding of the lipid A component of lipopolysaccharide (LPS) and disruption of the outer membrane, followed by the permeabilization and disruption of the inner membrane [25,26]. Octapeptins A and B display broad-spectrum activity against both Gram-positive and Gram-negative bacteria and have also antimicrobial activity against some filamentous fungi, protozoa and yeasts due to their ability to disrupt the cytoplasmic membrane. The iturin family compounds exerts fungicidal action through the interaction with sterol components in the fungal membrane, leading to an increase in K^{+} permeability [27]. It is generally believed that the first step in

the interaction between a surfactant and a bacterial cell consists of an ionic adsorption to the bacterial cell wall which is followed by damage to cell membrane leading to inactivation of metabolic processes and cell lysis. The role or preferential attachment of surfactants to the Gram-positive or negative cell wall may be a potential explanation to their selective activity on either types of cells but yet remains to be established.

2.1.2. Glycolipidic type compounds

Concerning glycolipidic compounds mode of action, Sotirova et al. [28] demonstrated that the exposure of *Pseudomonas aeruginosa* to rhamnolipids causes a multi-component response of the bacterial cells characterized by a reduction of total cellular LPS content, an increase in cell hydrophobicity and changes in membrane proteins and surface morphology. In the same way, antimicrobial activity of sophorolipids involves mechanisms that cause destabilization and alteration of the permeability of the cellular membrane [29]. Furthermore, Ortiz et al. [7] have recently reported on the interactions of bacterial biosurfactants trehalose lipids with phosphatidylserine and phosphatidylethanolamine membranes. Their results demonstrated that trehalose lipids, when incorporated into the bilayers, increased hydrocarbon chain conformational disorder and decreased the hydration of the interfacial region of the bilayer, leading to structural perturbations that might affect membranes functions.

The ability to reduce microbial cells adhesion to surfaces, thus limiting biofilm formation, is another well-known property of biosurfactants. Both numbers and initial deposition rates of microorganisms adhering to surfaces are determined by complex interactions of hydrophobicity (interfacial free energies), the presence of specific receptor sites on the microbial cell surfaces, electrostatic interactions and types of biosurfactants produced. In particular, biofilm formation on solid surfaces is generally directly proportional to the hydrophobicity of the surface, as long as the suspended medium is a simple buffer [30]. Microbial adhesion on hydrophobic substrates (e.g. silicone rubber) was speculated to be related to the removal of interfacial water between microorganism and interacting surfaces, which facilitates closer approach and adhesion [31]. The authors also advocated that biosurfactants reduce hydrophobic interactions which decrease surface hydrophobicity that ultimately hinders microbial adhesion to surfaces and subsequently interferes with biofilms development.

2.2. Antimicrobial activity of biosurfactants

2.2.1. Biosurfactant activity against human pathogenic bacteria and fungi

The most commonly reported class of biosurfactants with antimicrobial activity, are lipopeptides [24]. Antimicrobial lipopeptides include surfactin, iturin, fengycin, mycosubtilins and bacillomycins produced by *Bacillus subtilis*, [32], cyclic lipopeptides such as daptomycin, from *Streptomyces roseosporus* [33], polymyxin B, pumilacidin and lichenysin produced by *Bacillus polymyxa*, *Bacillus pumilus* and *Bacillus licheniformis*, respectively [34] and finally viscosin, from *Pseudomonads* [35]. Glycolipids, have also been reported to display antimicrobial activities, in particular, rhamnolipids from *P. aeruginosa* [36], sophorolipids from *Candida bombicola* [37,38], mannosylerythritol lipids (MEL-A and MEL-B) from *Candida antarctica* [39].

Ghribi et al. [40] reported a broad spectrum antimicrobial activity against bacteria and fungi and

effects against multidrug-resistant microbial strains for a biosurfactant produced by *B. subtilis* SPB1. The compound showed less activity against Gram-negative bacilli and higher activity against Gram-positive cocci with particularly significant effects against *Enterococcus faecalis.*

Using strain *Paenibacillus elgii* B69, Ding et al. [41] isolated two lipopeptide antibiotics, pelgipeptins C and D which were active against pathogenic *Candida* fungal strains and a number of Gram-negative and Gram-positive bacteria. In particular, pelgipeptin D exhibited effective rapid bactericidal action against a methicillin resistant strain of *Staphylococcus aureus* and with an intraperitoneal LD50 acute toxicity test values slightly higher than polymyxin B a structurally related antimicrobial agent. Tabbene et al. [42] also reported three anti-*Candida albicans* compounds (a1, a2 and a3) derived from *B. subtilis* B38 and resembling bacillomycin D-like lipopeptides. Compound a3 had strongest fungicidal activity exceeding amphotericin B activity against a pathogenic strain of *C. albicans* sp. 311 isolated from fingernail.

