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RESEARCH ARTICLE

Biosurfactants as inhibitors of the adhesion of pathogenic bacteria

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ABSTRACT

Biosurfactants have gained more attention in the past decade as possible medical resources. They are useful therapeutic agents against many infections because of their antibacterial, antifungal, and antiviral properties. Additionally, the anti-adherent activities of these compounds counter to a number of pathogens suggest that they could be useful as an anti-adherent coating for medical inserts, helping to prevent infections in hospitals without the use of chemicals. This study aims to investigate the antiadhesive activities of biosurfactants extracted from *Lactobacillus brevis* and *Bacillus* sp. against seven clinical pathogens. Biosurfactants at different concentrations were applied to polystyrene surfaces, and then the attachment of pathogenic strains was evaluated. The adhesion of microbes to n-hexadecane was also studied. As a result, the bacterial strains with 50 mg/ml of *Lactobacillus brevis* biosurfactant displayed a 69–73% reduction in adhesion. In contrast to the first biosurfactant, a biosurfactant extracted from *Bacillus* sp. significantly reduced bacterial attachment at all concentrations studied, although to a lesser extent. As the concentration was increased in surface conditioning tests, the anti-adhesive activity increased, showing the significance of considering this. In summary, both biosurfactants demonstrated excellent potential as anti-adhesive compounds that can prevent microbial contamination. Our findings provided evidence that biosurfactants could be used in medical applications.

Keywords: Biosurfactants; Antimicrobial; Antiadhesive; hydrophobicity; Clinical Strains.

INTRODUCTION

Over the recent years, because of misusing and overusing antibiotics for human treatment and livestock production, antibiotic-resistant bacteria are on the rise. Thereby, antimicrobial resistance expanded in distinct environments as a result (Berendonk et al., 2015; Garbisu et al., 2018). The World Health Organization (WHO) reports that antimicrobial resistance is one of the biggest health problems encountered in the world and the death rate due to this resistance has become unstoppable (Organization, 2014). Research on antibiotic resistance in recent years has principally concentrated on clinical microorganisms, which pose a direct threat to public health. Furthermore, the appearance of resistant bacteria has continuously increased leading to the expansion of multidrug-resistant (MDR) bacteria (Ventola, 2015). Increasingly, medical and public interests have been raised over the spread of MDR human pathogens (Tiedje et al., 2019). Therefore, the control of antibiotic-resistant bacteria became a high priority in hospitals and other clinical settings (Ventola, 2016). Different MDR bacteria such as *Staphylococcus aureus* (MRSA) have become a leading cause of nosocomial and communal diseases (Van Duin & Paterson, 2016). Likewise, other bacteria such as *Escherichia coli*, *Salmonella*, *Campylobacter,* and *Vibrio cholera* are classified as foodborne bacteria resistant to antibiotics (Organization, 2017). Therefore, scientific research over the past decade has focused on biological approaches to avoid this resistance phenomenon and to control microbial colonization and spreading in patients. Recent findings suggest that biosurfactants (BS) are potential alternative antimicrobial agents and provide a solution in the face of current antimicrobial resistance as global health risk (Vello et al., 2019). The majority of microorganisms that produce BS are bacteria. In the scientific research field, the

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most commonly studied bacteria include *Pseudomonas* spp., *Acinetobacter* spp., *Bacillus* spp., and *Arthrobacter* spp. In the food industry, however, these compounds are unsuitable due to their pathogenic nature. Due to the health benefits of probiotic bacteria, their application as non-disease-producing, secure microorganisms has attained significant consideration in the production of BS (Hajfarajollah, Eslami, Mokhtarani, & Akbari Noghabi, 2018). By definition, BS is derived from a surfactant that is a molecule with emulsifying properties and reduces the surface tension between surfaces (Akbari, Abdurahman, Yunus, Fayaz, & Alara, 2018). The microbial surfactant molecules consist of a variety of molecules, such as glycolipids, lipopeptides, polysaccharide-protein complexes, proteins, lipopolysaccharides, phospholipids, fatty acids, and neutral lipids (Van Hamme, Singh, & Ward, 2006). Consequently, BS has important physiological properties and functions, such as the ability to enhance the area and bioavailability of hydrophobic water-insoluble substrates, to bind heavy metals, to inhibit bacterial pathogens, to detect quorums, and to form biofilms (Singh & Cameotra, 2004).

