Potential Applications of Anti-Adhesive Biosurfactants

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Maria da Gloria Conceição da Silva, Italo José Batista Durval, Maria Eduarda Pereira da Silva, and Leonie Asfora Sarubbo

Abstract

There is a growing demand for materials covered with compounds that prevent the adhesion of microorganisms that form biofilms. Surface contamination is a concern of the biomedical and food industry, due to the risks to the health of patients and consumers. Thus, the interruption of microbial adhesion in its first moments is an excellent approach for the performance of anti-adhesive compounds. The microbial biosurfactants have the potential for the application on surfaces of economic interest as agents that inhibit microbial fixation. They comprise a variety of amphiphilic molecules that can be obtained directly, synthesized by plants and microbes, or indirectly, through chemical or genetic changes. Biosurfactant production from renewable substrates is possible, and there is a tendency for the substitution of synthetic surfactants of biological origin in industrialized countries. This chapter discusses the main classes of microbial biosurfactants with anti-adhesive action, the process of microbial adhesion for the formation of biofilms, and current studies involving the application of biosurfactants as biofilm disturbing agents on different surfaces.

Keywords

Biosurfactant · Medical instruments · Food industry · Contact surfaces · Antibiofilm · Antimicrobial

M. d. G. C. da Silva (✉) · I. J. B. Durval · L. A. Sarubbo
Northeast Biotechnology Network (RENORBIO), Federal Rural College of Pernambuco, Recife, Pernambuco, Brazil

Centre of Science and Technology (CCT), Catholic College of Pernambuco, Recife, Pernambuco, Brazil

M. E. P. da Silva
Centre of Science and Technology (CCT), Catholic College of Pernambuco, Recife, Pernambuco, Brazil

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10.1 Introduction

Advances in the area of biotechnology with the use of natural products that protect surfaces increase the economic interest in the generation of inputs against biofouling of the types that occur in medical devices or in places that come into contact with food (Gopikrishnan et al. 2015; Junter et al. 2016; Giri et al. 2019).

The attachment of microbial cells to surfaces covered by particles and colloids is the first stage in the development of the biofilm structure. As adhesion is still weak at this stage, this would be an excellent time for the application of anti-adhesive constituents. Microbial biosurfactants can change the surfaces they come in contact with. When adsorbing onto polystyrene surfaces, silicon and glass microbial surfactant changes the inherent hydrophobicity of such surfaces. In this way, the biosurfactant influences the effects of fixation and ease of removal of microorganisms depending on the type of the fouled surface (Janek et al. 2012).

Biosurfactants are versatile, stable, and biocompatible molecules, obtained from various sources such as bacteria, fungi, and yeasts (Gutnick and Bach 2017) and secondary compounds extracted from plants that exhibit surfactant characteristics (Cheok et al. 2014; Zhu et al. 2019).

Biosurfactants have the same properties as synthetic surfactants. Among the properties, we can highlight high biodegradability, low toxicity, and not inducing allergic reactions. They can be used in extreme environmental situations due to the stability of their properties when exposed to unusual pH occurrence, salinity, and temperature and specific bioactivity, which give them a great potential for practical applications in several areas (Freitas et al. 2016; Zhu et al. 2019; Liu et al. 2020).

In addition to these properties, they have antibiotic, antimicrobial, anti-biofilm, and anti-adhesive activities (Rivardo et al. 2011; Padmapriya and Suganthi 2013; Banat et al. 2014; Ndlovu et al. 2017). In this way, biosurfactants are used in the fields of industries, namely cosmetics and food, and in the biomedical and pharmaceutical areas, and they also expand their use in the oil industries to improve the recovery of this product (Jimoh and Lin 2019).

There is a tendency to substitute synthetic surfactants for those of biological origin in industrialized countries, stimulated by the sustainable advantages of biosurfactants, as they can be produced using renewable substrates, derived from industrial residues, which cheapens the cost of this bioactivity (Satpute et al. 2017; Araújo et al. 2019).

