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RESEARCH ARTICLE

Antibacterial effect of postbiotics derived from *Lactobacillus plantarum* against biofilm forming bacteria

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Abstract: The present study evaluated the antibacterial and antibiofilm potential of postbiotics derived from *Lactobacillus plantarum* (UBLP-40) against two biofilm-forming bacteria of significance in dairy processing environment: *Bacillus subtilis* (MTCC 441) and *Pseudomonas aeruginosa* (MTCC 424). Postbiotics were prepared by culturing *L. plantarum* in de Man, Rogosa, and Sharpe (MRS) broth, followed by heat inactivation, ultrasonication, centrifugation, sterile filtration and lyophilization. The biofilm-forming ability of the test organisms was assessed using the tissue culture plate method with crystal violet staining. Optical density measurements at 595 nm indicated that *B. subtilis* (OD = 0.122) was a moderate biofilm former, while *P. aeruginosa* (OD = 0.306) was a strong biofilm former. Minimum inhibitory concentration (MIC) values of the postbiotic preparation were determined to be 500 mg/mL for *B. subtilis* and 400 mg/mL for *P. aeruginosa*. The minimum biofilm inhibitory concentration (MBIC) was identified as 150 mg/mL for both strains. Furthermore, a dose-dependent inhibition of pre-formed biofilm was observed at postbiotic concentrations of 700, 800, and 900 mg/mL. At 900 mg/mL, the postbiotic reduced biofilm biomass by 68.97 per cent in *B. subtilis* and 80.20 per cent in *P. aeruginosa*, with all reductions being statistically significant ($p < 0.05$). These findings demonstrate the significant antimicrobial and antibiofilm activities of *L. plantarum*-derived postbiotics and suggest their potential application as natural and safe biocontrol agents for enhancing hygiene and microbial safety in dairy and food processing environments.

Keywords: Biofilm, *B. subtilis, L. plantarum*, Postbiotics, *P. aeruginosa*

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Introduction

Biofilm is defined as a community of microorganisms attached to an inert or living surface by a self-produced polymeric matrix. These biofilms have a base of minerals, such as calcium and iron and organic materials including proteins and butterfat. These deposits adhere to dairy processing equipment and provide an anchorage for bacteria to attach. Biofilm formation begins with bacteria adhering to a surface, a process influenced by the cell surface hydrophobicity of pathogens. Subsequently, these pathogens form multicellular structures, connected through auto-aggregation and exopolysaccharide (EPS) production. The resulting biofilm protects pathogens from antibiotics, desiccation, extreme temperatures and competing microorganisms ultimately making the biofilm difficult to remove. The main source of contamination of dairy products is often associated with the formation of biofilms on the surfaces of milk transport pipes, milking containers and accessories in the dairy industry. Biofilms are established mainly under conditions that allow bacteria to easily adhere to the walls of the pipes, for example, when the milk is found in transport pipes without flow. The bacteria may detach from biofilms and contaminate the milk as it passes surfaces. Moreover, biofilm forming bacteria can also increase the corrosion of metal pipes, reduce heat transfer and increase fluid frictional resistance. Effective control of biofilms in dairy processing facilities is critical to prevent contamination of food with spoilage and pathogenic bacteria. In the dairy industry, biofilms can be formed by heat-resistant spoilage and pathogenic bacteria. Consequently, the need for the development of new antibiofilm agents is of major importance in order to reduce the risk of contamination in dairy sector.

Probiotic bacteria produce substances known as postbiotics during the fermentation process. Postbiotics have generally demonstrated all the positive benefits of probiotics, including

immune system enhancement, antibacterial, antiviral, antioxidant and antifungal activities. Postbiotics refer to the metabolic by-products produced by probiotic microorganisms during their growth, which may have beneficial effects on human health. They include a variety of substances like short-chain fatty acids (SCFAs), proteins, enzymes and polysaccharides. Postbiotics is an emerging concept in the food industry, but exciting prospects of the application of Lactic Acid Bacteria (LAB) derived postbiotics lie ahead. Postbiotics as biologically active molecules with multi-functional properties can be designed as a novel, attractive and preventive strategy in the prevention of biofilm. However, studies related to the antibiofilm potential of postbiotics are relatively scarce. The aim of the present study was to determine the effect of postbiotics derived from *Lactobacillus plantarum* (*L. plantarum*) against biofilm forming bacteria in dairy industry.

