Asia-Pacific Journal of Science and Technology: Volume: 30. Issue: 04 Article ID: APST-30-04-02 Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by Research and Innovation Department,
Khon Kaen University, Thailand

Evaluation of the anti-oxidant, anti-microbial, antibiofilm potential of biosurfactants derived from *Pediococcus pentosaceus* S-2

Tamanna Kaundal[1], Anjali Sharma[2], and Navneet Batra[1*]

[1]Panjab University Research Centre, Goswami Ganesh Dutta Sanatan Dharma College (GGDSD) College, Department of Biotechnology, Sector-32, Chandigarh, India
[2]DAV College, Department of Biotechnology, Sector- 10, Chandigarh, India
[*]Corresponding author: Navneetbatra@ggdsd.ac.in

Received 15 September 2023
Revised 23 August 2024
Accepted 15 October 2024

Abstract

Numerous Lactic acid bacteria (LAB) tend to produce different types of biosurfactants i.e surface-active compounds. In the current investigation, biosurfactants derived from *Pediococcus pentosaceus* S-2 were explored for anti-oxidant, antimicrobial and antibiofilm activities against different test microorganisms. *Pediococcus pentosaceus* S-2 derived biosurfactants exhibited radical scavenging activity and antioxidant activity of 71% at a concentration of 600 µg. Antimicrobial activity was observed against different micro-organisms in the range between 0.8-5 mg/mL. At 4X minimum inhibitory concentration (MIC), there was 59-65 % inhibition of microbial biofilms in the presence of biosurfactants. Additionally, search engine marketing (SEM) microscopy provided additional evidence of the dispersion of different microbial biofilms in the presence of biosurfactants. Moreover, the biosurfactants also led to a reduction in the content of extracellular polymeric substances (EPSs), including proteins and carbohydrates, further contributing to the mitigation of microbial biofilms. These findings underscore the multifaceted potential of biosurfactants derived from *Pediococcus pentosaceus* S-2, suggesting their promising role as alternatives to synthetic surfactants across various applications, including antimicrobial, antibiofilm, and antioxidant functions.

Keywords: Antibiofilm activity, Antimicrobial activity, Biosurfactants, Lactic acid bacteria, *Pediococcous pentosaceus*

1. Introduction

Lactic acid bacteria (LAB) play a pivotal role in the fermentation of food products by acting as preservatives, texture, flavour and aroma enhancers. Different LAB tends to utilize different carbohydrates and synthesize various metabolites including lactic acid, acetic acid, ethanol, diacetyl, acetone, exopolysaccharide, specific proteases, biosurfactants and bacteriocin etc [1,2]. LAB such as *Lactobacillus, Bifidobacterium* and *Pediococcus, Lactococcus, Leuconostoc, Streptococcus, Bifidobacterium, Enterococcus, Vagococcus, Aerococcus, Tetragenecoccus,* etc are generally regarded as safe (GRAS) due to their non-pathogenic nature. Probiotics have gained attention nowadays owing to several health benefits so they can be explored as excellent microbial sources for the production of biosurfactants [3]. Biosurfactants are amphiphilic molecules consisting of two moieties, i.e hydrophilic moiety which comprises a carboxylic acid, alcohol, amino acid or carbohydrates whereas the second one is a hydrophobic moiety that mainly consists of fatty acids. They are synthesized by different micro-organisms on their surface or as extracellular secretion with tendency to reduce the surface and interfacial tension and have emulsification activity [4]. Biosurfactants are either lipopeptides, protein–polysaccharide complexes, glycolipids, phospholipids, neutral lipids or fatty acids in nature [5]. Due to their eco-friendly, biocompatibility, biodegradability and non-toxic nature, they can act as better replacements for synthetic surfactants for various applications in different sectors. A variety of biosurfactants are employed in different sectors including pharmaceutical, cosmetic food, petroleum, and agricultural sectors *etc* for multiple use [6].

LAB-derived biosurfactants play a crucial role in the pharmaceutical industry, particularly in the fight against antibiotic-resistant microorganisms that produce detrimental biofilms. These microbial biofilms are encapsulated by extracellular substances (proteins, lipids, and extracellular DNA), were developed as a result of microorganisms adhering to biotic or abiotic surfaces. Biofilms reduce bacterial susceptibility to host defence mechanisms, antibiotics, and other medications, which aids in bacterial persistence in chronic infections [7]. Even a lot of food-borne pathogens, including *Listeria monocytogenes, Salmonella, Escherichia coli,* and *Shigella,* may develop biofilm on a variety of surfaces, including plastic, glass, metal, and various equipment, belts, and floor drains found in food processing units [8]. Numerous research efforts were attempted to produce anti-biofilm strategies to get rid of detrimental biofilms. Innovative treatment approaches for the suppression of biofilms are currently showing a lot of interest in biological surface-active substances (biosurfactants). Several studies have shown that biosurfactants have anti-adhesive, antimicrobial, and biofilm disruption properties against a variety of micro-orgnisms [9]. Biosurfactants also have antioxidant properties with diverse applications in pharmaceuticals, cosmetics, and food industries for enhancing stability, shelf life, and offering health benefits. They protect against oxidative damage, improving efficacy in formulations while aligning with eco-friendly trends [10,11].