More recently, a lipopeptide produced by *B. licheniformis* M104 were investigated as antimicrobial agent against Gram-positive bacteria (*B. subtilis, B. thuringiensis, B. cereus, S. aureus* and *Listeria monocytogenes*), Gram-negative bacteria (*P. aeruginosa, Escherichia coli, Salmonella typhimurium, Proteus vulgaris*, *Klebsiella pneumoniae*) and *C. albicans* [43]. All the tested microorganisms, with the exception of *L. monocytogenes* and *K. pneumoniae*, were affected by the biosurfactant and *S. aureus* was the most susceptible. The antimicrobial effect of the lipopeptide was time and concentration-dependent. The maximum inhibitory activity was observed at a concentration of 48 μg ml^{-1} after 12h of treatment. The lipopeptide 6–2 produced by *Bacillus amyloliquefaciens* was also found to have interesting antifungal activity against *C. albicans*, *Metschnikowia bicuspidate*, *Candida tropicalis*, *Yarrowia lipolytica* and *Saccharomyces cerevisiae* [44]. Scanning electronic microscopy revealed the mode of action of lipopeptide 6–2 against *C. albicans* showing the presence of invaginations in the cell wall, disruption of the whole cells followed by the loss of integrity of the cell wall. They also reported that lipopeptide 6–2 biosurfactant damaged the plasma membranes of *C. albicans* protoplast leading to its lysis [44].

Very recently, Sharma et al. [45] purified and characterized a novel lipopeptide from *Streptomyces amritsarensis* sp. The antimicrobial activity of the biosurfactant was evaluated on a broad spectrum of bacteria and fungi. The MIC values of purified lipopeptide against *B. subtilis*, *Staphylococcus epidermidis*, *Mycobacterium smegmatis* strains and a methicillin resistant *S. aureus* (MRSA) were reported to be 10, 15, 25 and 45 μg ml^{-1}, respectively. No activity against any of the tested Gram-negative bacteria and against fungi was observed. The surface-active lipopeptide heat stability test established that exposure to 100 °C or 121 °C for 15min reduced the antimicrobial action by 13.7% and 18.2% respectively. It also showed both non-cytotoxic and non-mutagenic properties, which are important prerequisite for drug development.

Liang et al. [46] analysed the antimicrobial effect of a biosurfactant obtained by cultivating the strain *Paenibacillus macerans* TKU029 in a medium with 2% (w/v) squid pen powder as carbon/nitrogen source. The purified TKU029 biosurfactant displayed significant inhibitory effect on *E. coli* and *S. aureus* at concentrations of 2 and 1.5 mg ml^{-1} respectively and showed good antifungal activity against *Fusarium oxysporum* and *Aspergillus fumigatus*.

Serrawettin W1, first described as serratamolide [47], is reported to be an antimicrobial, antitumor and plant-protecting molecule, making this biosurfactant an interesting candidate for cosmetics or pharmaceuticals applications [48–50]. Very recently, Kadouri and Shanks [51] demonstrated the inhibitory activity of this compound against MRSA strains and other Gram-positive organisms. Furthermore, despite the cytotoxic activity of serratamolide, the authors suggest that

bacterial aminolipids may be a source for future antibiotics effective against MRSA and may play a role in microbial competition.

Samadi et al. [52] evaluated some biological activities of mono and di-rhamnolipids produced by *P. aeruginosa* MN1 and reported that the mono-rhamnolipid fraction was a more potent antibacterial agent than the di-rhamnolipid fraction, in particular, against Gram-positive bacteria that were inhibited at 25 μg ml^{-1} concentration. Moreover, the rhamnolipids remarkably enhanced oxacillin inhibitory effects against MRSA strains lowering its minimum inhibitory concentrations to 3.12–6.25 μg ml^{-1}.

Rhamnolipids were also examined to evaluate their antimicrobial potential against alone and when combined with nisin (a food preservative) against two wild-type strains of *L. monocytogenes* [53]. Rhamnolipids alone had an MIC values ranging from 78 to 2500 mg ml^{-1}, which was significantly reduced when in combination of nisin showing strong synergistic effect against *L. monocytogenes* isolates.

