Microbial biosurfactants: current trends and applications in agricultural and biomedical industries

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Abstract
Synthetic surfactants are becoming increasingly unpopular in many applications due to previously disregarded effects on biological systems and this has led to a new focus on replacing such products with biosurfactants that are biodegradable and produced from renewal resources. Microbially derived biosurfactants have been investigated in numerous studies in areas including: increasing feed digestibility in an agricultural context, improving seed protection and fertility, plant pathogen control, antimicrobial activity, antibiofilm activity, wound healing and dermatological care, improved oral cavity care, drug delivery systems and anticancer treatments. The development of the potential of biosurfactants has been hindered somewhat by the myriad of approaches taken in their investigations, the focus on pathogens as source species and the costs associated with large-scale production. Here, we focus on various microbial sources of biosurfactants and the current trends in terms of agricultural and biomedical applications.

Introduction
It is now accepted that widespread use of synthetic surfactants negatively affects the environment. An area of particular concern relates to the use of synthetic surfactants that are utilized in abundance by various industries, including pharmaceutical and medical manufacturing, the food and feed industry, agriculture, environmental remediation and the petroleum industry. Environmental concerns in developed countries and increasingly worldwide have resulted in increasing legal and societal pressure for these substances to be biodegradable and produced sustainably using renewable substrates. These requirements have led to intensification of research and more recently the development of new technologies involving biogenic surface-active substances of microbial origin, that is, biosurfactants, (Marchant and Banat 2012a; Santos et al. 2016).

Biosurfactants, have many advantages over chemically produced surfactants, such as high biodegradability and low ecotoxicity, and can be easily produced from renewal energy resources (Makkar and Cameotra 2002). These microbially derived surface-active substances are widely used in the pharmaceutical, food, cosmetic, textile, oil and agricultural industries (Fig. 1). They can be used as antifungal as well as antibiofilm agents (Gudiña et al. 2010; Banat et al. 2014a; Díaz De Rienzo et al. 2015; Haque et al. 2016). In a microbiological context, there is a particular interest in those biosurfactants produced by bacteria and their antibacterial, antifungal and antiviral properties. In addition, these compounds also have a range of possible therapeutic and biomedical benefits. Despite the potential of biosurfactants, the fact that the significant producers, namely Pseudomonas and Bacillus, are potentially pathogenic has proved a drawback, hence the interest in yeasts and yeast-like fungi including Starmerella bombicola and nonpathogenic bacteria which are generally seen as not posing a risk in terms of toxicity or pathogenicity. There is increasing evidence that biosurfactants, displaying the industrially valuable properties of detergency, emulsification and foaming, may also have significant bioactivities applicable to human and animal health (Fu et al. 2008; Shao et al. 2012; Fracchia et al. 2015).

The focus of many reviews in the area has been on the biosurfactants themselves and indeed recent reviews...
include those, which have focussed specifically on applications in agriculture or industry (Mnif and Ghribi 2016; Santos et al. 2016; Singh et al. 2019). This review focuses on microbial biosurfactants and current trends in agricultural and health-related applications.

Classification and structure of microbial biosurfactants of interest

Biosurfactants are classified according to their molecular weight and categorized by their microbial origin and composition. The high molecular weight biosurfactants include the lipopolysaccharides but those of main interest are the low molecular weight glycolipids and lipopeptides (LPs) and phospholipids. Of the glycolipids (Mnif and Ghribi 2016), which include trehalolipids, cellolbiose lipids, mannosylerthritol lipids (MELs), rhamnolipids, (derived from mainly Pseudomonas) and sophorolipids (SLs), (derived from Candida and related species) are of the most interest. The glycolipids (Marchant and Banat 2012b) and the LPs (derived mainly from Bacillus sp.) are the biosurfactants of most interest in terms of their therapeutic potential of those investigated thus far.

Rhamnolipids are amphipathic in nature comprising hydrophobic and hydrophilic moieties which enable them to reduce surface and interfacial tensions. The antimicrobial property of rhamnolipids is attributed to their permeabilizing effect which leads to disruption of the bacterial cell plasma membrane (Sotirova et al. 2008; Fracchia et al. 2015; Díaz de Rienzo et al. 2016a,b,c), their ability to compromise cell surface charge (Kaczorek 2012) and ability to change bacterial cell hydrophobicity (Sotirova et al. 2009). They also have the ability to prevent and obstruct biofilm formation making the constituent bacteria more susceptible to antimicrobial agents (for a comprehensive review of the potential applications of rhamnolipids see Chen et al. 2017).

