Ind. J. Pharm. Edu. Res., 2025; 59(2):738-746.
https://www.ijper.org

Original Article

Antibacterial Activity of Bacteriocin-Like Inhibitory Substances Produced by Lactic Acid Bacteria Isolated from Cheese against Pathogens

Amal Alghamdi, Kholoud Hesham, Shahad Alghamdi, Aya Kadi, Aeshah Basheer Aljohani, Amal Bakr Shori*

Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, SAUDI ARABIA.

ABSTRACT

Background: Lactic Acid Bacteria (LAB) produce bacteriocins, which have recently attracted considerable interest as alternatives to antibiotics. Cheese, a fermented food rich in LAB, is highly perishable and susceptible to contamination by foodborne pathogens. **Objectives:** This study aims to assess the antibacterial properties of Bacteriocin-Like Inhibitory Substances (BLIS) from four LAB i.e. *Lactobacillus fermentum* strain NBRC15885 (*L. fermentum*), *Lacticaseibacillus paracasei* strain NBRC 15889 (*L. paracasei*), *Pediococcus acidilactici* DSM 20284 (*P. acidilactici*), and *Enterococcus faecium* strain NBRC 100486 (*E. faecium*) isolated from Feta cheese sample against several antibiotics and bacterial pathogens. **Materials and Methods:** Antibacterial susceptibility test, and Agar well-diffusion test were conducted on LAB isolates. **Results:** *L. fermentum* and *E. faecium* displayed sensitivity to two types of antibiotics (imipenem and piperacillin). *E. faecium* BLIS showed the greatest effect on pathogenic bacteria, especially *E. coli* with an inhibition zone 1.5±0.14 cm. **Conclusion:** Bacteriocins produced by the four LAB isolates could effectively inhibit the growth of pathogen bacteria.

Keywords: Lactic acid bacteria, Bacteriocins, Feta cheese, Antibacterial activity, Pathogens, and Antibiotics.

Correspondence:
Prof. Amal Bakr Shori
Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, SAUDI ARABIA.
Email: shori_7506@hotmail.com

Received: 02-07-2024;
Revised: 10-09-2024;
Accepted: 12-11-2024.

INTRODUCTION

Cheese is made from milk, which is rich in nutrients. An average of 2000 variations of cheeses in the world with texture and flavor being the main determinants of variation.[1] Other factors, such as industrial enzymes, fermentation, and engineering, are also important. The European Union (EU) had the largest cheese consumption with 9135 metric tons in 2021.[2] Russia ranked third with 1367 metric tons, followed by the US with 5956 metric tons.[2] The value of the cheese market will be greater than $113,000 million.

Most of the cheeses found in grocery stores are produced commercially. To ensure safety and extend product shelf life by eliminating pathogens and enzymes, these cheeses are prepared from pasteurized milk, in small quantities, with minimal mechanization, and traditional and sustainable technologies are used to make artisanal cheeses.[3] Certain solutions have been put forth to protect the public's health and ensure the safe handling of artisanal cheeses.[4] It is important to keep in mind while choosing a strategy that consumers prefer goods with a minimal amount of chemical ingredients.

Food poisoning is a real problem for perishable dairy products like cheese, which poses a serious health risk for consumers.[5] Various nutrients found in cheese provide a suitable environment for pathogens to escape during manufacturing and survive in retail stores.[6] As a result of the overuse of antibiotics, resistant bacteria have spread, and effective alternative therapeutic approaches are needed to control their spread.[7] Several pathogenic bacteria that are foodborne exhibit antibiotic resistance. These infections, which are resistant to antibiotics, spread through the food chain and harm the public's health in several ways.[8] Furthermore, according to the World Health Organization (WHO), one in ten illnesses and 4,20,000 deaths worldwide each year are attributed to food contamination.[9] Various harmful bacteria can multiply based on the nutritional content of different types of food. These microorganisms release a variety of poisonous compounds that are hazardous to human health, including endotoxins, exotoxins, hepatotoxins, etc.,[10]

Advancements in food preservation technology have significantly improved human life. Natural bio-preservatives show extensive demand from consumers.[11,12] Bacteriocins have expanded research into discovering novel antimicrobial compounds

DOI: 10.5530/ijper.20250239

capable of combating pathogens in food.[13] Bacteriocin and Bacteriocin-Like Inhibitory Substances (BLIS) are proteinaceous short peptides synthesized by the bacterial ribosome, which can kill or inhibit the growth of related microorganisms.[14]

Since food safety has become a global concern, bacteriocins have shown great promise for controlling food spoilage or pathogen growth without severe adverse effects on the food itself.[15] Bacteriocins are considered promising alternatives and are committed to ensuring the quality and safety of ready-to-eat food, increasing product shelf life, and fresh taste for processed foods without using chemical preservatives.[16] Utilizing bacteria alone or in conjunction with bacteriocins as bio-preservatives offers a promising alternative to antibiotics and synthetic chemicals.[17]

Lactic Acid Bacteria (LAB) are a large group of gram-positive bacteria that are found naturally within maturing cheeses and are responsible for the aroma, flavor, texture, and microbial steadiness of these items.[18] Lactic Acid Bacteria (LAB) offer numerous health benefits such as lowering cholesterol and blood pressure, reducing oxidative stress, improving nutritional status, decreasing inflammatory cytokines, and treating mood disorders.[13] There are several antibacterial compounds produced by LAB, including reuttering, hydrogen peroxide, carbon dioxide, diacetyl, and acetaldehyde, as well as bacteriocins, such as Nicin.[11] LAB and their bacteriocins are considered safe bio-preservatives to be added as a food additive to eliminate the growth of pathogens and spoilage microorganisms.[19] In our lab, we have isolated four LAB from Feta cheese i.e. *Enterococcus facium, Pediococcus acidilactici, Lacticaseibacillus paracasei*, and *Limosilactobacillus fermentum*. Therefore, this study aims to assess the antibacterial properties of bacteriocin-like inhibitory substances from these four isolates against bacterial pathogens.

MATERIALS AND METHODS

Isolation and purification of lactic acid bacteria from cheese

Four samples of white feta cheese were acquired from a local market in Jeddah and kept at 4°C until analysis. The cheese sample was thoroughly homogenized before 10 g was weighed and 40 mL of De Man Rogosa Sharpe (MRS) broth was added to activate the microbial load of LAB. Then, the broth was incubated at 37°C for 48 hr under anaerobic conditions. Serial dilutions were then obtained using buffered peptone water at a concentration of 0.1% (w/v) ranging from 10^{-1} to 10^{-7} 100 µL. Each dilution was cultured on MRS agar and incubated at 37°C for 72 hr under anaerobic conditions.[20] For purification, single colonies were selected and streaked on MRS agar. Gram staining and microscopic examination of colony morphology were used to verify each isolate's purity.[21]

Molecular Characterization

DNA extraction of bacterial isolates

Bacterial DNA was extracted from fresh overnight LAB isolates using a manual protocol Azcárate-Peril and Raya,[21] with slight modifications. DNA samples were loaded in agarose gel using electrophoresis and then the gel was visualized using UV light.