Glycolipids and lipopeptides are well-established classes of biosurfactants. They exhibit broad-spectrum antimicrobial activity, anti-adhesive, and biofilm control. They are right now applied in various areas (food, beauty products, and pharmaceutical industries) as emulsifying, antimicrobial, and surfactant agents (Inès and Dhouha 2015; Mnif and Ghribi 2016).

Biosurfactants that have anti-adhesive activity can be produced by several microorganisms: *Pseudomonas aeruginosa* produces rhamnolipids, some species of *Candida* sp. produce sophorolipids, *Bacillus* sp. produces surfactin among other isoforms. Biosurfactants with anti-adhesive activity can also be released by lactic acid probiotic bacteria (LAB) (Yan et al. 2019).

This chapter deals with the main classes of biosurfactants with anti-adhesive action, microbial fixation for biofilm formation, and studies involving the use of microbial biosurfactants as disintegrating agents of this formation on different surfaces.

10.2 Biosurfactants That Display Anti-Adhesive Activity

The composition and type of microorganism are used to classify biosurfactants. Other forms of classification are low molecular weight biosurfactants (lipopeptides, glycolipids, and phospholipids) and high molecular weight biosurfactants (polysaccharides, lipopolysaccharides, proteins, and lipoproteins). Low molecular weight biosurfactants lower the surface and interfacial tension of different substances. The ones with high molecular weight are used as emulsifiers and stabilizers for different products (Sharma and Sharma 2018; Jahan et al. 2020).

The parameters of free energy and surface tension of the coated materials and the surfactant itself influence the development of the surfactant film on any solid surface. The surface orientation of the nonpolar and polar fractions of the film formed by the biosurfactants on some solids surfaces is crucial for the balance of the hydrophobic and hydrophilic properties of the covered solid. These behaviors of the biosurfactant configurations are of practical importance for the protection of areas that are often used in food handling, medical devices, and surgical implants (Zdziennicka and Jańczuk 2018).

Not all biosurfactants are of interest as anti-adhesive surfaces. The most representative classes that showed responses in reducing adhesion in different materials and against microorganisms of interest are lipopeptides, with surfactin standing out, and glycolipids being mostly represented by rhamnolipids (Cao et al. 2009; Nickzad and Déziel 2014; Abdelli et al. 2019; Ceresa et al. 2019).

Lipopeptide biosurfactants exhibit antibacterial, antifungal, antiviral, and anti-adhesive activities. They are divided into three main groups (surfactin, iturin, and fengycin). Each group presents various homologs and isoforms showing distinct constitutions of amino acids and fatty acid chains (Inès and Dhouha 2015). Due to its attractive surfactant properties and antimicrobial and antibiofilm activities, surfactin is the most powerful biosurfactant, with many isoforms that can be determined genetically or by structural chromatographic analysis techniques (Ibrar and Zhang 2020; Ohadi et al. 2020).

Another important class of interest is the glycolipids. Rhamnolipids produced by *Pseudomonas* sp. are the most important representative in this group. Rhamnolipids are easily produced as a blend of homologous molecules, specifically mono-rhamnolipids and di-rhamnolipids by *P. aeruginosa* (de Freitas et al. 2019). These biosurfactants manifest surface activities and emulsifying and biological activities. Due to this versatility, they were highlighted as versatile additives in food preparation (Nitschke and Silva 2018). Glycolipids have a polysaccharide in their main groups. So, when this group is impacted by electrolytes or undergoes pH changes, its micellar structure changes (Jahan et al. 2020). This can interfere with the process of anti-adhesion of surfaces.

