

Antibiofilm Activity of *Lactobacillus acidophilus* Derived Bacteriocin against Biofilm-Forming Clinical Pathogens

Sawas Younus Hammadi

Department of Pathological Analyses, College of Applied Sciences, University of Samarra, Samarra, Iraq.

*Corresponding authors' email: sshhfriends0770@gmail.com

ABSTRACT

Biofilm-producing bacteria infections have been shown to be a great challenge in the clinic because of the high tolerance of the biofilm to antibiotics and host immune system, hence resulting in antimicrobial resistance. Consequently, natural antibiotics with both antibacterial and antibiofilm abilities are greatly needed. In this study, we investigated the antibacterial and antibiofilm activities of the crude bacteriocin produced by *Lactobacillus acidophilus* against biofilm producing pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. Bacteriocin were prepared from overnight cultures using procedures that involved centrifugation, neutralization, catalase treatment, and membrane filtration of cultures. The activities of bacteriocin were determined using activity units (AU/mL) with *Lactobacillus plantarum* as an indicator strain. Antibacterial activity was performed using agar well diffusion method and minimum inhibitory concentration (MIC), while antibiofilm activity was done using crystal violet microtiter plate. The bacteriocin has the ability to inhibit all the tested pathogens with inhibition zones of 15.0 ± 0.9 to 22.0 ± 1.2 mm. The bacteriocin has the highest activity against *S. aureus* while *K. pneumoniae* was found to be the least susceptible. The MIC values for the strains tested ranged from 25 to 100 AU/mL. Also, the bacteriocin was able to inhibit biofilm formation by $81.5 \pm 2.1\%$, $72.3 \pm 1.8\%$, $68.4 \pm 2.0\%$, and $63.7 \pm 1.9\%$ against *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*, respectively ($P < 0.05$). Antibiofilm activity was found to increase dose-dependently with its maximum at 100 AU/mL.

Received: 02 Sept. 2026

Accepted: 09 Sept. 2026

Published: 07 Oct. 2026

Keywords: *Lactobacillus acidophilus*, Bacteriocin, Biofilm, Antimicrobial Peptides, Multidrug-Resistant Bacteria

INTRODUCTION

Antimicrobial resistance (AMR) has become a major threat to the effectiveness of modern antibiotic therapy. Although antibiotics have significantly reduced mortality from bacterial infections, the continued selection and spread of resistant organisms have gradually diminished the effectiveness of many established treatments (Murray *et al.*, 2022). Antimicrobial resistance is driven by bacterial survival strategies, including biofilm formation, which enables bacterial communities to adopt a protected lifestyle with increased tolerance to antimicrobial exposure and environmental stressors (Subramani *et al.*, 2026). During biofilm development, bacteria adhere, proliferate and produce extracellular polymeric substance (EPS) that contributes to the structural organization of the bacterial community (O'Toole, 2011). Such organization creates heterogeneous conditions where different bacterial cells have different metabolic activities, growth rates, nutrient supply, and sensitivity to the action of antimicrobials (Hall and Mah, 2017). The process of biofilm maturation includes several stages: from cell attachment to the surface, microcolony formation, matrix production, maturation and dispersion (O'Toole, 2011). Bacteria can regulate the process of biofilm maturation with chemical signaling through quorum sensing, coordinating their actions for virulence factors, matrix production, community organization and adaptation to environment (Subramani *et al.*, 2026). On the other hand, heterogeneous matrix of the biofilm and physiological characteristics of cells inside it may result in decreased efficiency of antimicrobials due to several reasons such as lack of penetration of the drug to the cells, change in

metabolism of the bacteria, stress response, efflux activity and persistence (Hall and Mah, 2017).

The clinical importance of biofilms becomes most pronounced in cases of opportunistic pathogens, which can produce infections that become persistent, and can colonize biological tissues and medical equipment. Bacteria such as *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*, which belong to bacteria producing biofilms, are clinically significant due to the problem of their treatment (Subramani *et al.*, 2026). In particular, biofilm-producing potential of *K. pneumoniae* is conditioned by multiple factors, which include surface structures, extracellular matrix formation, as well as other elements responsible for biofilm formation and drug resistance (Rahman *et al.*, 2023). Therefore, biofilm formation becomes a significant therapeutic problem due to the fact that bacteria persist even though there are enough antimicrobials to inhibit sensitive planktonic bacteria (Hall and Mah, 2017).