Therefore, the present study was designed to explore the antioxidant, antimicrobial, and antibiofilm activity of biosurfactants derived from *Pediococcus pentosaceus* S-2 which was isolated from wheat-based fermented food, Seera which was reported in our previous study [12]. Seera is a traditional fermented food from Himachal Pradesh, India, prepared from wheat grains soaked in water for 3-4 days with regular replacement of water. Then swollen grains are ground into a paste, left to settle for 1 hour, and the white starchy portion is collected aseptically after discarding surface water. It's known for its high nutritional value and medicinal properties, particularly beneficial in treating gynaecological disorders, including uterine haemorrhage postnatal [13, 14].

2. Materials and methods

2.1 Production and extraction of biosurfactants

MRS broth without Tween 80, was employed for biosurfactant production. This modified medium consists of Protease peptone, HM Peptone B, Yeast extract, Dextrose, Ammonium citrate, Sodium acetate, Magnesium sulfate, Manganese sulfate, and Dipotassium hydrogen phosphate procured from HiMedia Laboratories.

India150 mL of the modified MRS broth was inoculated with 1 mL of an overnight culture (3.0 x 10^8 CFU/mL) then incubated at 37°C. After 24 hrs, broth was centrifuged at 10000 rpm for 20 minutes to obtain the cell-free supernatant (CFS). For extraction of cell adhered biosurfactants, the cell pellet was resuspended in PBS (pH 7.4) and gently stirred for 2 hours and then again centrifuged at 9000 rpm for 10 minutes. The CFS was acidified to pH 2 using 2 N HCl and stored overnight at 4°C to precipitate the biosurfactants. The organic component of the biosurfactants was extracted thrice using chloroform/methanol (2:1) and dried after the addition of acetone. The recovered biosurfactants were mixed with PBS and filtered through a 0.22 µm pore size filter. Subsequently, the supernatant was dialyzed against demineralized water using dialysis membranes with a molecular mass cut-off of 12,000-14,000 Da and a pore size of 2.4 nm. Finally, the biosurfactants were freeze-dried and stored at -20°C until further use [15,16].

2.2 Antioxidant activity

Anti-oxidant activity of biosurfactants was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and Phosphomolybdate assay.

2.2.1 DPPH scavenging activity

For the determination of the DPPH free radical-scavenging activity of biosurfactants, 0.2 mM ethanolic DPPH radical solution (HiMedia Laboratories, India) was prepared. Different concentrations of biosurfants samples (1-7mg/mL) were prepared from lyophilized biosurfactants stock sample (10 mg/mL). An aliquot (0.1 mL) of biosurfactant sample (50-600 µg /mL) was added to 3.0 mL of ethanolic DPPH radical solution. The discoloration was measured at 517 nm after incubation in the dark for 30 min. The experiment was repeated in triplicates and ascorbic acid (50-600 µg /mL) (Sigma-Aldrich, Burlington, United States) was used as standard and control was prepared as above without any sample. The percentage of scavenged DPPH radical was calculated using the following Equation (1)[17].

$$Scavenging\ ability\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (1)$$

A_{sample} is the absorbance of the sample, $A_{control}$ is the absorbance of the control.

2.2.2 Phosphomolybdate assay (total antioxidant capacity)

The total antioxidant capacity of the biosurfactants was determined by the phosphomolybdate method using ascorbic acid as a standard. An aliquot of 0.1 mL of biosurfactant sample was mixed with 1 mL of reagent solution (0.6 M sulphuric acid (Lab Chem Pennsylvania, United States), 28 mM sodium phosphate (Molychem, Mumbai, India) and 4 mM ammonium molybdate (Sigma-Aldrich, Burlington, United States) and incubated in a water bath at 95°C for 1.5 hrs. The blank contained 1 mL of the reagent solution and the appropriate volume of the solvent and was incubated under the same conditions. Ascorbic acid (50-600 µg/mL) was used as a standard. Control was prepared as above without any biosurfactant sample and incubated under the same conditions. The absorbance of the sample was measured at 695 nm against a blank upon cooling. The antioxidant capacity was estimated using the following formula Equation (2) [18].

$$\% \text{ Anti-oxidant activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (2)$$

2.3 Antimicrobial activity of biosurfactants

The different concentrations of biosurfactants derived from *Pediococcus pentosaceus* S-2 were evaluated as antimicrobial agents against test micro-organisms including gram-positive, gram-negative bacteria and fungus. The test microorganisms were inoculated into appropriate media and incubated for 24 hrs at 37°C for Staphylococcus aureus (MTCC-96), *Micrococcus luteus* (MTCC-2470), *Escherichia coli* (MTCC-47), *Pseudomonas aeruginosa* (MTCC-424) and 28°C for *Enterobacter cloacae* (MTCC-9145) and *Candida albicans* (MTCC-27).