Molecular identification by 16S rRNA sequencing

Two universal primers were used to identify strains of LAB based on 16S rRNA amplification and sequencing 27F (5-AGAGTTTGATCCTGGCTCAG-3), and 1492R (5-AAGGAGGTGATCCAGCCGCA-3) with 1500 bp product. Thermocycler (Mastercycler® Gradient, Eppendorf, Hamburg, Germany) was used to perform polymerase chain reaction (PCR). The total volume of conducted reactions was 25 µL, containing 12.5 µL of EverGreen Universal 2× Master Mix, 0.5 µL each of forward and reverse primers, 10.5 µL of injection water, and 1 µL of DNA template. Macrogen performed sequence analysis, BLAST data were compared with the obtained data, and MEGA 11 software was used to analyze phylogenetic trees.[22]

Antibacterial susceptibility test of LAB isolates

An antibacterial susceptibility test was conducted using the Kirby-Bauer disk diffusion method. Bacterial suspensions were adjusted to McFarland's standard (10^8 CFU/mL). A sterile cotton swab was used to spread the suspension on Mueller Hinton agar plates. Antibiotic discs such as Imipenem (IMI) 10 µg, Piperacillin (PRL) 100 µg, Augmentin (AUG) 30 µg, Penicillin G (PG) 10 units, Clindamycin (CD) 2 µg, Cefoxitin (FOX) 30 µg, and Metronidazole (MZ) 5 µg were placed on plates using sterile forceps and incubated at 37°C for 48 hr before their inhibition zone was measured.[23]

Production of Bacteriocin-Like Inhibitory Substances (BLIS) from LAB isolates

MRS broth was used to grow LAB isolates at 37°C under anaerobic conditions for 24 hr.[24] The cultures were centrifuged for 15 min at 10,000 rpm to get Cell-Free Supernatant (CFS) which was considered as control. CFS was sterilized with a membrane filtration device equipped with a Millipore filter and a pore size of 0.22 µm. To eliminate the effects of organic acids, pH was adjusted to 6.5 by 1M NaOH, and hydrogen peroxide was eliminated by adding 1 mg/mL of the catalase enzyme. The treated supernatant was used as BLIS.

Agar well-diffusion test

To determine the inhibitory activity of BLIS produced by LAB, an agar well diffusion test was conducted against indicator pathogens (*S. aureus, E. coli, P. aeruginosa*, and MRSA).[25] Mueller Hinton agar plates were cultured with fresh overnight indicator pathogens (10^8 CFU/mL). Sterile cork borer was used (5 mm),

wells were made in the agar, and each well was filled with 100 µL of BILS and (CFS) as control and incubated at 37°C for 48 hr. Inhibition zones were examined, with positive inhibition defined by a clear zone surrounding the wells measuring at least 0.5 mm wide.

Statistical Analysis

All data are expressed as means ± Standard Error of the mean (SE). The data obtained were Analyzed for Variance (ANOVA) followed by Tukey's post hoc test for mean comparison. All analyses were performed using the SPSS statistical analysis program version 20.0 (SPSS Inc., Chicago, IL, USA), with a minimum level of significance of 5% (p<0.05).

RESULTS

Isolation of LAB and Molecular identification by 16S rRNA sequencing

A total of four LAB isolates were isolated from feta cheese (Figure 1). *Limosilactobacillus fermentum* (OR685530), *Lacticaseibacillus paracasei* (OR707101), *Pediococcus acidilactici* (OR707129), and *Enterococcus faecium* (OR707131). The isolated bacteria were morphologically small white creamy colonies, gram-positive, non-motile, coccus, and rod-shaped bacteria**.** The molecular identification of LAB strains was performed using 16S rRNA sequencing. Following agarose gel electrophoresis of the amplified PCR products, all four strains exhibited positive results with a size of 1500 bp (Figure 2). The nucleotide sequences have been blasted and deposited in the GenBank database (https://www.ncbi.nlm.nih.gov/genbank), revealing a similarity ranging from 99% to 100% with known strains. Specifically, the sequences showed similarity to *Limosilactobacillus fermentum* strain NBRC 15885 (OR685530), *Lacticaseibacillus paracasei* strain NBRC 15889 (OR707101), *Pediococcus acidilactici* DSM 20284 (OR707129), and *Enterococcus faecium* strain NBRC 100486 (OR707131). Additionally, Figure 3 depicts the phylogenetic tree generated from the alignment of the 16S rRNA genes obtained from the isolated LAB strains and those available in the GenBank database.

Antibacterial susceptibility testing of LAB isolates

In the present result, *L. fermentum* and *E. faecium* were resistant to five types of antibiotics PG, AUG, CD, FOX, and MZ with zero inhibition zone (Figures 4 and 5). However, *L. fermentum* was sensitive to two types of antibiotics IMI and PRL with inhibition zones 2.92±0.28 cm and 2.95±0 cm, respectively. Similarly, *E. faecium* was also sensitive to IMI and PRL with inhibition zones 2.75±0.07 cm and 2.4±0.28 cm, respectively. *P. acidilactici* was resistant to four types of antibiotics PG, CD, FOX and MZ with zero inhibition zone, and was susceptible to three types of antibiotics IMI, PRL, and AUG (2.85±0.07; 2±0.28; 1.2±0.14 cm, respectively). In addition, *L. paracasei* showed no growth in the plates and no inhibition zones (Figures 4 and 5).

Antibacterial activity of BLIS using agar well-diffusion

Agar well diffusion was used to test BLIS' inhibitory activity and its effect on selected pathogens (Figure 6 and 7). The *L. fermentum* BLIS inhibited MRSA and *P. aeruginosa* with inhibition zones 0.4±0.14 cm and 0.27±0.07 cm, respectively (Figure 7). However, *S. aureus* and *E. coli* were resistant (no inhibition zones; Figure 6 and 7). *L. paracasei* BLIS inhibited MRSA with 0.35±0.21 cm inhibition zone. *S. aureus, E. coli*, and *P. aeruginosa* were shown to be resistant (no inhibition zone). The *P. acidiolactici* BLIS strain showed no effect on all four pathogenic bacteria. *E. faecium* BLIS had a strong effect on all four pathogenic bacteria, with inhibition zones of 1.5±0.14 cm, 1.2±0.42 cm, 0.95±0.35 cm, and 0.65±0.63 cm for *E. coli, P. aeruginosa*, MRSA, and *S. aureus*, respectively (Figure 7).

