*Int.J.Curr.Microbiol.App.Sci* (2025) *14*(09): 8-15

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706* Volume 14 Number 9 (2025)**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2025.1409.002

# Probiotic Potential and Safety Assessment of Lactic Acid Bacteria from Naturally Fermented Milk in Karnataka: Molecular Characterization and Functional Traits

**D. C. Bhavya[1], Ajay Kumar Oli[2] and D. B. M. Virupakshaiah[1]***

*[1]Department of Microbiology, Davangere University, Davangere, Karnataka, India*
*[2]Department of Biomedical Science, SDM Research Institute for Biomedical Sciences, Shri Dharmasthala Manjunatheshwara University, Dharwad, Karnataka, India*

**Corresponding author*

**Keywords**
Lactic acid bacteria, Probiotics, Fermented milk, β-galactosidase, Exo-polysaccharides, Hemolysis

**Article Info**

***Received:***
09 July 2025
***Accepted:***
18 August 2025
***Available Online:***
10 September 2025

**A B S T R A C T**

The strains of the lactic acid bacteria that were identified in Karnataka, from naturally fermented milk, are the subject of this investigation to assess their probiotic potential and safety. Ten representative strains of LAB were identified through sequencing of 16S rRNA genes, includes *Lactiplantibacillus plantarum*, *Lactobacillus fermentum*, *Ligilactobacillus murinus*, *Leuconostoc mesenteroides*, *Enterococcus faecalis*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Enterococcus lactis*, *Lactobacillus sakei*, and *Lactobacillus paracasei*.. Functional characterization revealed that nine strains exhibited β-galactosidase activity, beneficial for lactose digestion, and all strains produced exopolysaccharides, which improve gut health and product texture. Hemolysis testing confirmed non-hemolytic in all strains, ensuring their safety. The study highlights the probiotic promise of these LAB strains for enhancing dairy products, particularly for lactose-intolerant individuals, and underscores the safety of most isolates for probiotic applications. Further research will focus on optimizing these strains for functional roles in human health, positioning traditional fermented milk as a valuable source of natural probiotics.

## Introduction

The fermented dairy products are the most significant in terms of quantity among fermented foods made with Lactic Acid bacteria (LAB). Around the world, people manufacture and eat yoghurt, kefir, sour cream and dips, cheese, cultured buttermilk, and many other delicacies. In the production of fermented dairy products, modern industrial procedures use specially prepared lactic acid bacteria called starting cultures, or starters, for sophisticated control (Cremonesi *et al.,* 2011). A wide range of microorganisms are referred to as lactic acid bacteria. In 1873, the first lactic acid bacteria pure culture was produced. Starting in the early 1900s, it was discovered that Lactic acid-producing bacteria, including those that cause milk to sour from different ecosystems

shared similarities (Huang *et al.,* 2021). The β-galactosidase enzyme is present in nature and can be derived from a number of sources, including microbes, plants, and animals. Today, the food and pharmaceutical sectors make extensive use of β-galactosidases because of their capacity to hydrolyse lactose, the most prevalent sugar in milk, and its byproducts. This promotes dairy product intake and helps to alleviate the issue of lactose intolerance, which impacts a significant percentage of the human population. Lactose's potent capacity to absorb flavours and odours is inhibited, improving the sensory characteristics of dairy products. But in some situations, such high lactose content, low water activity, and high temperatures, β-galactosidases can catalyse the galactosyde production reaction. Due to their strong prebiotic activity and possible health benefits, interest in this area of β-galactosidase utilisation has been increasing quickly, particularly in the field of galacto-oligosaccharide (GOS) synthesis (Devi *et al.,* 2011). Additionally, by blocking lactose's potent capacity to absorb flavours and odours, dairy products' sensory qualities are enhanced. However, under some circumstances, such as high lactose content, low water activity, and high temperatures, β-galactosidases can catalyse the galactosyde production reaction. Due to the significant prebiotic activity of galacto-oligosaccharides (GOS) and their possible health benefits, interest in this area of β-galactosidase utilisation has been increasing quickly. Furthermore, lactic acid bacteria strains are of particular interest among the many microbiological sources that are readily available, including moulds, bacteria, and yeasts. The neutral enzyme β-galactosidase, which is generated from LAB, is useful for the hydrolysis of milk lactose and sweet whey as well as the synthesis of GOS. LAB covers a broad category of industrial microorganisms with broad applications. Their full potential in the enzyme, primarily β-galactosidase, has not yet been realised in commercial production, despite their employment in large-scale lactic acid production. Only a small number of β-galactosidases from Lactobacillus sp. have been described and the most utilised strains are Streptococcus thermophiles and Bifidobacterium species (Hassan *et al.,* 2024).

