

PHARMACOLOGICAL POTENTIAL OF BIOACTIVE COMPOUNDS FROM LACTIC ACID BACTERIA ISOLATED FROM FERMENTED FOOD WASTE AGAINST *PSEUDOMONAS* SPECIES

Mahalakshmi S^{*1}, Devasena B², Swetha M³, Rubala Nancy⁴ and Janaki M⁵

¹Department of Nursing, PERI College of Nursing, Chennai -48

²Department of Physiotherapy, PERI College of Physiotherapy, Chennai -48

³Department of Pharmacy, PERI College of Pharmacy, Chennai -48

⁴Department of Microbiology, PERI Arts and Science, Chennai – 48

⁵Department of Computer Science and Engineering, PERI Institute of Technology, Chennai - 48

Corresponding mail id: publications@peri.ac.in

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ABSTRACT

Lactic Acid Bacteria producing bioactive compounds were isolated from semi rotten beans, idly flour and yoghurt. The isolated compound exhibited antibacterial activity against *Pseudomonas* species using agar spot test. The isolates that are obtained from the different sources grow in the medium nearly 6 to 7 pH, in which they produced bioactive compound extracellular at the highest level. The level of these compounds get increased in the medium with respect to the increase in bacterial growth. This study is to investigate the standardization of cultural conditions such as medium, pH and temperature that are needed for maximum antibacterial compound production and their stability against wide range of pH and temperature and also the evaluation of antibacterial compound activity.

INTRODUCTION

Antimicrobial resistance remains a global challenge in both clinical and food safety contexts, demanding innovative solutions beyond conventional synthetic drugs. Naturally occurring microorganisms, particularly lactic acid bacteria (LAB), have emerged as effective producers of bioactive metabolites with therapeutic potential. LAB are generally regarded as safe (GRAS) and are known for their ability to synthesize a variety of antimicrobial compounds including organic acids, hydrogen peroxide, and bacteriocins. Recent studies have demonstrated that LAB strains isolated from fermented and decomposing food products exhibit strong antagonistic activity against food borne and clinical pathogens. Among these, *Pseudomonas aeruginosa* and *P. fluorescens* are notable for their resistance to many conventional antibiotics and their role in opportunistic infections and food spoilage. Harnessing the antimicrobial capabilities of LAB against such pathogens can lead to the development of novel bio-preservatives or therapeutic agents.

This study aims to isolate LAB strains from semi-rotten food samples such as beans, idly flour, and yoghurt, and to evaluate their antibacterial potential against *Pseudomonas* species. In addition, the effects of culture conditions—including pH, temperature, and media composition—on antibacterial compound production and stability were assessed. The ultimate objective is to investigate the pharmacological relevance and industrial applicability of LAB-derived bioactive compounds as a natural and eco-friendly alternative to synthetic antimicrobials.

2. LITERATURE REVIEW

Lactic acid bacteria (LAB) have emerged as a promising source of natural antimicrobial agents due to their ability to produce bioactive compounds such as organic acids, bacteriocins, and hydrogen peroxide. These metabolites are gaining attention for their therapeutic and preservative roles in the food and pharmaceutical industries [1], [9], [21].

Several studies have demonstrated the antibacterial efficacy of LAB isolated from fermented dairy and vegetable sources. For instance, Joy and Olubukola [1] isolated probiotic *Lactobacillus* species from traditional beverages and demonstrated significant

inhibition of spoilage bacteria. Similarly, Sanni *et al.* [16] highlighted the antagonistic potential of LAB-derived bacteriocins from *ogi*, a traditional fermented food. LAB strains have also shown strong activity against multidrug-resistant *Pseudomonas aeruginosa*, a pathogen known for its role in nosocomial infections and food spoilage [26], [27], [30].

The mechanisms by which LAB exert antimicrobial activity include acidification of the environment, production of bacteriocins and competitive exclusion of pathogens [9], [21], [22]. Santos *et al.* [28] demonstrated that cell-free supernatants of various *Lactobacillus* species inhibited both growth and biofilm formation of *P. aeruginosa*. Likewise, Chelliah *et al.* [27] confirmed LAB efficacy in an in vivo *Caenorhabditis elegans* model.