The 24 hrs grown culture was diluted with sterilized media to match the test suspension with the 0.5 McFarland standard. 50µl of the diluted media was spread on the agar plate. Different concentrations of biosurfactants ranging from (800-5000 µg/ mL) were loaded in different wells, along with the positive control (Ampicillin, Flucozole), Himedia, Mumbai, India) and the negative control (PBS). The MIC was determined as the lowest concentration of biosurfactants where there was no visible growth [19].

2.4 Evaluation of the antibiofilm activity of biosurfactants using ELISA plate

The antibiofilm efficacy of biosurfactants was evaluated against various microorganisms in 96 well ELISA plate. The test micro-organisms were cultured in the presence of different concentrations of minimum inhibitory concentrations (MIC) i.e (0.5 MIC, 1 MIC, 2 MIC and 4 MIC) of biosurfactants, alongside a control, for a duration of 48 hours. Following incubation, planktonic bacteria were eliminated by washing the plates in PBS (pH 7.4) before letting them air dry. 0.1% Crystal violet solution (Himedia, Mumbai, India) was used to stain the biofilms. Plates were dried after being washed in deionized water to eliminate excess stains. To measure the percentage of inhibition of biofilm formation at various MICs, 250 µl of ethanol was added to each well and OD was detected at 570 nm using a micro–Bio Teck ELISA auto reader. Absorbance was compared with untreated test microorganisms.

2.5 Analysis of the anti-biofilm activity of biosurfactants on different micro-organisms by SEM microscopy

The test microorganisms were inoculated into petri plates containing coverslips in their respective media in the presence of different concentrations of biosurfactants (their respective MIC). Test micro-organism was grown without biosurfactants acting as a control. The media was decanted after incubation of 24 hrs and the coverslips were washed with PBS (pH 7.4). The biofilms formed on the coverslips were fixed for 2 hrs with 2.5% glutaraldehyde (Sigma-Aldrich, St. Louis) and air dried. The sample was then placed in an ion sputter, where it was made conductive by gold plating and examined using a JEOL6100 SEM [21].

2.6 Estimation of protein and carbohydrate content in microbial biofilms in the presence of biosurfactants.

The biofilm matrix is primarily composed of extracellular polymeric substances (EPS), including polysaccharides, proteins, nucleic acids, and lipids.

For estimation of protein and carbohydrate content in 200 µL of different micro-organism cultures with approximately 3x 10^8 CFU/mL were inoculated into test tubes containing 10 mL of fresh media. The test tubes were then kept at a suitable temperature for 36 hrs to form biofilms under static conditions in the presence of biosurfactant samples derived from selected strains separately. Test tubes containing test micro-organisms without biosurfactants act as a control.

After 48 hrs, the media was decanted and the biofilms were dissolved in PBS, then vortexed and sonicated. The protein content of biofilm was calculated using Lowry's method and the carbohydrate content of biofilms was calculated using the anthrone method [22].

2.7 Statistical analysis

SPSS software version 2.0 was used for all statistical analyses. All studies were carried out in triplicate and data were presented as mean values with standard deviation. One-way ANOVA was used for analysis of data statistically.

3. Results and discussions

3.1 Antioxidant activity of biosurfactants

3.1.1 DPPH activity of biosurfactant derived from Pediococcus pentosaceus S-2.

The DPPH assay is used to determine the free radical scavenging potential of a test compound. The free radicals are scavenged when DPPH comes into contact with a hydrogen-donating molecule resulting in colour change from purple to yellow with a decrease in absorbance [23]. In the present study, the experiment was conducted in triplicates and DPPH scavenging activity increased with an increased concentration of biosurfactants. It exhibited antioxidant activity of 71 %, while the antioxidant activity of standard ascorbic acid exhibited 86% at the same concentration of 600 µg (Figure 1). A similar study conducted by Giri et. al., reported that Bacillus-derived biosurfactants exhibited 69.1 % DPPH scavenging activity at a dosage of 5.0 mg/mL [24]. According to Jemil et al., lipopeptides derived from Bacillus methylotrophicus had radical scavenging of 80.6% at 1 mg mL^{-1}[25]. Biosurfactants derived from Pediococcus pentosaceus S-2 have better anti-oxidant activity even at low concentrations as compared to the reported studies.

