Azam et al | Article 9398
J Pure Appl Microbiol. 2024. doi: 10.22207/JPAM.18.3.64
Received: 21 March 2024 | Accepted: 01 July 2024

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Isolation and Characterization of Bacteriocin Producing *Levilactobacillus brevis* Strain ABRIINW-K from Buffalo Dung

Mohd Azam*, Rashi Srivastava and Tanzeel Ahmed

School of Biotechnology, IFTM University, Moradabad, Uttar Pradesh, India.

Abstract

Bacteriocins are proteins secreted by many species of bacteria to inhibit other bacteria, thus eliminating competitors to gain resources. Bacteria from the *Lactobacillus* group are known for their applications as probiotics and food preservatives. They have earned a reputation for producing substances that inhibit the growth of other microorganisms, which include organic acids, diacetyl, and bacteriocins. Produced by the ribosomes, bacteriocins are cationic proteins that inhibit other bacteria coexisting within a shared ecological habitat. Due to their potential uses in a variety of applications large-scale production of Bacteriocins would be necessary. The study aimed to identify and characterize *Lactobacillus* bacteria that produce potent bacteriocins and to analyze the antimicrobial activity and stability of the isolated bacteriocin under various physical and biochemical conditions. A total of 50 samples including buffalo dung, cheese, and rhizospheric region of plants were screened to isolate 8 *Lactobacillus* Li-1, Li-2, Li-3, Li-4, Li-5, Li-6, Li-7, and Li-8, confirmed by gram staining and other biochemical tests. The cell free supernatant from the Li-3 strain showed higher inhibition of *Escherichia coli* and *Staphylococcus aureus*, as compared to the other isolated strains. Li-3 strain was further identified as *Levilactobacillus brevis* strain ABRIINW-K by 16S rRNA gene sequencing. The bacteriocin isolated from this strain is a thermostable peptide (~6kDa), which is characteristic of class II bacteriocins, with potent antibacterial activity against *Lactobacillus rhamnosus*, *Escherichia coli*, and *Salmonella enterica*.

Keywords: Bacteriocin, *Lactobacillus brevis*, Antimicrobial Activity, 16S rRNA, Antimicrobial Peptides

***Correspondence:** mohdazaam0786@gmail.com

Citation: Azam M, Srivastava R, Ahmed T. Isolation and Characterization of Bacteriocin Producing *Levilactobacillus brevis* Strain ABRIINW-K from Buffalo Dung. *J Pure Appl Microbiol.* Published online 31 August 2024. doi: 10.22207/JPAM.18.3.64

INTRODUCTION

In recent years, the exploration of natural compounds with antimicrobial properties has garnered significant attention due to the escalating global concern over antibiotic resistance and its potential impact on public health. Bacteriocins, a class of ribosomally synthesized antimicrobial peptides, have emerged as a promising substitute for traditional antibiotics.[1-3] These peptide molecules exhibit a broad spectrum activity against pathogenic microorganisms, including bacteria, making them a subject of intense research within the fields of food preservation, pharmaceuticals, and clinical therapeutics.[4,5]

Among the sources of bacteriocins, lactic acid bacteria (LAB) have gained special attention for their ability to produce these bioactive compounds.[6-8] *Lactobacillus* species, a prominent group of LAB, has received wide attention for their probiotic properties and their role in maintaining gut health. In addition to their probiotic attributes, certain strains of *Lactobacillus* have shown the capacity to produce potent bacteriocins that exhibit inhibitory effects against closely related and even distantly related pathogenic bacteria.[4,9,10] This dual functionality of some *Lactobacillus* strains has spurred a growing interest in the characterization of the bacteriocins produced by them.[11-13]

The strain *Lactobacillus brevis* ABRIINW-K, isolated from an unexplored ecological niche, presents an intriguing opportunity for bacteriocin discovery. The genetic diversity of LAB strains, reflected in their distinct 16S rRNA sequences, contributes to variations in their metabolic profiles, antimicrobial capabilities, and probiotic potential. Understanding the genetic and functional attributes of *L. brevis* ABRIINW-K's bacteriocin holds promise for harnessing its antimicrobial properties for applications in food safety, microbial ecology, and biomedical interventions.[13]

This research paper aims to delve into the isolation and characterization of bacteriocin accomplished by *L. brevis* ABRIINW-K. By employing a combination of biochemical, molecular biology, and analytical techniques, we seek to elucidate the structural properties, mode of action, spectrum of activity, and potential applications of the isolated bacteriocin. The findings of this study may contribute to expanding the repertoire of natural antimicrobial agents available for combating bacterial infections and provide insights into the broader ecological roles of bacteriocin-producing LAB strains.[14-16]

In the following sections, we present the methodology employed for bacteriocin isolation, purification, and characterization from *Lactobacillus brevis* ABRIINW-K isolated from buffalo dung. Additionally, we will discuss the results of our investigations, drawing comparisons to existing literature and highlighting the significance of our findings in the context of antimicrobial research and application.[17-19]

MATERIALS AND METHODS

All laboratory-based experimental studies were conducted at the Microbiology laboratory, IFTM University, Moradabad, Uttar Pradesh, India. The indicator organisms used in the study are *Lactobacillus rhamnosus* (MTCC 1408), *Escherichia coli* (MTCC 1687), *Staphylococcus aureus* (MTCC 737), and *Salmonella enterica* (MTCC 3858) were obtained from MTCC, Chandigarh, India.

