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ISOLATION, CHARACTERIZATION, AND FUNCTIONAL ASSESSMENT OF LACTIC ACID BACTERIA FROM DAIRY SAMPLES WITH PROBIOTIC POTENTIAL

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ABSTRACT

This study aimed to isolate and characterize lactic acid bacteria (LAB) from locally sourced curd samples to evaluate their probiotic potential. Dairy samples were collected from domestic and commercial sources, followed by microbiological analysis using MRS (de Man, Rogosa, and Sharpe) medium for the selective cultivation of LAB. Isolates were subjected to both phenotypic and genotypic identification, including 16S rRNA sequencing. Functional characterization included assessments of acid and bile salt tolerance, NaCl resistance, and cholesterol assimilation capabilities. Optimal growth conditions were evaluated across varying pH levels. The acid and bile tolerance assays demonstrated the ability of several isolates to survive under gastrointestinal-like conditions. Cholesterol assimilation was measured using o-phthalaldehyde-based colorimetric analysis, revealing a significant reduction in cholesterol concentration in the culture supernatants. Furthermore, the adhesion ability of LAB strains to human intestinal epithelial HT-29 cells was examined, indicating strong adhesion in selected isolates. The study concludes that LAB strains isolated from curd possess desirable probiotic attributes, underscoring their potential for use in functional foods and therapeutic formulations.

Keywords: Lactic acid bacteria, Probiotics, Curd isolates, Adhesion assay, Cholesterol assimilation

Introduction

Probiotic lactic acid bacteria (LAB) have garnered significant attention in recent years due to their health-promoting benefits, particularly in gut health, immune modulation, and antimicrobial activity. These bacteria, widely found in fermented foods and the human microbiome, are now being explored for their dermatological applications, particularly for combating skin infections caused by pathogenic microorganisms [1, 2]. The skin, as the largest organ of the human body, serves as the first line of defence against environmental insults and microbial invaders. However, an imbalance in the skin microbiome can lead to various skin disorders, including acne, eczema, psoriasis, and infections caused by opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [3].

Role of Probiotic LAB in Skin Health

LAB have been extensively studied for their ability to inhibit pathogenic bacteria through several mechanisms, including competitive exclusion, production of antimicrobial peptides (bacteriocins), secretion of organic acids, and modulation of host immune responses [4, 5]. The production of lactic acid and hydrogen peroxide by LAB creates an acidic microenvironment that inhibits the proliferation of harmful bacteria, thereby preventing infections [6, 7]. Moreover, certain LAB strains produce extracellular polysaccharides (EPS), which have been reported to enhance skin hydration, reinforce the skin barrier, and promote wound healing [8].

Clinical studies have demonstrated that LAB can reduce symptoms of various dermatological conditions. For instance, the topical application of Lactobacillus plantarum significantly improved skin hydration and reduced inflammatory lesions in patients with acne and atopic dermatitis [9]. Similarly, *Lactobacillus paracasei*has been shown to alleviate redness and irritation in individuals with sensitive skin [10]. The anti-inflammatory effects of LAB are attributed to their ability to modulate cytokine production, particularly by increasing the levels of anti-inflammatory cytokines like IL-10 while reducing pro-inflammatory cytokines such as IL-6 and TNF-a [4, 7].

Antimicrobial Potential of LAB Against Skin Pathogens

LAB exhibit potent antimicrobial activity against a wide range of skin pathogens, including antibiotic-resistant strains of *S. aureus* and *Propionibacterium acnes* (formerly *Cutibacterium acnes*) [5, 6]. The production of bacteriocins such as plantaricins, lactocins, and enterocins further enhances their antimicrobial properties [9]. These bacteriocins act by disrupting bacterial cell membranes, inhibiting biofilm formation, and preventing the adhesion of pathogens to the skin [10].

Recent studies have also explored the synergistic effects of LAB with natural antimicrobial compounds. For example, *Lactobacillus reuteri* has been found to work in combination with plant-derived polyphenols to inhibit the growth of *P. acnes*, making it a promising candidate for probiotic-based acne treatments [3]. Furthermore, the ability of LAB to enhance

skin regeneration and wound healing has been demonstrated in in vitro and in vivo models, indicating their potential for therapeutic applications in dermatology [6, 8].

The identification and characterization of probiotic LAB involve a combination of phenotypic and genotypic techniques. Traditional microbiological methods such as Gram staining, catalase tests, and carbohydrate fermentation profiles are often employed to classify LAB strains [1]. However, molecular techniques like 16S rRNA sequencing, whole-genome analysis, and metagenomics have revolutionized the identification process by providing high-resolution taxonomic classification and insights into functional genes associated with probiotic activity [5].

Recent advancements in omics technologies, including transcriptomics and proteomics, have further expanded our understanding of LAB-host interactions [10]. These approaches have helped identify key bacterial metabolites involved in skin health, such as short-chain fatty acids (SCFAs) and exopolysaccharides (EPS), which contribute to maintaining skin homeostasis [2, 7].

METHODOLOGY

1. Screening and Characterization of lactic acid bacteria from dairy samples

- **Collection of samples**

Different curd samples were randomly collected from local households and commercially available milk parlors. These samples were collected in clean, sterile, wide-mouthed containers, ensuring they were free from disinfectant or detergent residue and had tight-fitting, leak-proof lids. Immediately after collection, the samples were transferred to the laboratory for microbiological analysis and stored aseptically at a low temperature (4°C) in a refrigerator to prevent contamination and deterioration.[11]

- **Isolation of probiotic bacteria**

In this study, bacteria were isolated from curd samples using MRS medium. Ten grams of each collected sample were diluted with sterilized phosphate-buffered saline (PBS) and transferred to 100 ml of MRS broth at pH 6.5. The MRS (de Man, Rogosa, and Sharpe) medium was used for the primary isolation of probiotic *Lactobacillus* bacteria by diluting the sample with normal saline solution. These solutions were added to the MRS broth and streaked onto MRS agar plates after 6 hours of incubation. The plates were aerobically incubated at 37°C for 18-24 hours. Cells were grown under a cool-white light. After incubation, white colonies were selected for single colony isolation and to isolate different strains of *Lactobacillus* species.[11]