DISCUSSION

The results of this study provide valuable insights into the antibacterial properties of LAB strains isolated from cheese and their bacteriocins against pathogenic bacteria i.e. *S. aureus, E. coli, P. aeruginosa*, and MRSA. The molecular identification of LAB strains using 16S rRNA sequencing confirmed their similarity to known strains, with *L. fermentum, L. paracasei, P. acidilactici*, and

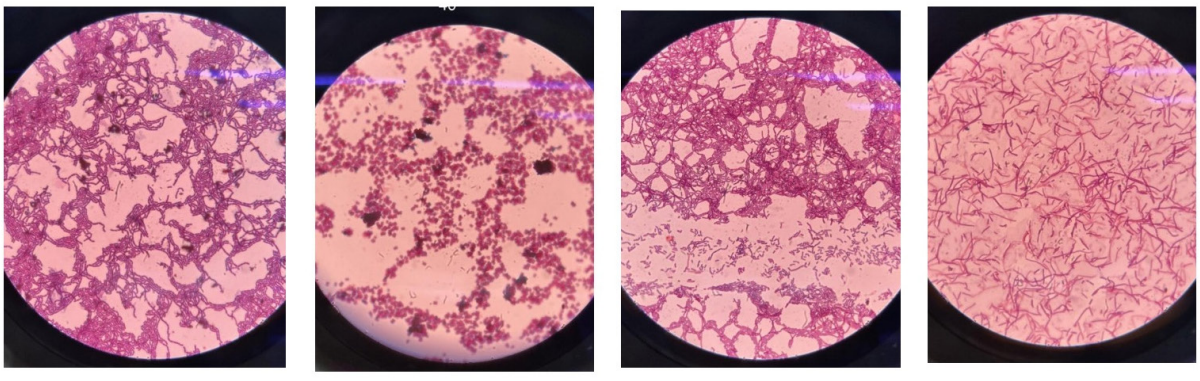

Figure 1: The morphology of lactic acid bacteria strains, *Enterococcus facium* (A), *Pediococcus acidilactici* (B), *Lacticaseibacillus paracasei* (C) and *Limosilactobacillus fermentum* (D). Under the light microscope (100X magnification).

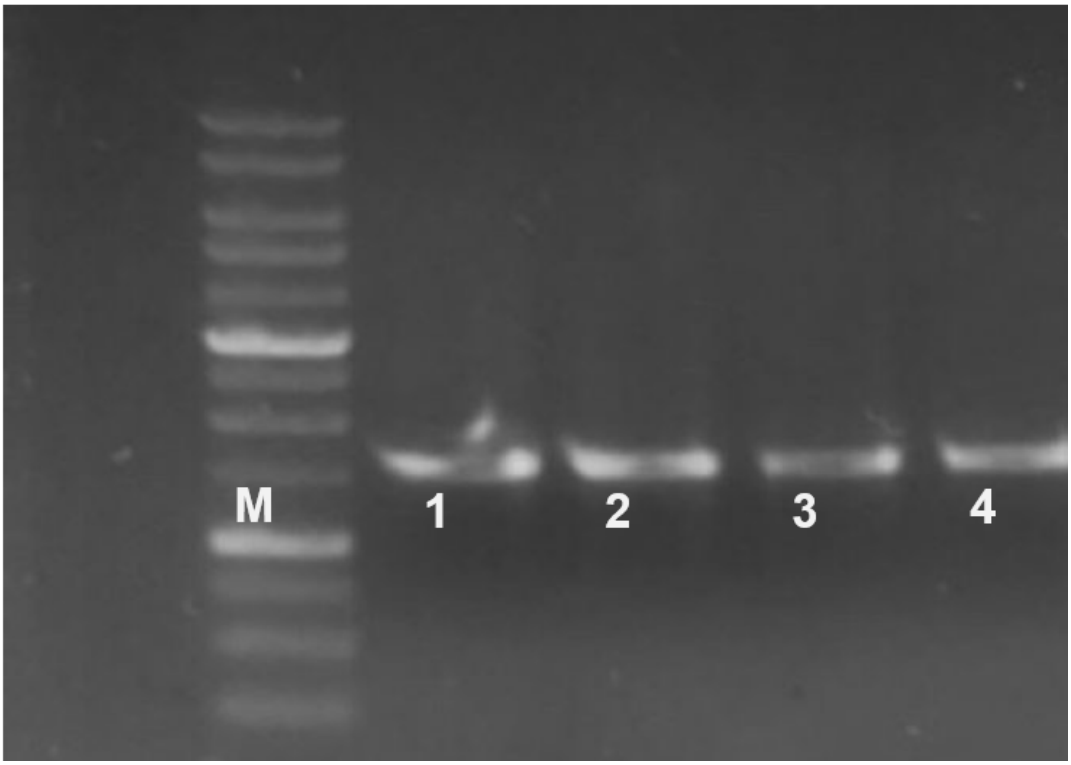

Figure 2: 16S rRNA of PCR products after amplification on agarose gel electrophoresis, lane M: 1 kb Marker (PROMEGA, USA), Lanes 1: *Limosilactobacillus fermentum* strain NBRC 15885, 2: *Lacticaseibacillus paracasei* strain NBRC 15889, 3: *Pediococcus acidilactici* DSM 20284, and 4: *Enterococcus faecium* strain NBRC 100486 are positive Lactic Acid Bacteria (LAB) strains at 1500 bp.