Isolation and screening of *Lactobacillus* strains

50 samples including buffalo dung, cheese, and rhizospheric region of plants were examined for the presence of bacteriocin-producing *Lactobacillus*. Buffalo dung is rich in organic matter and provides a nutrient-rich environment. Bacteria that produce bacteriocins can survive and thrive in such environments by limiting competition from other microorganisms that might utilize the available resources. Similarly, some bacteriocin-producing bacteria are naturally present in milk, the primary ingredient in cheese. These bacteria can survive the cheese-making process and become part of the cheese microbiota. In addition, the rhizosphere is a highly competitive environment with a diverse microbial community vying for resources such as nutrients and space. Bacteriocin-producing bacteria can inhibit or kill competing microbial species, giving them an advantage in establishing and maintaining their populations that's why these habitats were selected to isolate the bacteria. Strains were

identified by gram staining and other biochemical tests like Gram staining to confirm its Gram-positive nature, the catalase test, and the catalase test, both of which should yield negative results. Carbohydrate fermentation, H_2S, indole, motility, urease, MR-VP, and Oxidase were also assessed and developed in 50.0 mL MRS broth (HiMedia) at 30°C for 48 hours in a rotary shaker. After incubation, cell-free supernatant was obtained from each culture for screening of bacteriocin production. The broth was centrifuged at 10,000 rpm for 15 minutes at 4°C and the antibacterial capabilities of the supernatant against *E. coli* and *S. aureus* was assumed by well diffusion assay in *Mueller Hinton* agar (HiMedia) plates as described earlier.[20-22] As per our findings, *Lactobacillus* strain Li-3 was used for further studies due to the higher activity of its cell supernatant against both indicator organisms.

Molecular identification of Li-3 strain

Genomic DNA of the Li-3 strain has been purified utilizing a QIAamp DNA kit (Qiagen) and selective 16S rRNA gene has been amplified utilizing the forward as well as reverse primers 5'-GGATGAGCCCGCGGCCTA-3' and 5'-CGGTGTGTACAAGGCCCGG-3' respectively.[23] The PCR conditions were in the following order: the 5 minutes of initial denaturation at 96°C, denaturation at 96°C for 30 seconds, 30 seconds needed for annealing at 50°C, 1 minute and 30 seconds for the prolongation at 60°C.[24] Sanger dideoxy sequencing was carried out as previously described.[25,26] Applied Biosystems' ABI 3130 genetic analyzer was used for the sequencing process and chemistry big dye terminator version 3.1 cycle sequencing kit using a 50 cm capillary array, POP-7 Polymer, and BDTv3KB Denovo v5.2 protocol. Data was performed on the Applied Biosystems SeqScape v5.2 software. The organism has been observed by comparing the sequencing data to the NCBI database utilizing the BLAST (BasiciLocal Alignment Search Tool).[24-27]

Bacteriocin isolation and antibacterial activity

For bacteriocin isolation, 50 mL MRS broth with the Li-3 strain, hereon designated as *L. brevis* ABRIINW-K, was grown at 30°C for 48 hours with continuous shaking. To partially purify bacteriocin from the bacterial fermentation broth, begin by centrifuging the broth at 10,000 rpm for 10 minutes at 4°C to pellet the bacterial cells, and then filtered the supernatant through a 0.22 µm membrane filter to remove residual cells and debris. Next, gradually added solid ammonium sulfate to the filtered supernatant to achieve 60% saturation, stirring gently until fully dissolved. Allowed the solution to precipitate at 4°C for overnight, then centrifuged at 10,000 rpm for 10 minutes at 4°C to collect the protein pellet. Dissolved the pellet in a minimal volume of Tris-HCl buffer (10 mM, pH 7.5) and dialyzed against a large volume of distilled water or Tris-HCl buffer at 4°C, changing the buffer several times over 24 hours to remove ammonium sulfate.[22]

The activity of the *L. brevis* ABRIINW-K bacteriocin was assayed by the well diffusion test method. 50 µL crude bacteriocin was added into 8 mm diameter wells in Muller Hinton agar (HiMedia) plates. Inoculum size was estimated by the McFarland method. Approximately 1.0×10^8, *L. rhamnosus*, *E. coli*, and *Salmonella enterica* were plated onto the agar surface and incubated at 37°C for 24 to 48 hours. Further, loss of activity after treatment with proteases confirmed the active component was a protein. Activity units per milliliter (AU mL^{-1}) were determined by serial two-fold dilution of the Bacteriocin. One AU mL^{-1} shows the reciprocal of the maximum dilution at which a distinct zone of inhibition was observed.[22,27,28]

Effect of temperature, pH, and proteases on bacteriocin activity

The inhibitory activity of *L. brevis* ABRIINW-K bacteriocin was assayed against indicator organisms upon exposure to different physical and biochemical conditions. To study the effect of temperature bacteriocin was heated at 50, 60, 70, 80, 90, and 100°C for 2 hours and at 121°C for 20 minutes, then antibacterial activity was assayed against indicator organisms. The stability of purified bacteriocin was assayed at pH 4, 4.5, 5, 5.5, 6, 6.5, 7.0, and 9.0, pH was adjusted with sterile 1M HCL and 1M NaOH. The impact of proteases on Bacteriocin was studied by treatment with 0.1% w/v each of pepsin, proteinase K, chymotrypsin, and trypsin (all from HiMedia) at 30°C for 2 hrs in separate reactions. The impact of detergents like Triton X-100, Tween 20, and