In other works Luna et al. [54] and Rufino et al. [55] demonstrated antimicrobial activity of two biosurfactants derived respectively from *Candida sphaerica* UCP0995 and *Candida lipolytica* UCP 0988, known to produce sophorolipids (SL), against Gram-positive strains such as *Streptococcus mutans, Streptococcus sanguis, Streptococcus agalactiae, S. epidermidis, Streptococcus oralis,* and against *C. albicans*. Synergistic effects for sophorolipids biosurfactants (SL) with selected antibiotics were also reported by Joshi-Navare and Prabhune [56]. A strain of *S. aureus* was not totally inhibited by tetracycline at the concentration of 15 μg ml^{-1} after 6h exposure but was totally inhibited within 4h when combined with sophorolipids (at 300 μg ml^{-1}). Similarly, Cefaclor antibiotic showed better effects on *E. coli* when administered in combination with SL. Scanning electron microscopy revealed that the cells treated with mixtures of SL and antibiotics were characterized by cell membrane damage and pore formation, leading to enhanced leakage of the cytoplasmic contents and accumulation of cell debris. Similarly, a glycolipid biosurfactant from *Halomonas* sp BS4, containing 1, 2-Ethanediamine N, N, N', N'- tetra and (Z)-9-octadecenamide, showed antibacterial activity against *S. aureus*, *K. pneumoniae*, *Streptococcus pyrogenes* and *Salmonella typhi* and antifungal activity against *Aspergillus niger, Fusarium* sp, *Aspergillus flavus* and *Trichophyton rubrum* [57].

In spite of the high number of publication describing the antimicrobial activity of biosurfactants and of patents related to their usage, real applications in pharmaceutical, biomedical and health improvement related industries remains quite limited [4]. Some lipopeptides have reached a commercial antibiotic status, like echinocandins [58], micafungin [59], anidulafungin [60] and daptomycin [61]. Daptomycin a branched cyclic lipopeptide isolated from cultures of *S. roseosporus* and produced by Cubist Pharmaceuticals under the name Cubicin® [61], was approved in 2003 for skin infections treatment caused by MRSA and other Gram-positive pathogens and in 2006 for the treating endocarditis and bacteraemia usually caused by *S. aureus*. Daptomycin had also been reported to displays strong antibacterial activity against other important pathogens, such as penicillin-resistant *Streptococcus pneumoniae*, coagulase-negative *Staphylococci* (CNS), glycopeptide-intermediate-susceptible *S. aureus* (GISA) and vancomycin resistant *Enterococci* (VRE) [62].

Other lipopeptides such as micafungin, echinocandins and anidulafungin are low-toxic synthetically modified lipopeptides, usually obtained from the fermentation broths of various fungi [63]. Echinocandins can inhibit fungal cell wall formation particularly against *Aspergillus* spp. *Candida* spp. and *Pneumocystis carinii* [64]. The first licensed echinocandin was caspofungin; approved since 2001 for the treatment of invasive aspergillosis, esophageal and invasive candidiasis

particularly in difficult to treat cases [58]. Micafungin has been used to combat *Candida* and *Aspergillus* invasive infections in immune compromised children [59] whereas anidulafungin in the treatment of all forms of candidiasis [60]. Other lipopeptides suitable for the prevention or treatment of microbial infections have also been described as suitable antimicrobial agents with pharmaceutical applications [65]. For example, viscosin lipopeptides and congeners have been patented as therapeutic compounds capable of inhibiting *Trypanosoma cruzi, Mycobacterium tuberculosis* and a *Herpes simplex* virus [66].

2.2.2. Antiviral activity of biosurfactants

Antiviral activity of biosurfactants has also been observed, mostly against enveloped viruses, such as herpes viruses and retroviruses compared to non-enveloped viruses. This is believed to be due to the inhibitory action and physico-chemical interactions between the surfactants and the virus envelope [67]. Antiviral activity against bursal disease virus and newcastle disease virus was observed for lipopeptides produced by *B. subtilis* fmbj [68]. Similarly, sophorolipids and rhamnolipids alginate complex showed antiviral activity against HIV, human immunodeficiency virus [69] and herpes simplex viruses [70] respectively.

2.3. Biosurfactants role in biofilms and as anti-adhesives

The continuous increase in the use of medical devices is often associated with tangible risk of infectious complications, endocarditis, metastatic infections, septic thrombophlebitis and sepsis. These microbial infections are usually due to the formation of biofilms, complex biological structures adhering to the medical device consisting of a sessile and multicellular community encapsulated in a hydrated matrix of proteins and polysaccharides. Once a mature biofilm is established, the bacterial strains embedded within become greatly resistant to both antimicrobial agents [71] and host immune response. The Gram-positive bacteria *S. aureus, S. epidermidis*, *E. faecalis*, constitute >50% of the species isolated from patients with infections related to medical device biofilms such as catheter associated infections. *P. aeruginosa, Candida* spp. and uropathogenic *E. coli* are the remaining causal agents. Similarly, orthopedic metallic prostheses are associated with a significant risk of infection [72,73].