The intricate nature of biofilms has motivated researchers to look for therapies that go beyond traditional antibiotics. The current anti-biofilm methods include disrupting bacterial adherence to surfaces, interfering with quorum-sensing pathways, breaking down extracellular matrix molecules, using antimicrobial peptides and combination therapies aiming at enhancing the efficiency of antimicrobial treatments (Subramani *et al.*, 2026). This line of therapy is significant because the treatment of infections associated with biofilms can require intervention not only in bacteria themselves but also in the physiological and biochemical mechanisms that provide protection for these bacteria as part of an organized community (Høiby *et al.*, 2015). The biological agents studied

for such interventions include lactic acid bacteria (LAB) and the corresponding antimicrobial molecules produced by them. Species of *Lactobacillus* bacteria synthesize different biologically active molecules like exopolysaccharides, bacteriocins, organic acids, secretory proteins and other antimicrobial substances which can influence the growth of bacteria and biofilm formation (Li *et al.*, 2024). Hence, LAB-produced molecules can be considered as potential biofilm control agents because of their impact on bacterial growth and biofilm formation.

The bacteriocins produced by bacteria are ribosomal products that exhibit antimicrobial activity. Bacteriocins belong to the group of the most well-studied antimicrobials produced by LAB (Sugrue *et al.*, 2024). The mechanism of action of these compounds on bacteria depends on their structure and function. Thus, bacteriocins may exert their antimicrobial effect through interaction with the cell envelope, membrane permeabilization, pore formation, proton motive force inhibition, and interference with some of the processes associated with the cell wall biosynthesis (Arbulu and Kjos, 2024). Besides their antibacterial properties, some bacteriocins were found to possess the potential to inhibit the biofilm formation or alter its structure. This fact suggests the possibility of applying bacteriocins in inhibiting not only free bacteria but also bacteria in biofilm form (Ismael *et al.*, 2024). *L. acidophilus* is an especially interesting object of research because bacteriocins and other antimicrobial metabolites can be produced by strains of this species (Li *et al.*, 2024). Production of bacteriocin by *L. acidophilus* strains and their inhibitory effects on clinical strains of microorganisms, such as *P. aeruginosa*, were proved earlier (Mokoena, 2017). It should be noted that bacteriocins of *Lactobacillus* species could inhibit biofilm formation by more than one mechanism. These effects may include inhibition of bacterial growth, interference with bacterial aggregation and surface colonization, and alteration of biofilm-associated extracellular components (Ismael *et al.*, 2024).

Although there is considerable interest regarding bacteriocins and antimicrobial compounds produced by members of the genus *Lactobacillus* species, previous investigations have reported the ability of *Lactobacillus* derived compounds to exhibit antibacterial activity and inhibit biofilms produced by different bacteria, exhibiting different efficacies according to the bacterium and bacteriocin concentrations used (Li *et al.*, 2024; Ismael *et al.*, 2024). Nevertheless, the antibacterial and biofilm inhibiting activity of crude bacteriocin preparations, particularly those derived from *L. acidophilus*, against clinically relevant biofilm producing pathogens have not been fully exploited. It is believed that the assessment of the antibacterial activity and the MIC along with the effect of concentration on biofilm production may give an insight into the potential of such preparations as antimicrobial agents (Ismael *et al.*, 2024; Sugrue *et al.*, 2024). The current study is focused on the determination of the ability of the *L. acidophilus* preparation to inhibit the growth of bacteria and biofilm production.

MATERIALS AND METHODS

Study Design

The current lab experiment has been carried out in order to determine the capacity of the crude bacteriocin extract generated by *L. acidophilus* in demonstrating antibacterial effect on selected pathogenic bacterial strains and the inhibition of biofilms created by those bacteria. These experimental procedures involved the culturing of *L. acidophilus*, the generation and preparation of the crude bacteriocin extract, the determination of its antibacterial

effect using agar well diffusion test and determination of MIC, the inhibition of biofilm formation using crystal violet microtiter plate test, and analysis of the gathered data.

Clinical Bacterial Isolates

A total of 40 clinical bacterial isolates were used in this experiment where 10 isolates of each *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* bacteria were used. Ten clinical isolates were included for each bacterial species to provide an equal representation to all the four types of targeted bacteria and to facilitate comparison of their responses to the crude bacteriocin extract. The clinical isolates were obtained from wound swabs, urine, sputum, and blood samples collected from hospitalized patients. The bacterial isolates were initially identified based on colony morphology, Gram staining, and conventional biochemical tests, followed by confirmatory identification using the API identification system (bioMérieux, France). Pure cultures were maintained on nutrient agar slants at 4°C until further use.

