IJETIR
ISSN (Online) 2583-0554
International Journal of Emerging Technologies and Innovative Research (IJETIR)
Volume 6, Issue 6, June 2026
Impact Factor: 5.731

Isolation, Screening and Molecular Characterization of Bacteriocin-Producing Lactobacillus fermentum 28D1 from Environmental Sources

Dr. Hemlata Nishad and Vandita Gupta
Supervisor, Department of Microbiology
Research Scholar, Department of Microbiology
Dr. C.V. Raman University, Kargi Road Kota, Bilaspur (C.G.) INDIA

Abstract: *The growing emergence of antibiotic-resistant microorganisms has intensified the search for alternative antimicrobial agents. Bacteriocins, proteinaceous compounds synthesized by bacteria, have attracted considerable attention because of their antimicrobial properties and potential applications in food preservation and therapeutics. The present study aimed to isolate bacteriocin-producing bacteria from diverse environmental sources and identify isolates exhibiting broad-spectrum antibacterial activity. A total of 275 bacterial isolates were recovered from dairy products, food and vegetable waste, polluted river water, industrial waste, and garden soil. Initial screening using agar spot assay identified 69 isolates with antimicrobial activity, while agar well diffusion assay confirmed 48 isolates as bacteriocin producers. Among these, three isolates (28D1, 12VW1, and 10HR1) demonstrated broad-spectrum inhibitory activity against multiple indicator bacteria. Isolate 28D1 exhibited the widest antimicrobial spectrum, inhibiting both Gram-positive and Gram-negative bacteria, including Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, and Pseudomonas fluorescens. Biochemical characterization suggested that isolate 28D1 belonged to the genus Lactobacillus. Molecular identification through 16S rRNA gene sequencing confirmed the isolate as Lactobacillus fermentum 28D1 with 99.47% sequence similarity to reference strains. The bacteriocin produced by L. fermentum 28D1 exhibited broad-spectrum antibacterial activity but showed no inhibitory effect against yeast and mould species. The findings highlight the potential of L. fermentum* 28D1 as a promising source of natural antimicrobial compounds for food and pharmaceutical applications.

Keywords: Bacteriocin, Lactobacillus fermentum, antimicrobial activity, broad-spectrum bacteriocin, food preservation, probiotic bacteria.

I. INTRODUCTION

The widespread occurrence of antibiotic-resistant pathogens has become a significant challenge to public health worldwide. Consequently, considerable attention has been directed toward naturally occurring antimicrobial compounds produced by microorganisms. Among these compounds, bacteriocins represent a diverse group of ribosomally synthesized peptides or proteins capable of inhibiting the growth of competing bacteria.

Bacteriocin-producing microorganisms are commonly found in environments characterized by intense microbial competition, such as fermented foods, dairy products, soil, and decomposing organic matter. These antimicrobial peptides provide ecological advantages to producer strains by limiting the growth of neighboring microorganisms and enhancing survival under competitive conditions.

IJETIR
ISSN (Online) 2583-0554
International Journal of Emerging Technologies and Innovative Research (IJETIR)
Volume 6, Issue 6, June 2026
Impact Factor: 5.731

Several bacteriocins have demonstrated effectiveness against foodborne pathogens and spoilage organisms, making them attractive candidates for application in food preservation and alternative antimicrobial therapies. However, many bacteriocins possess a narrow spectrum of activity, restricting their practical utilization. Therefore, identification of bacterial isolates capable of producing broad-spectrum bacteriocins remains an important area of research.

The present investigation was undertaken to isolate bacteriocin-producing bacteria from different environmental sources, evaluate their antimicrobial spectrum, and identify the most potent broad-spectrum bacteriocin-producing isolate using biochemical and molecular techniques.

II. MATERIALS AND METHODS

Sample Collection and Isolation

Samples were collected from dairy products, food and vegetable waste, polluted river water, industrial waste, and garden soil. Bacterial isolates were obtained using standard microbiological techniques and purified through repeated subculturing.

Screening for Bacteriocin Production

The isolates were screened for antimicrobial activity using agar spot assay against selected indicator organisms. Positive isolates were subsequently evaluated using agar well diffusion assay to confirm bacteriocin production.