Coating medical surfaces with antimicrobial agents are the most common current biofilm preventive strategies, a process not always successful [74]. Surface modification strategies based on plasma, UV and corona discharge treatment of typical catheter materials, such as silicone and polyurethanes, have been developed with the aim to increase material hydrophilicity, thus decreasing microbial adhesion and biofilm formation [75]. Such modifications have a temporary effect on silicone, due to the rapid rearrangement of macromolecular chains, leading to surface hydrophobicity recovery [76]. Surface coatings releasing biocides (e.g. nitric oxide, antibiotics or silver) have been developed on metallic and polymer biomaterials, as short term antimicrobial strategies [77]. The main drawbacks of antimicrobial coatings arise from time limited effectiveness as in the case of PEG-based coatings, which are susceptible to oxidative degradation [78], development of microorganism resistance and potential toxicity towards human cells as in the case of quaternary ammonium salts coatings [79].

In this context, biosurfactants have recently emerged as a potential new generation of anti-adhesive agents with enhanced biocompatibility. Biosurfactants have demonstrated the ability to

disrupt biofilm formation, controlling microbial interaction with interfaces by altering the chemical and physical condition of the developing biofilms environments [30,80].

2.3.1. Anti-adhesives/biofilm lipopeptides biosurfactants

Rivardo et al. [81], reported that a lipopeptide biosurfactant produced by the strain *B. subtilis* V9T14 in association with antibiotics synergistically increased the efficacy of antibiotics against biofilm formation of the pathogenic *E. coli* CFT073. Some of the combinations used led to the complete eradication of its biofilm. This has been used to obtain an international patent on this application [82]. Combinations of the biosurfactant with biocides to act as adjuvants were designed to effectively prevent biofilms formation on biotic and abiotic surfaces and/or eradicating planktonic bacterial growth.

Janek et al. [83] investigated the role and applications of pseudofactin II, cyclic lipopeptide biosurfactant produced by *Pseudomonas fluorescens* BD5, as an anti-adhesive compound for therapeutic and medicinal applications. Pseudofactin II decreased the adhesion of *Enterococcus hirae, Proteus mirabilis, S. epidermidis, E. faecalis, E. coli,* and *C. albicans* to glass, polystyrene and silicone. In particular, pre-treatment of a polystyrene surface with pseudofactin II (0.5 mg ml^{-1}) reduced *C. albicans* adhesion by 92–99% and other bacterial adhesion by 36–90%. It also led to increased biofilm removal ability on pre-existing biofilms grown on untreated surfaces. Pseudofactin II also caused a significant inhibition of the initial adhesion of *E. coli, E. hirae, E. faecalis* and *C. albicans* strains onto silicone urethral catheters. At the highest concentration tested (0.5 mg ml^{-1}) total inhibition of growth was observed for *S. epidermidis* while partial growth inhibitions occurred on other bacteria and *C. albicans* yeast.

In other work, *Paenibacillus polymyxa* lipopeptide biosurfactants were able to inhibit mixed and single species biofilms [84]. This biosurfactant complex mainly composed of fusaricidin B and polymyxin D1, reduced the biofilm biomass for *P. aeruginosa, S. aureus, B. subtilis*, *Micrococcus luteus,* and *Streptococcus bovis.* Sriram et al. [85] also reported antimicrobial activity and biofilm inhibition using a lipopeptide biosurfactant produced by a soil strain of *Bacillus cereus* resistant to the heavy metals lead, iron and zinc. It also inhibited biofilm formation in pathogenic strains of *S. aureus* and *P. aeruginosa*. Maximum biofilm inhibition (57%) was observed against *S. epidermidis* at 15 mg ml^{-1}.

Zeraik and Nitschke [86] evaluated the anti-adhesive and attachment properties for *M. luteus, L. monocytogenes* and *S. aureus* on polystyrene surfaces at various temperatures upon treatment with rhamnolipids and surfactin. Rhamnolipids showed a slight decrease in the attachment of *S. aureus* but were generally not effective. Surfactin in comparison effectively inhibited adhesion of tested bacterial strains at all conditions with increased activity as temperature decreased with maximum 63–66% reduction in adhesion at 4 °C.