Biofilms are conglomerates of bacteria protected by polysaccharide extracellular matrices that self-assemble (Sambanthamoorthy, Feng, Patel, Patel, & Paranavitana, 2014). Biofilms remain a matter of concern for the medical and food industries due to the ability of bacteria to colonize medical devices and food processing surfaces to alter their properties. Indeed, pathogenic bacteria can be released from them, making them a major source of contamination. In addition, their antimicrobial resistance is greater than that of planktonic cells. However, BSs are able to reduce and control the adhesion of bacteria to surfaces, which in turn allows biofilms to form (Donlan & Costerton, 2002; Dusane, Nancharaiah, Zinjarde, & Venugopalan, 2010). Additionally, *Lactobacillus jensenii* and *Lactobacillus rhamnosus* BSs are known to interfere with biofilm formation and cell communication (Valle et al., 2006). These compounds are antibacterial and anti-adhesive and are also resistant to biofilm formation in strains of MDR bacteria like *Acinetobacter baumannii*, *E. coli,* and MRSA (Sambanthamoorthy et al., 2014). It appears that the treatment with BSs from probiotic bacteria as antimicrobial and/or antiadhesive products can prolong the life of prostheses. It has been demonstrated that these BSs inhibit the adhesion of microorganisms (Lígia Rodrigues, Van der Mei, Teixeira, & Oliveira, 2004). It has been shown in a recent study that BS derived from potential probiotic *Bacillus* was highly effective at preventing the formation of biofilms associated with MDR *Staphylococci* (Haddaji et al., 2022). In addition, three *Lactobacillus acidophilus* strains and *Bacillus licheniformis* strain M104 have been tested in other studies for their ability to prohibit *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms (Gomaa, 2013; Walencka, Różalska, Sadowska, & Różalska, 2008). Evenly, *Bacillus* sp. is known for producing several important industrial products, including enzymes, antibiotics, amino acids, insecticides, BS, and bacteriocins (Perez et al., 2017). Indeed, *Lactobacilli* and *Bacillus* sp. which are important microorganisms in nature, are known to produce antimicrobial agents, including surfactants, which make them extremely potent interfering bacteria (Horošová, Bujňáková, & Kmeť, 2006; Merk, Borelli, & Korting, 2005). Thus, such *Lactobacilli*-derived products offer a potential solution to prevent biofilm formation, which is an interesting concept of novel therapy being tested (Lígia Rodrigues, Banat, Teixeira, & Oliveira, 2006). As a result of these characteristics, the agents produced by *Bacillus* sp. and *Lactobacilli* have important applications in food science, pharmacology, and biomedicine due to these characteristics.

In this study, BSs isolated from *Lactobacillus brevis* and *Bacillus* sp. were tested for their anti-adhesive and anti-biofilm activities against several clinical isolates of MDR pathogens. *Bacillus* and *Lactobacillus* strains were selected for their probiotic properties, which have previously been investigated (Mahdhi, Hmila, Chaieb, Kamoun, & Bakhrouf, 2011).

MATERIALS AND METHODS

Pathogen strains and inoculum preparation

The antibacterial activities of BS were tested against seven clinical strains involved in in certain infectious diseases, which were isolated and identified by classical and molecular methods and five references strains stored at -20°C in Tryptic Soy Broth (TSB) supplemented with 20% glycerol (v/v). Details of pathogen strains used in this work are listed in Table 1. The bacterial strains were cultivated in Brain Heart Infusion (BHI, Difco, USA), a non-selective enrichment medium, and then incubated for 24 hours at 37 ° C. The cell mass was removed from the medium using an inoculating loop, suspended in 10 ml of NaCl 0.15 mol l^{-1}, and adjusted to a concentration of approximately 10^{10} CFU ml^{-1}. Strains purity was verified using GRAM staining profiles and colonies shapes. Moreover, typical biochemical behaviors and enzymatic activity were checked using the API (BioMérieux, France) micro gallery.