- **Identification of Lactic Acid Bacteria**

The isolated colony formed on the MRS agar (Hi-media) plates was identified by phenotypically (gram stain and biochemical tests) and genotypically (16s rRNA sequencing). The identification was performed according to Bergey's Manual of Determinative Bacteriology. The cultures were kept in MRS agar slants and stored at 4°C. For long-term storage, glycerol stocks were maintained and stored at -20°C.[11]

- **Determination of optimal growth at different pH**

To determine the optimal growth and pH of *Lactobacillus* spp., 1% (v/v) fresh overnight culture (a single isolated colony sub-cultured in MRS broth) of *Lactobacillus* was inoculated into MRS broth with varying pH levels ranging from 2 to 6.5. The pH was adjusted with concentrated acetic acid (99%) and 5 N NaOH. The inoculated broths were incubated under anaerobic conditions for 24 hours at 37°C. After incubation, bacterial growth was measured using a spectrophotometer, reading the optical density at 560 nm (OD560) against the uninoculated broth.[11]

- **Bile salt tolerance**

  Bile salt tolerance was examined for optimal growth by inoculating various isolates separately into MRS broth tubes containing 0.5%, 1%, 1.5%, 2%, and 2.5% bile salts. Bacterial growth was monitored by measuring absorbance at 600 nm after incubation for 18-24 hours at 37°C. Bile salt-free MRS was used as a control for this experiment.[12]

- **NaCl tolerance**

  For determining NaCl tolerance, all isolates were grown in MRS broth supplemented with different concentrations of NaCl (1-6%). The broth was inoculated with 10 ml of overnight culture of the isolates and incubated at 37°C for 18-24 hours. Bacterial growth was monitored by measuring absorbance at 600 nm, and NaCl-free MRS broth was used as a control.[13]

- **Cholesterol assimilation**

  Freshly prepared MRS broth was supplemented with 0.3% oxgall as a bile salt and filter-sterilized water-soluble cholesterol (100 mcg/ml). Each isolate was inoculated at a 1% level and incubated anaerobically at 37°C for 24 hours. After incubation, cells were removed by centrifugation (9000 rpm for 15 minutes), and the remaining cholesterol in the spent broth was determined colorimetrically using o-phthalaldehyde. One milliliter of the cell-free broth was added to 1 mL of KOH (33% wt/vol) and 2 mL of absolute ethanol, vortexed for 1 minute, and then heated at 37°C for 15 minutes. After cooling, 2 mL of distilled water and 3 mL of hexane were added and vortexed for 1 minute. One milliliter of the hexane layer was transferred into a glass tube and evaporated in a water bath at 65°C. The residue was immediately dissolved in 2 mL of o-phthalaldehyde reagent. After complete mixing, 0.5 mL of concentrated sulfuric acid was added, and the mixture was vortexed for 1 minute. Absorbance was read at 550 nm (T80 UV/Vis spectrometer) after 10 minutes. All experiments were replicated twice.[14]

- **Acid tolerance test**

  Acid tolerance capabilities of the isolates were carried out using the viable count method. One ml of the isolates grown in MRS broth for three generations, having an optical density of 0.280 at 600 nm, was inoculated into 9 ml of sterile MRS broth whose pH was adjusted to 3.5 with 0.5N HCl. Samples were incubated at 37˚C for 4 hours after inoculation. One ml of the sample was taken immediately after inoculation and after 4 hours, serially diluted with sterile saline solution, and inoculated onto MRS agar plates. The agar plates were incubated at 37˚C overnight, and the colonies were counted. The

reduction in log cycle after exposure to low pH for 4 hours, compared to the control, was considered the criterion for acid resistance. [15]

Adhesion assay: Adhesion assays were carried out after 60-90 passages for HT-29 cell lines. The cell suspension with 1 × 10^6 cells, prepared in 4 ml of complete DMEM medium, was transferred to each well of six-well tissue culture plates. The medium was changed every alternate day. When cells reached 80% confluency, the medium was replenished daily for 20 consecutive days for both cell lines. The spent medium was completely removed 24 hours before the adhesion assay, and cells were fed with DMEM medium lacking antibiotics. The cells were then washed twice with 3 ml phosphate-buffered saline (PBS, pH 7.4). An aliquot of 2 ml of DMEM (without serum and antibiotics) was added to each well and incubated at 37°C for 30 minutes. Different *Lactobacillus* cultures (at 1 × 10^7 cfu) suspended in 1 ml of DMEM medium (without serum and antibiotics) were added to different wells. The plates were incubated at 37°C in 5% CO_2 - 95% air for 2 hours. The monolayers were washed five times with sterile PBS (pH 7.4). The adhesion score was measured by enumerating adhered bacteria per 20 different microscopic fields. Percentage adhesion was determined by the plating method.

Adhesion score: Methanol were added to each well of six-well plate at the rate of 3 ml followed by incubation for 10 min at room temperature. Methanol was completely removed and fixed cells were stained with Giemsa stain (0.72% w/v) for 20 min at room temperature. The wells were washed with ethanol to remove excess stain. The plates were air dried and examined under oil immersion microscope. The number of bacteria were counted in 20 random microscopic fields and were grouped into non adhesive (≤40 bacteria), adhesive (41-100 bacteria) and strongly adhesive (>100 bacteria). [16,17]