Prevention of *C. albicans* biofilm formation on silicone disks and on acrylic resins for denture prostheses by lipopeptide biosurfactants produced by *Bacillus* sp. were reported by Cochis et al. [12]. Pre-coating with biosurfactants resulted in greater biofilm reduction and drop in cell number viability than did chlorhexidine disinfectant. This anti-adhesion activity was detected at fairly low concentrations (78–156 μg ml^{-1}) which were non-cytotoxic. In another work, the lipopeptide biosurfactant produced by *Bacillus tequilensis* CH (CHBS) was able to inhibit biofilm formation of pathogenic bacteria on both hydrophilic and hydrophobic surfaces [87]. *E. coli* and *S. mutans* biofilms were grown with different concentrations of biosurfactant on glass pieces or polyvinyl

chloride surfaces. Biofilms of *E. coli* and *S. mutans* were observed on the surfaces co-incubated with 0 and 25 μg ml^{-1} CHBS, whereas there was a complete absence of biofilm on the surfaces incubated with 50 and 75 μg ml^{-1} CHBS. Interestingly, CHBS did not inhibit the growth of *E. coli* and *S. mutans* planktonic cells under all tested concentrations, demonstrating that CHBS was not a bactericidal agent but only contrasted bacterial adhesion to different surfaces [87].

Recent research at the author's laboratory reported on a biosurfactant produced by *Lactobacillus brevis* CV8LAC, which significantly reduced biofilm formation and adhesion of *C. albicans* on silicone elastomeric disks [88]. In particular, co-incubation with CV8LAC biosurfactant significantly reduced biofilm formation by about 90%, whereas pre-coating of silicone disks reduced fungal adhesion of about 60%. The growth of *C. albicans* in both sessile and planktonic form was not inhibited; suggesting that biosurfactant CV8LAC remarkably affected cell-surface interactions making the surface less supportive for microbial adhesion.

Recent unpublished results from our laboratory also showed a significant reduction of biofilm formation by bacterial pathogens on polystyrene coated with a lipopeptide biosurfactant obtained from an endophytic strain, genotypically identified as *Bacillus subtilis* (Figure 1A). In particular, biofilms of three *P. aeruginosa* strains were reduced in a range of about 70–90%, whereas biofilms of *E. coli* and *S. epidermidis* strains were inhibited of about 70%.

The same lipopeptide also showed the ability to significantly reduce biofilm formation for *C. albicans* on biosurfactant-coated silicone elastomeric disks (Figure 1B). Chemical analysis of the crude extract revealed the presence of two families of lipopeptides, principally surfactin and a lower percentage of fengycin.

2.3.2. Anti-adhesives/biofilm glycolipid biosurfactants

Rhamnolipids and other surface-active plant oil extracts have recently been observed by some of the author's laboratories to have a significant role in the inhibition of complex biofilms and to act as adjuvants enhancing selected antibiotics microbial inhibitors [15]. In another study, a glycolipid biosurfactant from *P. aeruginosa* DSVP20 was evaluated for its ability to disrupt *C. albicans* biofilm. The treatment with the di-rhamnolipid (RL-2) at concentrations ranging from 0.04–5.0 mg ml^{-1} significantly reduced *C. albicans* adhesion on polystyrene surfaces (PS) in a dose-dependent manner. Data showed a reduction of the number of adherent cells, after 2h of treatment, of about 50% with 0.16 mg ml^{-1} RL-2, that gradually increased up to a complete inhibition of adherence at a concentration of 5 mg ml^{-1}. Moreover, *C. albicans* biofilm on PS surface was disrupted up to 70% and 90% with RL-2 treatment at concentrations of 2.5 and 5.0 mg ml^{-1}, respectively [89]. Also recently, Pradhan et al. [90] reported a new glycolipid obtained from *Lysinibacillus fusiformis* S9 with remarkable anti-biofilm activity against pathogenic *E. coli* and *S. mutans,* while not affecting microbial cell viability. In particular, the biosurfactant was able to completely contain the biofilms formation at a concentration of 40 μg ml^{-1}.

Recent unpublished data obtained at the author's laboratory investigating anti-biofilm activities of rhamnolipid biosurfactants against Gram-negative and Gram-positive pathogens on polystyrene are presented in Figure 2. The rhamnolipid extract, obtained from a *P. aeruginosa* isolated from cystic fibrosis patient (strain 89), was utilized at a concentration of 500 μg ml^{-1}.

