Check for updates

Original Research Article

Partial Purification and Characterizations of Bacteriocin from *Lactobacillus casei* Isolated from Goat Milk: A Novel Antimicrobial Agent

Journal of Pharmacology and Pharmacotherapeutics
17(2) 191–198, 2026
© The Author(s) 2025
Article reuse guidelines:
in.sagepub.com/journals-permissions-india
DOI: 10.1177/0976500X251347425
journals.sagepub.com/home/pha
Sage

Jumaila Kooriparambil[1], Vijaya Chitra Arumugam[2], Vajid Nettoor Veettil[3] and Shamla Alikadavath[1]

Abstract

Purpose/Background: This study aimed to isolate, purify, and characterize a novel bacteriocin produced by *Lactobacillus casei* strain GO3, isolated from goat milk. Bacteriocins are promising natural antimicrobial agents with potential applications in food preservation and therapeutics, addressing growing concerns over antibiotic resistance and synthetic preservatives.
Materials and Methods: *L. casei* GO3 was isolated from goat milk and screened for antimicrobial activity. The strain was identified through 16S rRNA sequencing, and the sequence was deposited in the NCBI database (accession number ON059683).
Purification: The bacteriocin was purified using a multi-step process involving 60% ammonium sulfate precipitation, dialysis (1 kDa cutoff), and ion-exchange chromatography (diethyl aminoethyl-cellulose). Antimicrobial activity was assessed against several pathogens (*Bacillus subtilis, Escherichia coli, Salmonella typhi*, and *Pseudomonas aeruginosa*) using the agar well diffusion method. Stability was evaluated under various conditions, including pH (2–12), temperature (30°C–121°C), enzymes (trypsin, pepsin, and protease K), and chemicals (ethylenediaminetetraacetic acid (EDTA), urea, NaCl, and dimethyl sulfoxide).
Results: The purification process yielded a 2.66% recovery rate with a 54.9-fold increase in specific activity (1,220.89 AU/mg). The bacteriocin demonstrated broad-spectrum inhibition (zone diameters >15 mm) and stability across a wide pH range (2–12) and temperatures up to 100°C. However, activity was lost at 121°C and with proteolytic enzymes, confirming its proteinaceous nature. Notably, EDTA enhanced antimicrobial activity, suggesting metal ion dependence.
Conclusion: The bacteriocin produced by *L. casei* GO3 exhibits robust stability and potent antimicrobial efficacy, making it a promising candidate for applications in food preservation and therapeutics. Future research should focus on optimizing production and evaluating safety for industrial use.

Keywords

Bacteriocin, biopreservative, goat milk, *Lactobacillus casei*, purification, therapeutic agent

Received 14 February 2025; **accepted** 10 May 2025

Introduction

Human health has been harmed by the excessive use of synthetic preservatives and antibiotic abuse. Lactic acid bacteria (LAB) isolated from milk and dairy products are known to contain a diverse array of bioactive peptides with potential health benefits and food preservation properties.[1] Bacteriocins are end products of ribosome synthesis and post-translationally modified peptides with potent antimicrobial properties.[2]*Lactobacillus* produced microbial peptides mainly focused on food applications in the early 2000s.[3] The rise of multiple drug-resistant microbes has sparked interest in searching for new treatment approaches. Bacteriocins, secreted by bacteria, have shown promise as effective agents against genetically related bacterial species, viruses, and

[1]Department of Microbiology, Sree Narayana Guru College, Coimbatore, Tamil Nadu, India
[2]Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamil Nadu, India
[3]IQRAA Centre for Research and Development, IQRAA International Hospital and Research Centre, Kozhikode, Kerala, India

Corresponding author:
Jumaila Kooriparambil, Department of Microbiology, Sree Narayana Guru College, Coimbatore, Tamil Nadu 641105, India.
E-mail: jumailakpvly@gmail.com

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (http://www.creativecommons.org/licenses/by-nc/4.0/) which permits non-Commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

