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Research Article

EXPLORING THE ANTIMICROBIAL ATTRIBUTE: CHARACTERIZATION OF LACTOBACILLUS ISOLATE AND IN VITRO ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF POSTBIOTIC CELL-FREE SUPERNATANTS AGAINST PATHOGENIC MICROORGANISMS

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ABSTRACT

This study examines the antimicrobial potential of the cell-free supernatant of a Lactobacillus isolate. The isolate was identified accurately by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Antimicrobial potential against four common pathogenic bacteria, namely *Escherichia coli, Enterococcus faecalis, Salmonella enterica*, and *Streptococcus bovis*, was determined by agar well diffusion assay. The results demonstrated concentration-dependent increases in inhibition zones for all tested pathogens; the postbiotic showed significant antimicrobial potential similar to reference antibiotics. This study demonstrates the potential of probiotic cell-free supernatant as a bioactive postbiotic agent that can inhibit pathogenic bacteria, thus providing alternative infection control. The results also suggest the potential of the postbiotic in being developed as a bioactive antimicrobial agent for infection management.

Keywords: Yogurt, Postbiotic, Cell free supernatant, Pathogenic bacteria and Antimicrobial activity.

INTRODUCTION

Fermented products like yogurt have gained widespread acclaim for their probiotic effects, a considerable amount of which has been attributed to the live and active cultures of helpful bacteria like *Lactobacillus* and *Bifidobacterium* with which yogurt is prepared. The probiotics ensure the establishment of a healthy gut microbiota that is vital for maintaining digestive health, enhancing nutrient assimilation, and preventing gastrointestinal upsets (Hadjimbei *et al.,* 2022). Daily intake of probiotic yogurt has been associated with improved immune function, less inflammation, and possibly a reduced risk of infections in the gastrointestinal and respiratory tracts. Yogurt also supplies vital nutrients, such as calcium, vitamin D, and protein, which help build bones and provide general nutrition (Yadav *et al.,* 2015). There is also evidence that probiotic yogurt can help control symptoms of lactose intolerance, decrease the duration of infectious diarrhea, and be involved in preventing chronic conditions like osteoporosis, diabetes, and cardiovascular diseases. The exclusive blend of live probiotic cultures and nutrient-dense matrix of yogurt makes it an ideal vehicle for transferring beneficial bacteria to the human gut (Meybodi *et al*., 2020).

Probiotics isolated from yogurt consist predominantly of strains such as *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactiplantibacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Lactobacillus casei*. Probiotics not only enrich the nutritional content of yogurt but also contribute towards health advantages such as the inhibition of pathogens and improved gut health. Literature shows that use of specific *Lactobacillus* and *Bifidobacterium* strains in yogurt results in products with probiotic viability at storage temperatures and reduces desired physicochemical and sensory characteristics. Particularly, yogurts containing *Lactobacillus plantarum* or *Lactobacillus casei* have improved texture and organoleptic attributes, while multi-strain mixtures can also increase

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probiotic functionality and quality of the product. The stability and effectiveness of these probiotic microorganisms make yogurt an excellent delivery vehicle for beneficial microbes to humans (Hoxha *et al*., 2023). Postbiotics are preparations from inanimate (non-viable) microorganisms and their fractions that confer a health benefit to the host. Differing from probiotics with the need for live microorganisms, postbiotics are made from inactivated microbial cells, cell fractions, or both—occasionally with their metabolic end products. This novel idea was developed in response to worries about the safety and viability of probiotics and hence interest in applying microbial cell components and metabolites that offer a beneficial effect without any living organisms. Postbiotics can improve health by modulating the immune response, improving epithelial barrier function, and influencing other physiological activities (Ma *et al.,* 2023).

Bioactive postbiotic metabolites have a diverse collection of molecules produced by probiotic bacteria during fermentation and include short-chain fatty acids (SCFAs), exopolysaccharides, bacteriocins, antioxidant enzymes, cell wall fragments, and other metabolic byproducts. Metabolites have many health-influential activities, such as antimicrobial, antioxidant, anticancer, anti-inflammatory, and immunomodulatory activities (Hijová E. 2024). Short-chain fatty acids such as butyrate, acetate and propionate Support intestinal health by reducing intestinal pH, regulating the immune response, and improving nutrient uptake (Asefa *et al.,* 2025). Exopolysaccharides that promote the growth of the beneficial gut bacteria and aid module immune function. Bacteriocins and cell-free supernatants show effective antimicrobial activity against numerous pathogens. Enzymes and peptides add antioxidant activity and can decrease inflammation. These bioactice molecules may be safely delivered as postbiotics without the potential negative consequences of live probiotic intake, providing a stable predictable method for enhancing health outcome (Pattapulavar *et al.,*2025) The purpose of this research is to isolate and characterize Lactiplanti bacillus plantarum from homemade yogurt, screen its probiotic attributes, and assess the antimicrobial efficacy of its cell-free supernatant (also known as postbiotic) against typical pathogens. The results join the increasing body of evidence that validates the use of postbiotics obtained from probiotics in health promotion, thereby opening the door to their safe use in therapeutic regimens.