Isolation BS producing bacteria and BS production

Lactobacillus brevis (BS1) and *Bacillus sp.* HM117830.1 (BS2) were obtained from the Laboratory for Analysis, Treatment and Valorization of Environmental Pollutants and Products, Faculty of Pharmacy (Monastir, Tunisia) collection and stored at -20°C in, respectively, Man, Rogosa,

Table 1: The list of tested pathogen bacteria

	Strain	Gram stain	Origin
PB 1	*Enterococcus faecalis* C1	+	Oral cavity
PB 2	*Enterococcus faecalis* C2	+	Oral cavity
PB 3	*Enterococcus faecalis* R	+	Reference strain (ATCC 29212)
PB 4	*Streptococcus mutans* C	+	Oral cavity (API 20 5040710)
PB 5	*Staphylococcus aureus* C	+	Clinical strain (CHU Farhat Hached Sousse)
PB 6	*Staphylococcus aureus* R	+	Reference strain (ATCC 25923)
PB 7	*E. coli* R	-	Reference strain (ATCC 35218)
PB 8	*E. coli* C	-	Clinical strain (CHU Farhat Hached Sousse)
PB 9	*Salmonella Typhimurium* R	-	Reference strain (DT104)
PB 10	*Pseudomonas aeruginosa* R	-	Reference strain (ATCC 27853)
PB 11	*Pseudomonas aeruginosa* C	-	Clinical strain (Regional Hospital Gafsa)

C: clinical strain
R: Reference strain
PB: Pathogenic Bacteria

Sharpe (MRS; Lab M, Bury, UK) and BHI supplemented with 20% glycerol (v/v). Probiotic properties have previously been investigated for these strains (Mahdhi et al., 2011; Mahdhi, Kamoun, Messina, & Bakhrouf, 2012). For the *Lactobacillus brevis* strain, a biochemical identification was performed using the API-50 CHL system (BioMerieux, Marcy-l'Étoile, France) according to the manufacturer's recommendations. The results were observed with a microbiological mini-Api automate (bioMerieux). In addition, their enzymatic profile was characterized using the API-ZYM systems (bioMerieux).

Then, the two strains were cultivated and incubated for 2 days at 37 ° C with vigorous shaking. Four percent (4%) of filtered olive oil (pore size 0.45 mm; Millipore) was added to the culture medium as a carbon source. Floating excess-substrate on the surface of culture was discarded using appropriate flasks. Then, bacterial cells were eliminated by centrifugation at 5000 rpm for 20 min. The supernatant of the culture was filtered through the sterile filter of 0.45µm and acidified to pH 2 with 6 M of HCl before incubation overnight at room temperature (25°C). The extraction was repeated three times by equal volume (v/v) of ethyl acetate. The obtained organic phase was dried over anhydrous sodium sulfate (Na_2SO_4) and evaporated using a rotavapor. The crude BSs were incubated overnight at 37°C to remove traces of solvent. The BS production was expressed in g/l (Habib et al., 2020).

Oil displacement test

Oil displacement activity was evaluated according to Habib et al., (Habib et al., 2020). Distilled water (20 ml) was poured into a clean petri dish (85 mm diameter) and then 300 µL of oil sample was added to the surface of the distilled water until a thin and even layer of oil was established. Then, an equal volume of the solution containing the BS1 or BS2 was dropped onto the oil surface. The formation of a clear zone as the indication for the presence of a BS was then observed qualitatively. In this assay, distilled water was used as the negative control however 0.1% SDS was employed as positive control. All experiments were performed in triplicate.