fungi.[4] These ribosomally synthesized peptides exhibit antibacterial properties and are distinct from antibiotics. Notably, when used as preservatives, many bacteriocins are rendered harmless to humans by digestive enzymes.[5] Due to their targeted action against pathogenic microorganisms, bacteriocins are used in the drug industry as antibacterial peptides and natural food preservatives.[6] Bacteriocin-producing strains can be cultured, or they can be introduced to food as partially or completely purified agents.[7,8] Bacteriocins provide a number of benefits, such as prolonging shelf life, preventing dangerous bacteria in food, and being chemical-free preservatives.[9] Bacteriocins have piqued the interest of the biomedical sector, which sees them as a promising new therapeutic strategy.[10] *Lactobacillus* bacteriocins can be categorized into four groups, as per Klaenhammer's classification.[11,12] Class I (lantibiotics) comprises complex proteins with lipid or carbohydrate components; Class II comprises tiny, heat-stable peptides; Class III comprises large, heat-sensitive proteins; and Class IV has unique amino acids. At the moment, only Class I nisin is produced industrially. But nisin efficacy is limited to Gram-positive bacteria.[8] Researchers continue to seek novel bacteriocins with broader antibacterial capabilities. Lantibiotics are an important subgroup of bacteriocin with low molecular weight and interact with bacterial cell membranes, inhibiting cell wall synthesis and DNA replication. Previous studies on lantibiotics highlight the current state of knowledge, mechanisms of action, and their significant application in different fields. The efficiency of conventional antibiotics has decreased due to the fast spread of antibiotic-resistant bacteria.[13] A new area of bacteriocin research has emerged as a result of the massive need for natural and safe preservatives,[14] which aims to identify a new class of antimicrobial chemicals that can effectively combat foodborne diseases. Bacteriocins are effective, safe additives against foodborne pathogens, with no major adverse effects. Globally, foodborne illnesses cause 600 million cases and 420,000 deaths annually due to food contamination.[15] When it comes to protecting food from microbial contamination, the food industry has traditionally relied on a wide variety of artificial preservatives. On the other hand, there is a rising concern regarding the potential toxicological effects that could result from prolonged exposure to these chemical preservatives.[16] Studies on natural food preservatives, which could be considered safer alternatives, have been initiated in light of this fact.[17] Bacteriocins can suppress other species that share an ecological niche, regardless of how closely related or unconnected they are and regardless of whether or not they are related to each other.[18] Finding novel bacteriocins that are safe, affordable, and maintain their antimicrobial activity in a range of environmental conditions and storage processes is still necessary, though.[19,20] To do this, a large range of LAB strains from various environmental niches for bacteriocin production must be isolated and tested.[21,22] The study aimed to screen antimicrobial peptides from *Lactobacillus casei*; partial purification and characterization of the bacteriocin helped to identify key information about its therapeutic applications. This would facilitate further development as a promising research tool for the future after increasing efficacy through optimization methods.

Materials and Methods

Isolation, Screening, and Molecular Identification of Strain

Thirty-five dairy and non-dairy samples from Ernakulam District were screened on De Man, Rogosa, and Sharpe (MRS) medium, and 26 LAB isolates were obtained. The most potent strain (GO3 from goat milk) showed antimicrobial activity against foodborne pathogens via agar well diffusion assay. Identified as *L. casei* (NCBI: ON059683) through biochemical tests and 16S rRNA sequencing. Genomic analysis studies previously published[23] revealed an antibiotic-class bacteriocin containing the cystathionine gamma-synthase *metB* gene.

Purification and Characterization of Bacteriocins

The selected LAB isolate was cultured for 72 h at 37°C in MRS broth. A modified protocol[24,25] was used to secrete bacteriocin. Since the bacteriocin was thought to be below this molecular mass, ultrafiltration was performed using the culture's cell-free supernatant (CFS) via a 10 kDa cutoff membrane cartridge filter. A range of saturated ammonium sulfate concentrations, from 30% to 60%, was used to precipitate the proteinaceous material in CFS, which was then stored at 4°C for the night. Centrifugation (10,000 *g*, 20 min, 4°C) was used to recover the precipitated protein. It was then resuspended in a small amount of 10 mM phosphate buffer (pH 6.0) and dialyzed (18 h, 4°C) in the same buffer using a 1 kDa cutoff pore size dialysis membrane (Himedia, LA387). Further purification of bacteriocin was carried out by the dextran-diethyl aminoethyl cellulose (Sigma Aldrich, Bengaluru) column chromatography equilibrated with 50 mM sodium phosphate buffer (pH 6.5) with slight modification.[26] Agar well diffusion assay was used for analyzing the collected fraction bacteriocin activity. After that, the effective one was collected and stored for further studies.

Antimicrobial Spectrum of Purified Bacteriocin

Antimicrobial activity was performed using a well diffusion assay.[27] Wells in plates inoculated with the bioassay strain were filled with bacteriocin at several doses. *Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli*, and *Bacillus subtilis* were the strains that were employed. The inhibitory zone diameters were measured after the plates were incubated overnight at 30°C.

Bacteriocin Sensitivity Assay

Thermal and pH stability, enzyme susceptibility to denaturation, storage stability, and organic solvent extraction of pure bacteriocin samples[26] were evaluated using the following procedure.

Effect of Enzymes

Purified bacteriocin was treated at 37°C for 1 h with 1 mg/mL concentrations of pepsin, trypsin, proteinase K, and lysozyme. All enzymes were dissolved in 50 mM phosphate buffer (pH 7) with a concentration of 10 mg/mL, except pepsin, which was dissolved in 0.02 N HCl. The reaction mixture was boiled for 10 min after incubation to inactivate the enzyme, and antibacterial activity was measured by agar well diffusion method against *B. subtilis*.