MATERIALS AND METHODS

Sample preparation and isolation

Overnight fermented homemade yogurt was collected. 10 ml of sample isolated in 100 ml of de Man, Rogosa, and Sharpe (MRS) Broth at 37 °C for 24–48 hours. Layter, it was streaked in de Man, Rogosa, and Sharpe (MRS) agar for 24 hours at 37°C. After subsequent culturing pure culture is obtained (Abdel *et al*., 20).

Morphological and Biochemical characterization

The identification and further morphological characterization of LAB was identified using microscopic observation that is by gram staining. For biochemical characterization IMVic, catalase and oxidase was performed.

MALDI-TOF

Followed by Biochemical characterization, MALDI-TOF MS (matrix-assisted laser desorption ionization time of flight mass spectrometry)-based VITEK MS PRIME for molecular identification of the isolated lactobacillus strain.

Cell free supernatant- postbiotic

Overnight fresh culture of isolated lactobacillus strain was centrifuged at 6000 rpm for 10 minutes. The cells was removed by 22µm membrane filter to obtain postbiotic cell free supernatant.

Antimicrobial activity

The antibacterial activity was done by agar well diffusion method. *Escherichia coli, Streptococcus bovis*, *Enterococcus faecalis* and *Salmonella enterica* was used for the antibacterial activity. These four strains were inoculated overnight in LB broth. Muller Hinton Agar was prepared and poured into sterile petri plates. Overnight incubated strains were swabbed in the freshely poured MHA agar plated. And well was cut for 4 different concentrations like 25, 50, 75 and 100 to load the postbiotic cell free supernatant along with the standard antibiotic disc. The zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

The isolated probiotic strain possessed standard morphological and microscopic features of lactic acid bacteria. Morphologically, the colonies were small, circular, and off-white in color. Microscopically, Gram staining revealed that the bacteria had a Gram-positive rod-shaped (bacilli) morphology. These results establish the identity of the isolate as a *Lactobacillus species*.

Biochemical analysis of the *Lactobacillus* strain confirmed a number of significant characteristics similar to those of probiotic lactobacilli. The test result for the methyl red was positive, showing that the bacteria were actively producing acid during fermentation. On the other hand, the test results for indole production, Voges-Proskauer reaction, citrate utilization, catalase, and oxidase activity were negative, indicating that the bacteria are incapable of producing indole, not fermenting citrate, and not producing catalase or oxidase enzymes. These biochemical properties are characteristic of *Lactobacillus* species, affirming their function as producers of lactic acid and substantiating their potential probiotic features (Table 1).

Table 1. Morphology, Microscopic and Biochemical characterization.

S.No	Characterization	Test	Observation
1.	Morphology	Colony morphology	Small round colonies
		Appearance	Off- white
2.	Microscopic	Gram staining	Gram positive
		Structure	Rod shaped bacilli
3.	Biochemical	Indole	-ve
		Methyl red	+ve
		Voges proskauer	-ve
		Citrate utilization	-ve
		Catalase	-ve
		Oxidase	-ve

The bacterial isolate was identified by molecular identification with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) facilitated by the VITEK MS PRIME system. The MALDI-TOF MS identification resulted in a distinct protein spectral pattern that corresponded with a large database of previously entered bacterial strains. The identification resulted in a confidence level of over 99.9%, thereby confirming the strain to be *Lactiplantibacillus plantarum*. The identification was validated by technical replicate tests, which affirm the reproducibility and reliability of the technique. The antimicrobial activity of the probiotic cell-free supernatant against selected pathogenic bacteria was evaluated using the agar well diffusion assay. The zones of inhibition were measured in mm for each pathogen at different concentrations of the supernatant of 25 µl, 50 µl, 75 µl, and 100 µl (Table 2).

Table 2. Zone of inhibition (mm) of postbiotics cell free supernatant.

S. No	Microorganisms	Zone of inhibition of postbiotic along with standard antibiotics					
		25µl	50 µl	75 µl	100 µl	Standard antibiotics	
1.	*Enterococcus faecalis*	-	10mm	11mm	14mm	Amplicillin	14.5mm
2.	*Escherichia coli*	8mm	11mm	13mm	15mm	Penicillin	17mm
3.	*Streptococcus bovis*	-	9mm	10mm	13mm	Vancomycin	15mm
4.	*Salmonella enterica*	-	8mm	12mm	16mm	Azithromycin	18mm

Among the pathogens, *Salmonella enterica* was the most sensitive to the postbiotic, with a maximum inhibition of 16 mm at 100 µL, almost reaching the zone of inhibition seen with azithromycin (18 mm). *Escherichia coli* was also the most sensitive, with a 15 mm zone at the highest concentration. No inhibition was detected at 25 µL for *Enterococcus faecalis*, *Streptococcus bovis*, and *Salmonella enterica*, indicating that a minimum effective concentration is necessary for antimicrobial activity. *Enterococcus faecalis* and *Streptococcus bovis* had moderate inhibition of 14 mm and 13 mm, respectively, at 100 µL against comparative antibiotic controls. The antimicrobial activity of the postbiotic supernatant increased progressively with volume, with a clear dose-response profile for all of the bacteria tested.