Sophorolipids are produced by yeasts. They have a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid through a glycosidic bond (for a
recent detailed review of SLs see De Oliveira et al. 2015). It is rapidly becoming apparent that the range of biosurfactant congeners produced by a micro-organism may have very different types and extents of bioactivity and therefore it is important to use highly purified individual congeners to assign unequivocally an activity to a specific congener. In the case of SLs, the acidic and lactonic (LT) forms show very different properties (Van Bogaert et al. 2007). In addition to the properties of detergency and bioactivity, the effectiveness of acidic SLs as a capping agent has been studied in the synthesis of various metal-based nanoparticles (Kasture et al. 2007; Dhar et al. 2011). Singh et al. (2013) reported the mesoscale molecular assembly of SL using the pulse UV laser processing technique. The available reports suggest that SL could be utilized as a carrier system for drug delivery by exploring its structure-forming attributes. Lactonic forms are more hydrophobic (Borsanyiova et al. 2016) and have been reported to have better biocide activities (Ito et al. 1980) as well as spermicide, cytotoxic and pro-inflammatory activities. Work by Shao et al. (2012) suggests that the LT form possessed anticancer activity; however, more recent work (Callaghan et al. 2016) suggests this is not the case in another model system when using highly purified congeners. The acidic forms are better foaming agents, have higher water solubility (Hirata et al. 2009) and have shown potential in the food, bioremediation and cosmetics industries (Ma et al. 2011). SLs bear two different polar heads on the two ends of the lipophilic core referred to as ‘asymmetric bolas’. Being amphiphilic in nature they tend to form self-assemblies or ‘liposomes’ (Rodrigues 2015) with unique structural and physiochemical properties as well as functionality (Duby et al. 2013) and biofilm disruption activity (Díaz de Rienzo et al. 2015), (for a review of the applications of SLs see De Oliveira et al. 2015).

Lipopeptides are composed of lipid moieties attached to a peptide chain and have biological activities including antimicrobial and anticancer. The most characterized LPs are daptomycin and polymyxin B, which are microbial-derived LP antibiotics. Surfactin (SUR), iturin and fengycin are among the best known LPs and have a myriad of potential applications (Fracchia et al. 2015) (for a comprehensive review of LP see Mnif and Ghribi 2015).

Antimicrobial and antifungal properties of biosurfactants

Given the rise in antibiotic resistance, the need to identify new antimicrobials and find a means of rehabilitating current antibiotics used in medicine has become clear. There has been a global call to arms (WHO, 2017) in terms of efforts both nationally (DoH and DEFRA 2013) and internationally (CDC, 2015) to meet the challenge of antibiotic resistance. Biosurfactants are ideally placed to answer the call in terms of their applications including: bactericidal, bacteriostatic, biofilm formation inhibition, biofilm disruption, synergistic and adjuvant effects with antibiotics.

Properties of biosurfactants include inhibition of bacterial and fungal growth (Kim et al. 1998; Lotfabad et al. 2010; Díaz de Rienzo et al. 2016a). Biosurfactants produced by Staphylococcus saprophyticus SBPS 15 showed antibacterial activity against Klebsiella pneumonia, Escherichia coli, Vibrio cholera, Bacillus subtilis and Staphylococcus aureus (Mani et al. 2016). Rhamnolipid has been reported to have biofilm disruptive capability against Bacillus pumilus (Dusane et al. 2010). The biosurfactant SUR can control the growth of Listeria monocytogenes in food (Sabaté and Audisio 2013) and some Gram-positive bacteria like B. pumilus, M. flavus (Das et al. 2007). LPs can damage and penetrate lipid containing negatively charged cell membranes. It has been suggested that a charge imbalance develops at the cell surface interface as a result of the polar element attempting to preserve solubility. This results in a loss of cell morphology leading to pore formation in the lipid-containing cell membrane of Gram-negative bacteria causing cell damage/death.

In the case of rhamnolipids, there is clear evidence that they reduce bacterial growth in the exponential phase, which suggests that these compounds may have an influence on normal cell division. Diaz de Rienzo et al. (2016a) suggest that rhamnolipids and SPs may have different mechanisms of action against different microorganisms. They postulate that rhamnolipids inhibit the growth in the exponential phase but that the antimicrobial effects of SPs occur between the exponential and stationary phases and, as evidenced by the enhanced effect produced by the inclusion of caprylic acid in this study, may be more comparable with conventional antibiotics than rhamnolipids. The differing results found when identical micro-organisms are challenged with biosurfactants in antimicrobial assays vs biofilm assays are a case in point. Often these assays give contradictory results for the same organisms in the presence of the same biosurfactant because of the different mechanism/mode of action at work.

The scientific literature also suggests that rhamnolipids may be more effective against Gram-positive bacteria than Gram-negative bacteria due to the presence of an outer membrane in Gram-negative bacteria which can work to exclude biosurfactant molecules (Sotirova et al. 2008; Bharali and Konwar 2011). Another suggestion is that rhamnolipids cause cell membrane damage by insertion of acyl tails causing cell leakage of cytoplasmic components (Yalçin and Ergene 2009). Sana et al. (2018)
showed that both *E. coli* and *S. aureus* were sensitive to rhamnolipid and that because of its’ hydrophilic and hydrophobic parts it interacts with the nonpolar part of the cell membrane. The membrane disintegrates leading to the penetration of the cell wall and plasma membrane by pore formation and subsequent leakage of inner cytoplasmic materials leading to cell death (Meincken et al. 2005; Ortiz et al. 2006). Another possibility is that rhamnolipid inserts its’ shorter acyl tails into the cell membrane and attacks the configuration of the cell wall and plasma membrane (Sánchez et al. 2006; Yalçın and Ergene 2009), alternatively, the membrane permeability produced by rhamnolipid may be enhanced by its interaction with the phospholipid component of the plasma membrane (Ortiz et al. 2006). In terms of SLs, the vigorous membrane-distorting potentiality of SUR is dependent on the size of the peptide ring with the peptide moiety penetrating into the cell membrane and generating a variance of charge at the site of action on the membrane surface (Heerklotz and Seelig 2001). These mechanisms might help explain how the LP produced by *B. stratosphericus* (Sana et al. 2018) has an antibacterial effect against both *S. aureus* and *E. coli*.