RESULTS

Identification of Lactic Acid Bacteria

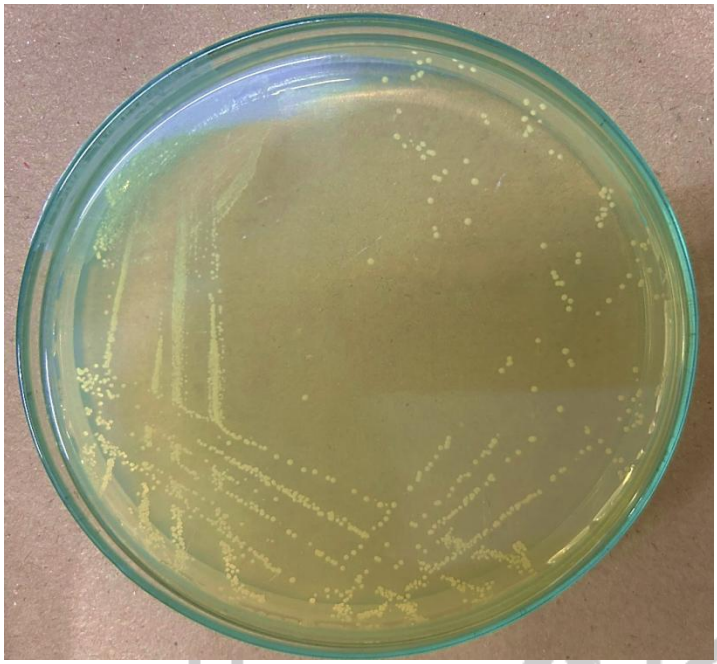


Fig 1. Typical characteristics of the isolates grown on MRS agar medium.

A total of 30 bacterial isolates were obtained from curd samples collected from local dairy and household curd. Among these, **13 isolates were catalase-negative**, suggesting a high probability that they belong to the **Lactobacillus** genus. Catalase-negative bacteria do not produce the enzyme catalase, which is a key characteristic of lactic acid bacteria.

Further identification through **Gram staining** revealed that all 13 catalase-negative isolates were **Gram-positive rods**. This morphological characteristic is consistent with Lactobacillus species, which are widely recognized as beneficial probiotic bacteria.

Table 1 Sugar fermentation chart of isolated Lactobacillus spp. from curd sample

Legend:

	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10	Isolate 11	Isolate 12	Isolate 13
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	–	–	+	+	–	+	–	–	+	–	–
Arabinose	+	–	–	–	+	–	+	–	+	–	–	+	–
Xylose	–	–	–	–	–	+	–	+	+	+	–	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+
Raffinose	–	–	–	–	–	–	–	+	+	+	–	–	+
Sorbitol	–	+	–	–	–	–	–	–	–	–	–	–	–
Trehalose	+	+	+	–	+	+	–	+	–	–	+	+	–
Ribose	+	+	+	+	+	+	+	+	+	+	+	+	+

- "+" indicates a positive result.
- "-" indicates a negative result.

The 13 **Lactobacillus isolates** demonstrate distinct sugar fermentation profiles, reflecting their metabolic diversity. Isolates 1, 2, and 5 show broad capabilities, fermenting most primary sugars like glucose, lactose, maltose, and sucrose, with Isolate 2 uniquely fermenting sorbitol. Isolates 3 and 4 have limited metabolic versatility, focusing on basic sugars and showing no fermentation of secondary sugars like xylose, arabinose, or raffinose. In contrast, isolates 8, 9, and 10 exhibit broader metabolic potential, fermenting secondary sugars such as arabinose, xylose, and raffinose. Isolates 6 and 7 stand out for their ability to ferment trehalose and xylose, respectively, but show limited utilization of mannitol and raffinose. Isolates 11 and 12 display moderate sugar utilization, focusing on primary sugars but lacking the ability to ferment most secondary sugars. Lastly, Isolate 13 exhibits an extended range, fermenting raffinose and xylose while being unable to utilize trehalose and sorbitol. These varied profiles underscore the strain-specific nature of Lactobacillus isolates, which can be

leveraged for targeted applications in nutrition and health.

To confirm the species-level identification of the promising probiotic isolates, 16S rRNA sequencing was performed. The sequencing results revealed that three isolates were successfully identified. Isolate I5 was identified as *Enterococcus durans* with a similarity of 99.78%, while Isolate I4 was also identified as *Enterococcus durans*, showing a 98.87% similarity. Additionally, Isolate I10 was identified as *Lactiplantibacilluspentosus* (formerly known as *Lactobacillus pentosus*) with 100% similarity. These results provide precise taxonomic classification of the isolates, strengthening their potential application in probiotic-based dermatological formulations.

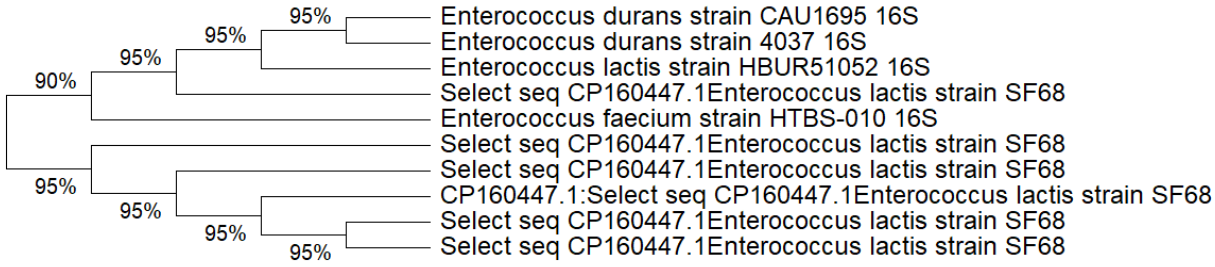


Fig 2.Phylogenetic Tree of Isolate 4(I4) and Isolate 5 (I5) of Enterococcus Strains Based on 16S rRNA Sequences. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 223.634 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. The analytical procedure encompassed 10 nucleotide sequences. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 960 positions. Evolutionary analyses were conducted in MEGA12.

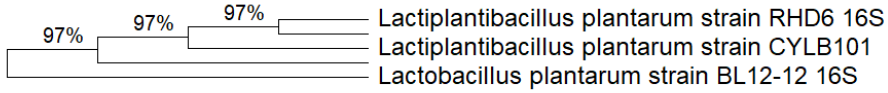


Fig 3.Phylogenetic Tree of Isolate 10 (I10) of Enterococcus Strains Based on 16S rRNA Sequences. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The tree is drawn to scale, with branch lengths (below the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The proportion of sites where at least 1

unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. The analytical procedure encompassed 5 nucleotide sequences. The pairwise deletion option was applied to all ambiguous positions for each sequence pair resulting in a final data set comprising 1,005 positions. Evolutionary analyses were conducted in MEGA12.