Effect of Temperature

Purified bacteriocin samples were kept at different temperatures (30°C, 40°C, and 100°C) for 1 h, and one sample was kept in an autoclave at 121°C under 15 lbs pressure for 15 min. The bacteriocin activity of the different heat-treated samples was measured using the agar well diffusion method against *B. subtilis.*

Effect of pH

In order to determine the effect of pH on the activity of bacteriocin, the sample pH value was set to different values (2–12) with 1 N NaOH or 1 N HCl, then incubated for 4 h at 37°C. Post incubation, pH was re-aligned to 6.0. Each of the bacteriocin samples was tested to study the effectiveness on antimicrobial activity at different pH levels by well-diffusion against *B. subtilis*.

Effect of Chemicals

Bacteriocin samples were incubated at 37°C for 1 h with 10% concentrations of different organic solvents, ethylenediaminetetraacetic acid (EDTA), urea, NaCl, and dimethyl sulfoxide (DMSO) in a 1:1 ratio. Post incubation, the remaining antimicrobial activity was examined by a 96-well diffusion assay plate against *B. subtilis*.

Statistical Analysis

Inhibition zones were consistent across triplicate assays. Future studies quantified with statistical tests, and analysis of variance (ANOVA) to confirm significance.

Results

Purification and Characterization of Bacteriocins

Partial purification was achieved by ammonium sulfate, and precipitated protein was collected through centrifugation at 10,000 rpm for 20 min (4°C), and then reconstituted in a minimum amount of phosphate buffer (10 mM; pH 6.0) and dialyzed in the same buffer for 18 h (4°C) in a tubular cellulose membrane using 1 kDa cutoff pore size dialysis membrane (HiMedia, LA387). After the dialyzed samples were gathered and kept, they had the most activity at 60% saturation and the least amount of activity at 30%–50% saturation. Further purification of the crude extract was achieved using diethyl aminoethyl (DEAE)-cellulose column chromatography. Notably, the protein content decreased, while the antimicrobial activity increased gradually, from the crude extract to the DEAE-cellulose purified fraction. The purification method (ammonium sulfate precipitation (60%), dialysis, and ion-exchange chromatography) reproducibly exhibited 54.9-fold purification and 2.66% yield in multiple experiments. This reproducibility is demonstrated by the apparent stepwise increase in specific activity (22.22–1,220.89 AU/mg) and the standardized methodology referenced from multiple pre-existing studies.[26,28] Protein concentration was determined using the Bradford method. The results are summarized in Table 1.

Antimicrobial Spectrum of Purified Bacteriocin

The inhibitory spectrum of the bacteriocin was evaluated using the agar well assay against a group of bacterial strains. Notably, the bacteriocin exhibited potent inhibitory activity against common pathogenic and spoilage bacteria. The inhibitory spectrum of bacteriocin contains approximately 0.2–1 mg/mL. This range is consistent with literature on lantibiotics,[26] where 10–100 µg doses show measurable inhibition zones. Here, the study was assessed by the agar well assay against the strains listed below, such as *B. subtilis, E. coli, S. typhi*, and *P. aeruginosa*, which showed an inhibition zone diameter above 15 mm (Figure 1). Future work will quantify exact concentrations via high-performance liquid chromatography (HPLC).

Bacteriocin Sensitivity Assay

The partially purified bacteriocins were analyzed for the effect of pH, temperature, enzymes, and organic solvents on their antimicrobial activity.

Table 1. Summary of Purification Profile for Bacteriocin from *L. casei.*

Purification Step	Soluble Protein	Specific Activity (AU/mg)	Fold Purification	Yield%
Crude	9,300	22.22	Nil	100
30%–60% $(NH_4)_2SO_4$ precipitation	640	39.77	1.79	12.32
Ion-exchange chromatography	4.5	1,220.89	54.94	2.66

Effect of Enzymes

Proteinase K, pepsin, and trypsin completely inactivated the bacteriocin, confirming its proteinaceous nature, while lysozyme treatment showed no effect, indicating that lipid and carbohydrate moieties are not essential for antimicrobial activity. The results are shown in Figure 2.

Effect of Temperature

The bacteriocin showed thermal stability from 30°C to 50°C but lost activity after autoclaving (121°C, 20 min), suggesting a heat-sensitive proteinaceous nature (Figure 3). This stability pattern aligns with lantibiotics containing unusual amino acids as reported in previous studies.[26] The results are shown in Figure 3.