Lactobacillus Acidophilus

L. acidophilus strain isolated from whey was cultivated in MRS broth (HiMedia, India) and incubated under microaerophilic conditions using a candle jar at 37°C for 24 h.

Production and Preparation of Crude Bacteriocin

Crude bacteriocin were produced based on the methods described by Gaspar *et al.* (2018) and Thuy *et al.* (2024), with some modifications. Briefly, 1 mL of a 24h old bacterial culture was inoculated into 50 mL of MRS broth contained in a 250 mL flask and incubated in a 10% CO₂ at 37°C for 24 h. Subsequently, the culture was centrifuged at 10,000 × g for 15 min at 4°C. After collecting the cell free supernatant (CFS), the pH was adjusted to 6.5 by adding sterile 1 N NaOH to neutralize the effects of organic acids present in the cell-free supernatant.

Furthermore, catalase was added at a concentration of 1 mg/mL, and the mixture was incubated at room temperature for 30 min to remove any residual hydrogen peroxide present in the medium. The medium was then filtered through a membrane filter with a pore size of 0.22 µm and stored frozen at -20°C until use in subsequent experiments.

Determination of Bacteriocin Activity (AU/mL)

The inhibitory effect of the crude bacteriocin were quantified in terms of AU/mL. The crude bacteriocin were subjected to serial two-fold dilutions in sterile PBS before each experiment was carried out using the agar well diffusion test against *L. plantarum* isolate as the indicator strain. The inhibition was then determined using the highest dilution showing inhibition zone using the following formula:

$$\text{AU/mL} = \frac{1000}{V} \times D$$

Where:

- i. V = volume of bacteriocin in µL
- ii. D = reciprocal of the highest dilution exhibiting complete inhibition.

Bacteriocin inhibitory activity was reported in AU/mL (Somsap *et al.*, 2015).

Standardization of Bacterial Inoculum

Fresh cultures of bacteria were resuspended in sterile saline solution and standardized to 0.5 MacFarland standards that

equal to approximately 1.5×10^8 CFU/mL prior to carrying out antimicrobial and antibiofilm studies.

Assessment of Antibacterial Activity

The antibacterial activity was determined by the agar well diffusion assay technique. Plates of Mueller-Hinton agar medium were evenly inoculated with bacterial cultures adjusted to the 0.5 McFarland standard. Wells (6 mm in diameter) were created using a sterile cork borer, and one hundred microliters of crude bacteriocin extract, was added to each pathogenic bacterium. The ciprofloxacin antibiotic discs (5 µg/disc) served as positive controls (Humphries *et al.*, 2019); on the other hand, 100 µL of sterile distilled water were used as negative controls. The plates were kept at 37 °C for 24 hours and the inhibition zone diameter was measured in millimeters (mm) using a digital caliper.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) was assessed using the method of broth microdilution as recommended by (CLSI, 2023). Two-fold serial dilutions prepared based on the bacteriocin activity (AU/mL) equation (1), were made in Mueller-Hinton broth in sterile 96 well microplates. Every well contained:

- i. 100 µL bacteriocin
- ii. 100 µL bacterial culture (0.5 McFarland)

Subsequently, after incubation at 37°C for 24 hours, growth inhibition of bacteria was assessed visually. All experiments were done in triplicates (Gaspar *et al.*, 2018).

Quantification of Biofilm Formation

The ability of bacterial isolates to form biofilms was evaluated using the crystal violet microtiter plate assay. Briefly, bacterial suspensions were prepared and adjusted to 0.5 McFarland standard. The bacterial suspension was added to sterile 96-well microtiter plates containing tryptic soy broth (TSB) supplemented with 1% glucose. The plates were incubated at 37°C for 24 h to allow biofilm formation. After incubation, planktonic cells were removed, and the wells were washed with sterile phosphate-buffered saline (PBS) to eliminate non-adherent cells. The attached biofilm biomass was fixed with methanol, stained with 0.1% crystal violet, and the bound stain was solubilized. The absorbance

was measured at 570 nm (OD570) using an ELISA microplate reader (O'Toole, 2011).