The antiadhesive activity of biosurfactants is also an important property particularly if you are seeking to prevent biofilm formation (Galí et al. 2018). Biofilm formation plays a key role in the survival of both pathogenic (Kumar et al. 2017) and nonpathogenic micro-organisms. The process of surface attachment and the growth of heterogeneous cells within a matrix can be considered generic, that is, common to both pathogenic and nonpathogenic micro-organisms. In pathogens, the mechanisms of attachment to and colonization of surfaces are key and there are numerous examples of clinically relevant biofilm formers, for example, *Pseudomonas* in the lungs (Lopes et al. 2015), *Pseudomonas* on contact lenses (El-Ganiny et al. 2017) and *Staphylococci* in orthopaedic implants and breast implants (Arciola et al. 2015; Seng et al. 2015). While biofilms can be composed of multiple species or a single species it is the case that many diseases including nosocomial infections are essentially biofilm-associated diseases associated with individual species, for example, *Mycoplasmata pneumonia*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Mycobacterium tuberculosis* and *Mycobacterium abscessus*. Key to the success of these biofilms is the advantages they afford to their pathogenic inhabitants principally: drug tolerance, avoidance of the host immune responses and recalcitrance of infection. The literature suggests that biosurfactants can play an important role in preventing biofilm formation on surfaces, for example, silicon (Rodrigues et al. 2006; Ceresa et al. 2015), titanium (Ciandrini et al. 2016) and polystyrene plates (Gómez et al. 2016). Gudiña et al. (2015) showed that a glycoprotein biosurfactant from *Lactobacillus agilis* inhibited the adhesion of *S. aureus* and Madhu and Prapulla (2014), in their evaluation of a glycoprotein from *L. plantarum* CFR2194, also showed the inhibition of *S. aureus* adhesion. Importantly, researchers (Gudiña et al. 2015) have also shown that the antiadhesive properties can also be affected by the carbon source in the medium in which the producer strain is grown. Hence, changes in the proportion of carbohydrate, lipid and protein present in polymeric fractions of microbial biosurfactants can play a role in their biological effectiveness.

Quinn et al. (2013) have shown that Rhamnolipid is effective in inhibiting *S. aureus*, *B. subtilis* and *M. luteus* single-species biofilms and that they were in fact more effective than broad-spectrum antibiotics used in the study. Rivardo et al. (2009) demonstrated the antiadhesive activity of two biosurfactants produced by *Bacillus* sp. therefore preventing human bacterial pathogens from producing bacterial biofilms. Rivardo et al. (2011) have also shown the synergistic effect of LP biosurfactant with antibiotics against *E. coli* CFT073 biofilm. It has been previously demonstrated that the use of biosurfactants preventively, that is, prophylactically, can prevent the formation of fungal biofilms (Dusane et al. 2012).

Immunocompromised and transplant patients and those with medical implants are highly susceptible to fungal infections such as those caused by *C. albicans* and other *Candida* species and *Candida auris* in particular (Schwartz and Patterson 2018). Haque et al. (2016) found the SL derived from *S. bombicola* MTCC 1910 inhibited *C. albicans* hyphal growth and biofilm formation as well as reducing the viability of preformed biofilms. SL when used in combination with amphotericin B (AmB) or fluconazole was found to act synergistically against both biofilm formation and preformed biofilm. Sarwar et al. (2018a,b) in their investigations of microbial biosurfactants from *Bacillus* species found that LP extracts displayed antifungal activity against *Fusarium moniliforme*, *Fusarium oxysporum*, *Fusarium solani* and *Tricoderma atroviride*. Additionally, the LP extracts showed haemolytic activity and potential as biocontrol agents against various *Fusarium* and *Trichoderma* species.

Fengycin is a cyclic lipodecapeptide produced by *B. subtilis* strains, and appears to act by increasing the plasma membrane permeability of the target cell (Vanitnanakom et al. 1986). Fengycin has been shown to exhibit strong fungitoxic activity specifically against filamentous fungi, inhibiting some enzymes (Loeffler et al. 1986; Steller and Vater 2000). The antifungal mechanism of fengycin may be as a result of its physicochemical properties due to its amphiphilic characteristics and affinity for lipid bilayers. Roy et al. (2013) in studies with fengycin did not show any
antibacterial effects but did show antifungal activity of a fengycin-like peptide from Bacillus thuringiensis strain SM1 against C. albicans and showed that treated cells displayed membrane blebs suggesting loss of contact between the cell membrane and the cell wall.

As previously mentioned the focus of research has now moved from the potential antimicrobial effects of biosurfactants themselves to how they might act in unison with current antibiotics to maintain or even improve their efficacy. In the face of antibiotic resistance, these may include inhibitory or antibacterial adjuvant activities against various micro-organisms (Fracchia et al. 2012; Borsanyiova et al. 2016).