Tween 80 was tested at 0.5% v/v concentration. Antibacterial activity for all tests was assayed in triplicates.[21,22,27]

Size estimation of Bacteriocin by SDS-PAGE

Polyacrylamide gel electrophoresis (SDS-PAGE) with sodium dodecyl sulfate was used to separate the partially purified bacteriocin with a 16% resolving gel utilizing the tris glycine buffer system, in a Mini-Protean Electrophoresis System (Bio-rad).[29] A pre-stained 2.5 to 45 kDa molecular weight marker (Amersham Biosciences) was used. Following electrophoresis, the gel was predicted by staining with Coomassie BrilliantiBlue R-250 (HiMedia).[21,30]

RESULTS AND DISCUSSION

Isolation and screening of *Lactobacillus* strains

In this study, eight *Lactobacillus* strains labeled Li-1 to Li-8 were isolated and identified through Gram staining and various biochemical tests. All strains demonstrated the ability to produce bacteriocins, aligning with the findings of previous studies on *Lactobacillus* bacteriocin production. The identification of these strains contributes to the growing body of research emphasizing the potential of *Lactobacillu*s species in antimicrobial applications.

The bacteriocin production observed in our strains is consistent with earlier reports by Todorov *et al*.,[13] who characterized bacteriocin-producing *Lactobacillus* strains isolated from traditional fermented foods. Similar to our findings, they reported the inhibitory activity of these bacteriocins against a range of pathogenic bacteria, suggesting a broad-spectrum antimicrobial potential.

The bacteriocin production observed in our *Lactobacillus brevis* strain UN is consistent with the findings reported by Gautam et al. [31], who also characterized bacteriocin production by *Lactobacillus brevis* isolated from Dhulliachar, a traditional food product of North East India. Their study demonstrated the purification and characterization of this bacteriocin, revealing its broad-spectrum antimicrobial activity against several pathogenic bacteria, which aligns with our observations. This suggests that the specific conditions and microbial communities in traditional fermented foods like Dhulliachar may foster the development of potent bacteriocin-producing strains with significant antimicrobial properties.

In addition, the study by Jang et al.[32] on *Lactobacillus brevis* KU15153, isolated from kimchi, supports our findings by highlighting the broad antimicrobial effects of bacteriocins produced by *Lactobacillus brevis*. They characterized the bacteriocin's inhibitory effects against a variety of pathogens, similar to the spectrum observed in our study. The work by Jang et al. also emphasized the probiotic characteristics of *Lactobacillus brevis*, further underlining the potential health benefits of strains isolated from traditional fermented foods.

Moreover, Elayaraja *et al*.[33] studied the bacteriocin produced by *Lactobacillus murinus* AU06 and reported a broad antibacterial spectrum, which echoes the broad-spectrum activity found in our *Lactobacillus brevis* strain UN. Their research, like ours, involved the purification and characterization of bacteriocins, adding to the body of evidence that bacteriocins from different *Lactobacillus* species can effectively inhibit a wide range of pathogens.

Finally, the work by Mounier *et al*.[34] on bacteriocins in smear-ripened cheeses provides additional context by exploring the role of bacteriocins in the complex microbial ecosystems of fermented foods. Although their focus was on cheese, the principles of bacteriocin interaction within microbial communities and their preservation qualities are relevant to our study, as they highlight the versatility and importance of bacteriocins in food safety and microbial balance.

In a comparative study by Ogunbanwo *et al*.,[35] the bacteriocins produced by *Lactobacillus* strains isolated from fermented food products exhibited notable antimicrobial activities. Their strains, like ours, were identified and characterized using Gram staining and biochemical tests, affirming the reliability and consistency of these methods for identifying bacteriocinogenic *Lactobacillus* species.

Furthermore, the study by Parvez *et al*.[36] highlighted the importance of bacteriocin-producing *Lactobacillus* in probiotic applications. Our isolated strains may have similar probiotic potential, given their ability to inhibit pathogenic

bacteria. This potential is further supported by our preliminary observations on the stability and activity of the bacteriocins under various conditions, akin to the stability characteristics reported by Yang *et al*.[37]

Our study adds to the existing literature by not only confirming the bacteriocin-producing capability of these eight *Lactobacillus* strains but also by preserving these strains for future research. This preservation is crucial for detailed genetic and proteomic analyses to understand the mechanisms underlying bacteriocin production and their applications in food preservation and human health.

In this study, we isolated eight *Lactobacillus* strains from diverse environments: the rhizospheric region of plants (Li-1 and Li-2), fecal samples of buffalo dung (Li-3 and Li-4), and cheese samples (Li-5 to Li-8). All strains demonstrated the ability to produce bacteriocins, as evidenced by the inhibitory activity of their supernatants against pathogenic bacteria. The protease sensitivity of these bacteriocins suggests that their antibacterial activity is proteinaceous, aligning with the characteristics of known bacteriocins.