Biofilm Formation Inhibition Assay by Bacteriocin

Biofilm formation inhibition was evaluated using the crystal violet microtiter plate assay as described by (O'Toole, 2011). Particularly, 100 µL of bacterial suspension that was prepared using TSB broth containing 1% glucose was added to the sterile flat-bottom 96-well microplates. The bacterial suspension was treated by add 100 µL from different concentrations of crude bacteriocin prepared according to the bacteriocin activity (AU/mL). After this, the plates were incubated at 37 °C for 24 h. After that, planktonic cells were discarded and wells were washed thrice with sterile PBS. Biofilm formation was fixed by treatment with methanol for 15 minutes, stained with 0.1% crystal violet for 15 minutes, rinsed thoroughly with distilled water and then dried under air. The stain was dissolved in 95% ethanol and the absorbance was determined at 570 nm by ELISA microplate reader. Biofilm inhibition assay calculation formula is as follows:

$$\text{Biofilm inhibition (\%)} = \frac{OD_{\text{control}} - OD_{\text{treated}}}{OD_{\text{control}}} \times 100$$

Statistical Analysis

All experiments were conducted independently in triplicate, and the results obtained were reported as mean $\pm$ standard deviation (SD). Statistical analyses were done using IBM SPSS Statistics software (version 26.0). Data normality was assessed using the Shapiro–Wilk test. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple comparison test when significant differences were detected. A value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Bacteriocin Activity

Bacteriocin activity was calculated using the equation (1), using *L. plantarum* isolate, which is considered an indicator strain. As shown in Table 1, the highest dilution of 1/8 was observed, indicating an inhibition zone. According to the equation (1), the bacteriocin activity was 100 AU/mL.

Table 1: Determination of Bacteriocin Activity by the Critical Dilution Method Using *L. plantarum* as the Indicator Strain

Dilutions	Formation inhibition zone
1/2	+
1/4	+
1/8	+
1/16	-
1/32	-
1/64	-

Antibacterial Activity of *L. acidophilus* Derived Bacteriocin

The crude bacteriocin extracted *L. acidophilus* were found to have antibacterial activities against all isolated pathogenic bacteria. They're existed significant differences in susceptibility of the tested bacteria isolates ($P < 0.05$). As depicted in Table 2 and Figure1, it was observed that the crude bacteriocin at a concentration of 100 AU/ml had antibacterial activities against all tested pathogenic bacteria isolates; and it was found that *S. aureus* was the most susceptible bacteria isolate to the crude bacteriocin with the highest inhibition

zone of (22.0 $\pm$ 1.2 mm), while *K. pneumoniae* was the least susceptible one (15.0 $\pm$ 0.9 mm). The Gram-negative bacteria such as *P. aeruginosa* and *E. coli* had moderate inhibitions zones (18.0 $\pm$ 1.0 mm) and (17.0 $\pm$ 0.8 mm), respectively. However, the positive control antibiotic Ciprofloxacin at a concentration of 5µg/disc had inhibitions zones of 26.0 $\pm$ 1.0, 24.0 $\pm$ 1.2, 25.0 $\pm$ 1.0 and 21.0 $\pm$ 0.8 mm against *S. aureus*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae*, respectively. The negative control did not generate any inhibition zone (0 mm) against any of the pathogens tested. Although Ciprofloxacin was found to have high inhibitions zones than the crude

bacteriocin, however, the bacteriocin had good activities against all tested bacteria isolates. In conclusion, the crude

bacteriocin have wide spectrum of antibacterial activities, especially against *S. aureus*.

Table 2: Antibacterial Effect of Bacteriocin Derived from *L. acidophilus*

Pathogen	Bacteriocin (mm)	Ciprofloxacin (mm)
<i>S. aureus</i>	22.0 ± 1.2 ^b	26.0 ± 1.0 ^a
<i>P. aeruginosa</i>	18.0 ± 1.0 ^b	24.0 ± 1.2 ^a
<i>E. coli</i>	17.0 ± 0.8 ^b	25.0 ± 1.0 ^a
<i>K. pneumoniae</i>	15.0 ± 0.9 ^b	21.0 ± 0.8 ^a

Data are Expressed as Mean ± SD (n = 3). Superscripts Denoting Different Letters within a Column Designate Statistically Significant Differences by one-way ANOVA and Post-hoc Tukey's Test (P < 0.05)

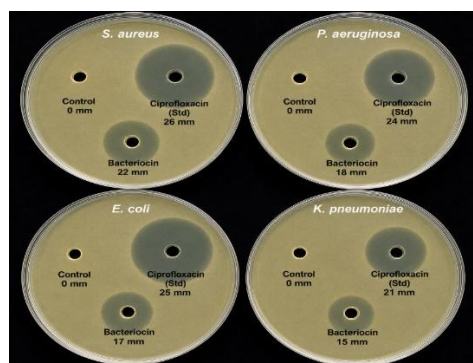


Figure 1: Example Agar Well Diffusion Assay Proving the Antibacterial Effectiveness of the Bacteriocin Produced by *L. acidophilus* Towards the Pathogenic Bacteria

