

FORMULATION OF NON-DAIRY PROBIOTIC BEVERAGE INFUSED WITH LACTIC ACID BACTERIA INDIGENOUS TO IDLI BATTER.**Ms.Taranoom Khan¹, Ms Pushpa Chaudhary² and Ms. Srushti Pansare³**¹Assistant Professor, Department of Microbiology, K.J. Somaiya College of Science and Commerce, Vidyavihar - 400077^{2,3}Department of Microbiology, K.J. Somaiya College of Science and Commerce, Vidyavihar - 400077**ABSTRACT**

Traditional Indian Food Culture has long emphasized on the importance of fermented food for improved digestive health and overall well being due to the presence of beneficial microbes in it, yet the potential application of these microorganisms in developing a plant - based probiotic drink still remains unexplored. By evaluating the feasibility of developing a non-dairy probiotic beverage with lactic acid bacteria (LAB) extracted from fermented idli batter and combining it with an amla (Amalaki) – sugarcane (Ikshu) juice matrix, the current work fills this gap. The probiotic potential and safety features of the isolate were examined.

*The indigenous isolate exhibited phenotypic and biochemical traits typical of *Lactiplantibacillus plantarum*. The probiotic potential of the strain was determined by evaluating its viability under gastric acidity (pH 3) and bile (1%) condition. Also the absence of hemolysis and other pathogenic activities confirms its food safe nature. A standardized blend of amla (*Phyllanthus emblica*) and sugarcane (*Saccharum officinarum*) juice was formulated as a plant-based fermentation substrate. The formulation supported substantial microbial viability during refrigerated storage, maintaining high cell counts over 72 hours. Physicochemical parameters including pH and titratable acidity remained within acceptable ranges, and ethanol estimation confirmed non-intoxicating levels. Antimicrobial compatibility testing showed that the intrinsic bioactive compounds present in the amla juice were not inhibitory to the obtained isolate.*

By blending the Indian food wisdom with microbiology the project demonstrates the feasibility to develop an affordable probiotic plant based food that meets the nutritional requirements of local low income groups and lactose intolerant individuals.

Keywords: Indian Knowledge Systems, Lactic Acid Bacteria, Probiotic Beverage, Sustainable Fermentation, Functional Nutrition.

1. INTRODUCTION

Living organisms known as probiotics can improve a person's health and maintain gut balance if they are consumed in adequate amounts.(Smolinska et al., 2025; Gul & Durante-Mangoni, 2024). Therefore,when we are encouraging the growth of beneficial bacteria and preventing the growth of harmful ones, these microorganisms help to keep the digestive system in balance. They also strengthen the intestinal barrier and control the immune system (Chandrasekaran et al., 2024; Smolinska et al., 2025). They have been investigated for potential benefits in the treatment of infections and inflammatory bowel disease, and they have an impact on both immunological responses and digestion (Vallejos et al., 2025; Nelwan et al., 2024). As more people prioritize gastrointestinal health and make informed dietary choices, there is growing interest in the roles of these beneficial bacteria beyond basic nutrition, which has led to their discovery in advanced food research (Sanders et al., 2023; Smolinska et al., 2025).

Lactic acid bacteria stand out within probiotic microbes because they've appeared safely in fermented dishes for ages while making helpful compounds like organic acids, bacteriocins, or antimicrobial peptides. These microbes aid gut processes - breaking down food, handling lactose, building vitamins, even blocking unwelcome germs from settling in (Tamang et al., 2016). Because of that track record, scientists still turn to nature, pulling and studying these bacteria to uncover new types capable of boosting health.

Fermented dishes made the old way host lively communities of microbes, guided by (Marco et al., 2017) regional crops, weather patterns, one step at a time. Scientists now look closely at them, they're full of lactic acid bacteria that might benefit us. (Tamang et al., 2016). In poorer nations, items built on grains or beans stand out; their worth shows up in meals people trust, can afford, and eat often. Their place in daily eating means good probiotics can provide natural benefits to us. (Marco et al., 2017). Fermented rice and black gram are used to make idli batter, which ferments naturally without the aid of specific starter cultures (Steinkraus, 1996). A mixed microbial population is present at the beginning of fermentation, but as the process progresses, lactic acid bacteria progressively take over (Mukherjee & Ramesh, 2015; Soni & Sandhu, 1990). According to Tamang et al. (2016), the metabolic activity of these bacteria produces organic acid, which lowers pH, improves

leavening, and gives the product its distinctive flavor and aroma. According to recent studies, lactic acid bacteria that were separated from idli batter also have antimicrobial and probiotic qualities (Sircar & Mandal, 2023). These microorganisms' steady dominance indicates that idli batter may be a good source of probiotic strains.