Materials and Methods

Collection and maintenance of bacterial cultures

The freeze-dried cultures of *Bacillus subtilis* and *Pseudomonas aeruginosa* procured from MTCC, Chandigarh were propagated in sterilized nutrient broth by incubating at 37°C for 24 h. After sufficient growth, it was sub cultured at weekly intervals on nutrient agar and stored at 5°C. Probiotic *Lactobacillus plantarum* (UBLP-40) procured from Unique Biotech was aseptically transferred in to skim milk and sub cultured at weekly intervals and stored at 4°C.

Preparation of postbiotics from *Lactobacillus plantarum*

L. plantarum (UBLP-40) culture was transferred to MRS broth and incubated at 37±1°C for 48 h in a CO_2 incubator. Then the bacterial suspension was inactivated at 121°C for 15 min. The inactivated suspension was ultra sonicated in an ice bath at 300 W, with the cycles of 10s on/10s off for 15 min. Microscopic examination was done to confirm the absence of intact bacteria. The suspension was centrifuged for 10 min at 6000 rpm at 4°C to remove the bacterial fragments. The cell free supernatant was harvested and filtered through a 0.45µm and 0.22µm membrane filter and the filtered cell free supernatant was lyophilized (freezing temperature: "40 °C, pump pressure: 100 mTorr and shelf temperature: "60 °C) and used as CFS (postbiotics containing material) in the next experiments (Moradi *et al*. 2019).

Biofilm forming ability of *Bacillus and Pseudomonas*

Isolates from fresh agar plates were introduced into Brain Heart Infusion (BHI) broth containing 2% sucrose and incubated under stationary conditions at 37°C for 18–24 hours. The turbid broth was then diluted 1:100 with fresh medium. Flat-bottom polystyrene plate wells were filled with 0.2 ml of the diluted cultures, while broth alone was used as a control to assess sterility and nonspecific medium binding (negative control). The plates were incubated at 37°C for 24 hours. After incubation, the well contents were carefully removed, and the wells were washed four times with 0.2 ml of PBS, (pH 7.2) to eliminate free-floating (planktonic) bacteria. Biofilm formed by adherent (sessile) bacteria were fixed using 2% sodium acetate for 30 minutes and subsequently stained with 0.1% (w/v) crystal violet for the same duration. Excess stain was removed by thoroughly washing with deionized water, followed by drying. Adherent bacterial cells typically formed biofilm along all sides of the wells and were uniformly stained with crystal violet. The optical density (OD) of stained adherent bacteria was measured at 595 nm using a microplate Enzyme Linked Immunosorbent Assay (ELISA) auto-reader (Panda *et al*. 2016). The cut-off OD value was defined by the negative control (ODc). Based on the bacterial biofilm-forming activity, the isolates were classified as follows: (Table 1).

Minimum Inhibitory Concentration (MIC)

The MIC of the postbiotic extract against *P. aeruginosa* and *B. subtilis* were measured using the broth microdilution method described by Ozma *et al*. (2024) with some modifications. This approach used 96-well microplates and nutrient broth medium. Postbiotic dilutions ranging from 300 to 1000 mg/ml were prepared. A 180µL of 0.5 McFarland turbidity standard (1.5×10^8CFU/mL) of *B. subtilis* and *P. aeruginosa* along with 20µL of different concentrations of postbiotics were added to each well and incubated for 24 hours at 37°C in an aerobic atmosphere. The MIC was calculated by checking each well for the presence of turbidity.