Sophorolipids are other important types of glycolipid, composed of a sugar dimer formed by the glycosidic bond to a hydroxylated fatty acid that are produced and released mainly by yeasts, such as *Candida bombicola* (Shah et al. 2007). The natural diversity of sophorolipids is triggered by variations in the acetylation standard of the sophorosis unit, by the incidence of inside esterification and by the attributes of the hydroxylated fatty acid. A sample of sophorolipids may contain over 20 congeners; however, few of these forms will be dominant. Sophorolipids are structurally classified into acidic and lactonic forms (Haque et al. 2017). Acidic forms are used for cleaning purposes, while lactonic forms are primarily responsible for bioactivity (Van Bogaert et al. 2007; Dhar et al. 2011). This biosurfactant has low cytotoxicity and has been approved for use in food and the pharmaceutical industry by the US FDA (Joshi-Navare and Prabhune 2013). They have antimicrobial and anticarcinogenic properties, in addition to antifungal activity against planktonic cells of pathogenic species (Haque et al. 2016).

Finally, we have the lactobacillus microorganisms, sometimes referred to as probiotics, which are outstanding producers of anti-adhesive biosurfactants. Also, they present antimicrobial, antibiofilm, and antioxidant activities in the same molecule. Therefore, they can be applied in different industrial sectors (Meylheuc et al. 2006; Sambanthamoorthy et al. 2014; Merghni et al. 2017; Yan et al. 2019).

Table 10.1 emphasizes the anti-adhesive activity mentioned in some recent studies in the literature in the area.

10.3 Biofilms and the Adhesion Process: Mechanisms and Effects

Biofilms are complex formations of microorganisms adhered to the surface of biogenic or inert materials. They are associated with each other through extracellular polymeric substances forming an aggregation of microbial cells. The extracellular substance produced by the biofilm, besides contributing to the access to nutrients, allows the existence of these microorganisms in adverse conditions, such as competition, lack of resources, and resistance to antimicrobial treatments. Thus, biofilms are responsible for making it difficult to treat chronic diseases with antibiotics (Roy et al. 2018; Prasad et al. 2020).

The formation of biofilm on any surface involves at least three different phases. In the first phase, microbial cells are bound to a surface previously covered by particles of glycoprotein origin, in the second phase, in this slime, more microbial cells colonize forming microcolonies, and, finally, the complete development of the biofilm through the formation of channels and the formation of firm structures. With the maturation of the biofilm, the disintegration will occur by mechanical and chemical processes and will influence the renewal of the biofilm by the dispersion of the colony (Payne and Boles 2016).

Table 10.1 Examples of action of the various biosurfactants and their target microorganisms

Biosurfactant or producing microorganism	Application field	Target	References
Pediococcus acidilactici and *Lactobacillus plantarum*	Biomedical	*Staphylococcus aureus*	Yan et al. (2019)
Pseudomonas aeruginosa	Biomedical	*Staphylococcus aureus* and *Staphylococcus epidermidis*	Ceresa et al. (2019)
Surfactin	Biomedical	*Staphylococcus epidermidis*	Abdelli et al. (2019)
Lipopeptides	Biomedical and food industry	*Staphylococcus aureus, Salmonella typhimurium, and Bacillus cereus*	Giri et al. (2019)
Lipopeptides	Agribusiness (disease control in plants)	*Agrobacterium tumefaciens*	Ben et al. (2018)
Lipopeptides	Biomedical and food industry	*Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, Salmonella entarica, Enterobacterium* sp., *Aspergillus Niger, Aspergillus flavus, Fusarium oxysporum, Pythium ultimum, Fusarium solani, and Rhizoctonia bataticola*	Jemil et al. (2017)
Lactobacillus plantarum subsp. plantarum	Food	*Escherichia coli, Staphylococcus aureus,* and *Salmonella enterica*	Bakhshi et al. (2017)
Rhamnolipids and surfactin	Food	*Listeria monocytogenes* and *Pseudomonas fluorescens*	Araujo et al. (2016)
Xylolipid	Biomedical	*L. monocytogenes*, *Escherichia coli* and *Bacillus cereus*	Sharma et al. (2015)
Glycolipid (glucose + palmitic acid)	Biomedical	*Candida albicans*, *Pseudomonas aeruginosa,* and the marine biofouling bacterium *Bacillus pumilus*	Dusane et al. (2011)
Surfactin/iturin A	Food	*Bacillus cereus*	Shakerifard et al. (2009)

The installation of bacteria is particularly intermediated by particle deposition, hydrodynamic forces, and Brownian motion. Adherence to the substrate is regulated by Lifshitz–Van der Waals, acid–base, hydrophobic, and electrostatic interaction forces (Van Oss 1995). Biofilms need to produce biopolymers and polymeric extracellular substances (EPS) rich in carbohydrates and proteins that function as a protective wrapper in which microorganisms are embedded. This ensures for biofilms their fixation and maintenance on surfaces in different environments (Donlan 2002).