The presence of a trans-envelope multidrug resistance (MDR) pump in some Gram-negative bacteria suggests that they may be resistant to a number of antibiotics (Girish and Smith 2008). This could be overcome since both rhamnolipids and LP act on cell surfaces only. LP biosurfactant antimicrobial properties are associated with their lytic membrane properties. Basit et al. (2018) revealed that cationic LP exhibited significant antibacterial and antifungal activity against S. aureus, E. coli, P. aeruginosa, K. pneumonia, A. niger and C. albicans. In addition, they showed antiviral activity against Newcastle disease virus. In susceptibility testing the largest zones of inhibition were found against S. aureus and the smallest against Aspergillus flavus. These results were in accordance with previously reported antibacterial, antifungal and antiviral activity of biosurfactants (Gomaa 2013; Borsanyiova et al. 2016; Jemil et al. 2017).

Diaz de Rienzo et al. (2016a) showed that preformed biofilms of P. aeruginosa PA01, E. coli NCTC 10418, B. subtilis NCTC 10400 and S. aureus ATCC 9144 on glass coverslips were disrupted with SLs (5%) in the absence of an adjuvant, that is, caprylic acid. Domalaon et al. (2018) in their investigation of short proline-rich LPs revealed an amphiphilic nonhaemolytic noncytotoxic L-LP that significantly potentiated the activity of minocycline and rifampicin against multidrug-resistant MDR and XDR clinical isolates of Pseudomonas aeruginosa. Ghribi and Ellouze-Chabouni (2011) isolated a biosurfactant-producing strain B. subtilis SPB1 (HQ392822) and identified antimicrobial activity against micro-organisms with multidrug-resistant profiles (Ghribi et al. 2012). Rossi et al. (2016) showed that some strains of biosurfactant-producing Staphylococcus haemolyticus had antimicrobial activity against a range of Gram-positive and Gram-negative bacteria and subinhibitory concentrations of the biosurfactant were able to decrease biofilm formation and showed synergistic effects with tetracycline.

The antimicrobial effects of SLs are dependent on the SL structure and class of bacteria examined. SLs have been shown to have virucidal and antibiotic adjuvant characteristics (Shah et al. 2005; Borsanyiova et al. 2016). A study using natural SL mixtures with a variety of sugar head groups reported antimicrobial activity against a range of predominately Gram-positive bacteria (Shah and Prabhune 2007). Equally important, given the renewed focus on maternal sepsis both in the developed and developing world, are biosurfactant studies carried out in rat models of peritonitis. Bluth et al. (2006) demonstrated that SLs block the lethal effects of septic shock in rats in a caecal ligation and puncture model of experimental sepsis and Hardin et al. (2007) showed that SLs derived from C. bombicola (now Starmerella bombicola) can improve sepsis survival. Di-rhamnolipid preparations have also been found to be successful in treating chronic decubitis ulcers (Piljac et al. 2008) and in the enhanced healing of full thickness burn wounds (Stipcevic et al. 2006).

**Inhibition of biofilm formation**

Some of the most promising candidates for the inhibition of biofilms have come from biosurfactants since they have strong antiadhesive, antimicrobial and biofilm disruption properties (Banat et al. 2014a; Sharma et al. 2014). It has been proposed that biosurfactants play an important role in organisms that produce them by partially disrupting the developing biofilm and maintaining channels for gas and nutrient diffusion and it is thus not surprising that they are effective in disrupting biofilms at appropriate concentrations. Researchers in this area point to the dispersal of a biofilm of pathogenic bacteria by decreasing bacterial cell viability and the reduction in bacterial adhesion properties as evidence of the effectiveness of biosurfactants. The suggested mechanism of action may be related to the binding of the biosurfactant molecules to cell wall components or the cell surface resulting in severe changes in outer membrane hydrophobicity. The insertion of biosurfactants into the bilayer structure of cell membrane may result in the disruption of its integrity. The effects on both Gram-negative and Gram-positive bacteria may be due to the release of LPS molecules from the outer membrane or due to the formation of transmembrane pores resulting in increased permeability of the cell wall (Sotirova et al. 2008; Rivardo et al. 2009), (for further discussion of the various roles of biosurfactants see Satpute et al. 2016a).

Previously, numerous studies have shown that biosurfactants inhibit biofilm formation by preventing adhesion of micro-organisms to solid surfaces (Kuiper et al. 2004; Rodrigues et al. 2004; Rivardo et al. 2009; Janek et al. 2012). Mukherji and Prabhune (2014) reported antibiofilm activity of SL against Vibrio cholerae, indicating that it is likely to be broad spectrum. The morphological
changes in microbial cells as a result of SL treatment (Haque et al. 2016) may go some way towards explaining the broad-spectrum nature of SLs and other biosurfactants (Haque et al. 2016). These changes could be associated with loss of cell membrane integrity resulting in cell death as reported previously for tetracycline-SL or cefaclor-SL combination treatment against S. aureus and E. coli respectively (Joshi-Navare and Prabhune 2013). Furthermore, deformation of cells and loss of cell membrane integrity have been reported as the mechanisms of antimicrobial activity of many biosurfactants (Gudiña et al. 2013).