Food-derived LAB strains from kefir [12], fermented vegetables [20], and Ethiopian dairy products [29] have been extensively characterized for their antimicrobial spectrum. Goa *et al.* [13] and Girma and Aemiro [10] found that LAB from traditional dairy products were effective against both Gram-positive and Gram-negative pathogens. The thermal and pH stability of these compounds enhances their practical application in food preservation [2], [11], [18].

In addition to food safety, LAB metabolites have immunomodulatory and gut microbiota-regulating effects, broadening their pharmacological relevance [5], [6]. Wang *et al.* [5] emphasized the role of probiotics in enhancing immune response via modulation of gut microbiota, while Xiang *et al.* [6] advocated for fermentation-enabled wellness foods as a frontier in functional nutrition.

Research by Bungenstock *et al.* [17] and Fhoula *et al.* [19] explored LAB from industrial and environmental niches, affirming their versatility. Their findings supported LAB application in bio-preservation, extending shelf life and reducing microbial contamination in meat and vegetable products [2], [11], [18]. Moreover, Kazemipour *et al.* [20] and Kumar [3] highlighted the relevance of traditional fermented food microbiota in sustainable antibacterial discovery.

Innovations in LAB screening and metabolomics have further uncovered strains like *Latilactobacillus sakei* with targeted inhibition against *Pseudomonas fluorescens* [23], reinforcing their niche potential in post-antibiotic alternatives. Reviews by Field *et al.* [21] and Barcenilla *et al.* [11] advocate the use of bacteriocins as next-generation biopreservatives.

Finally, the application of LAB in functional food development and their role in microbiome-based therapeutics are being increasingly acknowledged [4], [7], [15], [24]. Such studies affirm that fermented food waste can be a sustainable source of beneficial LAB strains with pharmacological and industrial significance.

Babu *et al.* [31] discussed the increasing concerns of microplastic accumulation in terrestrial and aquatic ecosystems. Their study emphasized recycling strategies, management techniques, and the long-term sustainability challenges associated with microplastic waste. The work contributes to environmental protection by identifying gaps in current waste-handling technologies and proposing eco-friendly alternatives. Rubala *et al.* [32] reviewed the histopathological impacts of environmental pollutants on living systems. The authors highlighted pathological changes caused by toxic exposure, underlining the importance of biomonitoring and early detection for preventive healthcare. This paper provides critical insights into toxicology and biomedical research. Ramya *et al.* [33] analyzed the growth trends and economic implications of *Penaeus monodon* aquaculture. Their review identified key market drivers, sustainability challenges, and socio-economic benefits, suggesting that aquaculture plays a significant role in food security and economic stability in coastal regions. Geetha *et al.* [34] presented a comprehensive review of ecotourism, emphasizing its applications in biodiversity conservation and environmental education. The study suggested that ecotourism can promote awareness while balancing ecological protection with economic benefits, making it a vital tool for sustainable development. Swetha *et al.* [35] provided a concise review of mosquito control measures, ranging from biological methods to chemical interventions. Their findings underline the importance

of integrated vector management (IVM) in reducing mosquito-borne diseases, thus supporting global public health initiatives. Mahalakshmi *et al.* [36] explored the health risks associated with inhalation of volatile paint fumes. Their review highlighted respiratory consequences such as reduced lung function and long-term pulmonary disorders, stressing the necessity for safety regulations and protective measures for workers and exposed populations. Farheen *et al.* [37] investigated medicinal plants as therapeutic candidates for hepatocellular carcinoma. Their mini-review pointed out the hepatoprotective properties of phytochemicals and their potential to provide affordable, accessible alternatives to conventional cancer treatments. Geetha *et al.* [38] used computational methods to evaluate natural bioactive compounds and their interactions with mosquito proteins. This research provides insights for novel insecticide design and biocontrol measures, advancing eco-friendly mosquito management strategies. Devasena *et al.* [39] highlighted sustainable biofuel production from fruit waste, offering a waste-to-energy approach. Their work underscored the dual benefit of reducing organic waste accumulation and providing renewable energy alternatives to fossil fuels. Krishnan *et al.* [40] quantified airborne microbial loads in clinical and adjacent environments. Their study demonstrated the importance of microbial monitoring for infection control and prevention, contributing to improved healthcare facility management. Krishnan *et al.* [41] studied the effect of aquarium wastewater irrigation on mustard and green gram plants. Results indicated enhanced growth responses, suggesting the feasibility of using treated wastewater in agriculture as a resource recovery and sustainability measure. Geetha *et al.* [43] discussed fabrication and analysis of nickel oxide nanoparticles for advanced applications. Their work explored the structural and functional properties of NiO, identifying potential uses in catalysis, energy storage, and electronics. Sindhuja *et al.* [44] synthesized and characterized spinel SrFe_2O_4 nanoparticles. Their review highlighted the application potential in magnetic storage, catalysis, and biomedical fields, demonstrating how nanostructuring enhances material properties. Geetha *et al.* [45] reported on the microwave-assisted synthesis and characterization of ZnO nanoparticles. Their findings revealed superior structural and functional performance, supporting ZnO's role in sensors, photocatalysis, and biomedical application