Humidity, temperature, environmental pH value, climatic conditions, and chemical composition of the nutritive substrate are the factors that affect the growth of biofilm. Besides, biofilms contain 80–90% water, and their depth can differ between 50 and 100 μm, depending upon the inhabited area (Kaali et al. 2012).

Materials with a hydrophobic surface favor bacterial fixation and biofilm formation, except those with a superhydrophobic surface such as Teflon that has surfaced with contact angles with water >150° (Zeraik and Nitschke 2012; Li et al. 2016; Yilgör et al. 2018).

The hydrophobicity of the microbial cellular membrane and the presence of extracellular filamentous annexes can affect the ratio and degree of bacterial binding. The progress of the hydrophobic type interaction between the exposed area of the material and the microbial cell surface tends to be greater with the increase in the nonpolar constitution of those involved (Donlan 2002; Krasowska and Sigler 2014). The hydrophobic regions of the bacterial cells are partially involved in the connection with a neighboring cell (Van Oss 1995; Krasowska and Sigler 2014). Studies carried out by many laboratories concluded that the susceptibility of materials to microbial adhesion is greater on wood and latex surfaces. A reduction occurs from silicone, PVC, Teflon, polyurethane, stainless steel, and titanium materials (Stoica et al. 2016).

Biofilms can cause microbiologically influenced corrosion (MIC). Some microorganisms cause MIC through extracellular electron transfer for energy. They secrete corrosive metabolites that lead to MIC (Jia et al. 2019).

As biosurfactants reduce the surface tension between liquids and the surface, they can wet surfaces and thus make them hydrophilic (Fig. 10.1), making microbial fixation difficult. Furthermore, they allow greater penetration of different fluids, including solvents and antimicrobial agents in biofilms, which can contribute to the removal of this and other fouling.

10.4 Applications of Biosurfactants as Anti-Adhesive Agents

The pre-contact of surfaces with surfactants can lead to the adsorption of these elements on the surfaces, which can affect the development of biofilm in two ways: (1) modification of the biofilm formation capacity, as surfactants can act against cellular metabolism, favoring or impairing the adhesion forces that maintain the mechanical stability of the biofilm, and/or (2) development of biofilms with less cohesive characteristics that can lead to the detachment of biomass (Rasulev et al. 2017).

Thus, bacterial adhesion to surfaces and the consequent development of biofilm are natural phenomena in different environments, such as marine, freshwater, hospital, food, and other industrial systems (Ricker and Nuxoll 2016; Galié et al. 2018; de Carvalho 2018), and biosurfactants prove to be an effective tactic to mitigate the establishment of biofilms and other fouling organisms.