Minimum Inhibitory Concentration (MIC)

Based on the bacteriocin activity, which was 100 AU/mL, two-fold serial dilutions of the crude bacteriocin extract were prepared to obtain concentrations of (100, 50, 25, and 12.5 AU/mL). The (MIC) was then determined by broth microdilution to identify the lowest bacteriocin concentration capable of inhibiting the visible growth of the pathogenic bacteria, as shown in Table 3. Based on the MIC results, *S. aureus* exhibited the lowest MIC of 25 AU/mL, indicating

that it was the most susceptible pathogen to the crude bacteriocin. Both *P. aeruginosa* and *E. coli* showed an MIC of 50 AU/mL, whereas *K. pneumoniae* exhibited the highest MIC of 100 AU/mL, indicating the lowest susceptibility among the tested pathogens. Overall, the MIC results were consistent with those obtained from the agar well diffusion assay, with *S. aureus* showing the greatest susceptibility to the crude bacteriocin.

Table 3: Minimum Inhibitory Concentration (MIC)

Pathogen	MIC (AU/mL)
<i>S. aureus</i>	25
<i>P. aeruginosa</i>	50
<i>E. coli</i>	50
<i>K. pneumoniae</i>	100

Effect of Bacteriocin Treatment on Biofilm Biomass

The inhibition of biofilm formation was tested through the use of the crystal violet microtiter plate test. Quantitative assessment of the impact of bacteriocin on the biofilm mass was performed based on the optical density (OD570) measurements of the untreated and bacteriocin treated biofilms. It was found that the bacteriocin treatment caused significant decrease of the biofilm mass in all of the bacterial strains tested (P < 0.05). The highest biofilm inhibition was observed against *S. aureus* (81.5 ± 2.1%), followed by *P. aeruginosa* (72.3 ± 1.8%), *E. coli* (68.4 ± 2.0%), and *K.*

pneumoniae (63.7 ± 1.9%) (Table 4). The reduction in OD570 following bacteriocin treatment shows a decrease in biofilm biomass. Since crystal violet staining measures the biomass associated with bacterial cells and extracellular polymeric substances (EPS), the lower absorbance values observed in treated wells indicate reduced biofilm biomass compared with untreated controls (O'Toole, 2011).

These findings demonstrate that the crude bacteriocin from *L. acidophilus* showed a notable ability to inhibit biofilms on all test pathogens, where the most prominent inhibition was seen on *S. aureus* while the least was on *K. pneumoniae*.

Table 4: Effect of Bacteriocin Treatment on Biofilm Biomass (Crystal Violet Method)

Pathogen	Control OD570	Treated OD570	Biofilm inhibition (%)
<i>S. aureus</i>	1.12 ± 0.04 ^a	0.21 ± 0.02 ^d	81.25 ± 2.1 ^a
<i>P. aeruginosa</i>	1.08 ± 0.05 ^a	0.30 ± 0.03 ^c	72.3 ± 1.8 ^b
<i>E. coli</i>	1.05 ± 0.03 ^a	0.33 ± 0.02 ^b	68.6 ± 2.0 ^c
<i>K. pneumoniae</i>	1.01 ± 0.04 ^a	0.37 ± 0.03 ^a	63.4 ± 1.9 ^d

Data are expressed as mean $\pm$ SD ($n = 3$). Superscripts denoting different letters within a column designate statistically significant differences by one-way ANOVA and post-hoc Tukey's test ($P < 0.05$).

Discussion

This study aimed to evaluate the ability of *L. acidophilus* to produce bacteriocin with antimicrobial activity. Clinical isolates were obtained from wound swabs, urine, sputum, and blood samples, and the mechanism of action was investigated in vitro for this strain. Bacteriocin production and detection were performed in vitro, accompanied by the production of other bacterial metabolites, such as lactic acid and hydrogen peroxide, which have been previously described for this strain. Therefore, to ensure that the assessed biological activity of the protein extract was not linked to these metabolites, their effect was neutralized. Accordingly, the experimental results indicate that an antimicrobial protein complex, bacteriocin, was produced and secreted by *L. acidophilus* into the extracellular culture medium. The diffusion method on agar medium showed that crude bacteriocin possesses a broad spectrum of antibacterial activity against all tested Gram-positive and Gram-negative pathogens. Its effect was greatest against *S. aureus* (a Gram-positive bacterium), followed by *P. aeruginosa* and *E. coli*, and finally *K. pneumoniae* (a Gram-negative bacterium). It also had an effect on the indicator strain. While the crude bacteriocin produced from *L. acidophilus* bacteria in the study presented by (Gaspar *et al.*, 2018) had an effect on the growth of *P. aeruginosa*, it did not affect the growth of *S. aureus* and *E. coli*. This may be due to the difference in strains. The broad-spectrum activity of crude bacteriocin produced by lactic acid bacteria was also observed in another study by Adedayo *et al.* (2025) where the researchers reported inhibitory activities of the bacteriocin against several Gram-positive and Gram-negative bacteria including *S. aureus*, *P. aeruginosa*, and *E. coli*. Variations in susceptibility patterns between the two studies might be attributed to differences in the bacteriocin-producing strains and the target organisms. Bacteriocin has an antibacterial effect through its interaction with the bacterial cell membrane. This is what Pérez-Ramos and his group (2021) stated, where they mentioned that most LAB bacteriocins work by causing a disturbance in the bacterial cytoplasmic membrane through the formation of pores, or by causing cell wall lysis.