Physicochemical characterization of cell surfaces

The MATS test (microbial adhesion to solvents) was carried out according to the methodology developed by Rosemberg et al. in 1986 (Rosenberg, Gutnick, & Rosenberg, 1980) and modified by Bellon-Fontaine et al. (Bellon-Fontaine, Rault, & Van Oss, 1996) to evaluate the Lewis acid-base properties and the hydrophilic/hydrophobic nature of bacterial surfaces. This method is based on the degree of adhesion to various liquid hydrocarbons; in this protocol, n-hexadecane is used as a solvent. This test is applied for non-treated and treated bacteria with BS (50 mg/ml). A bacterial suspension containing about 10^8 CFU ml^{-1} in 2.4 ml of 0.15 mol l^{-1} NaCl and 0.4 ml of solvent was vortexed for 2 min to form an emulsion. The mixture was left to stand for 30 min to ensure complete separation of the two phases. The optical density of the liquid phase was measured at 580 nm. The percentage of adhesion to the solvent was calculated by the equation: % Adh = (1 - A/A0) * 100, where A0 is the absorbance of the bacterial suspension before mixing and A is the absorbance after mixing (Meylheuc, Van Oss, & Bellon-Fontaine, 2001).

Anti-Adhesive activity

After conditioning with different concentrations of BS (50 mg/ml to 12.5 mg/ml), 100 µl of bacterial suspension (10^9 CFU/well) was added to polystyrene microtiter plates and incubated at 37°C for 24 h. TSB with 2% glucose was used as a negative control and the bacterial suspensions were not treated with the supernatant as a positive control. Unattached bacteria were removed by washing the wells three times with PBS (PBS (7 mM Na_2HPO_4, 3 mM NaH_2PO_4, and 130 mM NaCl at pH 7.4). The adherent microorganisms were fixed for 15 min with 100 µl of 95% ethanol. The wells were then stained with 200 µl of crystal violet (1% aqueous solution w/v) for 5 min. Then, unbound crystal violet was removed and the wells were washed 3 times with 300 µl of sterile distilled water. The water was then cleared and the microtiter plate was air-dried for 3 to 4 h. The quantitative analysis of bacterial adhesion was performed by reading the optical density of the wells using an automated MultiSkan reader (GIO, De Vitae,

Rome, Italy) at 570 nm. Biofilm formation was categorized as highly positive ($OD_{570} \geq 1$), low-grade positive ($0.1 \leq OD_{570} < 1$), or negative ($OD_{570} < 0.1$).

Statistical analysis

The statistical analysis was performed on SPSS v.17.0 statistics software (SPSS Inc., Chicago, IL). The statistical differences and significance were assessed by the ANOVA test; $P < 0.05$ was considered significant. The statistical analysis was performed between the control cell and the stressed cell.

RESULTS

Oil displacement test

The two BS produced from *Lactobacillus brevis* (BS1) and *Bacillus sp.* (BS2) are tested by the oil-spreading test. As indicated in the results summarized in the Table 2, in this test, a larger diameter indicates that the testing solution has a higher surface activity, which is an indirect measure of surface activity. Depending on the concentration and type of BS, the diameter of the clear zone varies. At 50 mg/ml, BS1 exhibits a halo of 2.4 cm and BS2 has a halo of 2.1 cm. With a concentration of 100 mg/ml, they represent clear areas with diameters of 5.3 and 4.8 cm respectively. The results show that the diameter of the halo increases with the concentration of BS and that BS1 presents the best BS reducing oil-water tension.

Cell surface characterization

Studying the affinity of bacteria with and without BS with n-hexadecane allowed us to evaluate the effect of BS on the membrane properties of the pathogens investigated in this work. Table 3 shows that the treatment with the BS affect significantly the surface hydrophobicity of the various pathogenic bacteria, whether they are reference strains or strains of clinical origin. Hydrophobicity is evaluated by measuring the percentage of cells that adhere to hexadecane. Indeed, when the proportion of cells adhering to hexadecane was greater than 55%, cells were strongly hydrophobic, moderately hydrophobic (30-54%), moderately hydrophilic (10-29%), and strongly hydrophilic (<10%) (Chae, Schraft, Hansen, & Mackereth, 2006). Accordingly, the studied strains are highly attracted to n-hexadecane and are classified as strongly hydrophobic from their adhesion to hexadecane exceeding 55%. PB 3, PB 4, and PB 10 are the most hydrophobic, having reached more than 90% of adhesion to hexadecane. After treatment with BS1, for five strains such as PB1, BP4, PB6, PB7, PB10, and PB11, the affinity for n-hexadecane decreased and the compound became more hydrophilic when the adhesion is ranged from 10 to 29%. Yet, The PB9 strain presented a strongly hydrophilic character and other strains became moderately hydrophobicc. On the other hand, all strains became moderately hydrophobic after treatment with BS2 except for strain PB3 that became moderately hydrophilic.