Comparative studies have similarly reported the isolation of bacteriocin-producing *Lactobacillus* strains from varied sources. For instance, Todorov *et al*.[13] isolated *Lactobacillus* strains from traditional fermented foods, which exhibited significant antimicrobial activity, akin to our findings with strains from cheese samples. Their study also emphasized the importance of the source in determining the bacteriocin spectrum and potency, a trend observed in our results, particularly with the superior activity of Li-3.

The inhibitory activity of Li-3, isolated from buffalo dung, was notably high against both *Escherichia coli* and *Staphylococcus aureus*, outperforming the other strains. This is consistent with Ogunbanwo *et al*.,[35] who reported robust bacteriocin activity from *Lactobacillus* strains isolated from animal sources, suggesting that such environments might harbor strains with potent antimicrobial properties. The enhanced activity of Li-3 could be attributed to the unique microbial interactions and competitive pressures in the gut microbiota of buffalo, which select for highly antagonistic bacteria.

Our findings are also in agreement with the work of Parvez *et al*.,[36] who highlighted the probiotic potential of bacteriocin-producing

Table 1. Biochemical identification of *Lactobacillus* strains

Characteristics	*Lactobacillus rhamnosus* (MTCC 1408)	Li-3-strain *Levilactobacillus brevis*
Gram stain	Gram-positive	Gram-positive
H_2S	Negative	Negative
Catalase	Negative	Negative
Indole	Negative	Negative
Motility	Negative	Negative
Urease	Negative	Negative
MR-VP	Negative	Negative
Oxidase	Negative	Negative

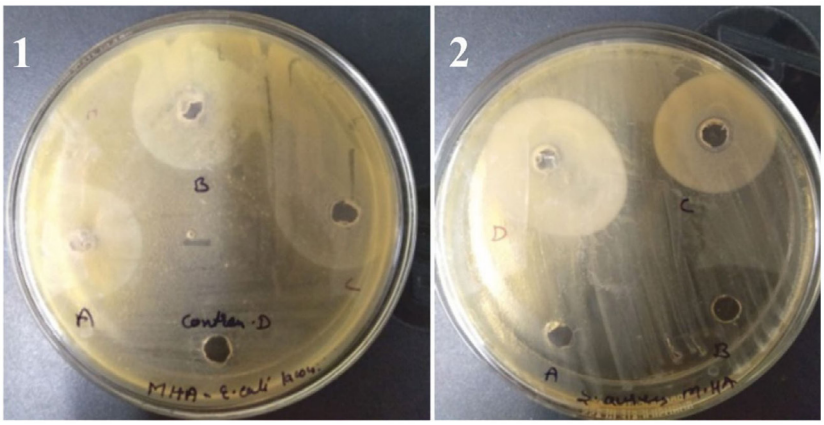

Figure 1. Antibacterial activity of *Lactobacillus* isolated from buffalo dung, (1) Well A: Li-3, Well B: Li-4, Well C: Li-4, and Well D: negative control. (2) Well A: negative control, Well B: Li-4, Well C: Li-3, and Well D: Li-3

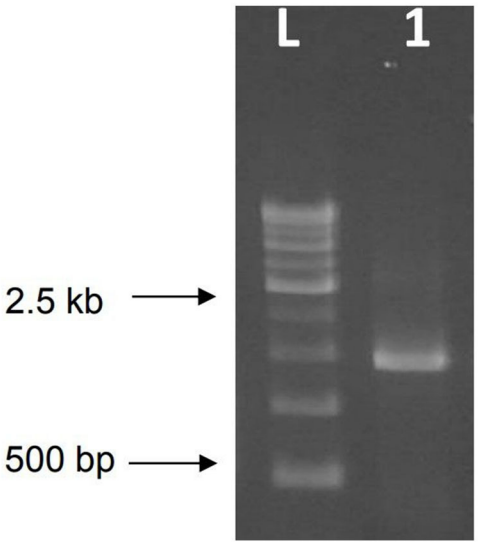

Figure 2. Agarose gel electrophoresis of (1) Amplified region of 16S rRNA gene of the Li-3 strain with ~1500bp size, (L) 500bp DNA ladder

Lactobacillus strains. The inhibition of common pathogens by Li-3 underscores its potential application in probiotic formulations aimed at improving gut health and preventing infections. Moreover, the study by Yang *et al*.[37] on the stability and activity of *Lactobacillus* bacteriocins under various conditions further supports the feasibility of using Li-3 in diverse applications, ranging from food preservation to clinical therapeutics.

The protease sensitivity of the bacteriocins produced by our strains indicates that their antimicrobial components are proteins, as demonstrated by previous research.[35,36] This characteristic is crucial for their functional categorization and potential application in environments where protein stability can be managed.