Of all the tested pathogens, *S. aureus* was found to be the most susceptible one to the crude bacteriocin, having inhibition zones of 22.0 ± 1.2 mm and the lowest MIC value of 25 AU/mL. Ciprofloxacin gave its maximum inhibition zone for the pathogen (26.0 ± 1.0 mm). The high sensitivity of *S. aureus* to crude bacteriocin is attributed, at least in part, to its lack of an outer membrane, which may facilitate the access of bacteriocin to the cytoplasmic membrane compared to Gram-negative bacteria (Sugrue *et al.*, 2024). Similar observations were obtained by Field *et al.* (2016), who proved that the bacteriocins synthesized by lactic acid bacteria are able to inhibit *S. aureus* and reduce its biofilm formation. On the other hand, *K. pneumoniae* showed lower susceptibility to crude bacteriocin, giving a zone of inhibition of 15.0 ± 0.9 mm and a MIC of 100 AU/mL. The larger zone of inhibition of 21.0 ± 0.8 mm was observed when ciprofloxacin was used against the same organism. Low susceptibility to bacteriocin by *K. pneumoniae* may be due to the possession of an outer membrane and a large polysaccharide capsule, which might hinder the penetration of antimicrobial peptides to the target site (Reuben and Torres, 2024). The results obtained from MIC were consistent with the results of the agar well diffusion test. Inhibition zone of the larger size meant smaller bacteriocin concentration was needed to inhibit the bacterial

growth. The consistency in both diffusion test and MIC measurement is the evidence of the reliable antibacterial activity and shows that bacteriocin is still effective even when used at relatively small concentration. Such dependence of the MIC on the inhibition zone was previously noted for bacteriocins produced by probiotics (Thuy *et al.*, 2024).

One of the significant discoveries of this research work is the potent inhibition of biofilm formation through the action of bacteriocin. Biofilms represent one of the major virulence factors used by pathogenic bacteria, as the extracellular polymer matrix shields the bacteria from external influence and helps them to withstand antibiotic treatments, immune system attacks, and harsh environmental conditions. Thus, bacteria in biofilms demonstrate high-level antimicrobial tolerance, which can exceed up to several hundred times compared to the planktonic bacteria (Vani *et al.*, 2023). Hence, disruption of biofilm formation is crucial for controlling the infections. The results obtained through crystal violet assays indicate a significant reduction in biofilm biomass. The stain binds both bacterial cells and extracellular polymers; thus, A decrease in the OD570 value reflects a decrease in the total biofilm biomass, which may result from decreased cell adhesion, inhibition of cell growth, or a reduction in the formation of matrix components. It can be concluded that these findings may suggest that bacteriocins interfere with one or more stages of biofilm development, including bacterial adhesion, microcolony formation, extracellular matrix production, and biofilm maturation. Such effects were observed by Li *et al.* (2024) as well.