Importantly, Rhamnolipids have been shown to be active against pre-existing bacterial biofilms of S. typhimurium (Leis et al. 2005). Salmonella remains an important cause of food-poisoning infections and has recently seen a resurgence in the EU primarily as a result of zoonotic infections (EFSA and ECDC 2017). Salmonella causes gastroenteritis and in some cases septicaemia (Wang et al. 2013a). Salmonella enterica is able to grow on stainless steel surfaces resulting in a 3D structure with several layers of cells, which may present different morphologies depending on the available nutrients (Wang et al. 2013b). Untreated steel is more easily colonized by Salmonella than polished or finished steel (Schliselberg and Yaron 2013). In dry conditions, S. enterica has been shown to survive in a biofilm on stainless steel for over a year (Morita et al. 2011). However, in contrast to other pathogens, glass surfaces are not as easily colonized by Salmonella (De Oliveira et al. 2014). Given the continued disease burden caused by Salmonella a number of researchers have investigated the potential of various biosurfactants against Salmonella including SURs produced by B. subtilis. SURs have been reported to inhibit the growth of biofilms of Salmonella spp cultivated on PVC microtitre plates and urethral catheters (Mireles et al. 2001).

**Nanoparticles**

Nanoparticle-based therapeutics have been considered as some of the most promising platforms in drug delivery applications due to their ability to increase drug accumulation in solid tumours by enhanced permeability and retention (EPR) and MDR reversal through bypassing or inhibiting P-gp activity (Bao et al. 2016). Furthermore, Basak et al. (2014) reported that SL-capped ZnO nanoparticle-mediated C. albicans cell death occurs via membrane bursting followed by oozing out of proteins and intracellular materials. In addition to functioning as a cyclic lipopeptide, the biosurfactant, SUR, has been found to exhibit versatile bioactive features including adjuvant for immunization and antitumour properties. Based on its unique amphipathic properties, SUR has the potential for self-assembly (under certain conditions) into nanoparticles to function as a drug carrier for loading hydrophobic drugs. Combining the anticancer activity of SUR and the characteristics of nanoparticles such as EPR effects and MDR reversal might improve cancer chemotherapy by designing SUR as a carrier to load anticancer drugs. In an investigation by Huang et al. (2018), SUR was assembled by a solvent-emulsion method to load the anticancer drug doxorubicin (DOX). The DOX@SUR assembly was shown to induce stronger cytotoxicity against DOX-resistant human breast cancer MCF-7/ADR cells compared to free DOX. The DOX@SUR nanoparticles exhibited enhanced cellular uptake and decreased cellular efflux. Moreover, in vivo DOX@SUR nanoparticles accumulated more efficiently in tumours than free DOX. The DOX@SUR showed stronger tumour inhibition activity and fewer side effects in MCF-7/ADR-bearing nude mice suggesting that SUR-based nanoparticles might be used as potential anticancer drug carriers to reverse MDR in cancer chemotherapy.

**Current trends and applications**

**Applications in agriculture**

Biosurfactants are integral components of many commercial products in a variety of agricultural applications, for both plant and farm animal production systems. Furthermore, biosurfactants, due to their low organisal and environmental impact, (low toxicity, low irritation response/hypo-allergenicity) while exhibiting high digestibility as well as high biodegradability appear to offer excellent advantages over their synthetic and other natural counterparts.

In farm animal production, nutritional/dietary manipulation is one of the main directions of biosurfactant applications. Natural biosurfactants, such as plant-derived alkyl polyglycosides (APG), have been shown to be effective in ruminant nutrition due to their positive effects on physiological and production parameters in, for example, ruminants. Both ruminal and intestinal digestibility of organic matter are increased together with ruminal microbial protein synthesis resulting in increased duodenal microbial flow of nitrogen (Yuan et al. 2010). Additionally, APG may have positive indirect effects in terms of its ability to modify the rumen microbial community as it increases total volatile fatty acid production in the rumen in vivo. APG has the ability to increase the activities of ruminal carboxymethyl cellulase and xylanase (Yuan et al. 2010), together with its ability to modify ruminal fatty acid composition and decrease the population of Ruminococcus albus in vivo (Zeng et al. 2012) hence providing a
favourable ruminal environment. Available research would indicate that microbial biosurfactants may have similar effects to those ascribed to APG in ruminant nutrition, for example, rhamnolipid (produced by \textit{P. aeruginosa}) has shown increased activity of xylanase, and overall increased degradation rates of organic matter \textit{in vitro} (Liu et al. 2011). Past research has also acknowledged that incorporation of yeast cultures with emulsified glycoprotein into ruminant diets can improve the digestibility of organic matter, including digestibility of cellulose and hemicellulose (Wiedmeier et al. 1987) and more recent work (Feye et al. 2016) suggests that \textit{Saccharomyces cerevisiae} fermentation products may mitigate faecal shedding of antibiotic-resistant \textit{Salmonella} in poultry (fed Original XPC™). Any development that can reduce the potential for the spread of antibiotic resistance in the agrarian environment (Conwell et al. 2017) is to be welcomed. Apart from improving the activity of fibrolytic enzymes in ruminant nutrition, microbial biosurfactants with their emulsifying properties have been suggested for improved digestibility of fats/oils in animal diets. Fats/oils are normally added to animal diets as an inexpensive source of energy; however, their use is limited by the animal’s physiological ability to digest high levels of dietary fats/oils. Thus, more recent livestock and poultry feed additives consisting of lysophospholipids of undisclosed origin have appeared on the market claiming enhanced effects on emulsification of nutritional fats/oils and hence improved digestion of fats/oils and improved absorption of other nutrients (for more information see: Lysoforte®, Kemin Industries, Inc., Des Moines, Iowa). It is possible that specific microbial biosurfactants could be introduced to emulsify fats/oils in animal feed for specific age groups of animals or to decrease the cost of feed by increasing the oil/fat content above the level of animal/physiological ability to effectively digest without the negative effects on animal health. Hence, the inclusion of biosurfactants may prove to be financially effective in animal production. Other avenues for further exploration may involve designer microbial biosurfactants that would aim to modify the ruminal microbiome and favour a bacterial ‘ruminotype’ associated with low methane production over those with high methane outputs, for example, species belonging to \textit{Ruminococcus} (Kittelmann et al. 2014).