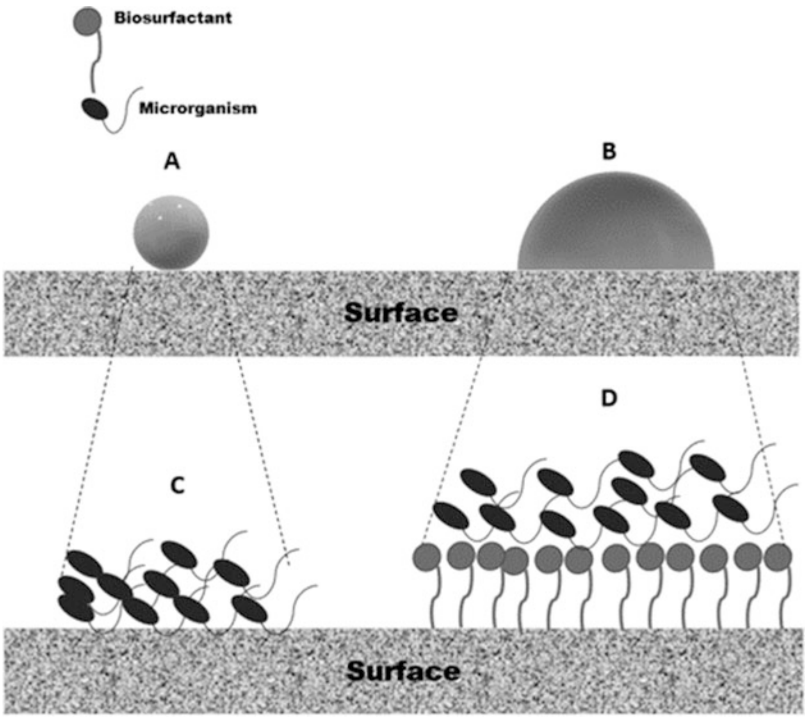

Fig. 10.1 Influence of biosurfactant on the surface hydrophilicity and microbial adhesion. (**a**) Solution without biosurfactant, hydrophobic surface; (**b**) Solution containing biosurfactant, surface becomes hydrophilic; (**c**) Emphasis on microbial adhesion influenced by surface hydrophobicity; and (**d**) Highlight on the inhibition of microbial adhesion caused by the adsorption of the biosurfactant on the surface

10.4.1 Anti-Adhesive Applications in the Biomedical Field

With the development of studies in the field of bacterial biofilms, the potential threats to health caused by infections caused by these biofilms have caused great public concern (Yan et al. 2019).

Synthetic surfactants are already used in the medical field, especially in cleaning infected lesions, in preparing the injured skin surface to receive surgical grafts (Percival et al. 2017). The presence of EPS in biofilms shows a reduced sensitivity to the host defense systems, antibiotics, among others, which contributes to bacterial persistence in chronic infections.

A lipopeptide from *Bacillus subtilis* AC7 combined with a farnesol molecule was able to neutralize biofilms of *Candida albicans* in silicone elastomer under simulated physiological conditions (Ceresa et al. 2018).

Using sophorolipid from *Candida bombicola* ATCC 22214, Ceresa et al. (2020) observed a significant reduction in the capacity of *Staphylococcus aureus* and

C. albicans to form biofilms and adhere to surfaces in 90–95% of silicone used in medical equipment. This research indicates the potential of biosurfactants as coating agents in biomedical materials to prevent infections by Gram-positive bacteria and fungi.

Satpute et al. (2019), using the glycolipoprotein biosurfactant produced by *Lactobacillus acidophilus*, observed antibiofilm and anti-adhesive activities against biofilm-producing microorganisms in medical implants based on PDMS (polydimethylsiloxane), considering a potential anti-adhesive agent on various surfaces of biomedical devices.

10.4.2 Anti-Adhesive Applications in the Food Industry Surfaces

The control of bacterial biofilms is one of the ways found by the food industry and related areas to reduce the undesirable effects of microbial contamination. The occurrence of biofilm can lead to food spoilage and disease transmission, which poses a risk to consumer health (Giri et al. 2019).

Several food manufacturing procedures present precarious sanitation environments, where microorganisms can successfully grow. These environments can include rubber surfaces, packaging machines, piping, valves, floor and walls, polystyrene materials, and stainless-steel materials (Faille and Carpentier 2009).

Several foodborne pathogens from different species of microorganisms such as *Bacillus cereus, Escherichia coli, Shigella* sp., *Staphylococcus aureus* (Sharma and Anand 2002; Sharma et al. 2015), *Listeria monocytogenes, Salmonella typhi, Pseudomonas fragi,* and *Leuconostoc citreum* (Dzieciol et al. 2016) among others are of great apprehension in food processing and preparation spaces.

Biosurfactants can either serve as a colonization factor for a specific microorganism or are also able to prevent or delay the establishment of other microorganisms. Scientific research with microbiological surfactants has already indicated anti-adhesive activities of these molecules against food-borne microbial pathogens. Therefore, microbiological surfactants, acting as antimicrobials, affected the growth of free and fixed forms of microbial cells of these organisms (Nitschke and Silva 2018).