3. MATERIALS AND METHODS:

3.1 Sample Collection

Semi-rotten vegetables (e.g., beans) and dairy products (yoghurt, idly batter) were collected from local households and markets in sterile polythene bags. Samples were immediately transported to the microbiology laboratory and stored at 4°C for further processing.

3.2 Isolation of Lactic Acid Bacteria (LAB)

Ten grams of each sample was homogenized in 90 mL of sterile saline (0.85% NaCl) and serially diluted up to 10^{-6} . Aliquots (0.1 mL) of appropriate dilutions were spread on de Man, Rogosa, and Sharpe (MRS) agar plates and incubated at 37°C for 48 hours under anaerobic conditions using GasPak systems. Distinct colonies with typical LAB morphology were sub-cultured and purified.

3.3 Preliminary Identification of LAB

Isolates were examined microscopically for Gram-staining and catalase activity. Only Gram-positive, catalase-negative, non-motile rod or cocci-shaped bacteria were considered presumptive LAB. Selected strains were preserved in 20% glycerol at -20°C for further analysis.

3.4 Antibacterial Activity Screening

Antibacterial activity of LAB isolates was evaluated against *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* using:

Agar Spot Method: LAB isolates were spot-inoculated on MRS agar and incubated at 37°C for 24 hours. Then, soft nutrient agar seeded with indicator strains (10^6 CFU/mL) was overlaid. After incubation, inhibition zones were measured.

Well Diffusion Assay: Cell-free supernatants (CFS) of overnight LAB cultures were obtained by centrifugation (10,000 rpm, 10 min). Wells (6 mm) were made in nutrient agar pre-inoculated with *Pseudomonas* spp., and 100 µL of CFS was loaded into each well. Plates were incubated at 37°C for 24 hours.

3.5 Optimization of Bioactive Compound Production

To assess optimal conditions for antibacterial compound production, selected LAB isolates were grown in MRS broth under varying:

Incubation periods: 24, 48, and 72 hours

pH values: 4, 5, 6, 7, and 8

Temperatures: 20°C, 30°C, 37°C, and 45°C

Supernatants were harvested and tested against *Pseudomonas* spp. as described.

3.6 Stability Analysis of Antibacterial Compounds

Thermal and pH stability of active compounds were evaluated by subjecting CFS to:

Heat treatment: 60°C, 80°C, and 100°C for 15 minutes

pH range: Adjusted between 2-10 and incubated for 1 hour before testing residual activity using the well diffusion method.

3.7 Proteinaceous Nature Assessment

The proteinaceous nature of the bioactive compound was assessed by treating the CFS with proteinase K (1 mg/mL) and trypsin for 1 hour at 37°C. Residual antibacterial activity was measured to determine enzyme sensitivity.

3.8 Estimation of Biomass and Optical Density

Cell biomass was measured by determining the dry weight of culture pellets after centrifugation and drying at 105°C. Optical

density (OD) was measured spectrophotometrically at 600 nm to monitor bacterial growth kinetics during fermentation.

3.9 Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using mean \pm standard deviation (SD). Statistical significance was assessed by ANOVA followed by Tukey's test, with $p < 0.05$ considered significant.