E. faecium exhibiting high sequence similarity to reference strains available in the GenBank database.

In terms of antibiotic resistance, *L. fermentum* and *E. faecium* demonstrated resistance to multiple antibiotics i.e. PG, AUG, CD, FOX, and MZ, while also displayed sensitivity to IMI and PRL. *P. acidilactici* exhibited resistance to antibiotics PG, CD, FOX and MZ but was susceptible to IMI, PRL, and AUG, highlighting strain-specific differences in antibiotic susceptibility. Notably, *L. paracasei* showed no growth on antibiotic plates, suggesting potential intrinsic resistance mechanisms or limitations in antibiotic diffusion. This is consistent with previous reports highlighting the variable antibiotic resistance profiles among LAB isolates.[19,26]

Bacteriocins are ribosomally synthesized antimicrobial peptides that inhibit or kill other closely related bacterial species.[7,10] BLIS produced by LAB exert their antibacterial effects primarily through mechanisms such as pore formation in bacterial membranes, inhibition of cell wall synthesis, and interference with essential metabolic pathways.[17] Many bacteriocins, like nisin and pediocin, target lipid II in bacterial membranes, disrupting cell wall biosynthesis and forming pores that cause ion leakage and cell death.[17,19] For example, nisin binds lipid II, inhibiting peptidoglycan synthesis while creating pores, leading to membrane depolarization.[19] Pediocin produced by *P. acidilactici*, follows a similar mechanism but depolarizes bacterial membranes, causing the release of intracellular components.[21] Lacticin 3147, another bacteriocin produced by *Lactococcus lactis*, also exhibits dual mechanisms, where it both forms pores and inhibits peptidoglycan biosynthesis by targeting lipid II.[21] In addition, some bacteriocins, like enterocin AS-48 from *E. faecium* disrupt membrane potential and affect intracellular ATP regulation.[14] LAB bacteriocins can exhibit both bactericidal and bacteriostatic effects, depending on their concentration and the susceptibility of the pathogen. Resistance mechanisms, such as membrane modifications by pathogens, can reduce the efficacy of bacteriocins by preventing their binding or action.[14] The results of the agar well diffusion assays in this study provide valuable insights into the inhibitory activity of Bacteriocin-Like Inhibitory Substances (BLIS) produced by the four LAB strains against selected pathogens. *L. fermentum* BLIS demonstrated inhibitory activity against MRSA and *P. aeruginosa* whereas *L. paracasei* BLIS exhibited inhibitory activity against only MRSA. This selective inhibitory activity suggests that the *L. fermentum* and *P. aeruginosa* BLIS may target specific cell structures or metabolic pathways of the targeted pathogens.[27] In contrast, no inhibition zones were observed for *S. aureus* and *E. coli*, indicating resistance to these two LAB. This result was inconsistent with a previous study that found *L. fermentum* (1 strain) isolated from Turkish dairy products demonstrated antibacterial activity against *S. aureus* and *E. coli* growth.[28] *Lb. fermentum* strain J23 isolated from Mexican Cocido cheese has been found to produce BLS that are effective against *S. aureus* ATCC 29213, *Listeria innocua* ATCC 33090, *Salmonella typhimurium* ATCC 14028, and *E.*

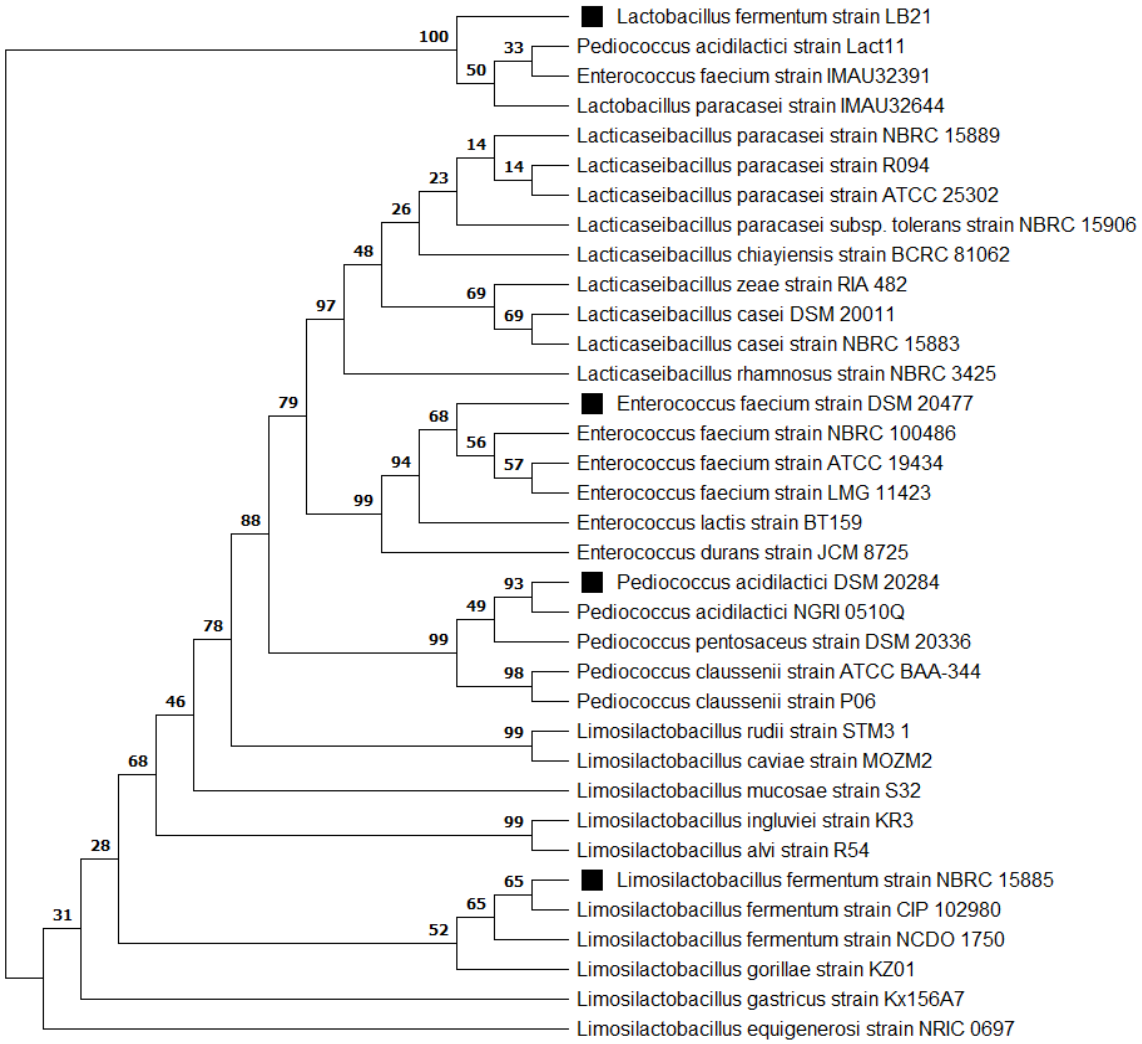

Figure 3: Based on 16S rRNA gene sequences, the phylogenetic tree of Lactic acid bacteria isolates is shown. Study isolates are marked in bold.