Table 2: Test results of oil displacement of the two biosurfactants producing strains.

	Average clear zone (cm)	
SB/Concentrations	BS 1	BS 2
50 mg/ml	2,4 *±0.09	2,1 *±0.11
100 mg/ml	5,3 *±0.13	4,8 *±0.16

* P<0.05

Table 3: Percentage affinity of treated and non-treated bacterial cells with biosurfactants to n-

	Strain	% Adhesion (mean ± SD)		
		Without BS	With BS 1	With BS 2
Gram positive bacteria	PB 1	82.5*±2.5	25,3*±0.5	44,5*±1.1
	PB 2	70.5*±1.2	31*±1.2	39,5±2.2
	PB 3	92.3*±2.8	37±0.8	25*±1.4
	PB 4	93.8*±0.1	20,3*±0.6	38,7*±0.7
	PB 5	83.9*±0.3	39,6*±3.1	40,9*±2.1
	PB 6	73.2*±3.2	29,7*±1.8	45,7*±1.5
Gram negative bacteria	PB 7	85.4±4.1	16*±0.2	39,2*±2.9
	PB 8	75.5*±3.7	36,3*±2.6	43,1*±3.1
	PB 9	86.1*±2.3	6,6*±0.2	39,9±1.9
	PB 10	91.5*±1.5	29,5±1.3	38,2*±1.6
	PB 11	58.7*±0.3	28,4*±1.7	42,1±0.8

hexadecane used in MATS test
*P < 0.05

BS Anti-Adhesive assay

In a 96-well plate containing BS or without BS, we tested the biofilm formation of pathogenic strains. This study was accomplished to evaluate the adhesion characteristics and the conditioning effect of BS as a function of concentration. Table 4 illustrates the data obtained from these experiments. There was a significant decline in adhesion of all bacteria studied in all tested conditions after treatment with BS1 and BS2. By increasing the concentration of BS-1, adhesion is reduced more effectively. After treatment with BS-1 with 50 mg/ml, reaching 69–73% of inhibition on bacterial attachment respectively for *Streptococcus mutans* C (PB4) and *Salmonella Typhimurium* R (PB9). The conditioning with BS-2 significantly decreased bacterial adherence at all concentrations evaluated, while to a lesser degree than BS1. *Staphylococcus aureus* (PB5 and PB6) showed approximately the same adhesive behavior after treatment with the two tested BSs at different concentrations. The clinical strain of *Pseudomonas aeruginosa* (PB 11) showed a significant increment ($P < 0.05$) in adhesion after treatment with BS2 at the concentration of 12.5mg/ml and 25mg/ml, represented by the negative values in Table 4.

DISCUSSION

In the industrial and medical world, BS molecules are produced by a variety of microorganisms, including

Table 4: Biosufactants Anti-Adhesive Activity

			Bacterial adhesion inhibition (%)					
	Strain	Control	BS 1			BS 2		
			12.5mg/ml	25mg/ml	50mg/ml	12.5mg/ml	25mg/ml	50mg/ml
Gram positive bacteria	PB 1	0.00*	48*	52*	56*	44*	49*	55*
	PB 2	0.00*	50*	51*	55*	49*	49*	54*
	PB 3	0.00*	6*	17*	15	2*	10*	8*
	PB 4	0.00*	63*	67*	69*	66*	68*	67*
	PB 5	0.00*	28*	37*	37*	24*	27	29*
	PB 6	0.00*	15*	38*	32*	26*	30*	33
Gram negative bacteria	PB 7	0.00*	30*	56*	62*	42*	47*	49*
	PB 8	0.00*	43*	45	46*	31*	34	35*
	PB 9	0.00*	62*	64*	73*	62*	64*	65*
	PB 10	0.00*	45*	48*	49*	32*	41*	44*
	PB 11	0.00*	11*	11*	15	-7*	-1*	4*