The exceptional performance of Li-3, in particular, warrants further investigation. Future research will focus on the molecular characterization of its bacteriocin, assessing its genetic basis, mode of action, and spectrum of activity. Additionally, its application in real-world scenarios, such as food safety and as a probiotic, will be explored. Understanding the precise

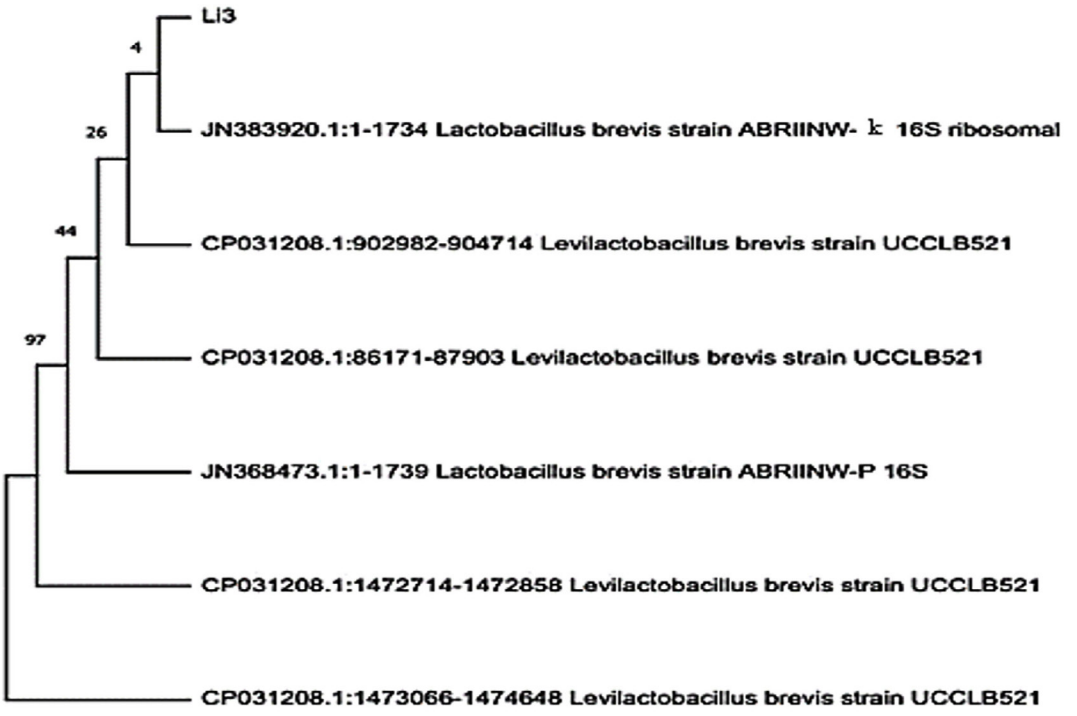

Figure 3. Phylogenetic tree of *Levilactobacillus brevis* strain ABRIlNW-K using the Fast Minimum Evolution method in the Blast Tree View Widget on NCBI server

mechanisms by which Li-3 exerts its antimicrobial effects will be crucial for developing effective strategies to harness its potential.

In conclusion, this study not only confirms the bacteriocin-producing capabilities of *Lactobacillus* strains from diverse sources but also identifies the Li-3 strain as a particularly potent candidate for further exploration. These findings contribute to the expanding knowledge of *Lactobacillus*-derived bacteriocins and their applications, with the potential to develop natural and effective antimicrobial solutions.

Isolation and screening of *Lactobacillus* strains

Eight *Lactobacillus* cultures were isolated and identified by gram staining and other biochemical tests (Table 1). All 8 strains were found to produce Bacteriocins and were preserved for future research. These strains were labelled from Li-1 to Li-8.

Li-1 and Li-2 were isolated from the rhizospheric region of plants, Li-3 and Li-4, were isolated from fecal samples of buffalo dung, and Li-5, Li-6, Li-7, and Li-8 were isolated from cheese samples. The inhibitory activity of the supernatant preparations from each organism containing crude bacteriocins is presented in Table 2. Treatment of the supernatants with proteases resulted in inhibition of their antibacterial activity. *Lactobacillus* strain Li-3 showed the highest inhibition of both *E. coli and S. aureus* (Figure 1). Therefore, the Li-3 strain was considered for further exploration of its bacteriocin properties.

Table 2. Screening of Bacteriocin activity of *Lactobacillus* strains

Indicator strain	Li-1	Li-2	Li-3	Li-4	Li-5	Li-6	Li-7	Li-8
E. coli	+	+	+++	+	++	-	-	-
S. aureus	-	-	+++	-	-	-	-	-

(-); No inhibition, (+); 1-5 mm low inhibition, (++); 5-10 mm moderate inhibition, (+++); 10 mmi high inhibition

Table 3. Antibacterial activity of *L. brevis* ABRIINW-K bacteriocin

Indicator strain	Antibacterial activity (AU mL^{-1})
L. rhamnosus	6400
Salmonella enterica	3200
E. coli	3200

Molecular identification of Li-3 strain

About 1,500 bp region of the Li-3 16S rRNA gene has been amplified (Figure 2) and sequenced. Analysis of the amplified sequence using BLAST search on the NCBI server showed 99.52% similarity to *Lactobacillus brevis* ABRIINW-K strain (Sequence ID JN368471.1). Phylogenetic analysis of the strain is presented in Figure 3. Owing to