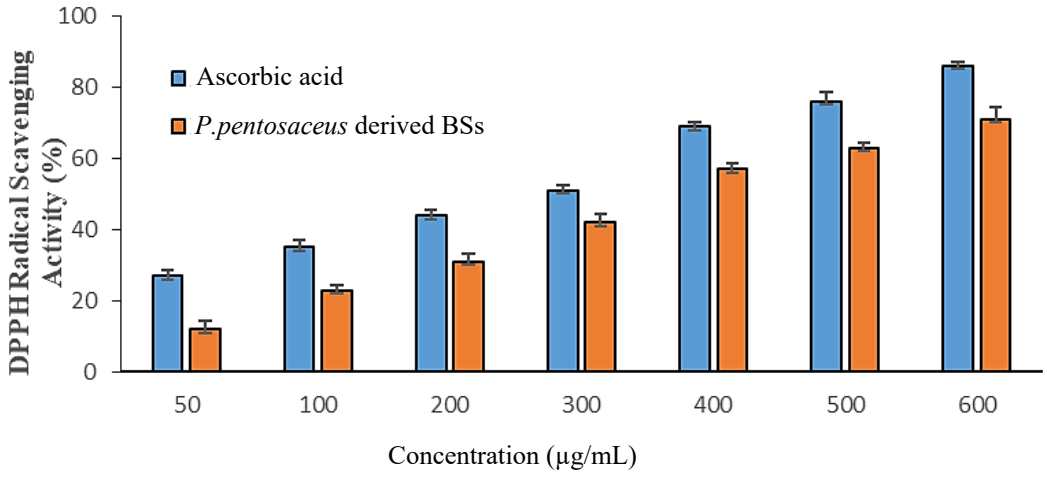

Figure 1 DPPH scavenging activity of biosurfactants derived from *Pediococcus pentosaceus* S-2.

3.2 *Antioxidant activity of biosurfactants*

Total anti-oxidant activity of derived biosurfactants was determined by Phospomolybdate assay. In the present study biosurfactants derived from *P. pntosaceus* S-2 have anti-oxidant activity of 71% while the standard was 88% at a concentration of 600 µg/ mL as depicted in Figure 2.This assay is based on the colour changing from light yellow to green as a result of the test ingredient reducing molybdenum VI to molybdenum V. A similar study was carried out by Bardone *et al.,*(2020) for determination of antioxidant activity (25.47%) of biosurfactants derived from *Candida bombicola* URM 3718 at a concentration of 2000 µg/mL [26]. The difference in the anti-oxidant activity was due to the distinct nature of biosurfactants and the producing micro-organisms. The antioxidant activity of strain *P. pentosaceus* S-2 was found to be more significant even at low concentrations of biosurfactants. Biosurfactants can be employed as antioxidant agents in the food and pharmaceutical sector after a complete analysis of their toxic.

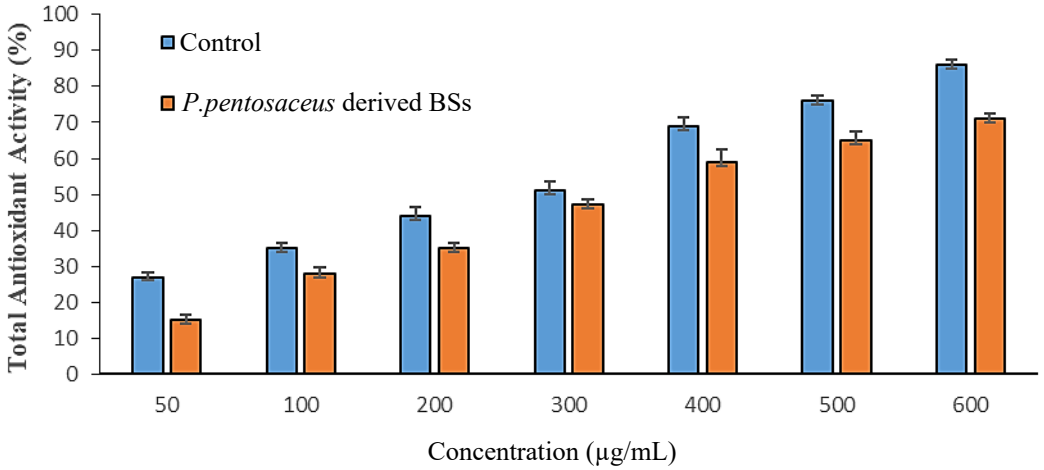

Figure 2 Antioxidant activity of biosurfactants by phosphomolybdate method.

3.3 Antimicrobial activity

The agar diffusion method was used to test the antimicrobial activity of different concentration of biosurfactants (800-5000µg/ mL) derived from *P. pentosaceus* S-2 against different test micro-organisms (*S. aureus*, *M. luteus*, *P. aeruginosa, E. cloacae, E. coli* and *C. albicans*).

Table 1 MIC of biosurfactants against different microorganisms.