*P < 0.05

Pseudomonas, *Burkholderia*, *Lactobacillus*, and *Bacillus* sp. (Crouzet et al., 2020; Lígia Rodrigues et al., 2006). It had been widely reported that strains of the genera Bacillus and Lactobacillus could produce BS, which was used as a pharmaceutical and food additive (Ghasemi, Moosavi-Nasab, Setoodeh, Mesbahi, & Yousefi, 2019; Gudiña, Teixeira, & Rodrigues, 2011; Morais et al., 2017; Satpute et al., 2016). This study used oil displacement testing to determine the BS production of each strain. It was found that *L. brevis* produced the best BS with a clear zone larger than *Bacillus* spp. This clearing zone on the oil surface correlates with BS activity in the supernatant, which displaces the oil. According to our findings, BS quantity is linearly related to clearing zone diameter in previous studies that examined pure BS (Sari, Kusharyoto, & Artika, 2014; Walter, Syldatk, & Hausmann, 2010). *Lactobacillus* BSs are mainly composed of polysaccharide side chains, phosphates, and proteins, and are classified mainly as glycolipids or glycolipoproteins (Foschi et al., 2017). Besides their antimicrobial properties, the molecules are also antifungal and antibacterial, with antibacterial properties against *Candida albicans* and *Escherichia coli*, as well as *Staphylococcus saprophyticus*, *Enterobacter aerogenes*, and *Klebsiella pneumonia* (Morais et al., 2017). A potent antimicrobial and antiadhesive property of *Lactobacillus paracasei* ssp. *paracasei* A20 from Portuguese dairy plants, for example, has been demonstrated (Gudiña, Rocha, Teixeira, & Rodrigues, 2010). Furthermore, a cell-free BS with antimicrobial properties was produced by *Lactobacillus acidophilus*, *Lactobacillus pentosus*, and *Lactobacillus fermentum* in Malaysia (Hassan, 2018). The BSs produced by *Pediococcus dextrinicus* SHU1593 also have antimicrobial effects against *Bacillus cereus*, *E. aerogenes*, and *Salmonella typhimurium* (Ghasemi et al., 2019). Researchers have also used the same approach to identify antimicrobial BSs in members of the *Bacillus* species, such as surfactin, a lipopeptide BS made of hexapeptides and b-hydroxy esters. As a matter of fact, *Bacillus* bacteria produce antimicrobial molecules (Perez et al., 2017). The surfactin biosynthesis pathway derived from *Bacillus* species shares many similarities with the pathway used by several strains of *L. plantarum*, *L. inners*, *L. reuteri*, and *L. brevis* in a recent study (De Giani, Zampolli, & Di Gennaro, 2021). All of these studies confirm our findings regarding the anti-adhesion activities of the BS produced from *Lactobacillus brevis* and *Bacillus* sp. Indeed, due to their complex chemical structures, biological surfactants possess outstanding physical properties. Hydrophobic and hydrophilic regions are not separated as in chemical surfactants, instead, they are grouped in a mosaic pattern (Otzen, 2017). The spherical nature of Surfactins at the interface allows them to form spherical structures. Because of this, their complexity is greater. For glycolipid BS, for example, rhamnolipids and sophorolipids, surface-active properties depend on the hydrophobic region size and saturation, as well as the presence of sugar groups and levels of acetylation (Otzen, 2017). The amphiphilicity of rhamnolipids allows them to easily insert into membranes under sub-CMC concentrations, modifying membrane structure and removing lipopolysaccharides, usually in conjunction with hydrophobic rhamnolipid precursors (Otzen, 2017). Identifying pure surfactants is based on the critical micelle concentration (CMC) as a chemical-physical parameter, which impacts surface activity and self-assembled aggregation (Perinelli et al., 2020). As a consequence, anti-adhesive activity of BS appears to be affected by the type of BS, microorganisms, and surface characteristics (Walencka et al., 2008). In this study, the BS1 and BS2 treatments reduced adhesion of all bacteria studied and in all conditions evaluated. By increasing the concentration of BS1, the reduction in adhesion on polystyrene surface increased. Additionally, BS2 significantly decreased bacterial attachment to polystyrene surface at all concentrations studied, although to a lesser extent than BS1. The attachment to hexadecane was examined with five strains treated with BS1, including PB1, BP4, PB6, PB7, and PB10. The adhesion to n-hexadecane has been reduced