Bile salt tolerance

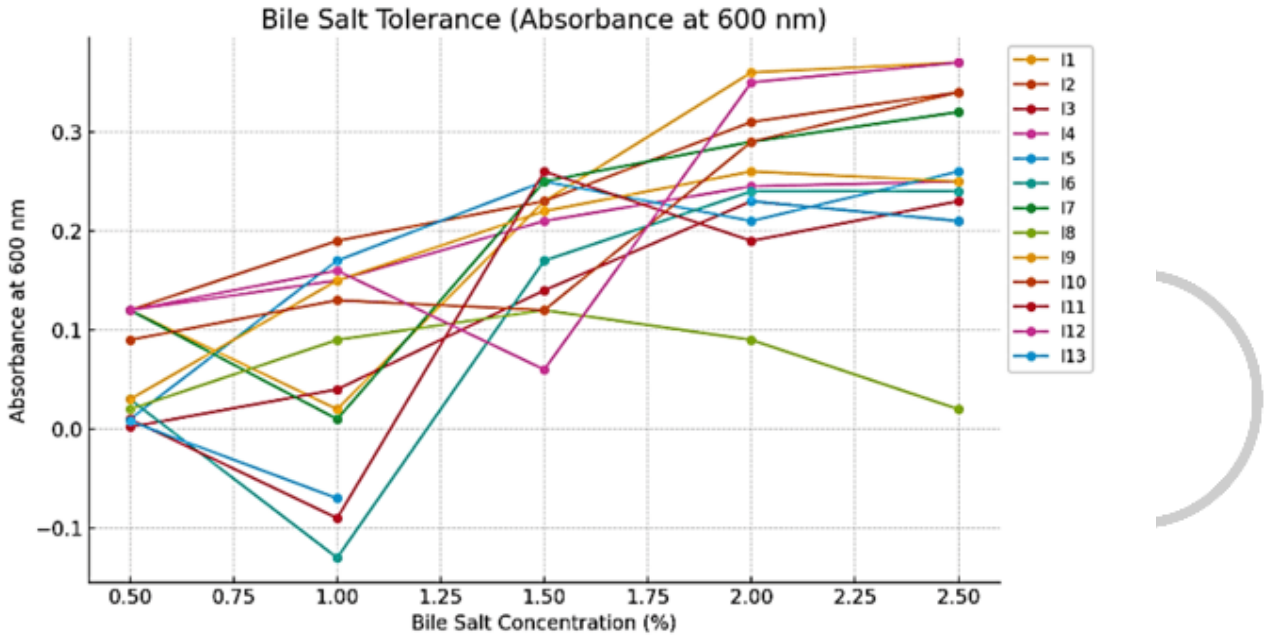


Fig. 4 Bile acid tolerance of Lactobacillus isolates from curd samples

Bile salt tolerance is a crucial trait for probiotic bacteria, as it determines their ability to survive and function in the human gastrointestinal tract. The study measured the tolerance of each isolate to different bile salt concentrations (**0.5%–2.5%**) by recording absorbance at **600 nm**.

The results indicated that most isolates showed increased absorbance as bile concentration increased. This suggests that these bacteria adapted well to bile salt exposure. **Isolate 12** demonstrated the highest absorbance at **2.5% bile salt**, suggesting excellent bile resistance, which is essential for probiotic efficacy. In contrast, isolates such as **I8 and I11** exhibited lower absorbance at higher bile concentrations, indicating weaker tolerance.

NaCl tolerance

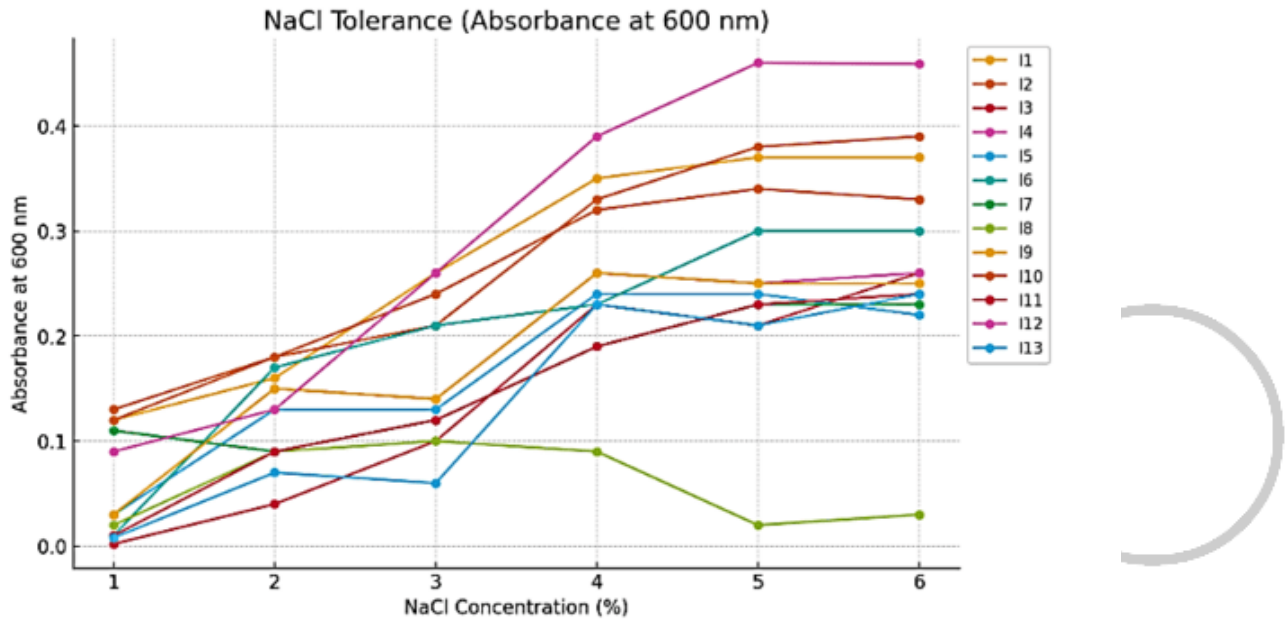


Fig. 5 NaCl tolerance of Lactobacillus isolates from curd samples

The ability of probiotic bacteria to tolerate **salt stress** is an important characteristic for their survival in food products and the human body. The study evaluated NaCl tolerance at concentrations ranging from **1% to 6%**, measuring bacterial growth through absorbance at **600 nm**.