	MIC of different compounds (µg/ mL)					
Compound	*Staphylococcus aureus*	*Micrococcus luteus*	*Escherichia coli*	*Enterobacter cloacae*	*Pseudomonas aeruginosa*	*Candida albicans*
P.pentosaceus biosurfactants	1000±5	800±5	1000±4	800± 2	3500±7	5000±7
Antibiotic	Ampicillin 10 ±0.4	6±0.5	8 ±0.3	6±0.4	12±0.4	Flucozole 60±0.6
PBS	-	-	-	-	-	-

The antimicrobial activity of different concentration biosurfactants derived from *P. pentosaceus* S-2 was tested against test micro-organisms. Lipopeptide biosurfactants derived from *P. pentosaceus* S-2 inhibited *M. luteus* and *E. cloacae* at a concentration of 800 µg/mL, while *S. aureus* and *E. coli* were inhibited at the concentration of 1 mg/mL. In the case of *P. aeruginosa* and *C. albicans* concentration of 3500 µg/ mL and 5000 µg/ mL were required respectively. A similar study was carried out by Adnan et al., for biosurfactants derived from *P. pentosaceus* reported MIC in the range from 6250–25000 µg/mL against *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. aureus* and our results were consistent with it [27].

There are very limited studies reported so far for the antimicrobial activity of LAB-derived lipopeptides, but many studies reported the antimicrobial activity of similar lipopeptide biosurfactants produced by *Bacillus* species against different micro-organisms [28]. The variation in MIC was due to the distinct nature of biosurfactants producing micro-organisms. The mechanism of antimicrobial action of biosurfactants is that it may disturb membrane structure through interaction with phospholipids as well as membrane proteins, Lotfabad *et al.,* reported that biosurfactants prevent the protein synthesis by inhibition of the peptidyl transferase by binding mainly the 23S rRNA in the 50s subunit of the bacterial ribosome [29].

3.4 Microscopic analysis of biofilm inhibition by biosurfactants derived from Pediococcus pentosaceus S-2

Figure 3 shows the SEM analysis of thick biofilms developed on untreated and control slides, but these biofilms were found to be reduced and disintegrated in the presence of biosurfactants derived from *P.pentosaceus S-2* the cells also looked to be lysed, indicating that the biosurfactants prevented adhesion and disrupted the biofilms.

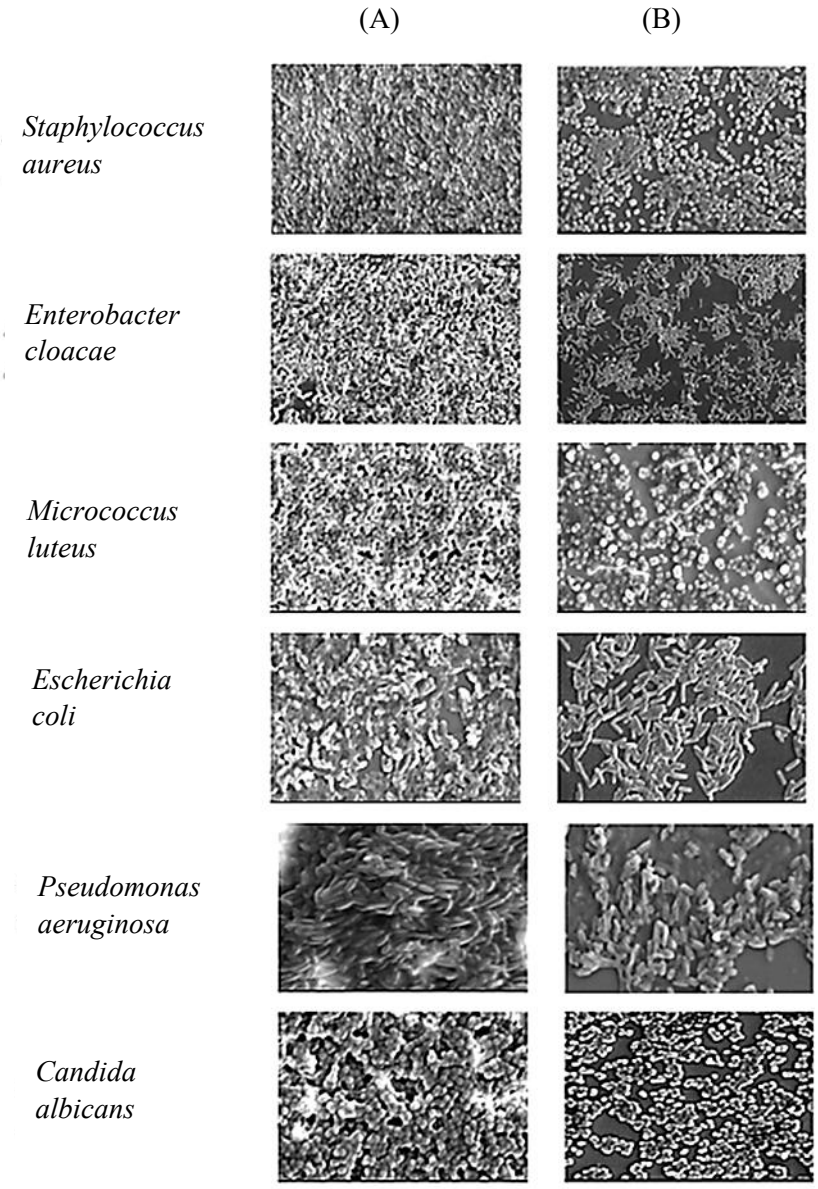

Figure 3 SEM images demonstrating (A) Control, (B) the antibiofilm potential of biosurfactants derived from *Pediococcus pentosaceus* S-2at their respective MIC.