Lactic acid bacteria, or LAB, are probiotics found in fermented foods like idli batter. Traditional idli fermentation research has identified a range of LAB populations with functional potential (Mukherjee and Ramesh, 2015; Sridevi and Halami, 2018). Certain strains can survive in highly acidic gastric conditions, like those found in the human stomach, according to Zhang et al. (2020) and Plaza-Díaz et al. (2019). In addition to being acid resistant, LAB isolated from fermented foods, such as idli batter, have been shown to be bile salt tolerant, allowing them to survive in the intestinal environment. Sanders et al., 2019; Mukherjee and Ramesh, 2015). This physiological resilience is believed to be essential for probiotic efficacy and host-microbe interactions (Gul and Durante-Mangoni, 2024). Bacteriocins, organic acids, and competitive exclusion mechanisms enable LAB to demonstrate antimicrobial activity against enteric pathogens such as *Escherichia coli* in addition to fermenting carbohydrates (Arena et al., 2018; Yang et al., 2020). LAB from traditional fermented foods in South India has also shown pathogen-inhibitory effects, claim Sridevi and Halami (2018). In the gut ecosystem, this antagonistic relationship boosts colonization resistance and inhibits the growth of pathogens (Zhang et al., 2020). Thus, fermented foods like idli may be used as dietary staples as well as natural carriers of live microorganisms that may have gut-protective properties.

Even though yogurt and fermented milk have long been go-to sources for probiotics, more people avoiding dairy - due to lactose issues, allergies, or choosing plants - has boosted interest in non-dairy options (Márquez-Morales et al., 2021). For those steering clear of animal products because of health, beliefs, or culture, plant-based drinks with live cultures open up new possibilities. These natural bases often come packed with useful nutrients like fiber, antioxidants, and polyphenols - not only feeding good bacteria but possibly helping them work better inside the gut (Martins et al., 2013). Looking into local lactic acid bacteria taken directly from idli batter could lead to affordable, eco-friendly probiotic drinks that fit well within traditional eating habits while meeting today's rising need for functional foods made without dairy.

2. MATERIALS AND METHODS

1. Isolation of Lactic Acid Bacteria from Idli Batter

Freshly fermented idli batter was aseptically collected and transported immediately to the laboratory for microbial analysis. Serial dilutions were prepared using sterile physiological saline, and appropriate dilutions were surface-plated onto de Man, Rogosa and Sharpe (MRS) agar, a selective medium widely used for the isolation and enumeration of lactic acid bacteria from fermented foods (Vinderola & Reinheimer, 2000).

The plates were incubated at 37°C for 24–48 h. Colonies exhibiting creamy, circular, and smooth morphology were selected and repeatedly streaked to obtain pure cultures. The purified isolates were maintained on MRS agar slants at 4°C for further characterization.

2. Morphological and Biochemical Characterization

The purified isolate was subjected to Gram staining and microscopic examination to determine cellular morphology and Gram nature. Catalase and oxidase tests were performed to confirm fermentative metabolism typical of LAB. Carbohydrate fermentation tests were conducted using glucose, sucrose, maltose, and galactose. Additional biochemical assays included citrate utilization, indole production, Voges–Proskauer reaction, and lysine decarboxylation.

Phenotypic traits were compared with established descriptions of LAB commonly isolated from cereal-based fermented foods (Tamang et al., 2016; Mukherjee & Ramesh, 2015).

3. Acid Tolerance Assay

To simulate gastric conditions, overnight-grown cultures were centrifuged, and the pellet was inoculated into MRS broth adjusted to pH 3.0. The suspension was incubated at 37°C for 2–3 h. Viability was assessed through optical density measurement and viable plate counts.

Survival at low pH is considered essential for probiotic functionality, as gastric acidity represents the first major barrier to intestinal colonization (Tripathi & Giri, 2014; Nawaz et al., 2017).