Many isolates displayed **strong growth at 4% NaCl**, indicating good salt tolerance. However, at **6% NaCl**, only a few isolates, such as **Isolate 12**, continued to thrive, demonstrating robust resistance to salt stress. This suggests that certain isolates could be used in probiotic formulations where salt tolerance is required.

Determination of optimal growth at different pH

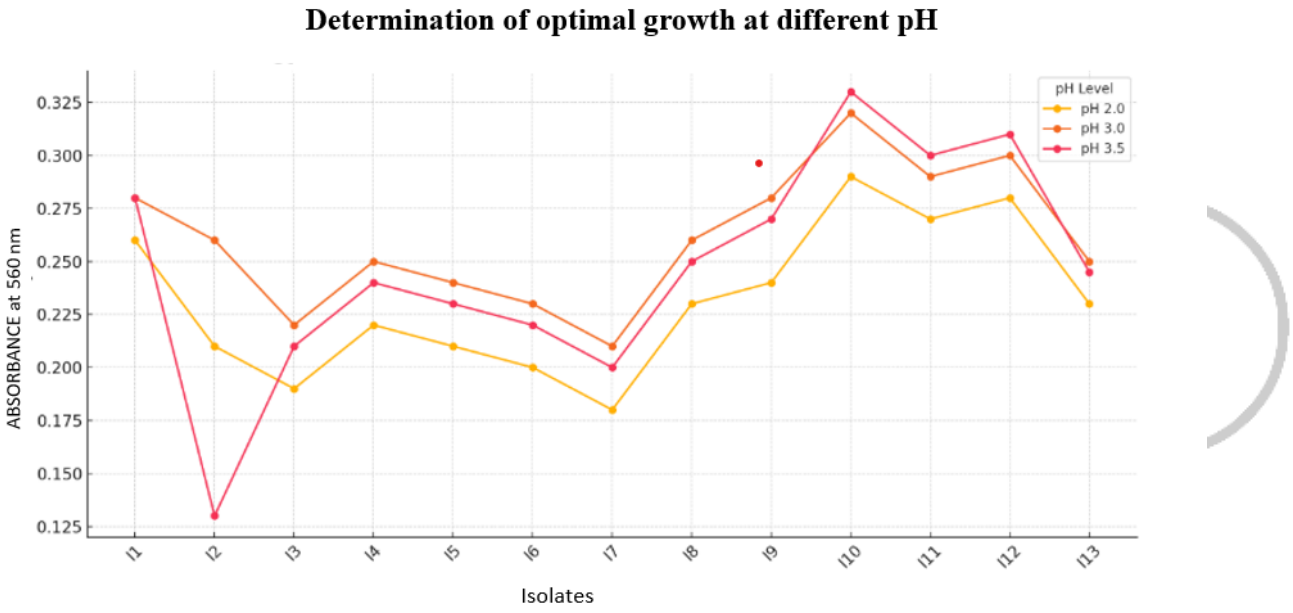


Fig. 6 Optimal growth and pH of isolated Lactobacillus isolates from curd samples.

The **optimal growth of an organism at different pH levels** by measuring absorbance at 600 nm. The pH values tested include **2.00, 3.00, and 3.50**, with growth measurements recorded under different conditions represented by **I_1 to $I_{1\ 3}$** . At pH 2.00, the absorbance values range from **0.09 to 0.29**, indicating moderate growth. At pH 3.00, the values increase slightly, ranging from **0.11 to 0.32**, suggesting improved growth. The highest growth is observed at pH 3.50, with absorbance values between **0.09 and 0.33**, indicating that this pH is the most favourable for the organism. Overall, the trend shows that growth improves as pH increases, suggesting that the organism thrives better in slightly less acidic conditions.

Acid tolerance test

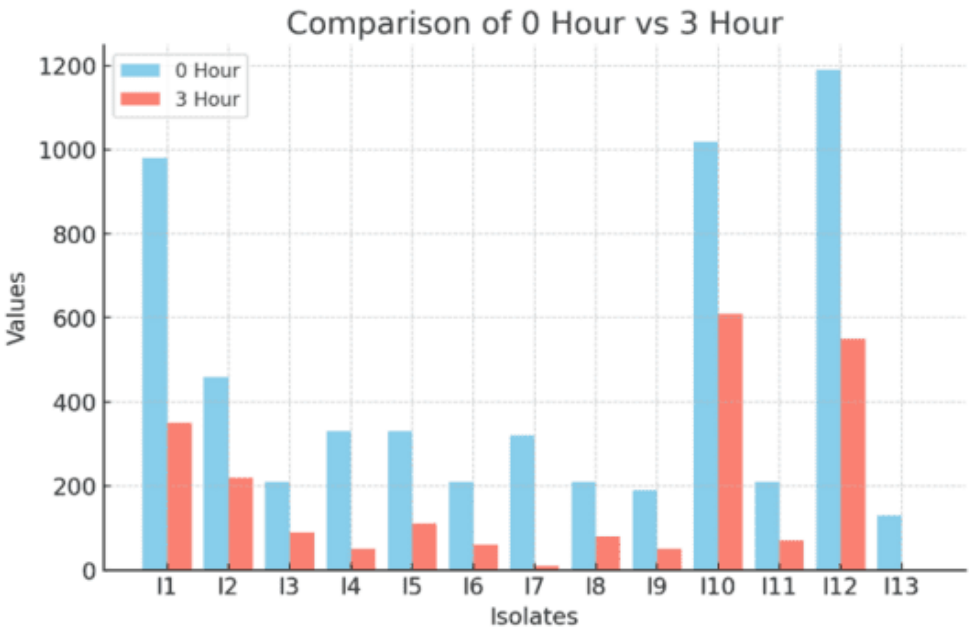


Fig. 7 Acid Tolerance of isolated Lactobacillus isolates from curd samples.