Bacteria produce exopolysaccharides (EPS), which are high molecular weight polymers of carbohydrates with nutritional components and defences against bacteriophages (Nowshin *et al.,* 2023). Food matrices can benefit from EPS's many properties, including its high water solubility, emulsification, flocculation, and water-holding ability, even its ability to remove heavy metals (Zhang *et al.,* 2023). EPS offers a number of health benefits, including cholesterol-lowering, immunomodulatory, hypoglycemic, antioxidant, and antibacterial qualities. Antioxidant, antimicrobial, hypoglycemic, immunomodulatory, and cholesterol-lowering are just a few of the health benefits of EPS (Zhang *et al.,* 2023). In the past 10 years, EPS has drawn increased attention due to its use in a variety of meals and its ability to isolate and characterise distinct bacteria. But the main origin of EPS produced from microbes, which is usually considered safe for human ingestion, is LAB (Zhang *et al.,* 2023; Kang *et al.,* 2022). LAB produced from EPS can be added to items that are fermentative to improve the Physical-chemical analysis and nutritional features of meals since the most widely utilised probiotics with positive effects for human health (Pramudito *et al.,* 2023). The majority of LAB species used to produce EPS are not productive enough for large-scale manufacturing (Zhang *et al.,* 2019; Yu *et al.,* 2021). Numerous experiments, including strain enhancement, have been conducted to enhance EPS commercial output (Wei *et al.,* 2022), moderate optimisation, (Yu *et al.,* 2021; Wei *et al.,* 2022; Bengoa *et al.,* 2018) and examining LAB strains (Lu *et al.,* 2023). However, the examined strains' inadequate yield of EPS production precludes both bulk production and food introduction. Hence, strains from various sources must be assessed for increased EPS yield production in order to generate and describe new polysaccharides for commercial uses. Customers are becoming more and more interested in functional foods. Therefore, it is now essential for the food business to produce new products or improve existing foods employing probiotic strains or functional ingredients obtained from LAB (Nemati *et al.,* 2023). The nutritional content of yoghurt its ability to improve lactose digestion, and its immune-boosting properties make it one among the most popular product made from fermented milk (Alizadeh *et al.,* 2023). The purpose of this investigation was to ensure that LAB produced exopolysaccarides and β-galactosidase.

## Materials and Methods

### Sample collection, isolation, identification of LAB

Ten lactic acid bacteria were identified by 16s rRNA sequencing, the lactic acid bacteria species are *Lactiplantibacillus plantarum*, *Lactobacillus fermentum*, *Ligilactobacillus murinus*, *Leuconostoc mesenteroides*, *Enterococcus faecalis*, *Lactiplantibacillus plantarum*,

*Lacticaseibacillus rhamnosus*, *Enterococcus lactis*, *Lactobacillus sakei*, *Lactobacillus paracasei*. These organisms are isolated from naturally fermented milk collected from different regions of Karnataka. Following a 24-hour incubation period at 37°C, all of the colonies from initial cultures were purified by subculturing on MRS agar. Biochemical assays and Gram staining were used to identify the suspicious colonies on MRS agar. Additional verification was carried out by DNA sequencing analysis. The possible activity of exopolysaccharide, beta galactisidase, and haemolysis production was then examined.