4. Bile Salt Tolerance Assay

Bile tolerance was evaluated by inoculating the isolate into MRS broth supplemented with 1% bile salts and incubating at 37°C for 2 h. Optical density was recorded at 620 nm, and viable counts were determined using the spread plate technique.

Resistance to bile salts is an important criterion for probiotic selection, as bile exposure occurs in the small intestine (Zhang et al., 2020).

5. Preparation of Amla–Sugarcane Juice Substrate

Fresh amla (*Phyllanthus emblica*) and sugarcane (*Saccharum officinarum*) were procured from a local market. Sugarcane was cleaned, peeled, and juiced, while amla fruits were washed, deseeded, and homogenized with sterile water in a 1:1 ratio. The extracts were filtered using a sterile muslin cloth to obtain clarified juice.

Equal volumes of amla and sugarcane juice were blended to form the fermentation substrate. The mixture was pasteurized and cooled before inoculation to eliminate undesirable microflora and ensure controlled fermentation (Pimentel et al., 2015).

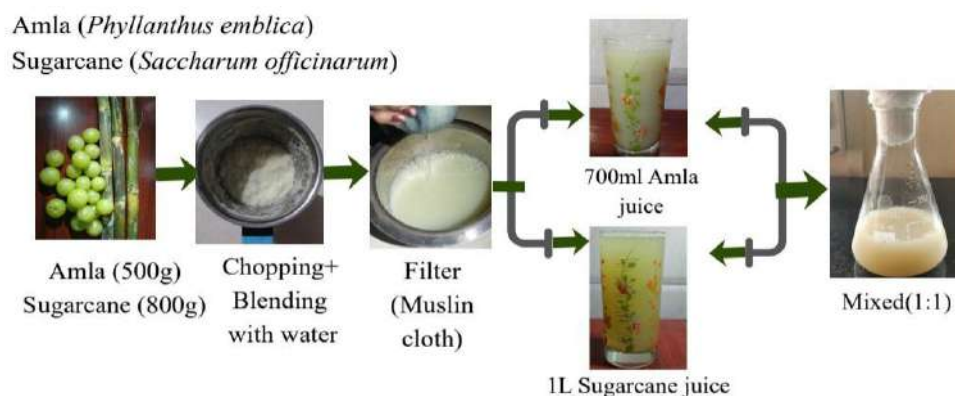


Fig. a) Procedure to make Amla and Sugarcane juice

6. Fermentation Process

Two formulations were prepared:

- Control – Juice blend without inoculum
- Experimental – Juice blend inoculated with 1 mL of LAB culture

Fermentation was carried out at ambient temperature for 24 h, followed by storage at 4°C. LAB-driven fermentation of fruit matrices typically results in acid production within 24 hr. (Swain et al., 2014).

7. Physicochemical Analysis

pH and Titratable Acidity

pH was measured using a digital pH meter. Titratable acidity was determined using standard titration methods and expressed as percentage lactic acid. Acid production reflects LAB metabolic activity and contributes to microbial stability (Marco et al., 2017).

Ethanol Estimation

Ethanol content was estimated using the potassium dichromate method to confirm compliance with non-alcoholic beverage standards. Results were interpreted according to the Food Safety and Standards Authority of India (FSSAI, 2011).

8. Viable Cell Count

Serial dilutions were plated on MRS agar and incubated at 37°C for 48 hr. Colonyforming units (CFU/mL) were calculated. Probiotic beverages are generally required to contain 10^6 – 10^9 CFU/mL to exert beneficial effects (Hill et al., 2014).

9. Safety Assessment

Hemolytic activity was assessed by streaking the isolate on blood agar and observing hemolysis patterns after 24 hr at 37°C. Gelatinase and DNase production were evaluated using standard plate assays to detect extracellular virulence-associated enzymes.

The assessment was performed according to probiotic safety guidelines, emphasizing the absence of hemolytic and pathogenic traits (Hill et al., 2014).

3. RESULTS AND DISCUSSION

The isolate obtained from idli batter produced smooth, creamy colonies on MRS agar. Microscopic examination revealed Gram-positive, rod-shaped, non-spore-forming cells.

The isolate was catalase-negative and oxidase-negative, consistent with the metabolic profile of lactic acid bacteria (Tamang et al., 2016).