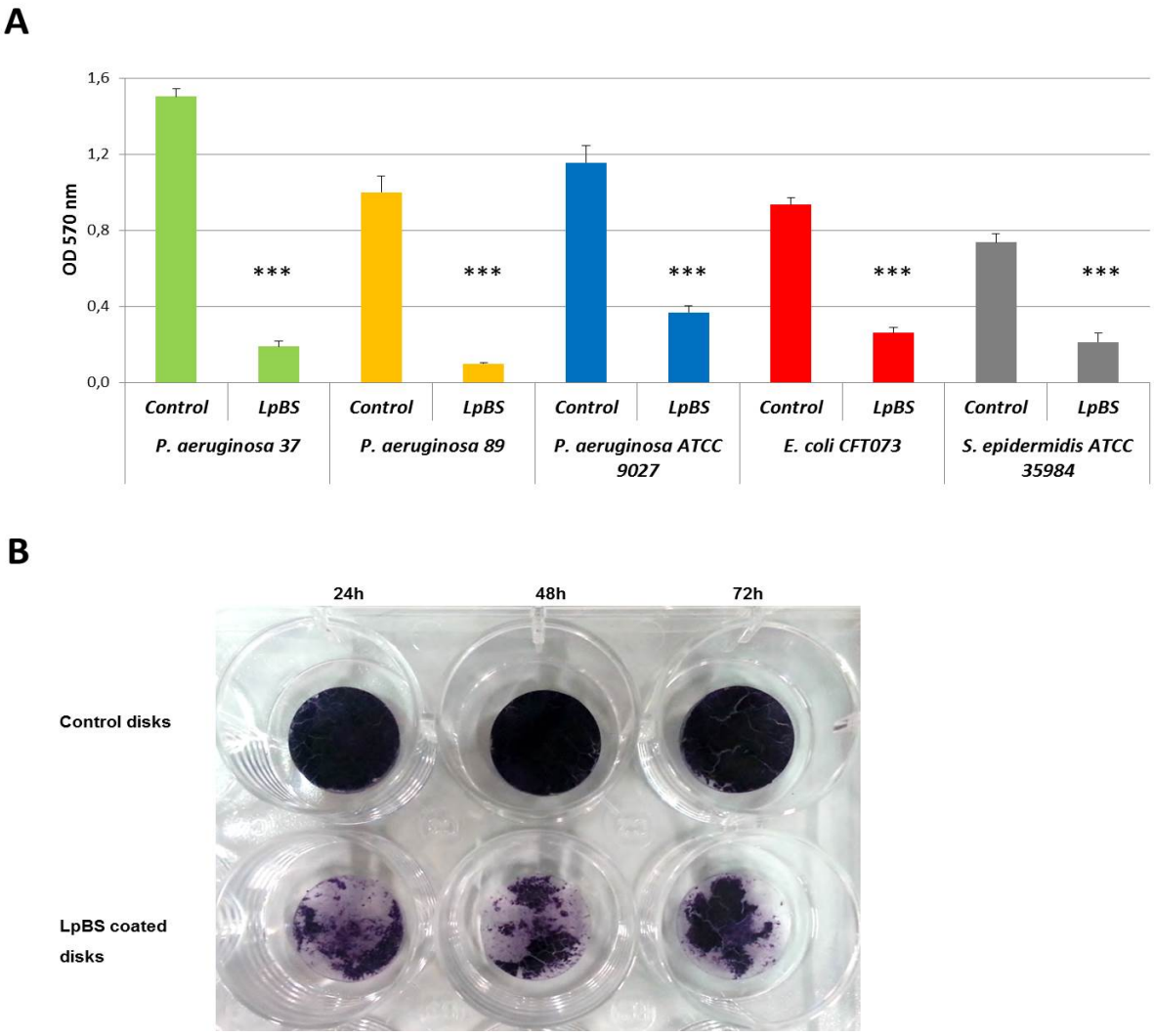

Figure 1. Anti-adhesion activity of a lipopeptide biosurfactant (LpBS) against bacterial and fungal biofilm. (A) Bacterial biofilm reduction on polystyrene coated by a LpBS after 24 hours incubation. The assay was carried out in Calgary Biofilm Device [MBEC Minimum Biofilm Eradication Concentration] AssayTM, Innovotech, St. Edmonton, Canada] by means of MTT method. The asterisks [*] indicate the level of statistical significance as determined by Student's t-test [* p < 0.001]; (B) *C. albicans* biofilm reduction on silicone disks coated with a lipopeptide biosurfactant at 24, 48 and 72 hours of incubation.**

It was observed that rhamnolipid significantly reduced biofilm formation abilities of the Gram-positive *S. epidermidis* and the Gram-negative *E. coli* respectively of 75% and 82%. The observed reductions of three related *P. aeruginosa* strains were at average of 31%. It is known that rhamnolipids play an important role at different stages of *P. aeruginosa* biofilm development and that their effect is concentration-dependent. While low amounts of rhamnolipids increase initial adherence of cells to a surface and microcolonies formation, the presence of high concentrations in the medium (as in the case of the anti-adhesion assay), limits attachment of the cells and further microcolonies formation [91], most likely leading to a reduction of biofilm.