## Molecular Identification

Bacterial cultures were centrifuged at 5000 × g to obtain pellets, which were then combined with 500 µl of CTAB buffer, vortexed, and incubated at 60°C for 30 minutes. The mixture underwent centrifugation at 14,000 × g, and the supernatant was mixed with isoamyl alcohol and chloroform before another round of centrifugation to separate the phases. DNA was precipitated from the aqueous phase using isopropanol at -20°C, rinsed with 70% ethanol, and reconstituted in elution buffer. For 16S rRNA gene PCR, thermal cycling included an initial denaturation at 95°C, annealing at 50°C, and extension at 72°C for 30 cycles, followed by a final elongation at 72°C for ten minutes. The desired DNA band was extracted from the gel, mixed with solubilization buffer and isopropanol, and applied to a purification column, followed by centrifugation and washing before elution with 20 µl of buffer. In Sanger sequencing PCR, denaturation at 95°C was followed by cycles of denaturation, annealing at 50°C, and extension at 60°C for 30 cycles, with samples held at 4°C. Post-sequencing, the reactions were mixed with ethanol and EDTA, centrifuged, air-dried, heat-denatured, and reconstituted in HiDi Formamide before sequencing. Sequence data were analyzed using FinchTV, aligned with BLAST on the NCBI database, and further processed using BioEdit, with the sequences obtained in this study submitted to GenBank NCBI and assigned accession numbers (Martín-Platero *et al.,* 2007).

## Screening for potential activity of LAB

## Beta (β)-galactosidase activity

To assess β-galactosidase activity, newly cultured LAB strains stripping onto Man Rogosa Sharpe Agar plates supplemented with 60 µL of X-gal and 10 mL of IPTG as an inducer, utilising the approach outlined by.[17] After 48 hours of incubation at 37°C, positive strains exhibited greenish to bluish colonies.

## Exopolysaccharide production

LAB strains were freshly cultured and streaked onto 10% skim milk agar with 1% w/v sucrose and 0.08 g/L of the indicator dye ruthenium red. The formation of white, ropy colonies indicated positive result, as outlined by Angmo *et al.,* (2016).

## Haemolysis of blood

The haemolytic activity of LAB strains was assessed using the approach outlined by Angmo *et al.,* (2016).[17] Fresh cultures were deposited onto Sheep Blood Agar plates and incubated for 24 to 48 hours at 37°C. Following incubation, the plates were inspected for signs of haemolysis.

## Results and Discussion

## Identification of lactic acid bacteria

Genomic DNA was extracted from 10 representative bacterial strains, and the 16S rRNA gene of each isolate was magnified utilising PCR. The amplified products were purified and sequenced using the Sanger method for genomic identification. Bacterial identification was carried out by utilising the BLAST 2.0 program to compare the acquired sequences with those in the GenBank NCBI database. This analysis identified various genera and species from naturally fermented milk samples of the Karnataka region. The bacterial species identified are summarized in Table 1.

## Screening of potential activity of LAB

## Beta-galactosidase Activity

The beta-galactosidase enzyme activity of all ten bacterial strains isolated from NFM was evaluated, and nine of them produced favourable results. They are, *Lactiplantibacillus plantarum*, *Lactobacillus fermentum*, *Ligilactobacillus murinus, Enterococcus faecalis, Leuconostoc mesenterides*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus, Enterococcus lactis,* and *Lactobacillus paracasei* showing that the

enzyme beta-galactosidase is active. After incubation, the MRS agar plates blue hue suggested that the betagalactosidase enzyme was being expressed. Whereas strain *Lactobacillus sakei*, did not exhibit the betagalactosidase enzyme (Figure 1, Table 2).