When adsorbed on the surfaces of different materials that come into contact with food, biosurfactants were able to inhibit adhesion and biofilm formation. On polystyrene and AISI 304 stainless steel surfaces, the bacterium surfactin *Bacillus subtilis* ATCC 21332 and rhamnolipid from *Pseudomonas aeruginosa* PA1 (Petrobras) were tested against Gram-positive and -negative microorganisms. These biosurfactants significantly reduced the formation of biofilm pathogens from Gram-positive food sources (*Listeria monocytogenes* ATCC 19112 and ATCC 7644) and Gram-negative microorganisms (*P. fluorescens* ATCC 13525) (Araujo et al. 2016).

The biosurfactants of *Bacillus subtilis* VSG4 and *Bacillus licheniformis* VS16 are demonstrated to be notable blockers of microbial adherence and biofilm generation of microorganisms associated with food contamination. Thus, the authors propose that both the biosurfactants have the potential to be exploited as an antioxidant, antimicrobial, and anti-adhesive and thus mitigate the development of microbial biofilms in the biomedical and food industries (Giri et al. 2019).

The DCS1 lipopeptide synthesized by *Bacillus methylotrophicus* DCS1 showed antimicrobial activity against several tested microorganisms. Besides, they interrupted the preformed biofilm and also presented anti-adhesive activity in the formation of biofilm. Thus, the authors suggested the viable use of the DCS1 lipopeptide as a substance that inhibits oxidation, acting as antimicrobial and anti-adhesive in reducing microbial adhesion and biofilm formation and its applicability also in biomedical devices and the food sector (Jemil et al. 2017).

Biosurfactants isolated from *Lactobacillus paracasei* showed antimicrobial properties and anti-adhesive and antimicrobial properties against various food pathogens at different levels of inhibition. Therefore, they recommend the biosurfactant tested against various food pathogens as an alternative antimicrobial agent (Gudiña et al. 2010).

Biosurfactants also show differences in anti-adhesive and antibiofilm action depending on the type of surface material treated. In a test carried out by Araujo et al. (2016), rhamnolipids reduced the fixation on the polystyrene surface up to 79% and on stainless steel up to 83%. Surfactin reduced 54% and 73%, respectively, in the same materials. pH is an important factor to be considered in the development of strategies based on rhamnolipids for the control of food pathogens. Rhamnolipid showed antimicrobial action against Gram-positive pathogens (*Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*). This activity was related to the increase in the acidity of the environment caused by the different pH levels. The susceptibility of these pathogens was associated with a reduction in the hydrophobicity of the microbial surface layer and consequent deterioration of the cytoplasmic membrane (de Freitas et al. 2019).

10.5 Future Trends and Conclusions

Due to a large number of applications, biosurfactants are exceptionally useful molecules. The surfactant, antimicrobial, and emulsifying properties of these molecules have been implemented in several industries, such as pharmaceutical, cosmetic, food, and biotechnological. The application of anti-adhesive activity is of great importance mainly in the pharmaceutical and food industries. Recent advances related to the identification of microorganisms that produce biosurfactants, purification, and characterization of their compounds, as well as cultivation with different residual raw materials and scale-up studies, have enabled the production of biosurfactants with different functionalities. However, the high production cost does not allow for large-scale synthesis, limiting the availability of these molecules.

The biosurfactants mentioned here can be used in the development of new strategies to delay the colonization of the surface, i.e., be used as antifouling agents.

Smart antibacterial coatings may contain fixed microorganisms that release biosurfactants with anti-adhesive action. These same coatings may also be antimicrobial by encapsulating these agents. Both are promising strategies since they can be doubly effective in presenting anti-adhesive and antimicrobial functions in the same product. Hence, investments in research for the development and industrial production of natural anti-adhesive products based on biosurfactants are necessary for the elucidation of chemical structures and their application in different sectors.

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