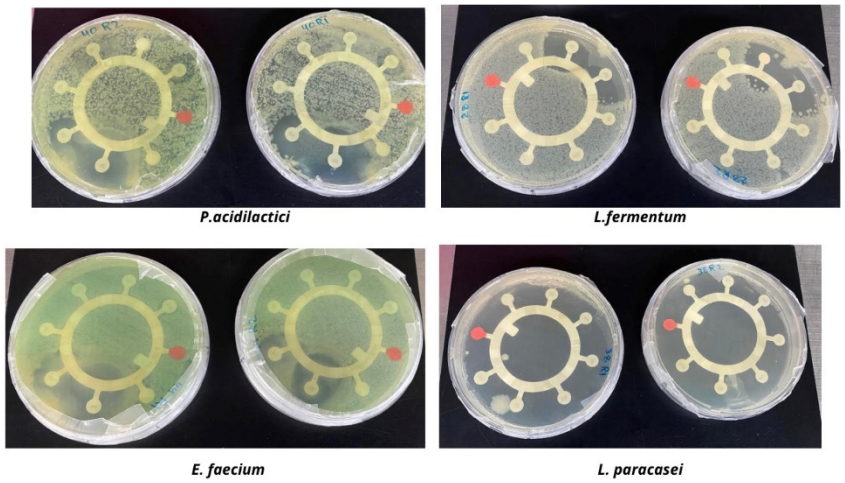

Figure 4: Antibacterial susceptibility test of LAB isolates growth against antibiotics i.e. Penicillin G (PG), Augmentin (AUG), Clindamycin (CD), Cefoxitin (FOX), Metronidazole (MZ), Piperacillin (PRL), and Imipenem (IMI).

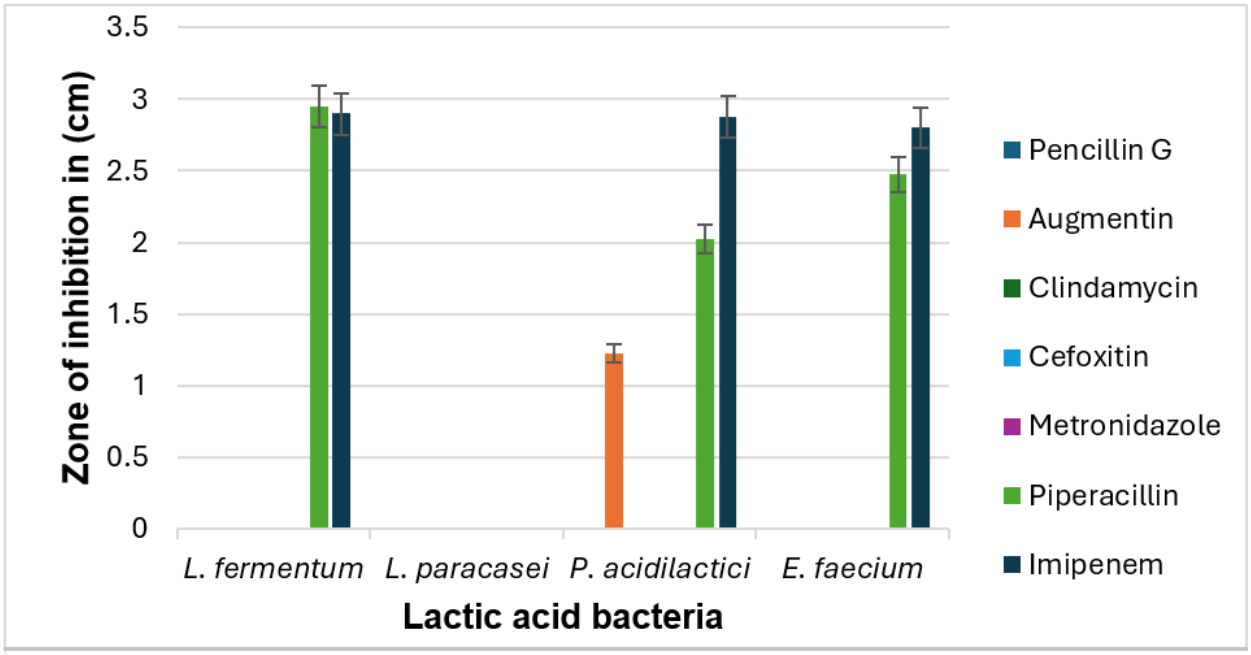

Figure 5: The effect of antibiotics on inhibition zone (cm) of LAB isolates growth. Data are presented as mean ± SEM.

coli ATCC 25922.[29] In contrast, *Lb. fermentum* strains J20, J28, and J32 exhibited the highest antimicrobial activity specifically against *E. coli* and *S. typhimurium*.[29] In addition, *L. paracasei* No. 244 isolated from spontaneous sourdough inhibited MRSA. Bendjeddou *et al.*[30] have also reported that *L. paracasei subsp. paracasei* BMK2005 isolated from infant faeces was highly active against MRSA. A previous study found that the cell-free supernatant of *Lacticaseibacillus paracasei* Zhang (*L. paracasei* Zhang) isolated from traditionally fermented horse milk was capable of inhibiting the growth of *S. aureus* but not *E. coli.*[31]

Once LAB isolates are activated, bacteriocin is produced in batch systems at the onset or end of exponential growth, often directly correlating with the amount of bacterial biomass produced, typically occurring within the first 24 hr.[32] Researchers have utilized several media for bacteriocin production, with MRS medium being the most frequently used due to its ability to induce bacteriocin synthesis owing to its high content of carbohydrates, proteins, and peptides, which is consistent with the medium used in our study.[33] In the current results, *P. acidilactici* BLIS did not show any inhibitory effect against the tested pathogenic bacteria. This result indicates that the BLIS produced by *P. acidilactici* may not possess antibacterial activity against the selected pathogens. However, *P. acidilactici* strain QC38 isolated from traditional cotija cheese from Mexico showed antimicrobial activity against 17 gram-negative and gram-positive pathogens that are found in food.[34] Remarkably, *E. faecium* BLIS exhibited potent inhibitory activity against all four tested pathogens, with significant inhibition zones observed for *E. coli* followed by *P. aeruginosa*, MRSA, and *S. aureus*, respectively. *E. faecium* T1 isolated from Chinese Tibet showed antibacterial activity against *Pseudomonas* spp. (3 species), *Shigella* spp. (2 species), *Salmonella typhimurium, Listeria monocytogenes, E. coli*, and *S. aureus*.[35] Enterocin-producing *E. faecium* strains H108 and H206, isolated from raw cow's milk, both exhibited inhibitory effects on the growth of *monocytogenes.*[36] On the other hand, *E. faecium* 130 isolated from mozzarella cheese exhibited no antibacterial activity against *Bacillus cereus, E. coli* CDC 02A.2B, *Proteus mirabilis* CDC 305, and *S. aureus* ATCC 29213.[37]