## Exopolysaccharide production

The plate assay for the synthesis of exopolysaccharides indicates that all strains *Lactiplantibacillus plantarum*, *Lactobacillus fermentum*, *Ligilactobacillus murinus, Enterococcus faecalis, Leuconostoc mesenterides*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus, Enterococcus lactis, Lactobacillus sakei* and *Lactobacillus paracasei* were found to be positive. (Figure 2, Table 2).

## Heamolysis Activity

One of the suggested qualities in the FAO/WHO (2002) criteria for the assessment of probiotics is safety. All the selected isolates showed no positive haemolysis activity. They could be regarded as safe with respect to absence of haemolytic action. The lactic acid bacteria haemolytic performance (LAB) isolates was assessed to determine potential pathogenicity. The isolates *Lactiplantibacillus plantarum, Lactobacillus fermentum, Ligilactobacillus murinus, Enterococcus faecalis, Leuconostoc mesenterides, Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus, Enterococcus lactis, Lactobacillus sakei, Lactobacillus paracasei* showed gamma-hemolysis, indicating no hemolytic activity. These findings suggest that the LAB isolates are generally safe for probiotic use due to their non-pathogenic nature in terms of hemolytic activity (Figure 3, Table 2).

The process of separating and identifying lactic acid bacteria (LAB) from milk that has been naturally fermented, the Karnataka region has revealed a diversity of species, including *Lactiplantibacillus plantarum*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Enterococcus faecalis*, *Lacticaseibacillus rhamnosus*, *Enterococcus lactis*, *Lactobacillus sakei*, and *Lactobacillus paracasei*. The high similarity scores in BLAST analysis (96.64–99.19%) confirm accurate identification, providing a foundational step for understanding the role of these LAB in traditional dairy fermentation. The diversity observed also suggests that naturally fermented milk in this region is an excellent source of potentially beneficial microbes for probiotic applications, a finding consistent with studies emphasizing the health-promoting qualities of these bacteria. The functional screening for probiotic characteristics, specifically beta-galactosidase and exopolysaccharide (EPS) production, provides insights into the metabolic capabilities of these isolates. Beta-galactosidase activity was positive in nine strains, including *Lactiplantibacillus plantarum*, *Lactobacillus fermentum*, *Ligilactobacillus murinus, Enterococcus faecalis, Leuconostoc mesenterides*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus, Enterococcus lactis,* and *Lactobacillus paracasei*, highlighting their potential to aid lactose digestion. This enzyme is crucial in probiotics, as it helps improve lactose intolerance symptoms by breaking down lactose into simpler sugars. The presence of blue colonies on X-gal and IPTG plates confirms enzymatic activity, demonstrating that these LAB strains could have potential applications in dairy product formulations aimed at lactose-intolerant populations. For instance, (Ibrahim, 2018) reported that β-galactosidase activity in LAB strains like *Lactobacillus reuteri* and *Lactobacillus delbrueckii* enhances survival in gastrointestinal conditions. Similarly, the presence of β-galactosidase in LAB isolates suggests that these strains could improve the texture of fermented dairy products and support gut colonization. Exopolysaccharide production, observed in all strains, is another important probiotic feature, as EPS can enhance gut health, improve the texture of fermented products, and support immune modulation. LAB strains such as *Lactiplantibacillus plantarum*, *Enterococcus lactis*, and *Lactobacillus sakei* exhibited mucoid colony morphology, indicating EPS production. This characteristic not only provides potential benefits for consumers but also improves the quality and consistency of fermented dairy products, contributing to better mouthfeel and viscosity. In terms of EPS production, (Patel *et al.,* 2021) have underscored that EPS enhances LAB survival and functionality as probiotics. The hemolysis test, a critical safety assessment for probiotics, indicated that all isolates displayed gamma-hemolysis, which indicates a lack of hemolytic activity, suggesting they are safe and non-pathogenic. According to FAO/WHO guidelines, non-hemolytic LAB strains are considered safe for use as probiotics, reinforcing the suitability of these strains for probiotic applications in food products.