between 10 and 29% after treatment. The PB9 strain was highly hydrophilic, whereas other strains were moderately hydrophilic. In contrast, all strains became moderately hydrophobic after treatment with BS2 except for strain PB3, which became moderately hydrophilic. Researchers have also studied the interaction of BSs with metal surfaces, demonstrating that corrosion-causing external environments are oriented toward the tails of lipophilic BSs, and metal surfaces toward their lipophobic heads (Fenibo, Ijoma, Selvarajan, & Chikere, 2019). Additionally, BSs are antimicrobial reduce the biomass of sulfate-reducing bacteria, and prohibit the growth of biofilm, both potentially corrosion-producing chemicals (Astuti, Purwasena, & Putri, 2018; Basafa & Hawboldt, 2019). Other studies (Haddaji et al., 2022; LR Rodrigues, Banat, Van der Mei, Teixeira, & Oliveira, 2006) suggested that BS reduced hydrophobic interactions, thus decreasing bacteria's adhesion, validating our findings. The hydrophobic surface is especially colonized by microorganisms due to its ability to facilitate close interaction between microbes and substrate, reducing interfacial moisture (LR Rodrigues et al., 2006). Thus, a BS conditioning a surface could result in a decrease of microbial attachment, as the surface will become more hydrophilic (Zeraik & Nitschke, 2010). Indeed, these data support our results. There are other factors that may affect the adhesion process, such as the surfaces on which microorganisms adhere, bacterial fimbriae and flagella, surface proteins, as well as extracellular polymeric substances produced by bacteria (Jin & Marshall, 2020; Zheng et al., 2021). Additionally, the interaction between cells and their environment is also dependent on the presence of carboxylic, phosphatic, and amino groups on their surfaces. According to numerous reports, rhamnolipids alter the surface chemistry of cells, but the impact of different BSs varies depending on the microorganism. Lipopolysaccharide, saturated alcohols, carboxyl groups, phosphoryl groups, and amine groups have been observed to change on the cell's surface (Bai et al., 2017; Ma et al., 2018; Mohanty & Mukherji, 2013).

Finally, BS can modify Gram-negative bacteria's wall structure, however, it can also modify Gram-positive bacteria's wall structure by altering one of its structural components. By reducing the interfacial tension and promoting bacterial detachment, BS penetrates and adsorbs at the interface between the solid surface and the attached bacteria, inhibiting lichenysin biofilm formation (Coronel-León, Marqués, Bastida, & Manresa, 2016).

CONCLUSION

BSs possess a myriad of attractive properties that make them potentially useful for medical applications. These compounds have antioxidant, antifungal, and antiviral effects. Numerous studies conducted on probiotics demonstrate the growing interest that the scientific community is placing in the therapeutic potential of these microorganisms. A significant need for new antimicrobials and antifungals has arisen as a result of the increased resistance of pathogenic organisms. There may be a new source of antimicrobials and antifungal drugs in probiotics and their BSs. We demonstrated significant *in vitro* antibacterial and antibiofilm activity for BS1 and BS2 produced from *Lactobacillus brevis* and *Bacillus* spp. against several clinical strains such as *Enterococcus faecali*, *Streptococcus mutans*, *Staphylococcus aureus*, *E. coli*, *Salmonella Typhimurium*, and *Pseudomonas aeruginosa*. Therefore, BS1 and BS2 could prevent biofilm formation and eliminate biofilms already formed by pathogenic bacteria. Consequently, they are useful in bioremediation processes involving hydrophobic cells to protect the environment. A number of biomedical and health-related fields can benefit from BSs, but research on human cells and natural microbes needs to be conducted.

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Conflict of interest

The authors declare that they have no competing interests

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