Effect of pH

The antimicrobial activity of the partially purified bacteriocin was tested across pH 2–12 against *B. subtilis*. The bacteriocin remained stable throughout this range, consistent with lantibiotics containing unusual amino acids that confer pH resistance. The literature reports[26] that unusual amino acids provide strength to the bacteriocin for stability at extreme pH ranges. The presence of unusual amino acids is the characteristic feature of the class lantibiotics. The results indicate that these bacteriocins belong to lantibiotics. The results are shown in Figure 4.

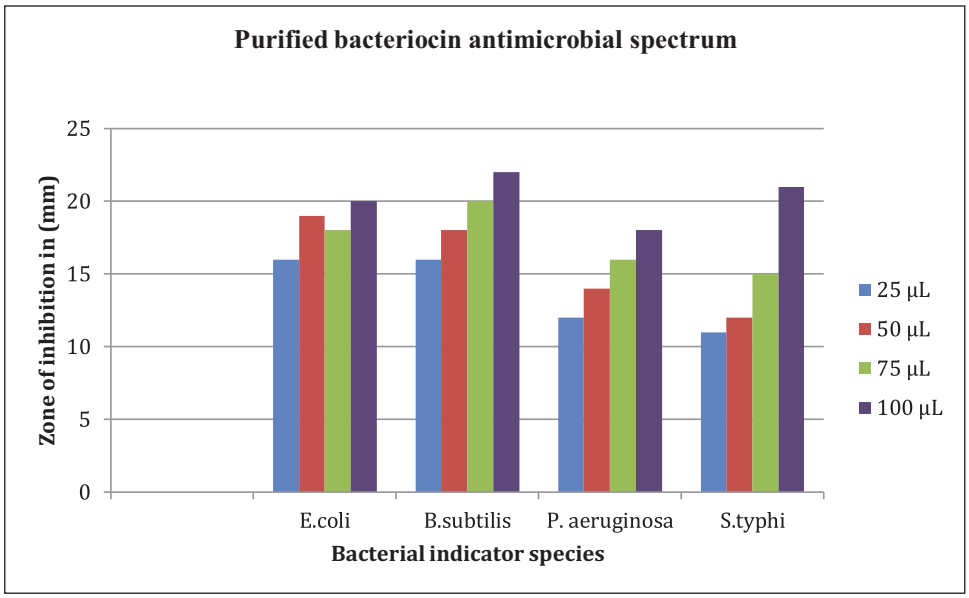


Figure 1. Showing Zone of Inhibition of Purified Bacteriocin Against Different Pathogens.

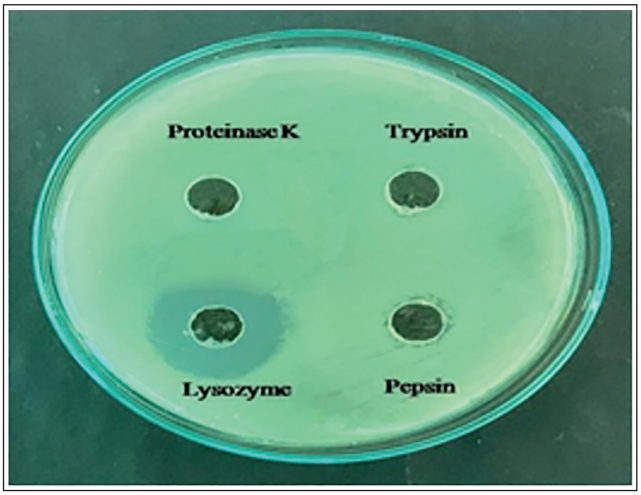


Figure 2. Effect of Various Enzymes on the Antimicrobial Activity of Purified Bacteriocin.

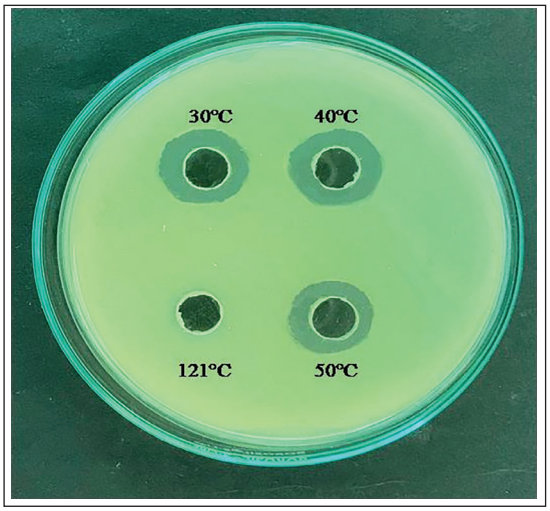


Figure 3. Effect of Temperature on the Antimicrobial Activity of Partially Purified Bacteriocin.