**Table.1** Bacterial strains from naturally fermented milk in Karnataka were found using 16S rRNA gene sequencing and BLAST analysis.

| Sl.No | Identified Organisms name | E value | Similarity (%) | Query coverage (%) | Base pair | Accession Number |
|---|---|---|---|---|---|---|
| **1** | *Lactiplantibacillus plantarum* | **0.0** | **96.64** | **99** | **1425** | PP338289 |
| **2** | *Lactobacillus fermentum* | **0.0** | **98.46** | **99** | **1252** | PQ451539 |
| **3** | *Ligilactobacillus murinus* | **0.0** | **97.90** | **99** | **1412** | PP411588 |
| **4** | *Leuconostoc mesenteroides* | **0.0** | **98.23** | **97** | **1199** | PP411605 |
| **5** | *Enterococcus faecalis* | **0.0** | **97.56** | **97** | **1198** | PP434577 |
| **6** | *Lactiplantibacillus plantarum* | **0.0** | **96.90** | **99** | **1258** | PP465467 |
| **7** | *Lacticaseibacillus rhamnosus* | **0.0** | **99.19** | **97** | **1379** | PP439469 |
| **8** | *Enterococcus lactis* | **0.0** | **98.30** | **97** | **1370** | PP439489 |
| **9** | *Lactobacillus sakei* | **0.0** | **98.41** | **97** | **1469** | PP439656 |
| **10** | *Lactobacillus paracasei* | **0.0** | **98.65** | **96** | **1436** | PP439702 |

**Table.2** Production of Beta galactosidase, Exopolysaccharide and Heamolysis activity

| Sl.No | Lactic acid bacterial strains | Results | | |
|---|---|---|---|---|
| | | β-galactosidase Production | Exopolysaccharide Production | Heamolysis Activity |
| **1** | *Lactiplantibacillus plantarum* | Positive | Positive | No heamolysis |
| **2** | *Lactobacillus fermentum* | Positive | Positive | No heamolysis |
| **3** | *Ligilactobacillus murinus* | Positive | Positive | No heamolysis |
| **4** | *Enterococcus faecalis* | Positive | Positive | No heamolysis |
| **5** | *Leuconostoc mesenteroides* | Positive | Positive | No heamolysis |
| **6** | *Lactiplantibacillus plantarum* | Positive | Positive | No heamolysis |
| **7** | *Lacticaseibacillus rhamnosus* | Positive | Positive | No heamolysis |
| **8** | *Enterococcus lactis* | Positive | Positive | No heamolysis |
| **9** | *Lactobacillus sakei* | Negative | Positive | No heamolysis |
| **10** | *Lactobacillus paracasei* | Positive | Positive | No heamolysis |

**Figure.1** *Lactiplantibacillus plantarum* (1), *Lactobacillus fermentum* (2), *Ligilactobacillus murinus* (3), *Enterococcus faecalis* (4), *Leuconostoc mesenterides* (5), *Lactiplantibacillus plantarum* (6), *Lacticaseibacillus rhamnosus* (7), *Enterococcus lactis* (8), *and Lactobacillus paracasei* (10) shows the β-galactosidase production in LAB.