Minimum biofilm inhibitory concentration (mg/mL)

Minimum biofilm inhibitory concentration is the minimum concentration of the antimicrobial agent required to produce an absorbance reduction less than or equal to 10 per cent of the mean positive control. The assay was performed using 96 well microtiter plate as per the procedure suggested by Haney *et al*. (2018) with some modifications. The wells were filled with 100µL of various dilutions of postbiotic solution from *L. plantarum*

Table 1: Categorization of biofilms based on optical density

OD ≤ ODc	No biofilm
ODc< OD < 2 OD	Weak biofilm
2 ODc< OD < 4 ODc	Moderate biofilm
OD > 4 ODc	Strong biofilm

ODc - OD value of negative control

Table 2: Biofilm forming ability of *B. subtilis*

Sl No.	Bacteria	Mean OD ±SE	P-value
1	*B. subtilis*	0.122±0.00	0.014*
2	Negative control	0.048±0.00	

* Significant at 0.05 level

and 20µL of diluted (0.5 Mc Farland) overnight cultures of *B. subtilis* and *P. aeruginosa* in Brain Heart Infusion (BHI) broth containing 2 per cent sucrose. Negative control wells were filled with 200µL of treated solutions of respective dilution only and positive control wells were inoculated with 200 µL of isolates only. Duplicate of each treatment were incubated for 48h at 37°C under aerobic conditions. The antimicrobial solutions were discarded and the wells were washed three times with PBS (pH 7.2) and stained by 200 µl of crystal violet solution (0.1% w/v) at 25°C for 20 min. After washing the crystal violet, 200 µl of 99 per cent ethanol was added to the well, and the absorbance was measured at 595 nm.

Antibiofilm potential of postbiotics against *Bacillus* spp. and *Pseudomonas* spp.

The anti-biofilm activity of postbiotics against the test organisms was determined as per the method described by Kim *et al*. (2024) with some modifications. To measure the anti-biofilm activity, 100 µL each of *Bacillus* and *Pseudomonas* cultures at 1×10^8 colony-forming units (CFU/mL) in nutrient broth were dispensed to the 96-well plates. Next, 100 µl of postbiotic solution was added and absorbance was measured at 595 nm after incubation at 37°C for 24 h. Negative control wells were added with 200µL of uninoculated broth only and positive control wells were inoculated with 200 µL of isolates only. Afterwards, the culture was removed and gently washed thrice with PBS. The adherent biofilm layer was stained with 200 µl of 0.1 percent crystal violet at 25°C for 20 min. After washing the crystal violet thrice with PBS, 200 µl of 99 percent ethanol was added to the well and the absorbance was measured at 595 nm.

Results and Discussion

Biofilm forming ability of *Bacillus* spp.

The biofilm forming ability of *Bacillus subtilis* (*B. subtilis*) was assessed by tissue culture plate method. The optical density (OD) of stained adherent bacteria was measured at 595 nm using a microplate Enzyme-Linked Immunosorbent Assay (ELISA) auto-reader (Fig.1). OD value of *B. subtilis* was 0.122. OD value of *B. subtilis* indicated a moderate biofilm forming ability. The mean OD values of the bacteria are presented in the Table 2. In a study conducted by Singh *et al.* (2016) *B. subtilis* showed OD values ranging from 0.12 to 0.46 at 595nm under nutrient-limited conditions, indicating moderate to strong biofilm formation. Similarly, Shukla and Rao (2017) reported OD value of 0.128 ± 0.03 at 595nm in LB medium after 48 h of incubation at 30°C. The results of the present research study are in agreement with previous research findings and confirm the moderate biofilm forming ability of *B. subtilis*.

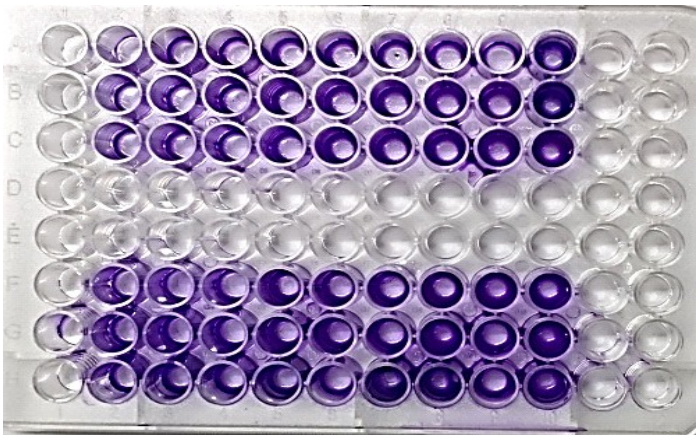

Fig. 1: Biofilm formation of *B. subtilis* and *P. aeruginosa* in 96 well plate using crystal violet assay

Biofilm forming ability of *Pseudomonas* spp.