Additional chemical analyses are underway to identify the type rhamnolipids produced by *P.*

aeruginosa 89 strain, however it most likely will be a mixture of the mono and di rhamnolipids with the 10 carbon fatty acids side chains typical of *P. aeruginosa* strain. The encouraging results obtained against biofilm producer strains make this biosurfactant a good candidate to prevent adhesion on plastic surfaces.

Padmapriya and Suganthi [92] have partially purified two biosurfactant produced by *C. tropicalis* and *C. albicans* and tested their anti-adhesive activity on different types of urinary and clinical pathogens. The results showed a reduction of adherent cells on the surface of urinary catheter pre-coated with biosurfactants and a higher activity of the biosurfactant synthesized by *C. tropicalis* in comparison with the biosurfactant synthesized by *C. albicans*.

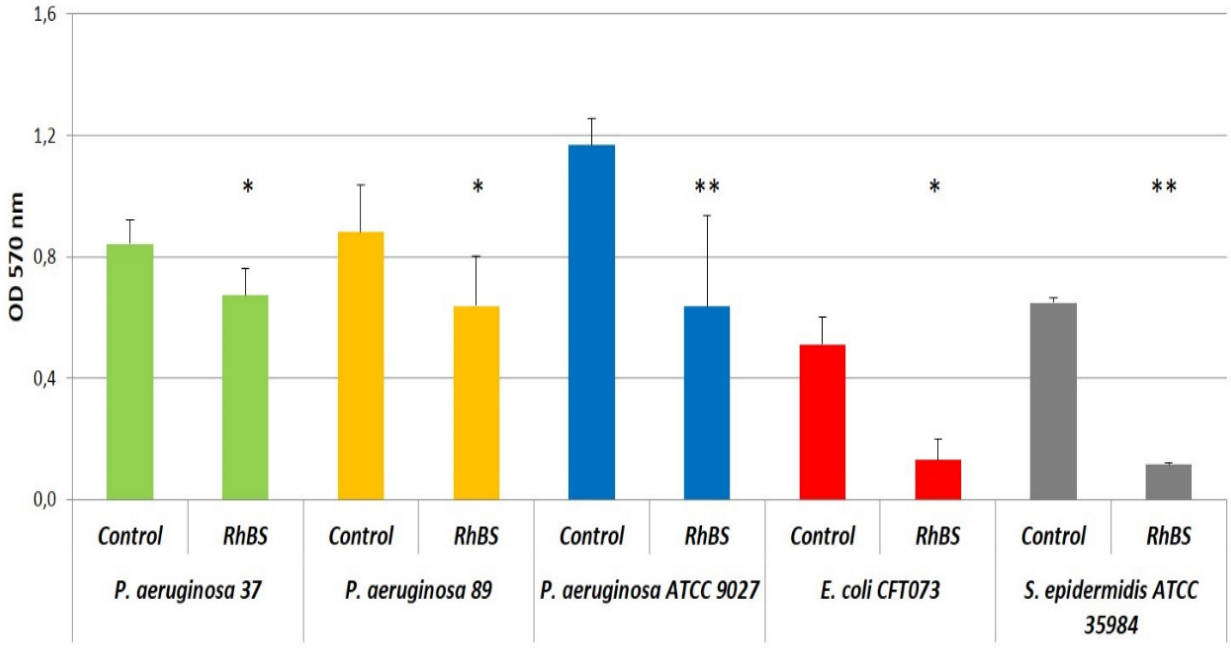

Figure 2. Bacterial biofilm reduction on polystyrene coated by a rhamnolipid biosurfactant (RhBS) after 24 hours incubation. The assay was carried out in Calgary Biofilm Device [MBEC Minimum Biofilm Eradication Concentration] Assay™, Innovotech, St. Edmonton, Canada] by means of MTT method. The asterisks [*] indicate the level of statistical significance as determined by Student's t-test [* $p < 0.05$; ** $p < 0.01$].

The effect of the *Lactobacillus acidophilus* DSM 20079 biosurfactant on adherence and on the expression level of the genes *gtf* B and *gtf* C in *S. mutans* biofilm cells was also analyzed by Tahmourespour et al. [93]. The *L. acidophilus* biosurfactant was able to interfere with the adhesion and biofilm formation of *S. mutans* to glass slide and led to shorter chains formation. Moreover, several properties of *S. mutans* cells (adhesion ability, biofilm formation, surface properties and gene expression) were altered as a result of treatment with *L. acidophilus* biosurfactant. A patent has been granted for *Lactobacillus* biosurfactants ability to inhibit bacterial pathogens attachment and colonization on medical devices particularly to prevent urogenital infection in mammals [94]. The anti-adhesive activity of a lipopeptide biosurfactant secreted by the probiotic strain *Propionibacterium freudenreichii* was analysed by Hajfarajollah et al. [95]. It showed a significant

anti-adhesive action against a wide range of pathogenic bacteria and fungi (*S. aureus, B. cereus, P. aeruginosa, E. coli*). The highest adhesion reduction was obtained for *P. aeruginosa* (67.1%) at the concentration of 40 mg ml^{-1}, whereas lower activities were observed for *S. aureus* (32.3%), *B. cereus* (39.1 %) and *E. coli* (47.7%), at the same concentration.