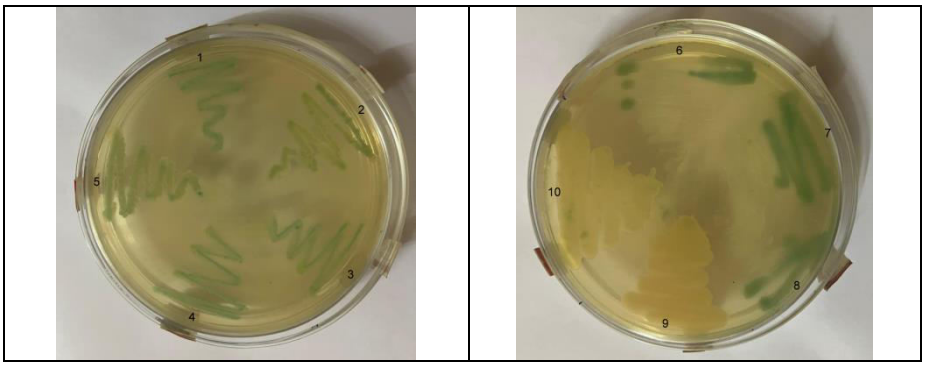

**Figure.2** Detection of the formation of exopolysaccharides synthesis detection (ropy colonies) in the *Lactiplantibacillus plantarum* (1), *Lactobacillus* fermentum(2), *Ligilactobacillus* murinus(3), *Enterococcus faecalis*(4), *Leuconostoc* mesenterides(5), *Lactiplantibacillus plantarum*(6) ,*Lacticaseibacillus* rhamnosus(7), *Enterococcus lactis*(8), *Lactobacillus sakei*(9), *Lactobacillus paracasei*(10) shows Exopolysaccharide production of LAB.

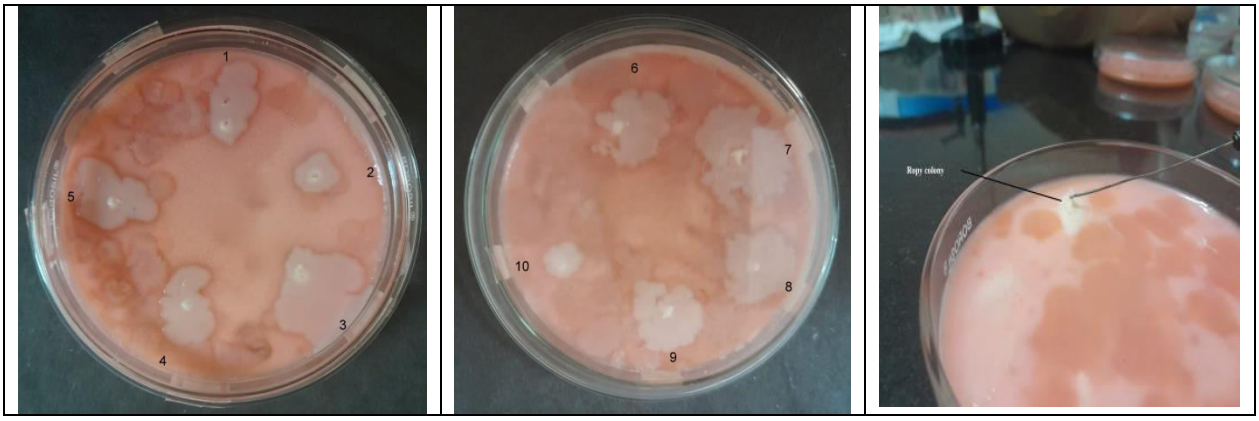


**Figure.3** Detection of Heamolysis activity *Lactiplantibacillus plantarum* (1), *Lactobacillus fermentum*(2), *Ligilactobacillus murinus*(3), *Enterococcus faecalis*(4), *Leuconostoc mesenterides*(5), *Lactiplantibacillus plantarum*(6), *Lacticaseibacillus rhamnosus*(7), *Enterococcus lactis*(8), *Lactobacillus sakei*(9), *Lactobacillus paracasei*(10) isolates showed gamma-hemolysis, indicating no hemolytic activity**.**

This study has successfully identified and characterized LAB strains taken out of milk that has naturally fermented in Karnataka, India, revealing their potential probiotic properties and safety profiles.