The ability of *Pseudomonas aeruginosa* (*P. aeruginosa*) to produce biofilm was tested using tissue culture plate method. The OD value of stained adherent bacteria was measured at 595 nm with a microplate Enzyme-Linked Immunosorbent Assay (ELISA) auto-reader (Fig. 1). OD value of *P. aeruginosa* was 0.306. *P. aeruginosa* formed a strong biofilm. The average OD value of *P. aeruginosa* was shown in Table 3. A study by Hassan *et al*. (2011) reported OD values ranging from 0.29 to 0.61 among *P. aeruginosa* isolates, with clinical strains generally showed stronger biofilm-forming tendencies. Moreover, Nagaveni *et al.* (2010) reported that all strains of *P. aeruginosa* associated with biomedical device infections were capable of producing biofilms. They tested 12 clinical isolates of *P. aeruginosa* for their ability to form biofilms using the microtiter plate assay method. Out of the 12 isolates, 5 (41.6%) were identified as strong biofilm producers, 4 (33.3%) as moderate producers and 3 (25%) as non or weak biofilm producers. The similarity between current and previous findings strengthens the evidence for the biofilm forming ability of *P. aeruginosa.*

Minimum inhibitory concentration (MIC) of postbiotics against *B. subtilis*

The MIC of postbiotics against *B. subtilis* was determined using the broth microdilution method. Among the tested concentrations, the least turbidity indicating maximum inhibition of bacterial growth was observed at 500 mg/mL (Fig 2). This suggests that a concentration of 500 mg/mL of postbiotics effectively inhibited the growth of *B. subtilis* under the experimental conditions. In a study by Khosravi *et al*. (2023), the antimicrobial potential of postbiotics derived from various lactic acid bacteria, including *L. plantarum*, was evaluated against several pathogens using the micro broth dilution technique. MIC values varied between strains but ranged from 125 to 500 mg/mL against Gram-positive bacteria, including *B. subtilis.* Similarly, in

a study conducted by Fakharian *et al*. (2024), postbiotics produced by *L. plantarum* in low-cost media such as cheese whey and skim milk showed inhibitory activity against *B. subtilis*, with MICs ranging between 250 and 500 mg/mL depending on the substrate. The relatively high MIC observed in this study suggests opportunities for optimizing postbiotic formulations to enhance potency and reduce effective dosage. The findings of this study are in agreement with the results reported by different researchers.

Minimum inhibitory concentration (MIC) against *P. aeruginosa*

The MIC assessment of postbiotics against *P. aeruginosa*, revealed that the least turbidity was achieved at a concentration of 400 mg/mL (fig 2). This finding indicated that 400 mg/mL of postbiotics was sufficient to substantially inhibit the growth of *P. aeruginosa* in the broth microdilution assay. According to Khiralla *et al.* (2015), postbiotics of *L. plantarum* showed the least amount of inhibition against *P. aeruginosa* at a dose of 90 mg/mL in 96-well plates using a micro-dilution method. Gutiérrez *et al.* (2020) reported that the minimum inhibitory concentration of a crude biosurfactant derived from *L. plantarum* against *P. aeruginosa* was 6.25 mg/mL. Together, these studies highlight the antimicrobial potential of postbiotics derived from *L. plantarum* with variations in efficacy depending on the method of preparation and experimental conditions. While postbiotics generally demonstrate promising activity at lower concentrations, the observed differences in MIC values between various studies indicate the need for further research in the area of optimization of the methodology for the extraction and formulation of *L. plantarum* postbiotics and biosurfactants.