This study also illustrated the concentration-dependent action of bacteriocin against biofilm formation. An increase in bacteriocin concentration resulted in significant biofilm inhibitory activity, which was maximal at 100 AU/mL. This result indicates that the activity of bacteriocin relies greatly on the availability of a sufficient concentration that can penetrate into the biofilm matrix and interact with bacteria. A similar dose-dependent biofilm inhibition activity has been documented by Ismael *et al.* (2024), showing progressive inhibition of biofilm formation and extracellular polymeric substance production with increasing bacteriocin concentration. However, the results revealed that even though *P. aeruginosa* was less susceptible compared to *S. aureus*, there was notable antimicrobial and antibiofilm activity. The significance of the results is especially evident considering that *P. aeruginosa* is known as one of the most challenging opportunistic pathogens because of its intrinsic resistance mechanisms, such as low outer membrane permeability, drug-efflux pumps, and efficient biofilm production. The effectivity of using the crude bacteriocin as a means of prevention of biofilm formation shows the potential usefulness of this bacteriocin in the treatment of different types of infections caused by this pathogen. The antibacterial effect observed during this experiment is mainly connected with the mechanism of action of the bacteriocins which is diverse and multifunctional. Comparing with many other antibiotics that target only one specific pathway of metabolic processes of bacteria, bacteriocins target the membrane permeability, proton motive force, cell wall biosynthesis, and intracellular metabolism. The diversification of the targets might reduce the probability of resistance development and increase the effectiveness of action of bacteriocins against various susceptible bacteria (Sugrue *et al.*, 2024). The diversity of the way of achieving antimicrobial effect by the bacteriocins is one of the main advantages of these natural

substances (Mokoena, 2017). Speaking about the practical application of the results of this experiment, they can be regarded as promising. The ability of the bacteriocin isolated from *L. acidophilus* to inhibit bacterial growth and biofilm formation suggests their potential suitability for developing antimicrobial coatings for urinary catheters, orthopedic implants, vascular catheters, wound dressings, and other medical devices where biofilm formation may cause infections and treatment failure (Reuben and Torres, 2024). Moreover, bacteriocins can be used in combination with antibiotics, however, these combined effects need to be verified experimentally (Field *et al.*, 2016).

On the whole, the present research contributes to the body of evidence supporting the potential of probiotic-derived bacteriocins as natural antimicrobial and antibiofilm agents (Sugrue *et al.*, 2024). Nonetheless, there is need for additional research on how to refine and study these bacteriocin peptides in order to define their molecular structure and mode of action, test their cytotoxicity and safety in mammalian cells, explore any possible synergies between them and traditional antibiotics, and establish their effectiveness in suitable in vivo models of infection (Reuben and Torres, 2024).

Limitations of the Current Study

There are certain limitations associated with the current study. First, only crude bacteriocin preparation was analyzed without the isolation and molecular identification of the active peptides. Second, antibacterial activity was tested using in vitro experiments; hence, the results may fail to fully depict in vivo efficiency. Third, the molecular mechanisms behind the effect of bacteriocin on biofilms changes were not explored. Finally, synergistic effects of bacteriocin and conventional antibiotics were not studied.

Future Perspectives

Future research directions should include the purification and structural analysis of bacteriocin by utilize chromatography and proteomics, identification of molecular weight and amino acid composition, determination of their cytotoxic activity towards mammalian cell cultures, and examination of synergistic effects in conjunction with existing antibiotics. Additionally, in vivo testing on animals as well as transcriptomic analysis of biofilm-related genes is recommended.

CONCLUSION

In conclusion, it was found in the current study that the bacteriocin extracted from *L. acidophilus* have good antibacterial and antibiofilm properties towards the tested pathogens. The bacteriocin displayed the highest antibacterial activity against *S. aureus*, followed by *P. aeruginosa*, *E. coli*, and *K. pneumoniae*. Despite the fact that ciprofloxacin had bigger zones of inhibition compared to the bacteriocin, the bacteriocin showed good broad-spectrum activity, whereas sterile distilled water served as a negative control and failed to produce any zones of inhibition. In addition, it was found that the bacteriocin could decrease the biofilm formation in a concentration-dependent manner, showing the highest inhibitory action against *S. aureus*. It can be stated that bacteriocin produced by *L. acidophilus* are promising antimicrobial and antibiofilm natural compounds.

REFERENCES

Adedayo, M. R., Akanbi, F. M., and Yusuf-Saliyu, B. O. (2025). Bactericidal potential of crude bacteriocins from lactic acid bacteria isolated from mouse gut against some food

spoilage bacteria. *FUDMA Journal of Sciences*, 9(8), 125–131. <https://doi.org/10.33003/fjs-2025-0908-3897>.

Arbulu S, Kjos M. Revisiting the multifaceted roles of bacteriocins. *Microb Ecol.* 2024. doi: <https://doi.org/10.1007/s00248-024-02414-5>.

Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2023. CLSI supplement M100.

Field D, O'Connor R, Cotter PD, Ross RP, Hill C. In vitro activities of nisin and nisin derivatives alone and in combination with antibiotics against *Staphylococcus* biofilms. *Front Microbiol.* 2016;7:508. doi: <https://doi.org/10.3389/fmicb.2016.00508>.