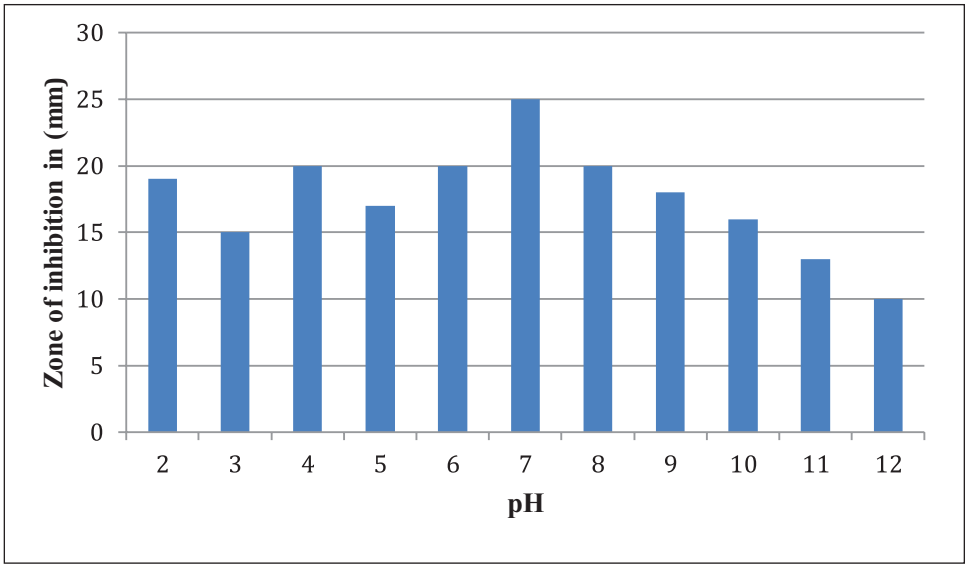


Figure 4. Effect of pH on the Antimicrobial Activity of Partially Purified Bacteriocin.

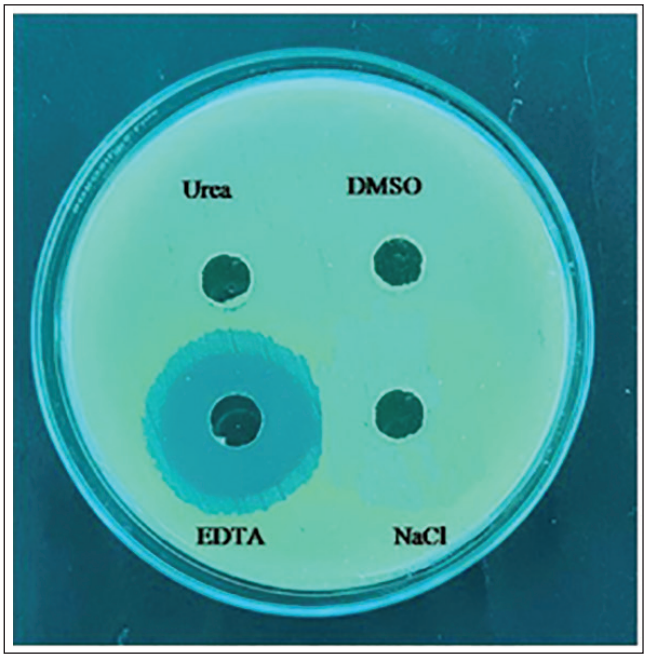


Figure 5. Effect of Chemicals on the Antimicrobial Activity of Partially Purified Bacteriocin.

Effect of Chemicals

The effect of EDTA, urea, DMSO, and NaCl at a concentration of 10% on the antimicrobial activity of partially purified bacteriocins was analyzed. Many of these chemicals are required for preservation or storage, and alteration in antimicrobial activity after treatment with the chemicals was analyzed. Results showed that partially purified bacteriocin's antimicrobial activity remained unaffected in the presence of EDTA but lost its activity after being treated with urea, NaCl, and DMSO (Figure 5). The study noted that antimicrobial activity could be enhanced with EDTA (a metal ion chelator). Future work could include EDTA-only controls to isolate the bacteriocin's metal-dependent effects.