More recently the potential of biosurfactants in seed protection and growth stimulation have been investigated, showing the effectiveness of LPs (Toral et al. 2018) against phytopathogens including \textit{Botrytis cinerea} and that of rhamnolipids (Borah et al. 2016) against \textit{Fusarium verticillioides} a major pathogen of maize. In addition, rhamnolipids have shown potential as biopesticides (Soltani Dashbozorg et al. 2016), fungicides (Shah et al. 2005) and as antizoospore agents (Miao et al. 2015). Sha et al. (2012) attributed the antifungal effect of cell-free culture broth of rhamnolipids to surface activity and rupture of plasma membranes.

**Health-related applications**

**Applications in wound healing**

A wide variety of bioactive metabolites, including biosurfactants, are viewed as having potential for dermatological applications including wound healing. Zouari et al. (2016b) evaluated the \textit{in vitro} antioxidant activities and the wound healing potential of \textit{B. subtilis} SPB1 LP on excision wounds induced in experimental rats. They found a significant increase in the percentage of wound closure compared with untreated and CICAFLORA™-treated groups. Biopris treated with SPB1 LPs showed entirely re-epithelized wounds with perfect epidermal regeneration. It has been, suggested that the free-radical scavenging properties of the LPs help to prevent inflammation and improve tissue formation, re-epithelization and differentiation of epidermis (Jemil et al. 2017). In addition, SPB1 has been shown previously to inhibit MDR bacteria (Ghribi et al. 2012) and show activity against phytopathogenic fungi (Mnif et al. 2016). Gupta et al. (2017) investigated accelerated wound healing in rat tissue \textit{in vivo} using a glycolipid produced by \textit{B. licheniformis} SV1 containing ointment and found re-epithelization and fibroblast cell proliferation in the early stage of wound healing with more rapid collagen deposition in the later stages. It has been suggested that the wound healing properties exhibited by those LPs investigated may be as a result of their ability to reduce oxidative stress through the prevention of reactive oxygen species production. Ohadi et al. (2017) in their study of wound healing in rats showed that the LP produced by \textit{Acinetobacter junii} B6 increased free-radical scavenging activities and improved histopathological remission. Lydon et al. (2017) tested a highly purified preparation of micelle-forming nonacylated acidic SL that contained 90% C18 congener suggesting that acidic SLs can be used as a component of antimicrobial creams to reduce the risk of wound infection during healing.

**Dermatological applications**

The antibacterial preservatives used in the majority of personal care products are synthetic and can cause skin irritation and allergic reactions by interaction with keratin or collagen and elastin and encourage the removal of lipids from the skin surface and affect the skin cells themselves (Bujak et al. 2015). On the other hand, biosurfactants are composed of lipid and proteins and are
compatible with the skin cell membrane (Stipcevic et al. 2013). While the majority of biosurfactant-related work is focussed on biosurfactants that are produced extracellularly by micro-organisms much less work has been carried out on cell-bound biosurfactants many of which are produced by, for example, probiotic Lactobacilli strains which have the added advantage of being nontoxic, biodegradable and environmentally friendly (Satpute et al. 2016b). Vecino et al. (2018) investigated the antimicrobial and anti-adhesive properties of cell-bound biosurfactants produced by Lactobacillus pentosus (PEB), which are characterized as glycolipid molecules against several micro-organisms found amongst human skin flora. The performance of PEB was compared against the glycolipids produced by Lactobacillus paracasei (PAB). The PEB showed antimicrobial activity against P. aeruginosa, Streptococcus agalactiae, S. aureus, E. coli, Streptococcus pyogenes and C. albicans, which was comparable with the results from PAB. Importantly, extracts prepared with phosphate-buffered saline (PBS) were more effective than phosphate buffer (PB) in the case of P. aeruginosa, S. aureus and E. coli. Those extracted in PBS had a higher lipid content while those extracted in PB had a higher carbohydrate content. Both PEB and PAB showed anti-adhesive properties against all the micro-organisms tested except for E. coli and C. albicans. PAB produced biosurfactants with a lower content of lipids than those produced by PEB. However, Sharma and Saharan (2016) investigated the antimicrobial activity of glycolipid from Lactobacillus helveticus and found higher antimicrobial activity against E. coli and S. epidermidis. On the other hand, Gudiña et al. (2015) working with L. agilis found no antimicrobial activity against E. coli or C. albicans. Ashby et al. (2011) investigated the potential of biopolymer embedded SLs to improve the antimicrobial potential of SLs against Propionibacterium acneus and found that the efficacy varied depending on the biopolymer matrix. Interestingly, when different carbon sources and different fermenting conditions are applied then the same strain can produce different biosurfactants with different antimicrobial properties (Singh et al. 2014).