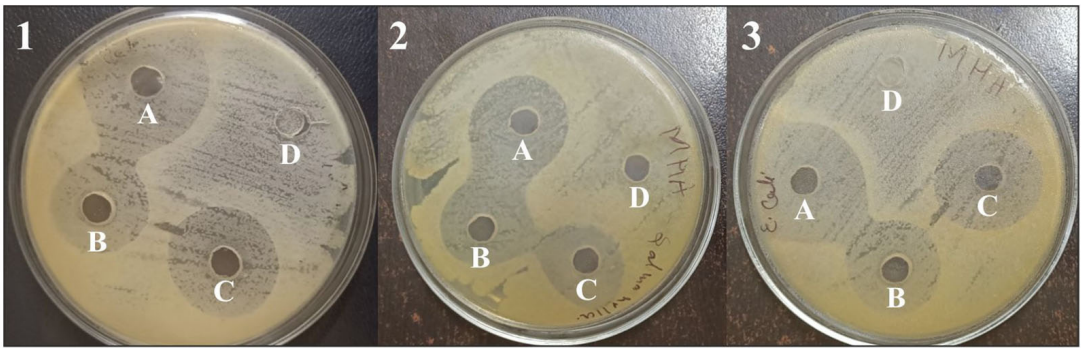

Figure 4. Antibacterial activity of *L. brevis* ABRIINW-K bacteriocin showing zone of inhibition against indicator strains (1) *L. rhamnosus,* (2) *Salmonella enterica*, and (3) *E. coli*. 0.1 mL of the bacteriocin was added to wells A, B, and C in each plate. 0.1 mL sodium acetate buffer was added to well D as a negative control.

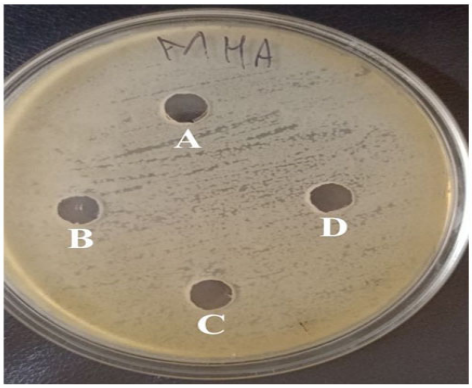

Figure 5. The antibacterial activity of bacteriocin after treatment with proteases. (A) Pepsin, (B) Trypsin, (C) Chymotrypsin, and (D) Proteinase K treated bacteriocin were added to each well in plates spread with *E. coli* culture

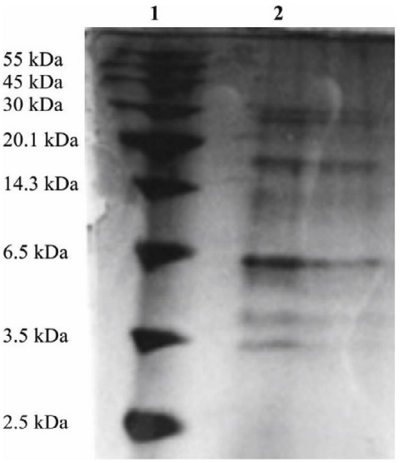

Figure 6. Size estimation of Bacteriocin by SDS-PAGE. Lane 1 Molecular weight marker, and Lane 2: Partially cleaned *L. brevis* ABRIINW-K bacteriocin

their probiotic potential, bacteriocin-producing *L. brevis* has also been isolated and characterized from diverse sources like traditional food products, various plants, animals, and human sources.[31,32]

Bacteriocin purification from *L. brevis* ABRIINW-K and antibacterial activity

The partially purified *L. brevis* ABRIINW-K bacteriocin obtained by dialysis showed active inhibition of all indicator strains *L. rhamnosus*, *Salmonella enterica*, and *E. coli* upon 24 to 48 hours of incubation at 37°C (Figure 4). Results are presented in Table 3. It is evident from past studies that bacteriocin from *Lactobacillus* species has the potential to inhibit or kill closely related as well as other groups of bacteria.

Effect of temperature, pH, and proteases on bacteriocin activity

The partially purified *L. brevis* ABRIINW-K bacteriocin heated at 50, 60, 70, 80, 90, and 100°C for 2 hours, showed no changes in their activity against indicator strains, but lost complete activity against indicator strains when heated at 121°C for 20 min, thus confirming its thermostable characteristics.[33] The bacteriocin remained stable between pH 5.5 to 8.5 and showed a decrease in its potency beyond this pH range. Complete bacteriocin activity has been eliminated after treatment with all of the proteases pepsin, proteinase K, chymotrypsin, and trypsin (Figure 5). Since bacteriocins are peptides that are sensitive to proteolytic enzymes.[24]

Size estimation by SDS-PAGE and classification of bacteriocin

SDS-PAGE analysis of the *L. brevis* ABRIINW-K bacteriocin compared to low molecular weight protein marker presented a clear band of approximately 6 kDa (Figure 6). Contaminant proteins were detected at lower concentrations. The low molecular weight and thermostability of the bacteriocin correspond to characteristics of class II bacteriocins as confirmed by earlier studies.[34]

CONCLUSION

Cell-free supernatants from eight *Lactobacillus* species showed antibacterial properties against various foodborne pathogens, suggesting potential use as a natural antimicrobial agent in food production. This could be a substitute for preservatives in organic foods. A potent bacteriocin from the *Lactobacillus* strain, *L. brevis* ABRIINW-K, was isolated and characterized, confirming its broad-spectrum activity and potential as a novel antimicrobial agent. Future research should focus on purification, structural elucidation, *in vivo* efficacy studies, and safety assessments. The data collected may also help explore other unexplored bacteriocins and

microorganisms, enriching our understanding of their diversity and functional significance within microbial ecosystems.