The acid tolerance test evaluated the survival of bacterial isolates under acidic conditions, mimicking the stomach environment. Bacterial counts were taken at **0 hours and 3 hours**, and results showed that some isolates maintained better survival over time.

Isolate 10 exhibited the highest survival rate, reducing from 102 × 10^1 CFU to 61 × 10^1 CFU, followed by Isolate 12, which reduced from 119 × 10^1 CFU to 55 × 10^1 CFU. These findings indicate that these isolates have strong acid resistance, which is essential for probiotics to survive in the digestive system.

Cholesterol assimilation

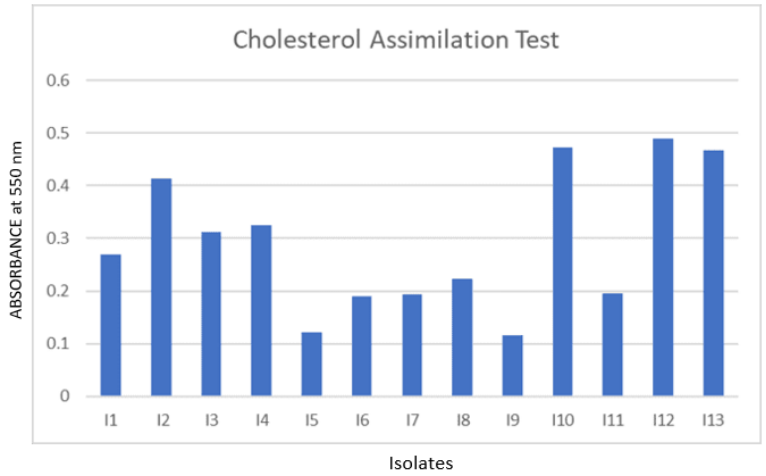
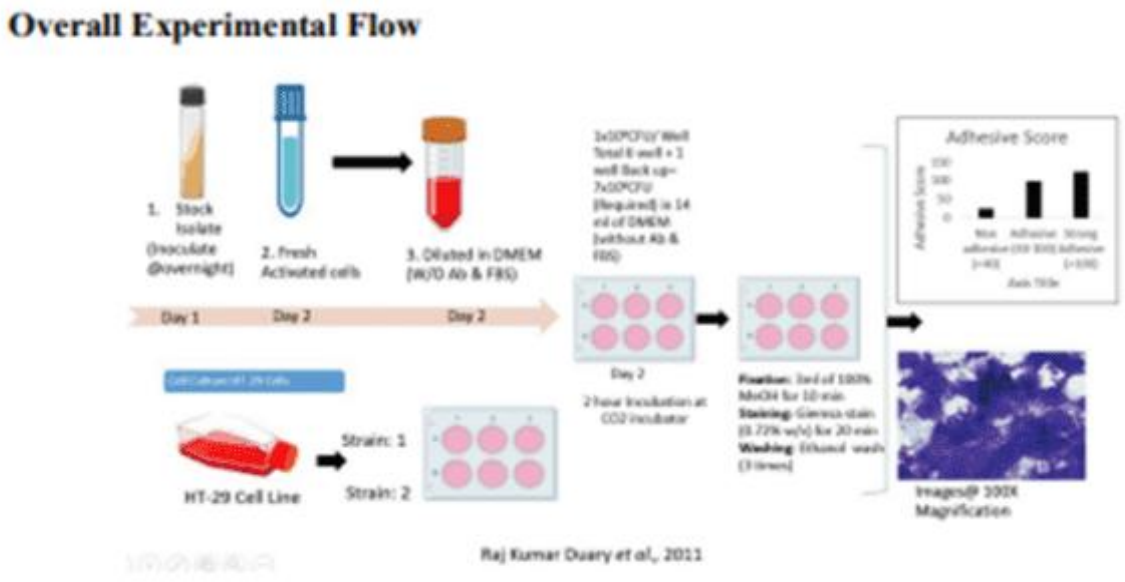


Fig. 8 Cholesterol Assimilation of isolated Lactobacillus isolates from curd samples.

Cholesterol assimilation was assessed by testing bacterial growth in a medium containing **0.3% oxgal and 100 mcg/mL cholesterol**, measuring absorbance at **550 nm**.

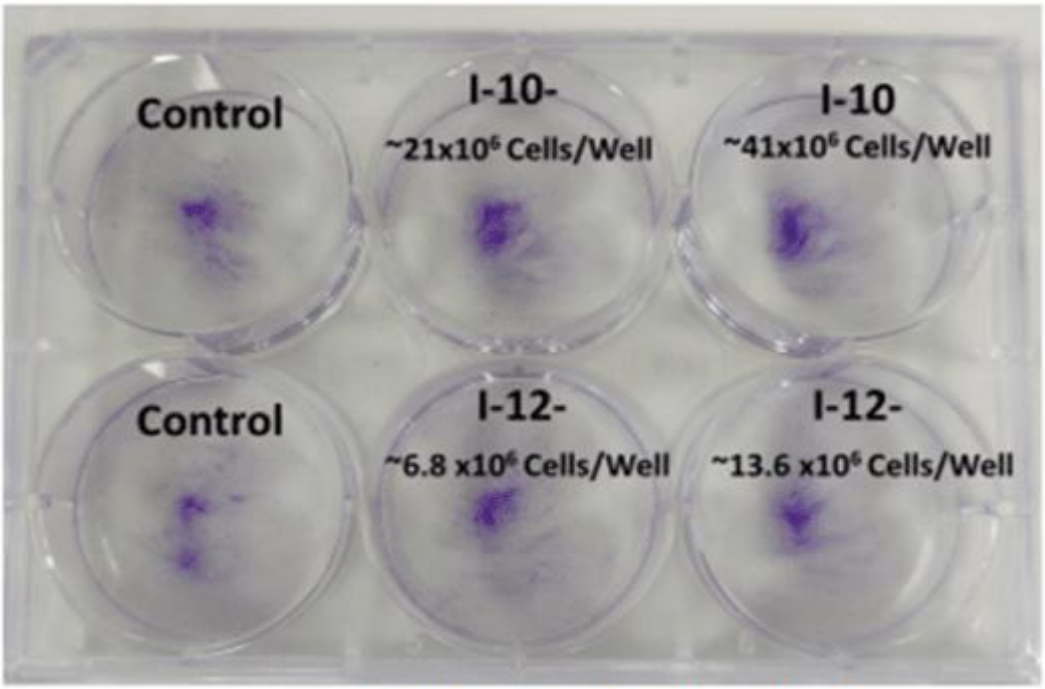
The results showed that isolates had varying abilities to assimilate cholesterol. **Isolate 12 recorded the highest absorbance (0.49), followed by Isolate 10 (0.472) and Isolate 13 (0.467)**. These results suggest that certain isolates have **potential cholesterol-lowering properties**, making them suitable for functional food applications aimed at improving heart health.