In nature P. aeruginosa releases rhamnolipids to form vesicles or micelles and sheds flagellin. Meyer-Hoffert et al. (2011) demonstrated that rhamnolipid secretion facilitates the expression of antimicrobial protein psoriasis in human healthy skin via flagellin. Flagellin will activate keratinocytes to induce the expression of the antimicrobial protein psoriasis, which can kill P. aeruginosa. Therefore, healthy skin can prevent colonization of pathogens before pathogens can develop strategies to disrupt the immune defence response. Antimicrobial hydrogels incorporating biosurfactants (Paniagua-Michel Jde et al. 2014) have been studied as an autodefence mechanism for combating drug-resistant infections associated with the skin, because polymeric gels exhibit many properties avoiding the freely dissolved condition, which enable them to remain in place on the skin while maintaining antimicrobial activity (Li et al. 2013). These characteristics suggest potential for wound healing, implant/ catheter coatings and skin infections.

**Oral care**

In the natural environment, biosurfactants have been found to contribute to innate oral care. Biosurfactant producers such as Strepococcus mitis in the oral cavity can discourage the adhesion of S. mutans. In their study of the effectiveness of rhamnolipids derived from nonpathogenic Burkholderia thailandensis E264, Elshikh et al. (2017a) identified a 3–4 log decrease in bacterial viability among oral pathogens (the potential of biosurfactants in oral cavity care has been reviewed in detail by Elshikh et al. 2016). Bouassida et al. (2017) examined the potential of B. subtilis SPB1 LP in toothpaste formulation and showed that an LP-based product exhibited an important antimicrobial activity against Enterobacter sp. and S. typhimurium. Previous reports on the effectiveness of B. subtilis SPB1 strain (HQ392822) revealed a wide spectrum of actions including antimicrobial activity towards micro-organisms with MDR profiles (Ghribi et al. 2012), antifungal activity against phytopathogenic fungi (Mnif and Ghribi 2016) and antidiabetic and antilipidemic properties in alloxan-induced diabetic rats (Zouari et al. 2016a).

**Drug delivery systems, including vaccines**

The use of biosurfactants as drug delivery agents offers attractive applications such as passive immunization particularly where drug treatment options are limited. For instance, the treatment of candidiasis is difficult due to the limited availability of antifungal drugs and their toxicities and severe side effects in humans (Laniado-Laborin and Cabrales-Vargas 2009; Nett 2014). These issues can be overcome by incorporating antifungal drugs into various drug delivery systems (Schinabeck et al. 2004; Ramage et al. 2013). Vesicular drug delivery systems including liposomes and noisomes are thought to be particularly important for targeted delivery of drugs and to minimize undesirable side effects (Jain et al. 2014).

Liposomes stand as promising candidates with wide applicability based on a drug delivery approach including vaccination (Loew et al. 2011; Davitt and Lavelle 2015). MEL-A, a type of glycolipid biosurfactant that contains cationic liposomes has been shown to promote gene transfection efficiency by five to seven times with mammalian cultured cells (Inoh et al. 2001). Liposomes are...
made up of two hydrophobic tails and may or may not contain cholesterol in the structure, whereas noisomes are nonionic surfactant-based vesicles made up of single hydrophobic chain, which makes them eminently suitable as carrier molecules in drug delivery applications (Kazi et al. 2010; Khan and Irichhaiya 2016). Noisomes are constructed by hydration with or without the amalgamation of cholesterol or other lipids (Kazi et al. 2010). The hydrophilic core of the noisome provides an ideal environment for hydrophilic drugs since hydrophobic drugs are mainly localized to the hydrophobic regions, that is, the lipid layer. Haque et al. (2017) compared the efficiency of SL-AmB niosome with a commercially available formulation of AmB and found fewer fungal hyphae in biofilm treated with the SL-AmB noisome, whereas more budding cells were found in biofilm treated with Phosome (AmB) alone. Fungal pseudohyphae/true hyphae are thought to be one of the most important virulence factors in C. albicans (Mayer et al. 2013). It is suggested that SL-AmB noisomes may interfere with gene expression, downregulating expression of hyphal genes. This is supported by other work indicating that antifungal drugs inhibit such genes (Cheng et al. 2009; Vediyappan et al. 2010).

Lipopeptide biosurfactants have also been shown to enhance the humoral immune response, additionally they are nontoxic and nonpyrogenic making them prospective adjuvants in vaccines. The WHI fungin has been shown to produce the SUR lipopeptide which has been suggested to be a potential adjuvant for immunization through the oral route (Gao et al. 2013). Additionally, Mittenbühler et al. (2003) have suggested that LPs increased the humoral immunity to the tetanus toxoid, without a decrease in serum IgG levels in a mouse model. Work by Basit et al. (2018) in an investigation of LPs as adjuvant in inactivated low pathogenicity avian influenza H9N2 vaccine suggest that biosurfactant-based vaccine increased the titre of antibodies in both broiler and layer chickens and showed comparable immunogenicity to oil-based vaccine.