4. RESULTS

4.1. Isolation and Screening of LAB

Out of 12 bacterial isolates obtained from fermented dairy and vegetable samples, six were confirmed as lactic acid bacteria (LAB) based on Gram-positive staining and catalase-negative reaction. Among them, isolates IS1 and IS4 exhibited strong antibacterial activity against *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* in both the agar spot and well diffusion methods.

4.2 Antibacterial Activity of LAB Isolates

The well diffusion assay revealed that isolates IS1 and IS4 produced clear zones of inhibition, with IS4 exhibiting the highest antibacterial effect. The mean zone of inhibition ranged from 10.4 mm to 22.6 mm, depending on the isolate and test organism.

Table 1. Antibacterial Activity of LAB Isolates Against *Pseudomonas* spp. (Well Diffusion Method)

Isolate	<i>P. aeruginosa</i> (mm)	<i>P. fluorescens</i> (mm)
IS1	19.3 \pm 0.47	17.8 \pm 0.23
IS2	11.0 \pm 0.00	10.4 \pm 0.47
IS3	12.5 \pm 0.82	11.3 \pm 0.23
IS4	22.6 \pm 0.23	20.8 \pm 0.47
IS5	15.8 \pm 0.47	13.5 \pm 0.47
IS6	13.8 \pm 0.23	12.3 \pm 0.23

Values are mean \pm SD (n = 3)

4.3 Effect of Incubation Time on Antibacterial Activity

The production of antibacterial compounds was observed to increase with incubation time, peaking at 48 hours. Activity

declined slightly at 72 hours, possibly due to compound degradation or nutrient depletion.

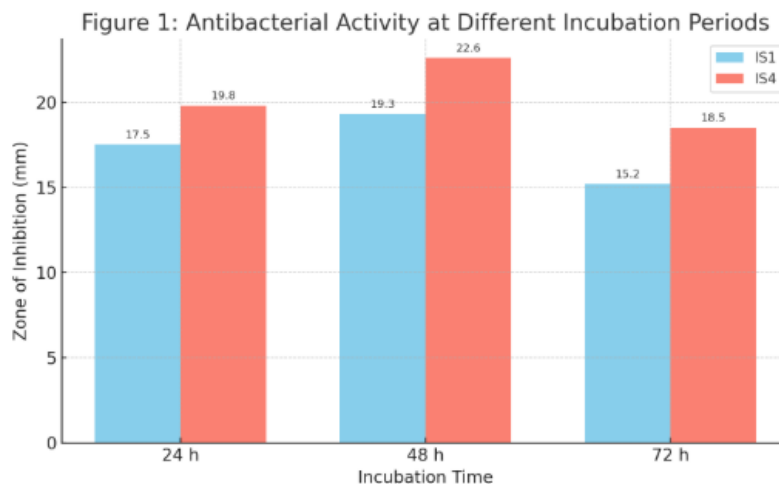


Figure 1. Antibacterial Activity at Different Incubation Periods (Well Diffusion Zones in mm)

[Bar graph recommended: X-axis = Time (24, 48, 72 h), Y-axis = Inhibition zone (mm), separate bars for IS1 and IS4]

4.4 Effect of Temperature and pH on Antibacterial Activity

The optimum temperature for antibacterial compound production was 30-37°C. Antibacterial activity declined

significantly at 45°C. Maximum inhibition was observed at pH 6-7.

Table 2. Optimization of Conditions for Antibacterial Compound Production

Condition	Variable	Zone of Inhibition (mm)
Temperature	20°C	14.2 \pm 0.23
	30°C	21.4 \pm 0.47
	37°C	22.6 \pm 0.23
	45°C	12.6 \pm 0.23

Condition	Variable	Zone of Inhibition (mm)
pH	4	10.5 ± 0.25
	5	15.2 ± 0.47
	6	21.3 ± 0.44
	7	22.6 ± 0.23
	8	18.4 ± 0.30

4.5 Thermal and pH Stability

The bioactive compound retained significant activity after heat treatment up to 100°C, suggesting it is heat-stable. It also remained active over a wide pH range (2-9), supporting its potential as a stable antimicrobial agent.

4.6 Proteinaceous Nature Confirmation

Treatment of the cell-free supernatant with proteolytic enzymes (proteinase K and trypsin) resulted in partial loss of antibacterial

activity, indicating that the active compound is proteinaceous in nature—likely a bacteriocin.