Adhesion Study of Probiotic Isolates on HT-29 Cell



Observation and Result

1. **HT-29:** Seeding Density= $3x10^5$ Cells/ Well for 48 hrs; Confluency: 70%;
2. **Probiotic Strains:** Seeding Density= 6-41x10^6 Cells/well; Adhesion time period= 2 hrs.



Plating images: After Gram Staining

Line

Fig 9Probiotic Adhesion to HT-29 Cells: Experimental Flow and Gram Staining Results of isolates 10,12

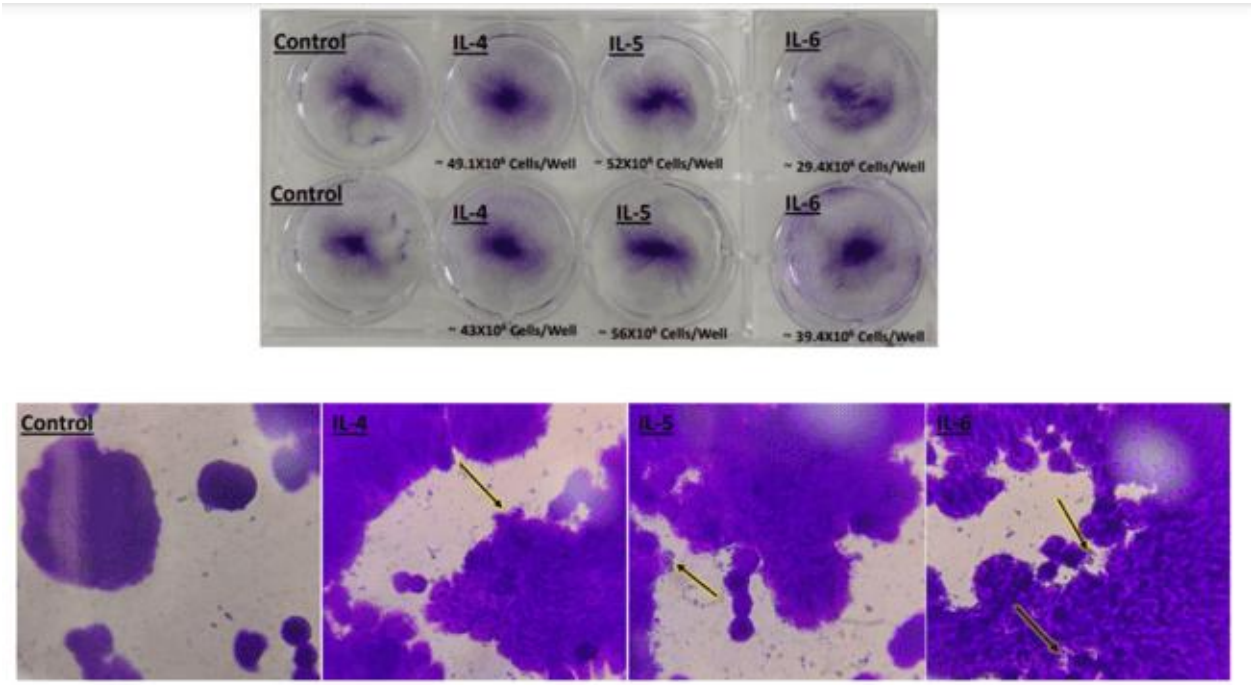


Fig. 10 Probiotic Adhesion to HT-29 Cells: Gram Staining Results of Isolates 4,5,6

The adhesion study was conducted to evaluate the ability of **Isolate 10, Isolate 12, Isolate 5, Isolate 6, and Isolate 4** to attach to **HT-29 human colon carcinoma cell lines**. Adhesion ability is crucial for probiotic strains as it determines their ability to colonize the intestinal lining, provide health benefits, and competitively exclude pathogens.

The adhesion ability of isolates was evaluated based on the number of bacteria adhering per microscopic field, with classification as **strongly adhesive (>100 bacteria per field), moderately adhesive (41–100 bacteria per field), and weakly adhesive (≤40 bacteria per field)**. The experimental setup included **HT-29 cell seeding at 3 × 10^5 cells/well with a 48-hour incubation period**, while probiotic strains were seeded at **6 × 10^6 – 41 × 10^6 cells/well**, with an adhesion time of **2 hours**. These criteria allowed for the accurate assessment of probiotic adhesion potential.