The identified strains, particularly those with beta-galactosidase and EPS production capabilities, Notably, nine LAB strains *Lactiplantibacillus plantarum*, *Lactobacillus fermentum*, *Ligilactobacillus murinus, Enterococcus faecalis, Leuconostoc mesenterides*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus, Enterococcus lactis,* and *Lactobacillus paracasei* displayed β-galactosidase activity.

This suggests that naturally fermented milk can be a valuable source of β-galactosidase-producing bacteria, beneficial for industrial applications. Similarly, ten isolates were screened for EPS production, in that *Lactiplantibacillus plantarum, Lactobacillus fermentum, Ligilactobacillus murinus, Enterococcus faecalis, Leuconostoc mesenterides, Lactiplantibacillus plantarum, Lacticaseibacillus rhamnosus, Enterococcus lactis, Lactobacillus sakei,* and *Lactobacillus paracasei* all ten showing positive results, underscoring their probiotic potential and resilience under gastrointestinal conditions shows promising potential for incorporation into dairy products to enhance nutritional benefits, especially for lactose-intolerant individuals.

Future research could further evaluate these strains in clinical settings to validate their health benefits and establish their functional roles in human health, enhancing the value of traditional fermented dairy products as sources of natural probiotics.

## Acknowledgements

The author sincerely thank Prof. Virupakshaiah D.B.M for his valuable guidance and supervision, and Prof. Ajay Kumar oli for his constant support in preparing manuscript.

## Author Contributions

D. C. Bhavya: Investigation, formal analysis, writing—original draft. Ajay Kumar Oli: Validation, methodology, writing—reviewing. D. B. M. Virupakshaiah:—Formal analysis, writing—review and editing.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

## References

Alizadeh B, Jooyandeh J, Vasiee A, *et al.,* Evaluation of anti-yeast metabolites. produced by Lactobacillus strains and their potential application as bio preservatives in traditional yogurt drink, *Lebensmittel-Wissenschaft und Technologie*, 2023;188 : 115428. https://doi.org/10.1016/J.LWT.2023.115428

Angmo K, Kumari A, and Bhalla TC. Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh, *LWT-Food Science and Technology,* 2016; 66: 428–435. https://doi.org/10.1016/j.lwt.2015.10.057.

Bengoa AA, Llamas MG, Iraporda C, *et al.,* Impact of growth temperature on exopolysaccharide production and probiotic properties of Lactobacillus paracasei strains isolated from kefir grains, *Food Microbiology*, 2018;69 : 212-218. https://doi.org/10.1016/J.FM.2017.08.012

Cremonesi P, Vanoni L, Marandi S, *et al.,* Development of pentaplex PCR assay for the stimultaneous detection of *Streptococcus Thermophillus, L. Delbrueckii,* in whey starter for grana padan cheese, *International journal of food microbiology,* 2011; 146:207-211. https://doi.org/10.1016/j.ijfoodmicro.2011.02.016

Devi BT, Meera NS, Theja PC, *et al.,* Production and optimization of β- Galactosidaze enzyme from probiotic lactobacillus spp, *Biotechnal Indian*, 2011; 5: 153- 159.

Hassan MH, Hussein MA, and Bakhiet EK. Screening of β Galactosidaze enzyme production by probiotic lactic acid bacteria isolated from raw and fermented milk, *Archives of agricultural science journal*, 2024; **7**: 36- 50. https://aasj.journals.ekb.eg/

Huang Li, Hanan A, Hamid AM, *et al.,* Partial characterization of probiotic lactic acid bacteria isolated from Chinese dairy product, *International journal of food properties*, 2021; 24: 446- 456. http://dx.doi.org/10.1080/10942912.2021.1900233

Ibrahim AH. Impact of hydrolysed lactose by $\beta$ galactosidaze enzyme on the physiochemical and organoleptic properties of fermented camel milk, 2018; 30: 778- 790. https://doi.org/10.9755/ejfa.2018.v30.i9.1801