Carbohydrate fermentation assays demonstrated utilization of glucose and sucrose, confirming fermentative metabolism. (Table no 1). These characteristics align with LAB populations previously reported in idli batter fermentation (Mukherjee & Ramesh, 2015; Soni & Sandhu, 1990). The phenotypic profile was comparable to *Lactiplantibacillus plantarum* strains isolated from cereal-based fermented foods (Agaliya & Jeevaratnam, 2013).

Table No 1: Biochemical profile of the isolate.

Biochemical Tests	Result
Glucose	+
Sucrose	+
Maltose	+
Galactose	+
Methyl Red	+
Voges-Proskauer	-
Citrate	-
Indole	-
Lysine	-
Catalase	-
Oxidase	-

1. Acid and Bile Tolerance

The isolate retained viability after 3 hours exposure to pH 3.0, indicating tolerance to simulated gastric conditions. Similarly, survival in 1% bile salts demonstrated intestinal resilience. Tolerance to gastric acidity and bile salts is widely recognized as a prerequisite for probiotic efficacy, as these stressors determine survival during gastrointestinal transit (Tripathi & Giri, 2014; Zhang et al., 2020). Comparable tolerance patterns have been reported in LAB isolated from traditional South Indian fermented foods (Sridevi & Halami, 2018; Sircar & Mandal, 2023).

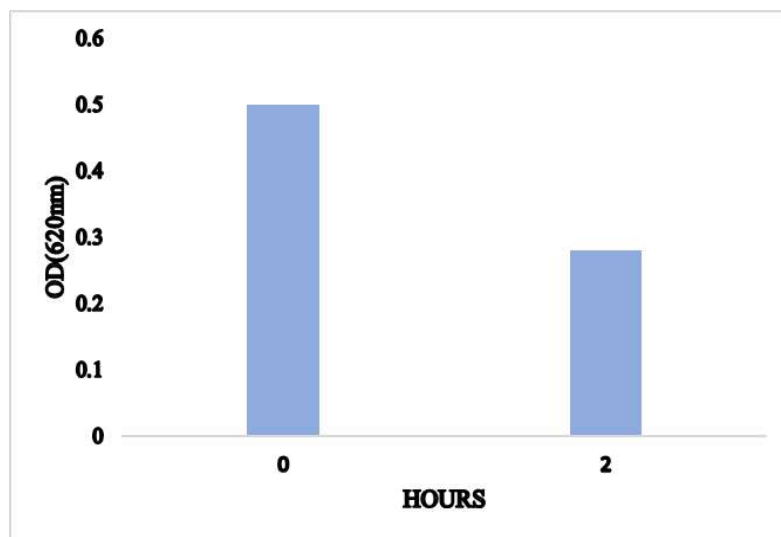


Fig: a) Graph of bile tolerance assay.

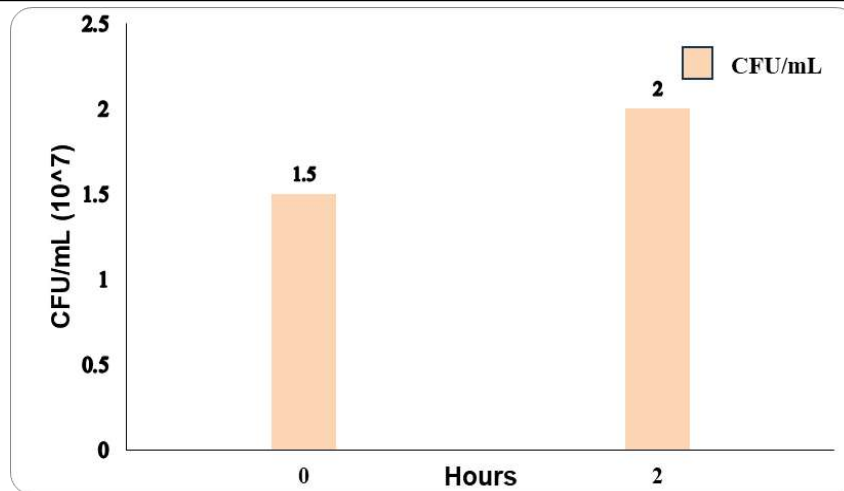


Fig: b) Graph of acid tolerance assay.