In the present study, *Lactobacillus* strain was isolated from homemade yogurt and confirmed through a combined approach of morphological, biochemical, and MALDI-TOF MS identification. The subsequent preparation of the cell-free supernatant (postbiotic) from this probiotic isolate demonstrated marked in vitro antimicrobial activity against major clinical pathogens, namely *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enterica*, and *Streptococcus bovis*. The activity observed was both concentration-dependent and substantial, approaching that of standard antibiotics in certain cases. The results of the present study complement and supplement previous work that identifies yogurt as a major source of probiotic strains, particularly of the *Lactobacillus* and *Lactiplantibacillus* genera. Subsequent publications (Harat *et al*., 2025; Liang *et al*., 2023) have described the importance of strain identification and the potential functional properties displayed by some yogurt-based isolates. Our work also complements the finding that traditional fermented foods remain a valuable reservoir for candidate probiotic screening, as documented in other global research (Goa *et al*., 2022).

The isolated postbiotic of *L. plantarum* was reported to possess strong antimicrobial activity. The finding supports the novel hypothesis among researchers that cell-free probiotic preparations of organic acids, bacteriocins, peptides, exopolysaccharides, and other metabolites can have strong inhibitory activity on a variety of pathogens (Aguilar-Toalá *et al*., 2018; Moradi *et al*., 2020). This study by (Gurunathan *et al*., 2023) points out postbiotics as a viable and safer alternative to conventional probiotics since their efficacy is sustained even in the absence of live microorganisms and with additional stability and predictability. Mechanistically, our findings echo those of (Ibrahim *et al*., 2021), who demonstrated that postbiotics not only lower environmental pH through organic acid release but may also disrupt pathogen cell membranes, impair quorum sensing, or even induce DNA damage, collectively contributing to bacterial cell death. Notably, the robust inhibition of *Salmonella enterica* and *Escherichia coli* by the postbiotic in this research supports its potential utility as promising results reported for *L. plantarum*-derived postbiotics in similar model systems (Tong *et al*., 2025).

However, there are some limitations to be noted. The method utilized, agar well diffusion, is standard for initial assessments but doesn't replicate the conditions found in complex food matrices or in the human gastrointestinal tract. Moreover, the specific range and molecular topology of the antimicrobial molecules present in our postbiotic extract have not been found. Future research involving in vivo efficacy trials, other pathogen assessments, and metabolomic profiling may shed light on these aspects and enable the identification of beneficial applications (Ji *et al*., 2023; Homayouni *et al*., 2020). The synergy of these antimicrobial and health-enhancing attributes highlights the potential value of postbiotic strategies to human health. However, with the optimistic nature of in vitro findings, are warranted to finally determine the safety, effectiveness, and functional advantages of postbiotics prior to their regular use in a clinical environment.

CONCLUSION

This research study provides comprehensive information on the isolation, characterization, and evaluation of the probiotic ability of *Lactiplanti bacillus* plantarum from homemade yogurt. Based on morphological, biochemical, and molecular identification methods, the strain was determined to possess characteristics of typical probiotic lactobacilli, such as a Gram-positive rod shape, ability to produce acid, and specific enzymatic activities. Cell-free supernatant (postbiotic) preparation expressed high antimicrobial activity against major foodborne pathogens such as Escherichia coli, Enterococcus faecalis, Salmonella enterica, and Streptococcus bovis, with the zones of inhibition being concentration-dependent, up to the level of effectiveness of standard antibiotics. The finding indicates the strong antimicrobial activity of probiotic postbiotics, which act by several modes, including cell membrane disruption of bacteria and induction of DNA damage to genomic DNA, leading to cell death of bacteria. The bioactive molecules, which are mainly organic acids and bacteriocins, are of high potential as replacements for synthetic preservatives and antibiotics at a time of growing concerns regarding antimicrobial resistance. Additionally, the study brings into focus fermented food, with yogurt being a prominent one, as a natural source of healthy microbes that possess high probiotic and antimicrobial activities. The efficiency and efficacy of probiotic cultures such as *Lactiplanti bacillus* plantarum in yogurt foods are of immense potential as effective modes for the delivery of health-improving microbes to consumers, thus providing benefits such as improved gastrointestinal health, immune system balance, and resistance to disease. The complete characterization and proof of antimicrobial activity constitute the solid foundation for the prospective utilization of postbiotics in therapeutic applications. Additional in vivo studies are required to authenticate the safety, bioavailability, and health-enhancing activity of these probiotic strains and their metabolites. In conclusion, this study highlights the potential in using natural probiotic materials and their bioactive molecules towards the improvement of food security, human health enhancement, and the resolution of antimicrobial resistance issues, thereby allowing the promotion of food functional technology and microbiological safety regulations.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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