**Anticancer potential of biosurfactants**

The LPs, glycolipids and other types of biosurfactants owing to their structural novelty and diverse biophysical properties have emerged as possible broad-spectrum agents for cancer chemotherapy/biotherapy and as safe vehicles or ingredients in drug delivery formulations. However, while it is possible to show cancer cell killing activity in vitro, the in vivo evidence is limited, and in many cases contradictory suggesting that in the short-term biosurfactants have limited clinical use except for topical or gut application. However, some studies have shown that LPs and glycolipids can selectively inhibit the proliferation of cancer cells and disrupt cell membranes causing their lysis through apoptosis pathways (Gudiña et al. 2013). Furthermore, the evidence from the literature suggests that the anticancer effects are based mostly on mixtures of congeners. There is a need to separate out these congeners in order to fully elucidate their individual anticancer effects.

The LPs and SLs are the biosurfactants most studied in terms of anticancer potential. The LPs are composed of a peptide and a fatty acid chain and have been shown to exhibit antitumour activity in vitro (Zhao et al. 2018). Reports on the Bacillus LPs, namely SUR, Iturin and Fengycin, suggest that they possess antitumour activities. Iturin has been shown to inhibit the proliferation of MDa-MB-231 cancer cells (Dey et al. 2015). Fengycin can block nonsmall cell lung cancer cell 95D and inhibit the growth of xenografted 95D cells in nude mice (Yin et al. 2013). Recently, Zhao et al. (2018) showed the B. subtilis LPs consisting of a majority of iturin exhibited promising potential in inhibiting chronic myelogenous leukaemia in vitro via simultaneously causing paraptosis, apoptosis and inhibition of autophagy. The anticancer mechanisms of Bacillus LPs have been extensively studied and SUR has been found to display an antiproliferative effect via apoptosis induction, cell cycle arrest and survival signalling suppression.

Among the suggested uses of SLs are their potential in human cervical cancer treatment. Li et al. (2017) showed induction of apoptosis of HeLa cells and inhibition of cancer cells in tumour-bearing mice but the vast majority of studies have been conducted in vitro (Table 1). However, the more recent studies have included xenograft and in vivo studies. In therapeutic and preventative xenograft models of B16-EGFRVIII melanoma cells, the self-adjuvant LP vaccine micelles effectively prevented tumour growth as well as tumorigenesis (Chen et al. 2018). Different anticancer mechanisms for SLs have been proposed including a role in differentiation and apoptosis. While it is well accepted that SLs have anticancer activity in vitro, Li et al. (2017) is one of the few studies to suggest antitumour activity in vivo. Moreover, there are conflicting reports in the literature including Callaghan et al. (2016) suggesting that LT SLs may increase tumour burden in Apc min± mice.

**Future trends and conclusions**

The two main obstacles to the further development of biosurfactant applications and unlocking their potential remain the large numbers of assays and approaches to this type of work. Microbial biosurfactants are produced as mixtures of congeners and the proportions of congeners will vary based on producer strain, growth conditions and growth medium (Singh et al. 2014; Izon de
Rienzo et al., 2016a). Since different congeners have different properties and activities, the use of ’mixtures’ in experiments leads to confusing results. There is also the problem of endotoxin contamination of biosurfactants produced by Gram-negative bacteria and very few investigators have taken steps to ensure that their experimental material is free of such highly bioactive molecules. Although expensive and time-consuming bioactivity needs to be determined with pure single congeners. The different assays currently employed may be providing different kinds of information on the mode of action of biosurfactants and the mechanism of action of biosurfactant either singly or in combination with other therapies against pathogenic micro-organisms. There is a need for the standardization of approaches and methodologies associated with biosurfactant research (recently reviewed in detail by Irorere et al., 2017).

The evidence of the efficacy of different biosurfactants from different micro-organism in differing contexts remains a challenge. There is good evidence of the effectiveness of biosurfactants in terms of antimicrobial activity and there is increasing evidence of the benefits of biosurfactants in terms of wound healing, dermatological applications and oral care (Elshikh et al., 2017b). There is promising work in the area of drug delivery but in the area of cancer treatment where biosurfactants might prove most efficacious there remains much conflicting data. It has to be pointed out, however, that their anticancer applications are likely to be limited to situations where topical application is possible, for example. skin or oral or for gastrointestinal administration.

The target market is of fundamental importance to any scale of biosurfactant production. To date developments have been limited for industrial applications such as bioremediation due to the deficit in the investment required and the feasibility of viable industrial production (Banat et al., 2014b). Therefore, the potential applications discussed here in terms of healthcare therapeutics are much more promising given the value-added nature of such products and their likely benefit to human health. The cost benefits would appear to be more favourable (Marchant and Banat 2012a) in terms of the biomedical applications because production is viable on a small scale. Of the range of potential applications discussed here, it is likely that the innate antimicrobial nature of many biosurfactants and the ability of some of these to act in synergy and/or as adjuncts to current therapeutics in the context of the ever increasing threat of antibiotic resistance may prove the most beneficial.

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Conflict of Interest

The authors declare no conflict of interest.

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