A possible mechanism employed by LAB to inhibit the growth of pathogens is the secretion of antibacterial substances, such as bacteriocins, which are known for their ability to inhibit microbial growth.[38] Some LAB strains produce bacteriocin capable of inhibiting the growth of harmful bacteria and foodborne pathogens such as *P. aeruginosa*, MRSA, E. *coli*, and *S. aureus*. Our results showed that among the four studied LAB-producing bacteriocins, *E. faecium* showed effectiveness towards all selected pathogens i.e. *E. coli, S. aureus*, MRSA and *P. aeruginosa*. It is promising that the bacteriocins produced by LAB can be used as antibiotic alternatives in the future.[39,40] However, understanding the specific mechanisms of action for each bacteriocin, such as pore formation, cell wall inhibition, or disruption of metabolic processes, is critical to elucidating their full potential as biopreservatives or therapeutic agents.[17] Future studies are required to conduct detailed biochemical characterizations of the bacteriocins produced by these LAB strains. This includes identifying target sites and mechanisms of resistance in pathogens.

BLIS produced by LAB have significant potential applications in food preservation due to their natural antimicrobial properties, which can inhibit the growth of spoilage microorganisms and foodborne pathogens.[21] Several studies have demonstrated the

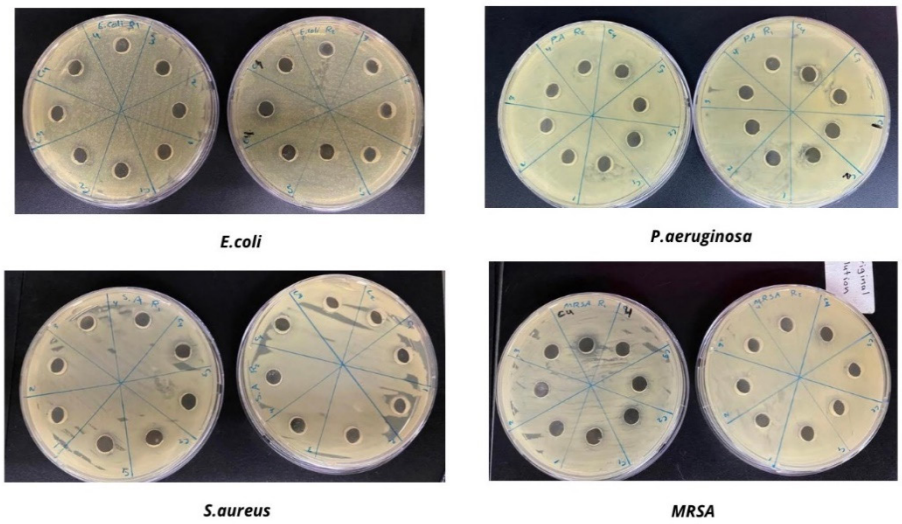

Figure 6: The inhibition zone (cm) in well agar diffusion test of Bacteriocin-Like Inhibitory Substances (BLIS) of LAB strains on pathogenic bacteria growth (*S. aureus*, MRSA, *E. coli*, and *P. aeruginosa*). C1: *L. fementum*, C2: *L. paracasei*, C3: *P. acidilactici* and C4: *E. faecium*.

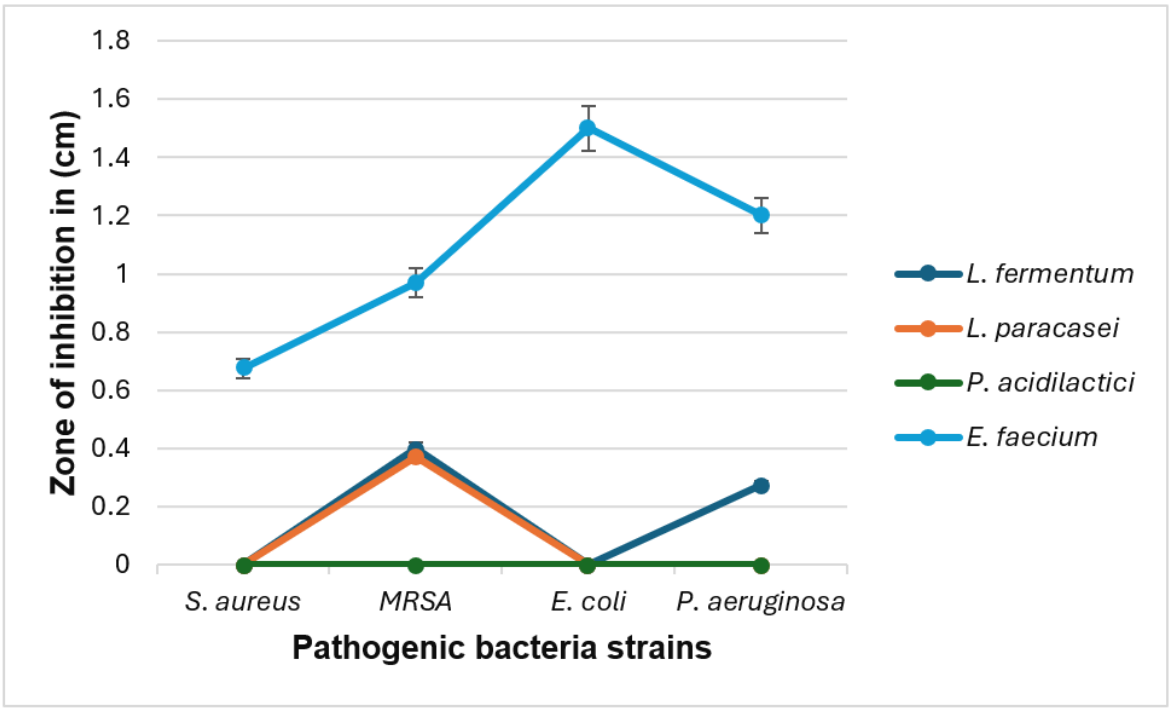

Figure 7: The inhibition zone (cm) of Bacteriocin-Like Inhibitory Substances (BLIS) of LAB strains on pathogenic bacteria growth (*S. aureus*, MRSA, *E. coli*, and *P. aeruginosa*). Data are presented as mean ± SEM.

effectiveness of BLIS as bio-preservatives in extending the shelf life of various food products.[24,26] For instance, BLIS from *Lactococcus lactis* has been successfully applied to preserve dairy products by inhibiting *L. monocytogenes* and *S. aureus*, which are common contaminants in cheese and yogurt.[17] Similarly, BLIS from *P. acidilactici* has been used in meat products to reduce the growth of *Salmonella spp* and *Clostridium perfringens*, significantly enhancing the safety and shelf life of these products.[18]

The incorporation of BLIS into food systems offers a natural alternative to chemical preservatives, aligning with consumer demand for cleaner, additive-free foods. Moreover, BLIS are Generally Recognized as Safe (GRAS) by food regulatory bodies, making them suitable for direct application in food preservation without adverse health effects.[14] Despite their promising potential, the feasibility of incorporating these BLIS into real-world food systems depends on factors like stability

during processing, interaction with food components, and the spectrum of antimicrobial activity.[14] Further research is needed to determine the most potent bacteriocins from the four LAB strains and evaluate their effectiveness under various food processing conditions and assess their long-term impact on product quality and safety.