Gaspar C, Donders GG, Palmeira-de-Oliveira R, Queiroz JA, Tomaz C, Martinez-de-Oliveira J, et al. Bacteriocin production of the probiotic *Lactobacillus acidophilus* KS400. *AMB Express.* 2018;8:153. doi:10.1186/s13568-018-0679-z. Hall, C. W., and Mah, T.-F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiol. Rev.* 41, 276–301. doi: <https://doi.org/10.1093/femsre/fux010>

Høiby, N., Bjarnsholt, T., Moser, C., Bassi, G. L., Coenye, T., Donelli, G., et al. (2015). ESCMID* guideline for the diagnosis and treatment of biofilm infections 2014. *Clin. Microbiol. Infect.* 21, S1–S25. doi: <https://doi.org/10.1016/j.cmi.2014.10.024>

Humphries RM, Hindler JA, Shaffer K, Campeau SA. 2019. Evaluation of ciprofloxacin and levofloxacin disk diffusion and Etest using the 2019 *Enterobacteriaceae* CLSI breakpoints. *J Clin Microbiol* 57:e01797-18. <https://doi.org/10.1128/JCM.01797-18>.

Ismael M, Huang M, Zhong Q. The bacteriocins produced by lactic acid bacteria and the promising applications in promoting gastrointestinal health. *Foods.* 2024;13(23):3887. doi: <https://doi.org/10.3390/foods13233887>.

Li J, Zhang Y, Wang X, Liu H, Chen Z, Zhao L. *Lactobacillus*-derived components for inhibiting biofilm formation and microbial adhesion. *World J Microbiol Biotechnol.* 2024;40:117. doi: <https://doi.org/10.1007/s11274-024-03948-y>.

Mokoena MP. Lactic acid bacteria and their bacteriocins: Classification, biosynthesis and applications against uropathogens: A mini-review. *Molecules.* 2017;22(8):1255. doi: <https://doi.org/10.3390/molecules22081255>.

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 399, 629–655. doi: [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)

O'Toole GA. Microtiter dish biofilm formation method. *J Vis Exp.* 2011;(47):2437. doi: <https://doi.org/10.3791/2437>.

Pérez-Ramos, A., Madi-Moussa, D., Coucheney, F., & Drider, D. (2021). Current knowledge of the mode of action and immunity mechanisms of LAB-bacteriocins.

Microorganisms, 9(10),
<https://doi.org/10.3390/microorganisms9102107>

2107.

Rahman MAA, Islam MA, Ahmed T, Biswas PK, Nahar S. Lactic acid bacteria biofilms and their antimicrobial potential against pathogenic microorganisms. *Biofilm*. 2023;5:100118. doi: <https://doi.org/10.1016/j.biofilm.2023.100118>.

Reuben RC, Torres C. Bacteriocins: Potentials and prospects in health and agrifood systems. *Arch Microbiol*. 2024;206:233. doi: <https://doi.org/10.1007/s00203-024-03948-y>.

Somsap O, Bangrak P, Bhoopong P, Lertcanawanichakul M. Antibacterial activity and purification of bacteriocin produced by *Brevibacillus laterosporus* SA14. *Walailak J Sci Technol*. 2016;13(1):55-65. doi: <https://doi.org/10.14456/WJST.2016.6>

Subramani T, George EA, Saju AE, Muralikrishnan E, De R, Siddharth AK, Ramaiah S, Anbarasu A. Biofilm-associated

antibiotic tolerance in the era of multidrug resistance: quorum-sensing mechanisms and emerging therapeutic strategies. *Front Cell Infect Microbiol*. 2026;16:1826282. doi: <https://doi.org/10.3389/fcimb.2026.1826282>.

Sugrue I, Ross RP, Hill C. Bacteriocin diversity, function, discovery and application as antimicrobials. *Nat Rev Microbiol*. 2024;22(9):556-571. doi: <https://doi.org/10.1038/s41579-024-01045-x>.

Thuy TTD, Nguyen HT, Tran TN, Nguyen PH, Le HM. Characterization of the broad-spectrum antibacterial activity of bacteriocin-like inhibitory substance-producing probiotics isolated from fermented foods. *BMC Microbiol*. 2024;24:115. doi: <https://doi.org/10.1186/s12866-024-03245-0>.

Vani S, Vadakkan K, Mani B. A narrative review on bacterial biofilm: Its formation, clinical aspects and inhibition strategies. *Future J Pharm Sci*. 2023;9:50. doi: <https://doi.org/10.1186/s43094-023-00499-9>.



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