In summary, these reports add to the expanding knowledge of bacteriocins and their promise as natural substitutes for conventional antibiotics. The combination of molecular, biochemical, and biophysical techniques has allowed us to unravel the intricate details of this bacteriocin's properties, providing a foundation for further research in areas such as food preservation, medical therapeutics, and biopreservation.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

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

REFERENCES

1. Gunasekaran T, Gopalakrishnan A, Balaraman D, Kathirkaman P. Chitinolytic Bacterial Disease Infected in *Scylla serrata* (Forsskal, 1775) Fattening Farm of Tamil Nadu. *Int J Life Sci.* 2018;7(2):90-95. doi: 10.5958/2319-1198.2018.00011.8
2. Mathur H, Field D, Rea MC, Cotter PD, Hill C, Ross RP. Bacteriocin-Antimicrobial Synergy: A Medical and Food Perspective. *Front Microbiol*. 2017;8:1205. doi: 10.3389/fmicb.2017.01205
3. Turgis M, Vu KD, Jamshidian M, Maherani B, Lacroix M. Synergistic antimicrobial effect of combined bacteriocins against food pathogens and spoilage bacteria. *Microbiol Res Int.* 2016;4(1):1-5.
4. Pieterse R, Todorov SD. Bacteriocins - exploring alternatives to antibiotics in mastitis treatment. *Braz J Microbiol.* 2010;41(3):542-562. doi: 10.1590/S1517-83822010000300003
5. Ortiz-Rivera Y, Sanchez-Vega R, Gutierrez-Mendez N, Leon-Felix J, Acosta-Muniz C, Sepulveda DR. Production of reuterin in a fermented milk product by *Lactobacillus reuteri:* Inhibition of pathogens, spoilage microorganisms, and lactic acid bacteria. *J Dairy Sci.* 2017;100(6):4258-4268. doi: 10.3168/jds.2016-11534
6. Hammami R, Zouhir A, Le Lay C, Ben Hamida J, Fliss I. BACTIBASE second release: a database and tool platform for bacteriocin characterization. *BMC Microbiol.* 2010;10:22. doi: 10.1186/1471-2180-10-22
7. 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
8. Kabuki T, Uenishi H, Watanabe M, Seto Y, Nakajima H. Characterization of a bacteriocin, Thermophilin 1277, produced by *Streptococcus thermophilus* SBT1277. *J Appl Microbiol.* 2007;102(4):971-980. doi: 10.1111/j.1365-2672.2006.03159.x
9. Tagg JR, Dajani AS, Wannamaker LW. Bacteriocins of gram-positive bacteria. *Bacteriol Rev.* 1976;40(3):722-756. doi: 10.1128/br.40.3.722-756.1976
10. Lelise A, Belaynesh G, Mulubrhan M, Kedija S, Endashaw B, Abebe B. Isolation and screening of antibacterial producing lactic acid bacteria from traditionally fermented drinks ("Ergo" and "Tej") in Gondar town, Northwest Ethiopia. *Global Research Journal of Public Health and Epidemiology*; 2014;1(3):18-22
11. Asare PT, Greppi A, Stettler M, Schwab C, Stevens MJA, Lacroix C. Decontamination of Minimally-Processed Fresh Lettuce Using Reuterin Produced by *Lactobacillus reuteri. Front Microbiol.* 2018;9:1421. doi: 10.3389/fmicb.2018.01421
12. Cotter PD, Ross RP, Hill C. Bacteriocins - a viable alternative to antibiotics? *Nat Rev Microbiol*. 2013;11(2):95-105. doi: 10.1038/nrmicro2937
13. Todorov SD, Furtado DN, Saad SMI, Tome E, Franco BDGM. Potential beneficial properties of bacteriocin-producing lactic acid bacteria isolated from smoked salmon. *J Appl Microbiol.* 2011;110(4):971-986. doi: 10.1111/j.1365-2672.2011.04950.x
14. Cocolin L, Foschino R, Comi G, Fortina MG. Description of the bacteriocins produced by two strains of *Enterococcus faecium* isolated from Italian goat milk. *Food Microbiol.* 2007;24(7-8):752-758. doi: 10.1016/j.fm.2007.03.001
15. Mathara JM, Schillinger U, Kutima PM, Mbugua SK, Holzapfel WH. Isolation, identification and characterisation of the dominant microorganisms of kule naoto: the Maasai traditional fermented milk in Kenya. *Int J Food Microbiol.* 2004;94(3):269-278. doi: 10.1016/j.ijfoodmicro.2004.01.008
16. Kang JH, Lee MS. Characterization of a bacteriocin produced by *Enterococcus faecium* GM-1 isolated from an infant. *J Appl Microbiol*. 2005;98(5):1169-1176. doi: 10.1111/j.1365-2672.2005.02556.x
17. O'Shea EF, O'Connor PM, O'Sullivan O, Cotter PD, Ross RP, Hill C. Bactofencin A, a new type of

cationic bacteriocin with unusual immunity. *mBio.* 2013;4(6):e00498-00413. doi: 10.1128/mBio.00498-13