4.7 Growth Kinetics and Biomass Correlation

The optical density (OD600) and dry biomass were measured over time. Maximum biomass and antibacterial activity were observed at 48 hours of incubation.

Figure 2: Growth Curve and Bioactive Production Kinetics of Isolate IS4

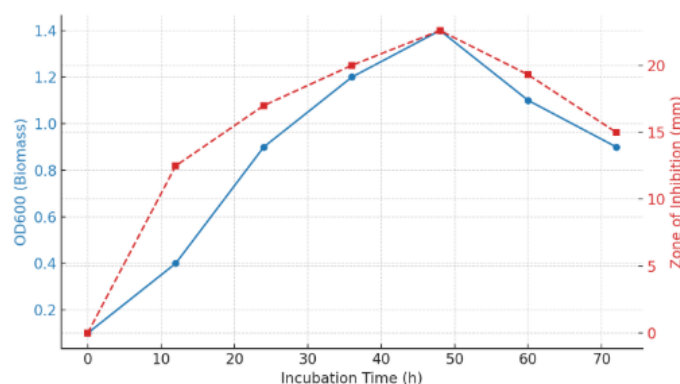


Figure 2. Growth Curve and Bioactive Production Kinetics of Isolate IS4

[Line graph: X-axis = Time (h), Y-axis = OD600 and inhibition zone (dual axis), showing peak at 48 h]

DISCUSSION

This study successfully demonstrates that lactic acid bacteria (LAB) isolated from semi-rotten vegetables and fermented dairy products are capable of producing bioactive compounds with potent antibacterial activity against *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Among the isolates, IS4 displayed the highest antagonistic effect, with inhibition zones comparable to or exceeding those reported in similar studies on probiotic LAB strains [1], [26], [28].

The production of antibacterial metabolites by LAB appeared closely correlated with bacterial growth kinetics, peaking at 48 hours and slightly declining thereafter. This is consistent with prior findings indicating that bacteriocin-like substances are often synthesized during the late logarithmic to early stationary growth phases [9], [21]. The loss of activity beyond 48 hours may be due to compound degradation or nutrient depletion.

The study also revealed that optimal antibacterial production occurred at neutral to slightly acidic pH (6-7) and moderate temperatures (30-37 °C), aligning with physiological conditions of LAB fermentation [11], [18]. The bioactive metabolites retained considerable thermal and pH stability, suggesting their utility in food systems where processing conditions may vary.

Proteolytic enzyme treatment led to a reduction in activity, confirming the proteinaceous nature of the antimicrobial compound—most likely a bacteriocin [19], [27]. These findings align with the growing body of research proposing bacteriocins as a next-generation biopreservative and therapeutic alternative to synthetic antibiotics [21], [22], [28].

Furthermore, the wide inhibitory range of the compounds and their effectiveness against resistant strains of *Pseudomonas* support the potential use of these LAB isolates in pharmaceutical and food safety applications. This is especially significant in the context of increasing antibiotic resistance and the need for eco-friendly, low-cost alternatives derived from agro-industrial or domestic waste [6], [7], [13].

Overall, this study contributes valuable evidence for the exploitation of underutilized fermented food waste as a sustainable source of health-promoting LAB with broad-spectrum antimicrobial activity.

CONCLUSION

The results of this study confirm that LAB isolated from semi-degraded vegetables and fermented dairy products possess strong pharmacological potential as natural antibacterial agents. The isolates, particularly IS4, exhibited effective inhibition against *Pseudomonas* spp., with optimal bioactive production at 48 hours, pH 6-7, and 30-37 °C. The bioactive metabolites produced were proteinaceous in nature, heat-stable, and retained efficacy over a broad pH range, reinforcing their suitability for food preservation and biomedical applications.

The use of such LAB strains not only provides a cost-effective and eco-friendly approach to combat pathogenic bacteria but also supports the valorization of food waste. Future work should focus on purifying the active compound, determining its molecular structure, and validating its safety and efficacy in in vivo systems. These findings pave the way for the development of novel antimicrobial formulations derived from LAB, contributing to food safety, functional foods, and alternative therapies against resistant pathogens.

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