Discussion

LAB were isolated from dairy and non-dairy sources and screened for antimicrobial properties against foodborne pathogens such as *Salmonella, Escherichia, Staphylococcus, Klebsiella*, and *Pseudomonas* and the spoilage organism *Bacillus*. Of 26 LAB isolates, 14 showed antagonistic activity with GO3 (from goat milk), exhibiting the broadest inhibitory spectrum. 16S rRNA sequencing identified GO3 as *L. casei*.[23] To purify bacteriocin from *L. casei* GO3, CFS was ultrafiltered (10 kDa cutoff), precipitated with 60% ammonium sulfate, dialyzed, and further purified by ion-exchange chromatography. Similar protocols reported optimal activity at 60% saturation.[26,28] The final yield was 2.66% with a 54.9-fold purification increase across multiple experiments,[29–31] and a specific activity of 1,220.89 AU/mg (from 22.22 AU/mg) using standardized protocols referenced from prior studies.[26,28]In our study, significant inhibitory activity of the *L. casei* (GO3) bacteriocin concentrations in tested samples was 0.24–1 mg/mL against both Gram-positive and Gram-negative foodborne pathogens. This was found upon evaluation of the antimicrobial spectrum. Similar characteristics were reported,[32–34] compatible with bioactive ranges reported for lantibiotics (e.g., nisin at 0.1–1 mg/mL). Different factors affect bacteriocin activity, including pH, temperature, proteolytic enzymes, and chemicals to determine the bacteriocin effectiveness. In our study, purified bacteriocin, even after being exposed to a broad

pH range,[33] indicates the presence of an unusual amino acid-containing lantibiotic-type bacteriocin. Physicochemical properties—stability across a wide range of pH (2–12) and moderate temperature (≤100°C), and sensitivity to proteolytic enzymes (trypsin, pepsin)—further support them being lantibiotics, identified from a previous study.[11,35,36] With mild heat treatment up to 50°C and proteolytic enzymes,[32,37] the inhibitory activity remained functional. The inhibitory activity persisted despite denaturants like urea, NaCl, and DMSO, but was lost at 121°C, eliminating bacteriocin activity. This was similarly reported in other studies[38,26] for isolating bacteriocin from *Lactobacillus plantarum* J23, which was isolated from Chinese traditional fermented milk. In our study, antimicrobial activity of bacteriocin was enhanced following EDTA treatment.[39] When combined, these findings demonstrate the potential bacteriocin from *L. casei* (GO3) as a viable natural biotherapeutant to manage infections and spoilage organisms that contaminate food. Furthermore, the *L. casei* (GO3) isolation obtained from goat milk might be formulated as a protective culture to enhance quality, safety, and probiotic efficacy using optimization parameters[40] because lantibiotics typically target bacterial membranes by pore formation or inhibit cell wall synthesis using lipid II binding in cell wall synthesis. The observed synergy with EDTA indicated membrane disruption that was dependent on metal ions. Future studies utilizing assays such as electron microscopic imaging and membrane permeability are required. However, researchers face some challenges, including low yield,[40] formulation stability, or lyophilization[10] to enhance shelf life and safety product formulation[17] for pharmacokinetics and industrial applications that need to be addressed.

Conclusion

In conclusion, this study demonstrates the potential of a bacteriocin derived from *L. casei* as a valuable antimicrobial agent. Following purification and characterization, the bacteriocin exhibited notable stability and activity, suggesting its suitability for applications in food and pharmaceutical industries. However, further research is warranted to elucidate the mechanism of action and fully explore the potential uses of this bacteriocin. Moreover, optimization of the purified product could improve the activity of the bacteriocin for further studies. Lack of cost-effective production, stable formulation, and safety evaluation of bacteriocin are the major challenges of bacteriocin development; new strategies and studies can overcome such challenges in the future.

Abbreviations

AU/mg: Activity units per milligram; **CFS:** Cell-free supernatant; **DEAE:** Diethyl aminoethyl; **DMSO:** Dimethyl sulfoxide; **EDTA:** Ethylenediaminetetraacetic acid; **HPLC:** High-performance liquid chromatography; **kDa:** Kilodalton; **LAB:** Lactic acid bacteria; **MRS:** De Man, Rogosa, and Sharpe (culture medium); **NCBI:** National Center for Biotechnology Information; **rRNA:** Ribosomal RNA.

Authors' Contributions

All authors contributed to the work, reviewed the manuscript, and approved it for publication. Each author has made significant,direct and intellectual contribution to the work.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This article does not contain any studies on human participants or animals performed by any of the authors.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Informed Consent

Not applicable.