Kang JY, Lee B, Kim CH, *et al.,* Enhancing the prebiotic and antioxidant effects of exopolysaccharides derived from Cordyceps militaris by enzyme-digestion, *Lebensmittel-Wissenschaft und -Technologie*,2022; 167 : 113830. https://doi.org/10.1016/J.LWT.2022.113830

Lu J, Mao Y, Liu X, *et al.,* Screening and genome analysis of lactic acid bacteria with high exopolysaccharide production and good probiotic properties, *Food Bioscience*, 2023; 56 : 103211. https://doi.org/10.1016/J.FBIO.2023.103211

Martín-Platero AM, Valdivia E, Maqueda M, *et al.,* Fast, convenient, and economical method for isolating genomic DNA from lactic acid bacteria using a modification of the protein "salting-out" procedure, *Anal. Biochem*. 2007; 366: 102–104. https://doi.org/10.1016/j.ab.2007.03.010

Nemati V, Mirza AA, Varzakas T, *et al.,* Production of traditional torba yogurt using lactic acid bacteria isolated from fermented vegetables: Microbiological, physicochemical and sensory properties, *Journal of Agriculture and Food Research*, 2023; 14 :100850. https://doi.org/10.1016/j.jafr.2023.100850

Nowshin T, Tanim JH, Ferdausi A, *et al.,* Antioxidant, antimicrobial and emulsification properties of exopolysaccharides from lactic acid bacteria of bovine milk, *Insights from biochemical and genomic analysis, LWT,* 2023; 186: 115263. https://doi.org/10.1016/j.lwt.2023.115263

Patel A, Prajapati JB, and Holst O. Exopolysaccharide production by lactic acid bacteria: Technological benefits and biotechnological applications, *Frontiers in Microbiology*, 2021; 12. https://doi.org/10.3389/fmicb.202 1.707358.

Pramudito TE, Desai K, Voigt C, *et al.,* Dextran and levan exopolysaccharides from tempeh-associated lactic acid bacteria with bioactivity against enterotoxigenic Escherichia coli (ETEC), *Carbohydrate Polymers*, 2023; 121700. https://doi.org/10.1016/J.CARBPOL.2023.121700

Wei L, Mao Y, Liu H, *et al.,* Effect of an inorganic nitrogen source (NH4)2SO4 on the production of welan gum from Sphingomonas sp. mutant obtained through UV-ARTP compound mutagenesis, *International Journal of Biological Macromolecules*, 2022; 210: 630-638. https://doi.org/10.1016/J.IJBIOMAC.2022.04.219

Yu Z, Xiaofei D, Haonan J, *et al.,* The effect of optimized carbon source on the synthesis and composition of exopolysaccharides produced by Lactobacillus paracasei, *Journal of Dairy Science,* 2021; 104(4). https://doi.org/10.3168/jds.2020-19448

Zhang G, Zhang W, Sun L, *et al.,* Preparation screening, production optimization and characterization of exopolysaccharides produced by Lactobacillus sanfranciscensis Ls-1001 isolated from Chinese traditional sourdough, *International Journal of Biological Macromolecules,* 2019;139 :1295-1303. https://doi.org/10.1016/J.IJBIOMAC.2019.08.077

Zhang J, Yue X, Hongchao W, *et al.,* Lactic acid bacteria-derived exopolysaccharide: Formation, immunomodulatory ability, health effects, and structure-function relationship, *MicrobiologicalResearch*, 2023; 274: 127432. https://doi.org/10.1016/j.micres.2023.127432

**How to cite this article:**

Bhavya, D. C., Ajay Kumar Oli and Virupakshaiah, D. B. M. 2025. Probiotic Potential and Safety Assessment of Lactic Acid Bacteria from Naturally Fermented Milk in Karnataka: Molecular Characterization and Functional Traits. *Int.J.Curr.Microbiol.App.Sci.* 14(09): 8-15. **doi:** https://doi.org/10.20546/ijcmas.2025.1409.002