2.4. Therapeutic and biotechnological applications

Mannosylerythritol lipids (MELs), surfactin and trehalose lipids, all often reported as very powerful biosurfactant molecules, are known to have immunosuppressive and immunomodulating, anti-tumour and anti-inflammatory activity in addition to other properties such as cells stimulation and differentiation, cell-to-cell signalling, self-assembling, interaction with stratum corneum lipids, membrane perturbation and haemolytic activity [6]. Antitumor activities were described for surfactin by Cao et al. [96] and for other lipopeptides by Saini et al. [35]. Significant effects against tumour cell lines were also observed for serratamolide AT514, a cyclic depsipeptide from *Serratia marcescens* [97] and for glycolipids, in particular mannosylerythritol lipids (MELs) [98] and sophorolipid [99].

Surfactin also showed interesting anti-inflammatory activities due to its inhibitory properties on phospholipase A2, on the release of Interleukin (LK-6) and the overproduction of nitric oxide [100]. Park et al. [101] explored the mechanisms by which surfactin induced anti-inflammatory actions in relation to serious gum infection caused by *Porphyromonas gingivalis*. These authors also observed that surfactin significantly reduced the pro-inflammatory cytokines, including interleukin IL-6, IL-12 and IL-1β and tumour necrosis factor-α, through suppression of nuclear factor κB activity in *P. gingivalis.* The role of surfactin in the inhibition of the immunostimulatory functions of macrophages through blocking the NK-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and MAPK (mitogen-activated protein kinases) and Akt (serine/threonine kinase Akt, also known as protein kinase) cell signalling pathway suggests important immunosuppressive capabilities for this molecule [102].

Properties such as emulsification, foaming, detergency, and dispersion render biosurfactants curious molecules with several potential application in areas of drug delivery [103]. Rhamnolipids liposomes have been patented some time ago as drug and other molecules delivery system as microcapsules containing these drugs, proteins, nucleic acids and dyes and with an the ability to biomimetic biological membranes and acting as sensors for the detection of pH variations. Nguyen et al. [104] reported on using sophorolipids and rhamnolipids mixed with lecithins to prepare biocompatible micro-emulsions suitable for both cosmetic and drug delivery applications. Other biosurfactants such as fengycin and surfactin were also reported suitable as enhancers for the skin accumulation and transdermal penetration of antiviral drug acyclovir increasing its concentration in the epidermis by a factor of two [105].

Finally, biosurfactant mediated nanomaterial synthesis and/or stabilization has recently been emerging as a "green chemistry" clean, non-toxic and environmentally acceptable procedure [106]. Reddy et al. [107] successfully synthesized gold and silver nanoparticles by using surfactin from the bacterium *B. subtilis* while Singh et al. [108] synthesized a highly stable cadmium sulphide nanoparticles using surfactin from *B. amyloliquefaciens* KSU-109 and both sophorolipids and rhamnolipids were successfully used in the synthesis and stabilization of metal-bound nanoparticles. Palanisamy and Raichur [109] and Kumar et al. [110] synthesized spherical nickel oxide and silver nanoparticles using rhamnolipids as alternative surfactant through microemulsion technique and

reported antimicrobial activity with the silver nanoparticles. Sophorolipids were also successfully used to attach silver nanoparticles to polymer scaffolding and passing on antibacterial activity [111].

3. Conclusions

Microbial biofilms are recalcitrant environments often providing shelter and protection to producing and inhabiting microbial flora. They also are mainly responsible for many persistent infections in clinical environments, the dissemination of airborne pathogens and the fouling of industrial surfaces in clinical, food and environmental settings. These problems are progressively challenged by the increase in resistant microbial biofilm populations and the scarcity of alternative eradication solutions. Biosurfactants represent a group of emerging surface-active agents which have inherent anti-microbial (bacterial, fungal and viral) properties and ability to act as anti-adhesive, disruptive and dispersant for such biofilm structures. Their uses either on their own or as adjuvants to other antimicrobial, chemotherapies may represent a possible way forward in tackling infections, biofilms formation and microbial proliferation in the future.

Conflict of Interest

The authors report no conflicts of interest.

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