In the present study, biosurfactants derived from *P. pentosaceus* S-2 were investigated for antibiofilm activity against different microorganisms. Derived biosurfactants were found to be effective in the eradication of biofilms of tested micro-organisms. Visualizing under SEM provided the confirmation that cell wall integrity was ruptured and the thickness of the multi-layered biofilm of micro-organisms were greatly reduced. Disruption of biofilms was clearly visible, as all microbial strains failed to form biofilms (Figure 3). Our results were consistent with the results reported by Adnan *et al.,* in terms of disruption of biofilms by biosurfactants [30].

3.6 Evaluation of antibiofilm activity of biosurfactants derived from Pediococcus pentosaceus S-2

The antibiofilm activity of biosurfactants was evaluated by crystal violet staining methods against different microorganisms. The test microorganisms were grown in the presence of different MIC of 0.5 MIC, 1 MIC, 2 MIC and 4 MIC. It was found that biosurfactants derived from *P. pentosaceus* S-2 inhibited the biofilm formation in its presence at MIC of 0.5X MIC, 1X MIC, 2 X MIC, and 4X MIC as compared to the control in all test microorganisms. At 4X MIC, there was 59-65 % of inhibition of the microbial biofilms by *P. pentosaceus* S-2.

Table 2 Antibiofilm activity (%) of biosurfactants derived from *Pediococcus pentosaceus* S-2 at different MIC.

Test microorganisms	0.5 x MIC	1.0x MIC	2.0x MIC	4.0x MIC	*p*-value
Staphylococcus aureus	20±0.3	47±0.5	58±0.7	65±0.3	<0.01
Micrococcus luteus	35±0.4	42±0.6	65±0.8	72±0.5	>0.01
Escherichia coli	37±0.7	46±0.7	59±0.6	65±0.9	<0.01
Enterobacter cloacae	41±0.4	52±0.8	68±0.5	75±0.8	>0.01
Candida albicans	26±0.6	33±0.4	45±0.8	56±0.6	<0.01
Pseudomonas aeruginosa	29±0.5	35±0.5	46±0.4	59±0.5	<0.01

Note: Statistical analysis by one- way ANOVA. The result is significant is significant at p<0.01 except for *Micrococcus luteus and Enterobacter cloacae*

A similar study conducted by Siddiqui *et al,* reported that biosurfactants derived from *L. rhamnosus* inhibited biofilms produced by a variety of pathogens (*B. subtilis*, *P. aeruginosa*, *S. aureus* and *E. coli*) [31]. *Streptococcus thermophilus* and *Lactococcus lactis-derived* biosurfactants have shown inhibitory action on the biofilm growth of harmful microorganisms on vocal prostheses [32]. In this case, *L. paracasei* A20 derived biosurfactants that inhibit gram-positive and gram-negative bacteria, yeasts and filamentous fungi [33]. Biosurfactants act as an emerging therapeutic that efficiently disperses or disrupts microbial biofilms.

3.7 Estimation of protein and carbohydrate content in the extracellular matrix of biofilms treated with biosurfactants derived from Pediococcus pentosaceus S-2

The polymeric matrix of microbial biofilms comprises of extracellular proteins, polysaccharides and DNA. Biosurfactants disrupt biofilm formation by reducing surface tension to prevent initial bacterial attachment and by penetrating the biofilm matrix to destabilize EPS structure, inhibiting biofilm development. To observe the inhibitory effect of biosurfactants on the microbial biofilm formed in test tubes, a reduction in protein and carbohydrate content was determined.

Table 3 Reduction of protein and carbohydrate content after treatment with biosurfactants derived from *Pediococcus pentosaceus* S-2 at their respective MIC.

Microbial biofilm	Reduction of protein content in biofilm (%)	Reduction of the carbohydrate content in biofilm (%)
Enterobacter cloacae	35.6±0.04	29.0±0.07
Candida albicans	20.0±0.13	18.5±0.05
Pseudomonas aeruginosa	20.3±0.20	16.6±0.01
Micrococcus luteus	37.6±0.03	32.4±0.03
Staphylococcus aureus	28.0±0.06	24.0±0.04
Escherichia coli	32.6±0.12	29.4±0.02

Values are mean ± standard error of means

The biofilm matrix of microorganisms mainly consists of polysaccharides, proteins, lipids, and nucleic acids [34]. These extracellular polysaccharide substance (EPS) of the biofilm protects the microorganisms from various antimicrobial agents, so it is very important to reduce the extracellular matrix to reduce the occurrence of biofilms. A similar study conducted by Adnan *et al*., revealed that biosurfactants derived from *P. pentosaceus* significantly reduce the exopolysaccharide matrix of microbial biofilms [35]. Another study conducted by Kim *et al.* reported that biosurfactants derived from *Pseudomonas sp* reduced the carbohydrate and protein content by 31.6 and 79.6%, respectively [36].