2. Fermentation Performance and Physicochemical Changes

Fermentation of the amla–sugarcane substrate resulted in a noticeable decrease in pH accompanied by increased titratable acidity, indicating active lactic acid production. Acidification enhances product safety by suppressing undesirable microorganisms (Marco et al., 2017).

Ethanol concentration was 0.56%, remaining within permissible limits for non-alcoholic beverages under FSSAI regulations (FSSAI, 2011). Although sugar-rich substrates can favour ethanol formation in mixed fermentations, LAB-dominated systems primarily convert sugars into organic acids rather than alcohol (Swain et al., 2014).

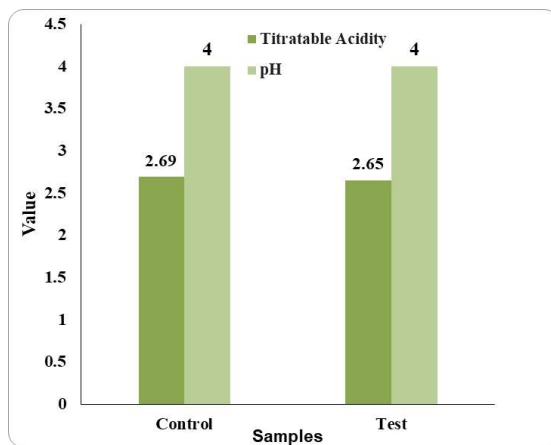


Fig c) Graph of Titratable Acidity and pH

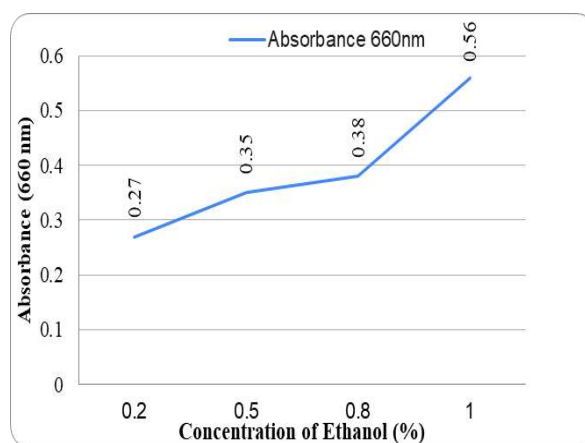


Fig d) Graph of ethanol estimation.

3. Viability in Plant-Based Matrix

The fermented beverage maintained a viability of ~90% (1.35×10^8 CFU) after 72 hours of fermentation, satisfying the minimum recommended probiotic threshold (Hill et al., 2014).

Fruit matrices often contain phenolic compounds that may inhibit microbial growth; however, the present isolate remains stable within the amla–sugarcane system. Similar observations have been reported in fruit-based probiotic beverages (Pimentel et al., 2015; Martins et al., 2013).

4. Safety Evaluation

The isolate exhibited γ -hemolysis on blood agar, indicating the absence of hemolytic activity. No gelatinase or DNase production was detected, suggesting a lack of extracellular virulence factors.

According to international probiotic consensus guidelines, the absence of hemolysis and pathogenic enzymatic activity is essential for probiotic qualification (Hill et al., 2014). The isolate satisfied these safety criteria, supporting its suitability for application in functional beverage development.

4. CONCLUSION

The current study shows that lactic acid bacteria isolated from a traditional Indian fermented food can be used to create a safe and effective non-dairy probiotic beverage. The potential probiotic characteristics of the native LAB strain included compatibility with a plant-based amla-sugarcane substrate, safety conformity, and resistance to gastrointestinal stressors. The prepared beverage demonstrated acceptable sensory quality and sustained significant microbiological viability throughout storage without going over allowable alcohol levels.

In addition to its scientific value, this work demonstrates how traditional fermentation knowledge can be translated when analysed using contemporary microbiological frameworks. Utilizing indigenous microbial cultures and locally accessible plant resources, the formulation promotes cost-effective functional nutrition while lowering reliance on dairy-based probiotic systems. These resource-conscious, plant-based methods support sustainable development goals (SDG 12: Responsible Consumption and Production) and public health (SDG 3: Good Health and Well-Being), two worldwide concerns. The study offers a fundamental framework for combining evidence-based functional food development with culturally based culinary wisdom.

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