Minimum biofilm inhibitory concentration (MBIC) of postbiotics against *B. subtilis*

The MBIC of postbiotics against *B. subtilis* biofilm following 48 h of incubation is presented in Table 4. A reduction of biofilm formation by less than or equal to 10 per cent of the mean OD value of the positive control was considered indicative of MBIC. The results demonstrated that postbiotics effectively inhibited *B. subtilis* biofilm at a concentration of 150 mg/mL, as evidenced by the significant reduction in OD values when compared to the control group. Gudiña *et al*. (2015) found that biosurfactants and metabolites produced by *L. plantarum* significantly reduced the biofilm biomass of several Gram-positive strains, although *B. subtilis* was not specifically tested Similarly, Choi *et al*. (2018) reported inhibition of *Listeria monocytogenes* biofilm by *L. plantarum* derived postbiotics at concentrations ranging from 50–150 mg/mL The findings of the current study are in agreement with earlier reports. *L. plantarum* postbiotics possessed significant biofilm-inhibiting activity against *B. subtilis*. The effectiveness of these postbiotics against *B. subtilis* at an MBIC of 150 mg/mL is consistent with the reported MBIC in other Gram-positive bacteria, including *Listeria monocytogenes* and *Staphylococcus aureus*.

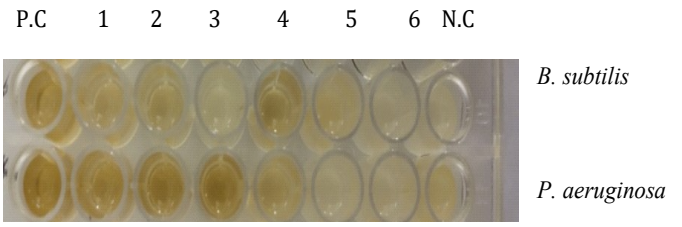

Fig. 2 Minimum Inhibitory Concentration (mg/mL) against *B. subtilis* and *P. aeruginosa*

P.C- Positive control, N.C- Negative control, 1-6 are different concentrations of postbiotics from *L. plantarum* 1-200 mg/mL, 2-300mg/mL, 3-400mg/mL, 4-500mg/mL, 5-600mg/mL, 6-700mg/mL

Table.3 Biofilm forming ability of and *P. aeruginosa*

Sl No.	Bacteria	Mean OD ±SE	P-value
1	*P. aeruginosa*	0.306±0.01	1.00^{ns}
2	Negative control	0.048±0.00	

ns not-significant at 0.05

Minimum biofilm inhibitory concentration (MBIC) of postbiotics against *P. aeruginosa*

Similarly, the MBIC of postbiotics against *P. aeruginosa* biofilm is presented in Table 5. The results revealed that postbiotics effectively suppressed the *P. aeruginosa* biofilm at a dosage of 150 mg/mL, as seen by the considerable drop in OD values when compared to the control group. A comparable study by Cramton *et al*. (2019) found that *L. plantarum* derived postbiotics disrupted biofilm formation by various Gram-negative bacteria, including *P. aeruginosa*. Their results showed that CFS from *L. plantarum* significantly reduced biofilm formation, with MBIC values ranging between 50 and 150 mg/mL Pereira *et al.* (2020) explored the effect of *L. plantarum* postbiotics on biofilm formation by *Staphylococcus aureus*. Their study revealed that MBIC of *L. plantarum* postbiotics on *S. aureus* biofilm was 150 mg/mL. The relatively low concentration required for biofilm inhibition suggests that *L. plantarum* postbiotics may offer an alternative to traditional detergents, which are often less effective against biofilms due to poor penetration and bacterial resistance mechanisms.