4. Conclusions

Biosurfactants are amphiphilic molecules synthesized extracellularly by microorganisms as secondary metabolites. The present study specifically investigates their multifaceted functions ranging from disrupting the extracellular polymeric components of microbial biofilms, to serving as antimicrobials, and antibiofilm agents against many pathogenic micro-organisms. Biosurfactants demonstrate a remarkable ability to strongly inhibit bacterial growth at different concentrations. The antioxidant activity of biosurfactants derived from *P.pentosaceus* S-2 suggests their potential as effective substitutes for synthetic antioxidants. The outcome of the present study suggested that biosurfactants could be exploited further for possible use as potential antioxidant, antimicrobial and anti-adhesive agents against biofilm-forming micro-organisms in the biomedical and food industry.

5. Acknowledgments

The Indian Council of Medical study (ICMR), Delhi, India, provided financial assistance in the form of a fellowship to the first author for study in the present field.

6. Conflicts of interests

No conflict of interest.

7. References

[1] Gaspar P, Carvalho AL, Vinga S, Santos H, Neves AR. From physiology to systems metabolic engineering for the production of biochemicals by lactic acid bacteria. *Biotechnol Adv*. 2013;31(6):764–88.

[2] Bintsis T. Lactic acid bacteria: their applications in foods. J Bacteriol Mycol. 2018;6(2):89–94.

[3] Hajfarajollah H, Eslami P, Mokhtarani B, Akbari Noghabi K. Biosurfactants from probiotic bacteria: A review. Biotechnol Appl Biochem. 2018;65(6):768–83.

[4] Nayarisseri A, Singh P, Singh SK. Screening, isolation and characterization of biosurfactant producing *Bacillus subtilis* strain ANSKLAB03. Bioinformation. 2018;14(6):304–314.

[5] Santos DK, Rufino RD, Luna JM, Santos VA, Sarubbo LA. Biosurfactants: Multifunctional Biomolecules of the 21st Century. Int J Mol Sci. 2016;17(3):401.

[6] Sarubbo LA, Silva MG, Durval IJB, Bezerra KGO, Ribeiro BG, Silva IA, Twigg MS, Banat IM. Biosurfactants: Production, properties, applications, trends, and general perspectives. Biochem Eng J. 2022; 181:108377.

[7] Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. Science. 2002;295(5559):1487.

[8] Davey ME, O'Toole GA. Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev. 2000;64(4):847-867.

[9] Banat IM, Satpute SK, Cameotra SS, Patil R, Nyayanit NV. Cost effective technologies and renewable substrates for biosurfactants' production. Front Microbiol. 2014; 5:697.

[10] Kumar A, Singh SK, Kant C, Verma H, Kumar D, Singh PP, Modi A, Droby S, Kesawat MS, Alavilli H, Bhatia SK, Saratale GD, Saratale RG, Chung SM, Kumar M. Microbial biosurfactant: A New Frontier for sustainable agriculture and pharmaceutical industries. Antioxidants (Basel). 2021;10(9):1472.

[11] Bjerk TR, Severino P, Jain S, Marques C, Silva AM, Pashirova T, Souto EB. Biosurfactants: Properties and applications in drug delivery, biotechnology and ecotoxicology. Bioengineering (Basel). 2021;8(8):115.

[12] Kaundal T, Sharma A, Batra N. Isolation of Biosurfactant Producing *Pediococcus pentosacaeus* from Laboratory Controlled (Simulated) Fermentation of Indian Wheat-based Seera. Curr J Appl Sci Technol. 2022;41(26):12–23.

[13] Kanwar SS, Bhushan K. Ethnic Fermented Foods and Beverages of Himachal Pradesh. In: Tamang J, editor. Ethnic fermented foods and beverages of india: Science history and culture. Singapore: Springer; 2020.

[14] Savitri, Thakur N, Kumar D, Bhalla TC. Microbiological and biochemical characterization of Seera: A traditional fermented food of Himachal Pradesh. Int J Food Ferment Technol. 2012;2(1):49–56.

[15] Augustin M, Majeste P, Hippolyte M, Leopold T. Effect of Biosurfactants Extracted from a locally fermented milk (Pendidam) on Its Shelf Life. J Adv Biol Biotechnol. 2015;3(1):12–22.

[16] Satpute S, Mone N, Das P, Banpurkar A, Banat I. Lactobacillus acidophilus Derived Biosurfactant as a Biofilm Inhibitor: A promising investigation using microfluidic approach. Appl Sci. 2018;8(9):1555.

[17] Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. Molecules. 2022;27(4):1326.