Spectrum of Antimicrobial Activity

Confirmed bacteriocin-producing isolates were tested against a panel of pathogenic, spoilage, and probiotic microorganisms, including Gram-positive bacteria, Gram-negative bacteria, yeast, and mould species.

Biochemical Characterization

Morphological observations, Gram staining, catalase reaction, indole test, methyl red–Voges-Proskauer tests, arginine hydrolysis, aesculin hydrolysis, and carbohydrate utilization patterns were employed for preliminary identification of broad-spectrum bacteriocin-producing isolates.

Molecular Identification

Genomic DNA was isolated from the most potent bacteriocin-producing strain. The 16S rRNA gene was amplified by PCR and sequenced. Sequence similarity was determined using BLAST analysis, and phylogenetic relationships were established using reference sequences available in GenBank.

III. RESULTS

Isolation and Screening of Bacteriocinogenic Bacteria

A total of 275 bacterial isolates were recovered from various environmental samples. Preliminary screening identified 69 isolates exhibiting antimicrobial activity. Further confirmation by agar well diffusion assay established that 48 isolates were true bacteriocin producers.

Food and vegetable waste and dairy products represented the richest sources of bacteriocinogenic bacteria. In contrast, industrial waste samples failed to yield bacteriocin-producing isolates.

Determination of Antimicrobial Spectrum

Evaluation of the antimicrobial spectrum revealed considerable variation among bacteriocin-producing isolates. Most isolates displayed activity against only a limited number of test organisms. However, three isolates, designated 28D1, 12VW1, and 10HR1, demonstrated broad-spectrum antibacterial activity.

Among these, isolate 28D1 exhibited the highest inhibitory potential. The bacteriocin produced by this strain inhibited several Gram-positive and Gram-negative bacteria, including Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Listeria monocytogenes, Enterobacter aerogenes, Bacillus cereus, Lactococcus lactis, Lactobacillus rhamnosus, Lactobacillus acidophilus, and Pseudomonas fluorescens.

Yeast and mould species such as Saccharomyces cerevisiae, Rhodotorula glutinis, Aspergillus flavus, and Aspergillus niger showed complete resistance to the bacteriocin preparations.

Biochemical Identification

Isolate 28D1 was characterized as a Gram-positive rod-shaped bacterium. The strain was catalase-negative, indole-negative, methyl red-negative, Voges–Proskauer-negative, and capable of aesculin hydrolysis. Growth occurred at elevated temperatures, whereas no growth was observed at lower temperatures. These characteristics were consistent with members of the genus Lactobacillus.

Molecular Characterization

PCR amplification of the 16S rRNA gene generated an amplicon of approximately 1314 bp. Sequence analysis demonstrated 99.47% similarity with Lactobacillus fermentum reference strains available in the GenBank database. The nucleotide sequence was deposited in GenBank under accession number MT071668.

Phylogenetic analysis further confirmed the placement of isolate 28D1 within the Lactobacillus fermentum clade.

IV. DISCUSSION

The present study demonstrates that environmental habitats rich in microbial diversity are valuable reservoirs of bacteriocin-producing bacteria. The predominance of bacteriocinogenic isolates in food and vegetable waste and dairy samples may be attributed to intense microbial competition and abundant nutrient availability.

The broad-spectrum activity exhibited by Lactobacillus fermentum 28D1 is particularly significant because most bacteriocins are characterized by narrow inhibitory spectra. The ability of this isolate to inhibit both Gram-positive and Gram-negative pathogens suggests the production of a potent antimicrobial compound with potential industrial relevance.

The absence of activity against yeast and mould species indicates that the antimicrobial mechanism is primarily directed against bacterial targets. Similar observations have been reported for several bacteriocins produced by lactic acid bacteria.

Molecular identification through 16S rRNA sequencing provided reliable taxonomic confirmation of the isolate. The high sequence similarity with known L. fermentum strains supports its classification and further emphasizes the importance of lactic acid bacteria as sources of antimicrobial metabolites.