Antibiofilm potential of postbiotics against *B.subtilis*

The antibiofilm activity of postbiotics derived from *L. plantarum* against *B. subtilis* and *P. aeruginosa* biofilm were evaluated at concentrations of 700, 800 and 900 mg/mL. The mean OD values and biofilm reduction per cent are presented in table 6. At a postbiotics concentration of 900 mg/mL, maximum anti biofilm activity was observed (Fig. 3). The results demonstrated a clear

concentration-dependent inhibitory effect, with higher concentrations leading to a more pronounced reduction in biofilm biomass. The mechanism of prevention of biofilm could be attributed to interference with initial adhesion, disruption of extracellular polymeric substance integrity, acidification of the microenvironment and inhibition of quorum sensing signals. Dimopoulou *et al*. (2018) highlighted the potential of postbiotics from *L. plantarum* in inhibiting biofilm formation by Gram-positive bacteria. Their study reported substantial inhibition at concentrations above 700/ mg/mL, consistent with the present results showing inhibition at 700/ mg/mL and above. Wang *et al*. (2019) found that the postbiotics of *L. plantarum* significantly delayed and reduced biofilm formation by *Bacillus subtilis* at concentrations at or above 500 mg/mL, indicating that bioactive metabolites such as lactic acid, bacteriocins and small peptides might have triggered the observed inhibition. Moreover, Yang *et al*. (2021) demonstrated that postbiotics produced by *L. plantarum* strains were capable of disrupting bacterial adhesion at concentrations starting from 500/ mg/mL. De Souza *et al*. (2022) reported that antimicrobial peptides produced by *L. plantarum* inhibited *Bacillus subtilis* biofilm formation at concentrations between 500 and 1000/ mg/mL. Pham *et al*. (2022) observed a significant reduction in *B. subtilis* biofilm density when treated with postbiotics at 400–800/ mg/mL. From these findings, it can be concluded that postbiotics derived from *L. plantarum* are highly effective in inhibiting biofilm formation by *B. subtilis*. The postbiotic concentrations evaluated in the present study were effective in preventing the biofilm. The results obtained in the present study reaffirm the earlier findings.

Antibiofilm potential of postbiotics against *P. aeruginosa*

The antibiofilm activity of postbiotics derived from *L. plantarum* against *P. aeruginosa* biofilm was evaluated at concentrations of 700, 800 and 900 mg/mL. The mean OD values and biofilm reduction per cent are presented in table 7. At a postbiotic concentration of 900 mg/mL, maximum anti biofilm activity was observed (Fig. 3). Previous research has similarly highlighted the ability of *L. plantarum* derivatives to interfere with biofilm development. Khiralla *et al*. (2015) demonstrated a significant reduction in *P. aeruginosa* biofilm after exposure to 600/ µL of *L. plantarum* postbiotics, as reflected by a low optical density

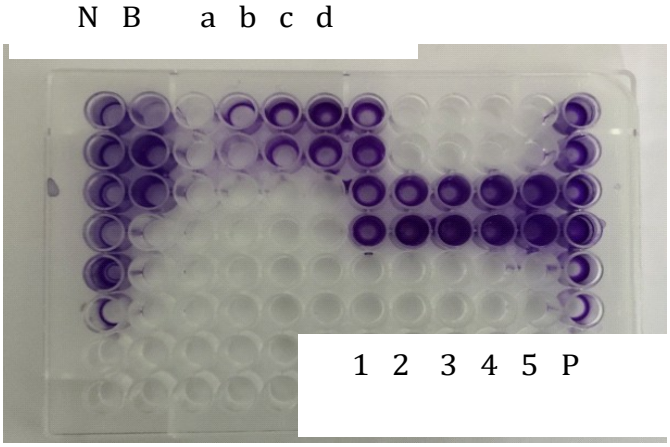

Fig .3 Antibiofilm activity of postbiotics against *B. subtilis* and *P. aeruginosa*

N-Negative control, B- Positive control of *B. subtilis*, a-d are different concentrations of postbiotics from *L. plantarum*against *B. subtilis* a-600mg/mL, b -700mg/mL, c-800mg/mL, d-900mg/mL, 1-5 are different concentrations of postbiotics from *L. plantarum*against *P. aeruginosa*1-600mg/mL, 2 -700mg/mL, 3-800mg/mL, 4-900mg/mL,5-500mg/mL P-Positive control of *P. aeruginosa*

Table 4. Minimum Biofilm Inhibitory Concentration values of postbiotics from *L. plantarum* against *B. subtilis*

Sl. No.	Concentration of postbiotics derived from *L. plantarum* (mg/mL)	Mean OD value ±SE
1	150	0.086^{a}±0.00
2	100	0.105^{b}±0.00
3	75	0.111^{b}±0.00
4	Negative control	0.032^{c}±0.00
5	Positive control	0.109^{b}±0.00

Means bearing different superscripts between columns differ significantly ($p<0.05$).