CONCLUSION

The main goal of this study was to screen the ability of several LAB isolates to produce bacteriocin and test their inhibitory activity against several pathogens. *L. fermentum* and *E. faecium* were sensitive to two types of antibiotics (IMI, PRL). In addition, *E. faecium* BLIS had a strong effect on all four pathogenic bacteria. In conclusion, bacteriocins produced by the four LAB isolates could effectively inhibit the growth of pathogen bacteria. These results highlight the varied antibacterial capabilities of the four LAB strains and their bacteriocins, suggesting potential for developing novel antibacterial agents to improve food safety. Further investigation is required to understand the mechanisms behind the antibacterial activity of these LAB strains and their bacteriocins, as well as to explore their applications in food safety.

ACKNOWLEDGEMENT

Express our gratitude to King Fahd Medical Research Center and Faculty of Science, Department of Biological Sciences at King Abdulaziz University.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

.ABBREVIATIONS

LAB: Lactic acid bacteria; **BLIS:** Bacteriocin-like inhibitory substances; **EU:** European Union; **WHO:** World Health Organization; **MRS:** De Man Rogosa Sharpe; **PCR:** Polymerase chain reaction; **IMI:** Imipenem; **PRL:** Piperacillin; **AUG:** Augmentin; **PG:** Penicillin G; **CD:** Clindamycin; **FOX:** Cefoxitin; **MZ:** Metronidazole; **CFS:** Cell-free supernatant; **SE:** Standard Error.

REFERENCES

1. Centi V, Matteucci F, Lepidi A, Del Gallo MD, Ercole C. Microbiological and biochemical aspects of inland Pecorino Abruzzese cheese. Heliyon. 2017;3(2):e00258. doi: 10.1016/j.heliyon.2017.e00258, PMID 28280791.
2. Artiga-Artigas M, Acevedo-Fani A, Martín-Belloso O. Improving the shelf life of low-fat cut cheese using nanoemulsion-ased edible coatings containing oregano essential oil and mandarin fiber. Food Control. 2017;76:12-1.
3. Johnson ME. A 100-year Review: cheese production and quality. J Dairy Sci. 2017;100(12):9952-65. doi: 10.3168/jds.2017-12979, PMID 29153182.
4. Bezerra JS, Rangel AH, Marques Júnior S, Sales DC, Galvão Júnior JG, Brito AS, *et al.* Diagnosis of the impact of Covid-19 on artisanal cheese production in the semi-arid region of Brazil. Food Sci Technol. 2023;43:e83322. doi: 10.1590/fst.83322.
5. Cheng T, Wang L, Guo Z, Li B. Technological characterization and antibacterial activity of *Lactococcus lactis* subsp. cremoris strains for potential use as starter culture for cheddar cheese manufacture. Food Sci Technol. 2022;42:e13022. doi: 10.1590/fst.13022.
6. Kaur R, Kaur L. Encapsulated natural antimicrobials: a promising way to reduce microbial growth in different food systems. Food Control. 2021;123:107678. doi: 10.1016/j.foodcont.2020.107678.
7. Soltani S, Hammami R, Cotter PD, Rebuffat S, Said LB, Gaudreau H, *et al.* Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. FEMS Microbiol Rev. 2021; 45(1):fuaa039. doi: 10.1093/femsre/fuaa039, PMID 32876664.
8. Dye C, Bartolomeos K, Moorthy V, Kieny MP. Data sharing in public health emergencies: A call to researchers. Bull World Health Organ. 2016;94(3):158. doi: 10.2471/BLT.16.170860, PMID 26966322.
9. Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, *et al.* World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLOS Med. 2015;12(12):e1001923. doi: 10.1371/journal.pmed.1001923, PMID 26633896.
10. Zhang Y, Yang J, Liu Y, Wu Y, Fang Z, Wang Y, *et al.* A novel bacteriocin PE-ZYB1 produced by Pediococcus pentosaceus zy-B isolated from intestine of Mimachlamys nobilis: purification, identification and its anti-listerial action. LWT. 2020;118:108760. doi: 10.1016/j.lwt.2019.108760.
11. Amiri S, Moghanjougi ZM, Bari MR, Khaneghah AM. Natural protective agents and their applications as bio-preservatives in the food industry: an overview of current and future applications. Ital J Food Sci. 2021;33(SP1):68-55.
12. Hussein AR. Foods bio-preservation: a review. Int J Res Appl Sci Biotechnol. 2022;9(3):217-2.
13. Amiri S, Aghamirzaei M, Mostashari P, Sarbazi M, Tizchang S, Madahi H. The impact of biotechnology on dairy industry. In: Microbial biotechnology in food and health. Academic Press; 2021. p. 79-53.
14. Aljohani AB, Al-Hejin AM, Shori AB. Bacteriocins as promising antimicrobial peptides, definition, classification, and their potential applications in cheeses. Food Sci Technol. 2023;43:e118021. doi: 10.1590/fst.118021.
15. Shori AB. Awareness and knowledge about food spoilage and principles of food preservation among Saudi women in Jeddah. J Food Microbiol Saf Hyg. 2017;02(2):120. doi: 10.4172/2476-2059.1000120.
16. Silva CC, Silva SP, Ribeiro SC. Application of bacteriocins and protective cultures in dairy food preservation. Front Microbiol. 2018;9:594. doi: 10.3389/fmicb.2018.00594, PMID 29686652.
17. Kirtonia K, Salauddin M, Bharadwaj KK, Pati S, Dey A, Shariati MA, *et al.* Bacteriocin: A new strategic antibiofilm agent in food industries. Biocatal Agric Biotechnol. 2021;36:102141. doi: 10.1016/j.bcab.2021.102141.
18. Prpich NZ, Camprubí GE. Cayr´e ME, Castro MP. Indigenous microbiota to leverage traditional dry sausage production. International J Food Sci. 2021:15-1.
19. Mokoena MP. Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. Molecules. 2017;22(8):1255. doi: 10.3390/molecules22081255, PMID 28933759.
20. Albayrak ÇB, Duran M. Isolation and characterization of aroma producing lactic acid bacteria from artisanal white cheese for multifunctional properties. LWT. 2021;150:112053. doi: 10.1016/j.lwt.2021.112053.
21. Zou J, Jiang H, Cheng H, Fang J, Huang G. Strategies for screening, purification and characterization of bacteriocins. Int J Biol Macromol. 2018;117:781-9. doi: 10.1016/j.ijbiomac.2018.05.233, PMID 29870810.
22. Azcárate-Peril MA, Raya RR. Methods for plasmid and genomic DNA isolation from *Lactobacilli*. In: Food microbiology protocols Methods in biotechnology. Spencer J.F.T. and A.L. DSpencer (eds.). Argentina: Humana Press; 2001. p. 139-5.
23. Ruppitsch W, Nisic A, Hyden P, Cabal A, Sucher J, Stöger A, *et al.* Genetic diversity of*Leuconostoc mesenteroides* isolates from traditional montenegrin brine cheese. Microorganisms. 2021;9(8):1612. doi: 10.3390/microorganisms9081612, PMID 34442691.
24. Hefzy EM, Khalil MA, Amin AA, Ashour HM, Abdelaliem YF. Bacteriocin-like inhibitory substances from probiotics as therapeutic agents for candida vulvovaginitis. Antibiotics (Basel, Switzerland). 2021;10(3):306. doi: 10.3390/antibiotics10030306, PMID 33802636.
25. Tatsinkou FB, Goghomu S, Tongwa M, Ndjouenkeu R, Cho-Ngwa. Screening for bacteriocins producing probiotic bacteria from fermented sap of palm trees (*Elaeis guineesis and Raffia sudanica*): production and partial characterization of bacteriocins. J Appl Biotechnol Amp Bioeng. 2017;2(1):8-1.
26. Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus* brevis OG1. Afr J Biotechnol. 2003;2(8):227-19.
27. Zhu J, Chen Y, Imre K, Arslan-Acaroz D, Istanbullugil FR, Fang Y, *et al.* Mechanisms of probiotic Bacillus against enteric bacterial infections. One Health Adv. 2023;1(1):21. doi: 10.1186/s44280-023-00020-0.
28. Aslim B, Yuksekdag ZN, Sarikaya E, Beyatli Y. Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products. LWT Food Sci Technol. 2005;38(6):691-4. doi: 10.1016/j.lwt.2004.08.001.
29. Heredia-Castro PY, Méndez-Romero JI, Hernández-Mendoza A, Acedo-Félix E, González-Córdova AF, Vallejo-Cordoba B. Antimicrobial activity and partial characterization of bacteriocin-like inhibitory substances produced by *Lactobacillus* spp. isolated from artisanal Mexican cheese. J Dairy Sci. 2015;98(12):8285-93. doi: 10.3168/jds.2015-10104, PMID 26476937.
30. Bendjeddou K, Fons M, Strocker P, Sadoun D. Characterization and purification of a bacteriocin from *Lactobacillus* paracasei subsp. paracasei BMK2005, an intestinal