V. CONCLUSION

The study successfully identified Lactobacillus fermentum 28D1 as a potent broad-spectrum bacteriocin-producing bacterium. Among 48 confirmed bacteriocinogenic isolates, strain 28D1 exhibited the widest range of antibacterial activity against both Gram-positive and Gram-negative bacteria. Molecular characterization confirmed its identity as L. fermentum with 99.47% sequence similarity to reference strains. The broad inhibitory spectrum and probiotic nature of the isolate highlight its potential application in food preservation, biotechnology, and the development of alternative antimicrobial agents. Further studies involving purification, characterization, and safety evaluation of the bacteriocin are recommended to facilitate commercial utilization.

REFERENCES

[1]. Tagg, J. R., Dajani, A. S., & Wannamaker, L. W. (1976). Bacteriocins of Gram-positive bacteria. Bacteriological Reviews, 40(3), 722–756.

[2]. Nes, I. F., Diep, D. B., Håvarstein, L. S., Brurberg, M. B., Eijsink, V., & Holo, H. (1996). Biosynthesis of bacteriocins in lactic acid bacteria. Antonie van Leeuwenhoek, 70(2–4), 113–128.

[3]. Riley, M. A., & Wertz, J. E. (2002). Bacteriocins: Evolution, ecology, and application. Annual Review of Microbiology, 56, 117–137. https://doi.org/10.1146/annurev.micro.56.012302.161024

[4]. Kloepper, J. W., Ryu, C. M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology, 94(11), 1259–1266.

[5]. Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. Nature Reviews Microbiology, 3(10), 777–788. https://doi.org/10.1038/nrmicro1273
[6]. Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology, 68(1), 1–13.
[7]. Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. Annual Review of Microbiology, 63, 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918
[8]. Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., & Moënne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil, 321(1–2), 341–361. https://doi.org/10.1007/s11104-008-9568-6
[9]. Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizosphere: Mechanisms and applications. Journal of Advanced Research, 1(3), 219–229. https://doi.org/10.1016/j.jare.2010.03.003
[10]. Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications.
[11]. Scientifica, 2012, 1–15. https://doi.org/10.6064/2012/963401
[12]. Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial microorganisms. FEMS Microbiology Reviews, 37(5), 634–663. https://doi.org/10.1111/1574-6976.12028
[13]. Perez, R. H., Zendo, T., & Sonomoto, K. (2014). Novel bacteriocins from lactic acid bacteria. Current Opinion in Microbiology, 19, 152–158. https://doi.org/10.1016/j.mib.2014.05.003
[14]. Chalasani, A. G., Dhanarajan, G., Nema, S., Sen, R., & Roy, U. (2015). An antimicrobial metabolite from Bacillus species isolated from rhizosphere soil. Journal of Applied Microbiology, 119(5), 1349–1360.
[15]. Sugrue, I., Ross, R. P., & Hill, C. (2024). Bacteriocin diversity, function, discovery and application as antimicrobials. Nature Reviews Microbiology, 22(9), 556–571. https://doi.org/10.1038/s41579-024-01045-x
[16]. Reuben, R. C., & Torres, C. (2024). Bacteriocins: Potentials and prospects in health and agrifood systems. Archives of Microbiology, 206, 233. https://doi.org/10.1007/s00203-024-03948-y
[17]. Historical advancements in understanding bacteriocins produced by rhizobacteria for their application in agriculture. (2024). Rhizosphere, 31, 100908. https://doi.org/10.1016/j.rhisph.2024.100908
[18]. Wang, X., Chi, Y., & Song, S. (2024). Important soil microbiota's effects on plants and soils: A comprehensive 30-year systematic literature review. Frontiers in Microbiology, 15, 1347745. https://doi.org/10.3389/fmicb.2024.1347745
[19]. Thepbandit, W., & Athinuwat, D. (2024). Rhizosphere microorganisms supply availability of soil nutrients and induce plant defense. Microorganisms, 12(3), 558. https://doi.org/10.3390/microorganisms12030558
[20]. Zhang, Y. C., Zhan, X., Chen, J. Y., Yu, D. T., Zhang, T., Zhang, H., & Duan, C. G. (2025). Reduced fungal protein acetylation mediates the antimicrobial activity of a rhizosphere bacterium against a phytopathogenic fungus. Nature Communications, 16, 5644. https://doi.org/10.1038/s41467-025-60870-7