ORCID iD

Vajid Nettoor Veettil https://orcid.org/0000-0002-5194-9051

References

1. Veettil VN and Chitra AV. Probiotic lactic acid bacteria from goat's milk potential producer of bacteriocin: evidence from liquid chromatography-mass spectrometry. *J Pure Appl Microbiol* 2022; 16(1): 305–317. doi: 10.22207/JPAM.16.1.19
2. Huang F, Teng K, Liu Y, et al. (2021). Bacteriocins: potential for human health. *Oxid Med Cell Longev* 2021: 5518825. doi: 10.1155/2021/5518825
3. López-Cuellar MDR, Rodríguez-Hernández AI, and Chavarría-Hernández N. LAB bacteriocin applications in the last decade. *Biotechnol Biotechnol Equip* 2016; 30(6): 1039–1050. doi: 10.1080/13102818.2016.1232605
4. Cheruvari A and Kammara R. Bacteriocins future perspectives: substitutes to antibiotics. *Food Control* 2024: 110834. doi: 10.1016/j.foodcont.2024.110834
5. Meade E, Slattery MA, and Garvey M. (2020). Bacteriocins, potent antimicrobial peptides and the fight against multi drug resistant species: resistance is futile? *Antibiotics* 2020; 9(1): 32. doi: 10.3390/antibiotics9010032
6. Soltani S, Hammami R, Cotter PD, 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
7. Lü X, Hu P, Dang Y, et al. Purification and partial characterization of a novel bacteriocin produced by *Lactobacillus casei* TN-2 isolated from fermented camel milk (Shubat) of Xinjiang Uygur Autonomous region, China. *Food Control* 2014; 43: 276–283. doi: 10.1016/j.foodcont.2014.03.020
8. Deegan LH, Cotter PD, Hill C, et al. Bacteriocins: biological tools for bio-preservation and shelf-life extension. *Int Dairy J* 2006; 16(9): 1058–1071. doi: 10.1016/j.idairyj.2005.10.026

9. Lahiri D, Nag M, Dutta B, et al. Bacteriocin: a natural approach for food safety and food security. *Front Bioeng Biotechnol* 2022; 10: 1005918. doi: 10.3389/fbioe.2022.1005918
10. Allémann E, Leroux JC, and Gurny R. Polymeric nano-and microparticles for the oral delivery of peptides and peptidomimetics. *Adv Drug Deliv Rev* 1998; 34(2–3): 171–189. doi: 10.1016/s0169-409x(98)00039-8
11. Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev*, 1993; 12(1–3): 39–85. doi: 10.1111/j.1574-6976.1993.tb00012.x
12. Rea MC, Ross RP, Cotter PD, et al. Classification of bacteriocins from Gram-positive bacteria. In: *Prokaryotic antimicrobial peptides: From genes to applications*. New York, NY: Springer New York; 2011. p. 29–53. doi: 10.1007/978-1-4419-7692-5_3
13. Mihaylova-Garnizova R, Davidova S, Hodzhev Y, et al. Antimicrobial peptides derived from bacteria: classification, sources, and mechanism of action against multidrug-resistant bacteria. *Int J Mol Sci* 2024; 25(19): 10788. doi: 10.3390/ijms251910788
14. Surati S. Bacteriocin, antimicrobial as A new natural food preservative: its potential and challenges. *Eruditio Indones J Food Drug Saf* 2020; 1(1): 63–82. doi: 10.54384/eruditio.v1i1.34
15. Tashakor A, Hosseinzadehdehkordi M, Emruzi Z, et al. Isolation and identification of a novel bacterium, *Lactobacillus sakei* subsp. dgh strain 5, and optimization of growth condition for the highest antagonistic activity. *Microb Pathog* 2017; 106: 78–84. doi: 10.1016/j.micpath.2017.02.008
16. Mills S, Griffin C, O'Connor PM, et al. A multibacteriocin cheese starter system, comprising nisin and lacticin 3147 in *Lactococcus lactis*, in combination with plantaricin from *Lactobacillus plantarum*. *Appl Environ Microbiol* 2017; 83(14): e00799-17. doi: 10.1128/aem.00799-17
17. Field D, Begley M, O'Connor PM, et al. Bioengineered nisin A derivatives with enhanced activity against both gram-positive and gram-negative pathogens. *PLoS One* 2012; 7(10): e46884. doi: 10.1371/journal.pone.0046884
18. O'Shea EF, O'Connor PM, Cotter PD, et al. Synthesis of trypsin-resistant variants of the listeria-active bacteriocin salivaricin P. *Appl Environ Microbiol* 2010; 76(16): 5356–5362. doi: 10.1128/AEM.00523-10
19. Zacharof MP and Lovitt RW. Bacteriocins produced by lactic acid bacteria: a review article. *APCBEE Procedia* 2012; 2: 50–56. doi: 10.1016/j.apcbee.2012.06.010
20. Mathur H, Rea MC, Cotter PD, et al. The sactibiotic subclass of bacteriocins: an update. *Curr Protein Pept Sci* 2015; 16(6): 549–558. doi: 10.2174/1389203716666150515124831
21. Stevens KA, Sheldon BW, Klapes NA, et al. Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. *Appl Environ Microbiol* 1991; 57(12): 3613–3615. doi: 10.1128/aem.57.12.3613-3615.1991