isolate active against multidrug-resistant pathogens. World J Microbiol Biotechnol. 2012;28(4):1543-52. doi: 10.1007/s11274-011-0958-1, PMID 22805936.

31. Huang T, Li Z, Qu X, Yao G, Kwok LY, He Q, *et al.* Preliminary purification and partial characterization of a functional bacteriocin of *Lacticaseibacillus paracasei* Zhang and mining for its gene cluster. Probiotics Antimicrob Proteins. 2024:1-12.
32. Tawangalun AT, Maskoen AM, Rachmawati E, Arumimaniyah C, Asarina S, Safitri R, *et al.* Optimized Novel antibacterial Production from Geobacillus kaustophilus Tm6T2 (a) as Treatment for *Salmonella* typhimurium. Indonesian J Clin Pathol Med Lab. 2024;30(3):250-4. doi: 10.24293/ijcpml.v30i3.2202.
33. Garmasheva IL, Oleschenko LT. Screening of bacteriocin-producing dairy Enterococcus strains using low-cost culture media. Front Microbiol. 2023;14:1168835. doi: 10.3389/fmicb.2023.1168835, PMID 37333643.
34. Morales-Estrada AI, Lopez-Merino A, Gutierrez-Mendez N, Ruiz EA, Contreras-Rodriguez A. Partial characterization of bacteriocin Produced by Halotolerant *Pediococcus acidilactici* Strain QC38 Isolated from Traditional Cotija Cheese. Pol J Microbiol. 2016;65(3):279-85. doi: 10.5604/17331331.1215607, PMID 29334047.
35. Liu H, Zhang L, Yi H, Han X, Gao W, Chi C, *et al.* A novel enterocin T1 with anti-Pseudomonas activity produced by *Enterococcus faecium* T1 from Chinese Tibet cheese. World J Microbiol Biotechnol. 2016;32(2):21. doi: 10.1007/s11274-015-1973-4, PMID 26745981.
36. Meral-Aktaş H. Bacteriocin characterization of Enterococcus faecium isolates and evaluation of their *in situ* anti-listerial activity in Beyaz cheese. Food Biosci. 2024;61:104741. doi: 10.1016/j.fbio.2024.104741.
37. Tulini FL, Gomes BC. Martinis ECPde. Partial purification and characterization of a bacteriocin produced by *Enterococcus faecium* 130 isolated from mozzarella cheese. Food Sci Technol. 2011;31(1):159-5.
38. Ibrahim SA, Ayivi RD, Zimmerman T, Siddiqui SA, Altemimi AB, Fidan H, *et al.* Lactic acid bacteria as antimicrobial agents: food safety and microbial food spoilage prevention. Foods. 2021;10(12):3131. doi: 10.3390/foods10123131, PMID 34945682.
39. Mokoena MP, Omatola CA, Olaniran AO. Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. Molecules. 2021;26(22):7055. doi: 10.3390/molecules26227055, PMID 34834145.
40. Nisa M, Dar RA, Fomda BA, Nazir R. Combating food spoilage and pathogenic microbes via bacteriocins: A natural and eco-friendly substitute to antibiotics. Food Control. 2023;149:109710. doi: 10.1016/j.foodcont.2023.109710.

Cite this article: Alghamdi A, Hesham K, Alghamdi S, Kadi A, Aljohani AB, Shori AB. Antibacterial Activity of Bacteriocin-Like Inhibitory Substances Produced by Lactic Acid Bacteria Isolated from Cheese against Pathogens. Indian J of Pharmaceutical Education and Research. 2025;59(2):738-46.