[18] Khatoon M, Islam E, Islam R, Rahman AA, Alam AHMK, Khondkar P, et al. Estimation of total phenol and *in vitro* antioxidant activity of *Albizia procera* leaves. BMC Res Notes. 2013;6(1):121.

[19] Magaldi S, Mata-Essayag S, Hartung C. Well diffusion for antifungal susceptibility testing. Int J Infect Dis. 2004; 8:39–45.

[20] Kahlmeter G, Brown D, Goldstein F, Macgowan A, Mouton J, Odenholt I, et al. European committee on antimicrobial susceptibility testing (EUCAST) technical notes on antimicrobial susceptibility testing. Clin Microbiol Infect. 2006; 12:501–503.

[21] Yan X, Gu S, Cui X, Shi Y, Wen S, Chen H, Ge J. Antimicrobial, anti-adhesive and anti-biofilm potential of biosurfactants isolated from *Pediococcus acidilactici* and *Lactobacillus plantarum* against *Staphylococcus aureus* CMCC26003. Microb Pathog. 2019; 127:12–20.

[22] Wilson C, Lukowicz R, Merchant S, Valquier-Flynn H, Caballero J, Sandoval J, et al. Quantitative and qualitative assessment methods for biofilm growth: A Mini-review. Res Rev J Eng Technol. 2017;6(4):1-12.

[23] Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthone on the auto oxidation of soybean in cyclodextrin emulsion. J Agric Food Chem. 1992;40(1):945–948.

[24] Giri S, Ryu E, Sukumaran V, Park S. Antioxidant, antibacterial, and anti-adhesive activities of biosurfactants isolated from *Bacillus* strains. Microb Pathog. 2019; 132:66–72.

[25] Jemil N, Ayed HB, Manresa A, Nasri M, Hmidet N. Antioxidant properties, antimicrobial and anti-adhesive activities of DCS1 lipopeptides from *Bacillus methylotrophicus* DCS1. BMC Microbiol. 2017;17(1):144.

[26] Bardone E, Marzocchella A, Bravi M, Silva I, Gercyane K, Bezerra O, et al. Evaluation of the emulsifying and antioxidant capacity of the biosurfactant produced by *Candida bombicola* URM 3718. Chem Eng Trans. 2020; 79:355–360.

[27] Adnan M, Siddiqui AJ, Hamadou WS, Ashraf SA, Hassan MI, Snoussi M, et al. Functional and structural characterization of *Pediococcus pentosaceus*-derived biosurfactant and its biomedical potential against bacterial adhesion, quorum sensing, and biofilm formation. Antibiotics (Basel). 2021;10(11):1371.

[28] Jenny K, Kappeli O, Fiechter A. Biosurfactant from *Bacillus licheniformis*: structural analysis and characterization. Appl Microbiol Biotechnol. 1991;36(1):5–13.

[29] Lotfabad TB, Abassi H, Ahmadkhaniha R, Roostaazad F, Masoomi HS, Zahiri G, et al. Structural characterization of a rhamnolipid-type biosurfactant produced by *Pseudomonas aeruginosa* MR01: Enhancement of di-rhamnolipid proportion using gamma irradiation. Colloids Surf B Biointerfaces. 2011;81(2):397–405.

[30] Adnan M, Siddiqui AJ, Hamadou WS, Ashraf SA, Hassan MI, Snoussi M, et al. Functional and structural characterization of *Pediococcus pentosaceus*-derived biosurfactant and its biomedical potential against bacterial adhesion, quorum sensing, and biofilm formation. Antibiotics. 2021;10(11):1371.

[31] Siddiqui AJ, Hamadou WS, Surti M, Awadelkareem AM, Ashraf SA, et al. Inhibition of bacterial adhesion and antibiofilm activities of a glycolipid biosurfactant from *Lactobacillus rhamnosus* with its physicochemical and functional properties. Antibiotics (Basel). 2021;10(12):1546.

[32] Rodrigues L, Teixeira JA, Oliveira R. Biosurfactant from *Lactococcus lactis* 53 inhibits microbial adhesion on silicone rubber. Appl Microbiol Biotechnol. 2004;66(3):306–311.

[33] Gudiña EJ, Teixeira JA, Rodrigues LR. Isolation and functional characterization of a biosurfactant produced by *Lactobacillus paracasei.* Colloids Surf B Biointerfaces. 2010;76(1):298–304.

[34] Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623–633.

[35] Adnan M, Siddiqui AJ, Hamadou WS, Ashraf SA, Hassan MI, Snoussi M, et al. Functional and structural characterization of *Pediococcus pentosaceus*-derived biosurfactant and its biomedical potential against bacterial adhesion, quorum sensing, and biofilm formation. *Antibiotics (Basel).* 2021;10(11):1371.

[36] Kim LH, Jung Y, Kim SJ, Kim CM, Yu HW, Park HD, et al. Use of rhamnolipid biosurfactant for membrane biofouling prevention and cleaning. Biofouling. 2021;31(2):211–220.