22. Gong X, Martin-Visscher LA, Nahirney D, et al. The circular bacteriocin, carnocyclin A, forms anion-selective channels in lipid bilayers. *Biochim Biophys Acta* 2009; 1788(9): 1797–1803.
23. Jumaila KP, Chitra AV, and Veettil VN. Isolation and molecular characterization of therapeutic probiotic *Lactobacillus* from different sources in Ernakulam-Kerala. *J Pure Appl Microbiol* 2024; 18(3): 1752–1767. doi: 10.22207/JPAM.18.3.24
24. Muriana PM and Klaenhammer TR. Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. *Appl Environ Microbiol* 1991; 57(1): 114–121. doi: 10.1128/aem.57.1.114-121.1991
25. Gonzalez CF and Kunka BS. Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. *Appl Environ Microbiol* 1987; 53(10): 2534–2538. doi: 10.1128/aem.53.10.2534-2538.1987
26. Zhang J, Yang Y, Yang H, et al. Purification and partial characterization of bacteriocin Lac-B23, a novel bacteriocin production by *Lactobacillus plantarum* J23, isolated from Chinese traditional fermented milk. *Front Microbiol* 2018; 9: 2165. doi: 10.3389/fmicb.2018.02165
27. Gupta H, Malik RK, Bhardwaj A, et al. Purification and characterization of enterocin FH 99 produced by a faecal isolate *Enterococcus faecium* FH 99. *Indian J Microbiol* 2010; 50(2): 145–155. doi: 10.1007/s12088-010-0039-4
28. Goyal C, Malik RK, and Pradhan D. Purification and characterization of a broad spectrum bacteriocin produced by a selected *Lactococcus lactis* strain 63 isolated from Indian dairy products. *J Food Sci Technol* 2018; 55(9): 3683–3692. doi: 10.1007/s13197-018-3298-4
29. Ge J, Sun Y, Xin X, et al. Purification and partial characterization of a novel bacteriocin synthesized by *Lactobacillus paracasei* HD1-7 isolated from Chinese sauerkraut juice. *Sci Rep* 2016; 6(1), 19366. doi: 10.1038/srep19366
30. Elayaraja S, Annamalai N, Mayavu P, et al. Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum. *Asian Pac J Trop Biomed* 2014; 4(Suppl. 1): S305–S311. doi: 10.12980/APJTB.4.2014C537
31. Srinivasan R, Kumawat DK, Kumar S, et al. Purification and characterization of a bacteriocin from *Lactobacillus rhamnosus* L34. *Ann Microbiol* 2013; 63: 387–392. doi: 10.1007/s13213-012-0486-8
32. Chen SY, Yang RS, Ci BQ, et al. A novel bacteriocin against multiple foodborne pathogens from *Lacticaseibacillus rhamnosus* isolated from juice ferments: ATF perfusion-based preparation of viable cells, characterization, antibacterial and antibiofilm activity. *Curr Res Food Sci* 2023; 6: 100484. doi: 10.1016/j.crfs.2023.100484
33. Huang T, Li Z, Qu X, et al. Preliminary purification and partial characterization of a functional bacteriocin of *Lacticaseibacillus paracasei* Zhang and mining for its gene cluster. *Probiotics Antimicrob Proteins* 2025; 17(2): 487–499. doi: 10.1007/s12602-024-10249-9
34. Yang E, Fan L, Jiang Y, et al. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *AMB Express* 2012; 2(1): 48. doi: 10.1186/2191-0855-2-48
35. Simons A, Alhanout K, and Duval RE. Bacteriocins, antimicrobial peptides from bacterial origin: overview of their biology and their impact against multidrug-resistant bacteria. *Microorganisms* 2020; 8(5): 639. doi: 10.3390/microorganisms8050639
36. Flórez AB and Mayo B. Genome analysis of *Lactobacillus plantarum* LL441 and genetic characterisation of the locus for the lantibiotic plantaricin C. *Front Microbiol* 2018; 9: 1916. doi: 10.3389/fmicb.2018.01916

37. Refay RM, Abushady HM, Amer SA, et al. Determination of bacteriocin-encoding genes of lactic acid bacteria isolated from traditional dairy products of Luxor province, Egypt. *Future J Pharm Sci* 2020; 6: 22. doi: 10.1186/s43094-020-00031-3
38. Islam KN, Akbar T, Akther F, et al. Characterization and confirmation of *Lactobacillus* spp. from selective regional yoghurts for probiotic and interference with pathogenic bacterial growth. *Asian J Biol Life Sci* 2016; 9: 1–9. https://scialert.net/abstract/?doi=ajbs.2016.1.9
39. Yuliana T, Pratiwi AR, Zahratunnisa S, et al. Purification and partial characterization of a bacteriocin produced by *Lactobacillus pentosus* 124-2 isolated from dadih. *Appl Sci* 2023; 13(7): 4277. doi: 10.3390/app13074277
40. Veettil VN. Optimization of bacteriocin production by *Lactobacillus plantarum* using response surface methodology. *Cell Mol Biol* 2022; 68(6): 105–110. doi: 10.14715/cmb/2022.68.6.17