Table 5. Minimum Biofilm Inhibitory Concentration values of postbiotics from *L. plantarum* against *P. aeruginosa*

Sl.No.	Concentration of postbiotics derived from *L. plantarum* (mg/mL)	Mean OD value ±SE
1	150	0.137^{a}±0.00
2	100	0.151^{b}±0.00
3	75	0.157^{b}±0.00
4	Negative control	0.032^{c}±0.00
5	Positive control	0.165^{d}±0.00

Means bearing different superscripts between columns differ significantly ($p<0.05$)

Table 6. Antibiofilm activity of posbiotics against *B.subtilis*

Sl No.	Concentration of postbiotics derived from *L. plantarum* (mg/mL)	Mean OD Value ± SE	Reduction (%)
1	700	0.056 [a]±0.00	51.72
2	800	0.044 [b]±0.00	62.07
3	900	0.036 [b]±0.00	68.97
4	Negative control	0.043 [c]±0.00	
5	Positive control	0.116 [b]±0.00	

Means bearing different superscripts between columns differ significantly ($p<0.05$).

Table 7. Antibiofilm activity against *P. aeruginosa*

Sl No.	Concentration of postbiotics derived from *L. plantarum* (mg/mL)	Mean OD Value ± SE	Reduction (%)
1	700	0.071[b] ±0.00	64.85
2	800	0.046[a] ±0.00	77.23
3	900	0.040[a] ±0.00	80.20
4	Negative control	0.045[a] ±0.00	
5	Positive control	0.202[c] ±0.00	

Means bearing different superscripts between columns differ significantly ($p<0.05$)

value, suggesting strong antibiofilm activity. In another study, Shokri *et al*. (2018) reported a variable inhibition of *P. aeruginosa* biofilm mass, ranging from 0 to 64 per cent, at a concentration of 400/ mg/mL of postbiotics from Lactobacillus strains. Furthermore, Li *et al*. (2020) observed notable biofilm suppression at concentrations between 50 and 100/ mg/mL of *L. plantarum* cell-free supernatants. Compared to these studies, the concentrations used in the current research work were considerably higher, which might have contributed to the enhanced antibiofilm effect observed. The increased presence of antimicrobial compounds such as organic acids, bacteriocins and other bioactive metabolites at higher concentrations might have caused more effective disruption of the extracellular polymeric matrix and interference with microbial adhesion and communication. These findings reinforce the potential of *L. plantarum* postbiotics as promising biofilm control agents, particularly in the context of *P. aeruginosa*, a pathogen well-known for its resistance to conventional detergents.

Conclusion

This study highlights the novel application of postbiotics derived from *Lactobacillus plantarum* (UBLP-40) as effective antibacterial and antibiofilm agents against two significant biofilm forming bacteria, *Bacillus subtilis* and *Pseudomonas aeruginosa*, commonly found in dairy processing environments. Unlike traditional antimicrobial approaches that often rely on chemical sanitizers or antibiotics, this research underscores the potential of heat-inactivated, cell-free postbiotic preparations as natural, safe and sustainable alternatives for biofilm control. The demonstration of strong, dose-dependent inhibition of both biofilm formation and established biofilms especially against the notoriously resilient *P. aeruginosa* sets this study apart from previous works that have largely focused on live probiotic cultures or crude extracts. The identification of specific MIC and MBIC values adds quantitative depth to the understanding of postbiotic efficacy. Overall, this work provides absolute evidence for the incorporation of *L. plantarum* derived postbiotics in food industry hygiene protocols, contributing both to food safety and to the growing field of postbiotic-based biocontrol strategies. The findings pave the way for future investigations into the mechanistic aspects and industrial scalability of postbiotic applications in biofilm management

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