Table 2: Results of Adhesion Study

Isolate	Adhesion Score (Bacteria per Field)	Adhesion Category
Isolate 4 (I-4)	**49.1×10^6 CFU/well**	**Strongly Adhesive**
Isolate 5 (IL-5)	**56×10^6 CFU/well**	**Strongly Adhesive**
Isolate 10 (IL-10)	41×10^6 CFU/well	Moderately Adhesive
Isolate 6 (IL-6)	39.4×10^6 CFU/well	Moderately Adhesive
Isolate 12 (I-12)	13.6×10^6 CFU/well	Weakly Adhesive

The adhesion study on HT-29 cell lines revealed that **Isolate 5 (IL-5)** and **Isolate 4 (I-4)** were the **strongest adhesive isolates**, with adhesion scores of **56×10^6 CFU/well** and **49.1×10^6 CFU/well**, respectively. Their strong adhesion is likely due to the presence of surface proteins, extracellular polysaccharides (EPS), and bacterial aggregation factors that enhance their colonization efficiency and competitive exclusion of pathogens. **Moderately adhesive isolates**, **Isolate 10 (IL-10)** and **Isolate 6 (IL-6)**, showed adhesion scores of **41×10^6 CFU/well** and **39.4×10^6 CFU/well**, indicating fair binding capacity but slightly lower colonization potential than the strongly adhesive strains. In contrast, **Isolate 12 (I-12)** exhibited the **lowest adhesion** at **13.6×10^6 CFU/well**, classifying it as **weakly adhesive**, and suggesting limited persistence in the gut environment despite possible probiotic benefits. These results highlight **Isolate 5 (IL-5)** and **Isolate 4 (I-4)** as the most promising candidates for probiotic applications targeting gut health, while **Isolate 10 (IL-10)** and **Isolate 6 (IL-6)** show moderate potential. **Isolate 12 (I-12)**, however, may require further optimization to improve its adhesion characteristics for effective use in probiotic formulations.

Discussion

The identification and characterization of lactic acid bacteria (LAB) from curd samples revealed significant probiotic potential among the isolates, particularly in terms of bile salt tolerance, NaCl resistance, acid tolerance, cholesterol assimilation, and antagonistic activity against skin pathogens. These findings provide valuable insights into the application of these isolates in functional foods, gut health, and dermatological formulations.

The study identified 13 catalase-negative and Gram-positive rod isolates, confirming their classification as Lactobacillus spp. The ability of these bacteria to ferment various sugars highlights their metabolic versatility, a critical trait for probiotic functionality. Notably, isolates exhibited differential fermentation capabilities, with some strains (e.g., Isolate 2, 5, 8, 9, and 10) capable of fermenting a broad range of sugars, while others (e.g., Isolate 3 and 4) showed limited metabolic potential. This metabolic diversity suggests potential applications in the production of dairy and non-dairy probiotic products [18].

The 16S rRNA gene sequencing results provided a deeper understanding of the probiotic strains' identities. Two isolates (I4 and I5) were identified as *Enterococcus durans*, and one isolate (I10) as *Lactiplanti bacilluspentosus*.*Enterococcus durans* has been reported to produce bacteriocins and lactic acid, contributing to antimicrobial activity against skin pathogens. Moreover, *Lactiplantibacillus pentosus* is widely recognized for its skin benefits, including enhancing skin barrier function, promoting hydration, and exerting anti-inflammatory effects. Previous studies have demonstrated that *L. pentosus* can modulate skin immunity and suppress pro-inflammatory cytokines, suggesting potential for therapeutic use in conditions like acne and eczema. Thus, the identification of *Lactiplanti bacillus pentosus* among the isolates strengthens the potential application of these strains in probiotic-based skincare formulations.[27]

Bile salt tolerance is a crucial determinant of probiotic survival in the gastrointestinal (GI) tract. The human intestine typically contains bile salt concentrations ranging from 0.3% to 2% [19]. In this study, most Lactobacillus isolates demonstrated substantial growth at bile salt concentrations up to 2.5%, with Isolate 12 showing the highest tolerance. This suggests that these isolates can survive passage through the small intestine, which enhances their ability to colonize the gut and exert beneficial effects on host health [20].

Sodium chloride (NaCl) tolerance is another important factor influencing the stability of probiotics in food matrices and the human body. The normal NaCl concentration in the human body ranges between 0.9% (physiological saline) and 3% in specific digestive secretions [21]. The study showed that most isolates thrived at 4% NaCl, and some, such as Isolate 12, even at 6%, indicating their robustness for incorporation into probiotic supplements and fermented foods.

The stomach presents a highly acidic environment, with a pH ranging from 1.5 to 3.5 [22]. For a probiotic to be effective, it must withstand this extreme acidity to reach the intestine. The acid tolerance test indicated that Isolate 10 and Isolate 12 exhibited the highest survival rates, suggesting their superior resilience to gastric conditions. This characteristic enhances their potential for gut colonization and prolonged probiotic efficacy [23].Cholesterol metabolism is a key consideration for probiotic efficacy in promoting heart health. The ability of certain isolates to assimilate cholesterol suggests their potential to reduce serum cholesterol levels, thereby mitigating the risk of cardiovascular diseases. Isolate 12 exhibited the highest cholesterol assimilation ability, making it a strong candidate for functional food applications aimed at lipid regulation [24].Probiotic adhesion to intestinal epithelial cells is essential for effective colonization and pathogen exclusion. Isolate 4 and Isolate 5 demonstrated the strongest adhesion to HT-29 cells, a human intestinal cell line model. The high adhesion capacity of these isolates is likely due to their surface proteins and extracellular polysaccharides, which facilitate stronger interactions with the intestinal lining [25]. These findings suggest that these isolates could serve as effective probiotics for gut health maintenance and protection against enteric infections. Previous studies have shown that the antimicrobial activity of probiotics is crucial for maintaining skin health by competitively excluding pathogenic microorganisms and modulating the skin's immune response. *Lactobacillus plantarum*, a close relative of the isolates studied, has been reported to

inhibit *P. acnes* and *S. aureus*, supporting skin barrier function and reducing inflammation. Additionally, *Enterococcus durans*, identified among the isolates, is known for its bacteriocin production and its role in inhibiting Gram-positive pathogens. The robust activity of Isolate 4 and Isolate 5 indicates their potential as active ingredients in probiotic-based dermatological products aimed at treating infections like acne and impetigo.

These results highlight the importance of strain selection in probiotic research and suggest that targeted application of specific isolates could enhance the efficacy of topical treatments for bacterial skin infections.[26,28,29].

Conclusion

This study highlights the potential of LAB as effective probiotic agents for dermatological applications. Their ability to inhibit skin pathogens, withstand harsh environmental conditions, and promote skin hydration positions them as promising candidates for probiotic-based skincare formulations. Further research into their clinical applications could lead to innovative probiotic treatments for various skin conditions.

Competing Interests:

The authors declare no competing interests.

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