18. Serna CL, de Stouvenel AR. Lactic acid production by a strain of *Lactococcus lactis* subs lactis isolated from sugar cane plants. *Electron J Biotechnol.* 2006;9(1). doi: 10.2225/vol9-issue1-fulltext-10
19. Rantsiou K, Drosinos EH, Gialitaki M, Metaxopoulos I, Comi G, Cocolin L. Use of molecular tools to characterize *Lactobacillus* spp. isolated from Greek traditional fermented sausages. *Int J Food Microbiol.* 2006;112(3):215-222. doi: 10.1016/j.ijfoodmicro.2006.09.001
20. Alias R, Idrus ES, Lani M, et al. Antibacterial Activity of Lactic Acid Bacteria Isolated from Goats' Milk. 2012.
21. Hassan MU, Nayab H, Rehman TU, et al. Characterisation of Bacteriocins Produced by *Lactobacillus* spp. Isolated from the Traditional Pakistani Yoghurt and Their Antimicrobial Activity against Common Foodborne Pathogens. *BioMed Res Int.* 2020;e8281623. doi: 10.1155/2020/8281623
22. Malheiros PS, Sant'Anna V, Todorov SD, Franco BDGM. Optimization of growth and bacteriocin production by *Lactobacillus sakei* subsp. *sakei*2a. *Braz J Microbiol.* 2015;46:825-834. doi: 10.1590/S1517-838246320140279
23. Verma M, Mishra V. Bioelectricity Generation Using Sweet Lemon Peels as Anolyte and Cow Urine as Catholyte in a Yeast-Based Microbial Fuel Cell. *Waste Biomass Valorization.* 2023;14(8):2643-2657. doi: 10.1007/s12649-023-02050-6
24. Rossi E, Ali A, Efendi R, et al. Characterization of Bacteriocin Produced by Lactic Acid Bacteria Isolated from Solid Waste of Soymilk production. *IOP Conf Ser Earth Environ Sci.* 2021;709(1):012020. doi: 10.1088/1755-1315/709/1/012020
25. Santos NC, Cabrera MEB, Flores JD, et al. Molecular Identification and Phylogenetic Analysis of Bioluminescent Bacteria Isolated from Selected Marine Crustacean in Manila Bay, Philippines. *J Pure Appl Microbiol.* 2023;17(4):2119-2130. doi: 10.22207/JPAM.17.4.08
26. Singh RK, Tiwari S, Mishra VK, Tiwari R, Dhole TN. Molecular epidemiology of Chikungunya virus: Mutation in E1 gene region. *J Virol Methods*. 2012;185(2):213-220. doi: 10.1016/j.jviromet.2012.07.001
27. Sevim E, Karaoglu SA, Sevim A, Canakci S. Antimicrobial Activity of *Bacillus* Strains Isolated from Spring Water and A Novel Bacteriocin: RS108. *J Pure Appl Microbiol.* 2013;7(4):2757-2765.
28. Ribeiro SC, O'Connor PM, Ross RP, Stanton C, Silva CCG. An anti-listerial *Lactococcus lactis* strain isolated from Azorean Pico cheese produces lacticin 481. *Int Dairy J.* 2016;63:18-28. doi: 10.1016/j.idairyj.2016.07.017
29. Merzoug M, Dalache F, Zadi Karam H, Karam NE. Isolation and preliminary characterisation of bacteriocin produced by *Enterococcus faecium* GHB21 isolated from Algerian paste of dates "ghars." *Ann Microbiol.* 2016;66(2):795-805. doi: 10.1007/s13213-015-1165-3
30. Tulini FL, De-Martinis ECP. Improved adsorption-desorption extraction applied to the partial characterization of the antilisterial bacteriocin produced by *Carnobacterium maltaromaticum* C2. *Braz J Microbiol Publ Braz Soc Microbiol.* 2010;41(2):493-496. doi: 10.1590/S1517-83822010000200032
31. Gautam N, Sharma N, Ahlawat OP. Purification and Characterization of Bacteriocin Produced by *Lactobacillus brevis* UN Isolated from Dhulliachar: a Traditional Food Product of North East India. *Indian J Microbiol.* 2014;54(2):185-189. doi: 10.1007/s12088-013-0427-7
32. Jang HJ, Lee NK, Paik HD. Probiotic characterization of *Lactobacillus brevis* KU15153 showing antimicrobial and antioxidant effect isolated from kimchi. *Food Sci Biotechnol.* 2019;28(5):1521-1528. doi: 10.1007/s10068-019-00576-x
33. Elayaraja S, Annamalai N, Mayavu P, Balasubramanian T. Production, purification and characterization of bacteriocin from Lactobacillus murinus AU06 and its broad antibacterial spectrum. *Asian Pac J Trop Biomed.* 2014;4(Suppl 1):S305-311. doi: 10.12980/APJTB.4.2014C537
34. Mounier J, Coton M, Irlinger F, Landaud S, Bonnarme P. Chapter 38 - Smear-Ripened Cheeses. *Cheese* (Fourth Edition). 2017:955-996. doi: 10.1016/B978-0-12-417012-4.00038-7
35. Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by Lactobacillus plantarum F1 and Lactobacillus brevis OG1. *African Journal of Biotechnology,* 2003;2(8):219-227.
36. Parvez S, Malik KA, Ah-Kang S, Kim HY. Probiotics and their fermented food products are beneficial for health. *Journal of applied microbiology,* 2006;100(6):1171-1185. doi: 10.1111/j.1365-2672.2006.02963.x
37. Yang E, Fan L, Jiang Y, Doucette C, Fillmore S. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *AMB express,* 2012;2:1-12.