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In Vitro Characterization of Probiotic-Associated and Safety-Related Properties of Five Lactic Acid Bacterial Cultures

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ABSTRACT: Probiotic microorganisms, particularly lactic acid bacteria (LAB), have been investigated for their ability to tolerate gastrointestinal conditions and inhibit pathogenic bacteria. In this study, five strains, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum and Lactococcus lactis, were characterized for a range of probiotic-associated properties. Acid and bile tolerance, sodium chloride tolerance, antimicrobial activity against Escherichia coli and Staphylococcus aureus, antioxidant activity and antibiotic susceptibility were examined. All five strains remained viable at pH 2.0 and tolerated bile salts at concentrations of up to 1.0%. During exposure to simulated gastric conditions, L. rhamnosus showed the highest survival, followed by L. plantarum and L. acidophilus. Antimicrobial testing resulted in inhibition zones of 8-11 mm against the two indicator pathogens, with L. rhamnosus producing the largest zones. All strains also showed DPPH radical-scavenging activity, ranging from 54.6% to 72.3%, and the highest value was recorded for L. plantarum. Preliminary phenotypic screening showed that all cultures were non-hemolytic, catalase-negative and non-motile under the conditions tested. The combined results demonstrate differences among the strains in gastrointestinal stress tolerance and functional activities, with L. rhamnosus and L. plantarum showing comparatively stronger performance in several of the evaluated assays. These findings provide a basis for further strain-specific assessment of their suitability for functional food and dietary applications.

INTRODUCTION

Food safety and preservation have become one of the key issues of the food industry and consumers, especially as the most demanded are foods with minimum processing involving longer shelf life and fewer references to chemical preservatives (Chauhan and Rao, 2024). This has caused an increasing interest in natural and biologically produced alternatives, in particular, those which are related to the traditional fermentation procedures. Of these, a key role is taken by lactic acid bacteria (LAB) because of their Generally Recognized as Safe (GRAS) status and because of the variety of antimicrobial metabolites that they generate (Abdul *et al.*, 2023). Application of organic acids is the major cause of the antimicrobial action of LAB, as it reduces the amount of the surrounding environment pH. Moreover, LAB also synthesizes a number of bioactive components including hydrogen peroxide, diacetyl, bacteriocins and low-molecular-weight metabolites like phenyl lactic acid and cyclic dipeptides (Bangar *et al.*, 2022). LAB can be used in the food preservation and therapeutic processes because of their broad-spectrum action against foodborne and clinical pathogens. The growing resistance of microorganisms to antibiotics has further caused the necessity of alternative antimicrobial approaches, and probiotic LAB have high potential to be applied here (Ibrahim *et al.*, 2021) Probiotics are described as viable organisms that provide beneficial physiological effects to when provided to the host in

sufficient quantities. *Lactobacillus* organisms are some of the most researched probiotic organisms of interest because of their presence in the gastrointestinal tract and fermented food (Maftei *et al*., 2024). Nevertheless, probiotic properties are very specific to the strain, and so functional and safety properties of individual strains must be assessed in detail. To be an effective probiotic, a microorganism ought to be able to endure the harsh environment of the digestive system, including acidic pH and the presence of bile salts and withstand conditions during transit (Fijan, 2014). Also, probiotic strains must possess antimicrobial properties against pathogenic microorganisms and have safety properties of non-hemolytic character and lack of harmful enzymatic activity. This capacity to endure osmotic stress, the generation of bioactive metabolites, and their antioxidant potential base more on their utility in the functional food systems (Bustos, 2025; Staniszewski and Kordowska, 2021).

Regarding this, the current investigation was done to evaluate the probiotic characteristics of the selected lactic acid bacteria strains in terms of their ability to tolerate acidic pH, bile salts, and sodium chloride, antimicrobial, antioxidant and antibiotic resistance provisions. The results of the research work are used in the discovery of potential probiotic candidates with a high probability of use in the functional foods and nutraceutical particles.

MATERIAL AND METHODS

Bacterial Strains

Five bacterial cultures designated as Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum and Lactococcus lactis were obtained from the Research and Development Department of Century Pharmaceutical Ltd. and maintained as glycerol stocks at −20°C until use. Cultures were revived in MRS broth and incubated at 37°C for 24 h. The identification work performed in this study was phenotypic and comprised Gram staining and microscopic examination of cell morphology, together with the biochemical tests described below, including catalase activity and carbohydrate-fermentation profiling. The cultures were not identified by 16S rRNA sequencing or another molecular method; accordingly, the species designations in this study are based on the supplied culture labels and the phenotypic characteristics examined, rather than molecular confirmation.

Revival and Maintenance of Cultures

De Man, Rogosa and Sharpe (MRS) broth and agar revived all strains of lactic acid bacteria. Each culture was placed in 10 ml of MRS broth and kept at 37°C 24 h. Plates of MRS agar were also streaked with cultures and incubated under the same conditions. To study comparative growth, additional propagation of cultures was done in sterile soy milk medium and incubated for 24 h at 37°C. All further experiments were done with actively growing cultures (Gokmen *et al*., 2024).

Media and Growth Conditions

MRS agar and MRS broth were made using standard formulations with a pH and then sterilized. The Soy milk was made by soaking soybean over 24 h and later grind using distilled water in a 9:1 proportions (water:soybean). The slurry was filtered through sterile cheesecloth, autoclaved for 5 min, and incubated at 37°C for fermentation (Piazentin *et al*., 2020).

Evaluation of Probiotic Characteristics

Acidic Test

The capacity of the strains to tolerate pH variations was determined by examining their growth in MRS broth adjusted to different pH levels. Sterile MRS broth (5 mL) was made using 1 N HCl or 1 N NaOH at pH levels of 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 before sterilization.

An aliquot of 50 µL from each overnight culture was transferred into the respective tube, followed by incubation at 37°C for 24 h. Growth of bacteria was quantified by measuring turbidity at 600 nm using spectrophotometer. Uninoculated MRS broth adjusted to the respective pH values served as the blank (Dahou *et al*., 2024).

Bile Salt Test

Growth assessment using agar plate was carried out in order to assess the bile salt tolerance of the strains. Overnight cultures were picked and placed on MRS agar plates to which 0.1%, 0.3% 0.5% and 1.0% (w/v) bile salts were added. MRS control agar with no bile salts were stored. The prepared plates were maintained at 37°C in 24 hours. Afterwards the strains were incubated.

Their growth was measured by observation in terms of the formation of the visible colonies. on the plates. Then the degree of growth was objectively registered and indicated as +++ (maximum). growth), + (poor growth) - (no growth), which implies the susceptibility of the strains to the gradual. increase in the bile salt concentration (Gajjar et al., 2019).

NaCl Tolerance

Strains were subjected to salt tolerance test by growing them in different dilution of NaCl in MRS broth. NaCl concentration of 3% and 5% and 7%, 10%, 12% and 15% (w/v) were added to sterile MRS broth (5 mL). A culture that had been grown overnight in each tube was inoculated using 1% (v/v) and incubated at 37°C during a 24 h period. Optical density at 600 nm was used to measure bacterial growth with the help of a spectrophotometer. The control was uninoculated MRS broth with the corresponding concentration of NaCl (Kamaliya *et al*., 2023).

Antimicrobial Activity Assay

The antimicrobial activity of the strains against *Escherichia coli* and *Staphylococcus aureus* was determined by the agar well diffusion assay. The cell-free supernatants were centrifuged at 6000 × g in the 15 min centrifugation run and filtrated through a 0.2 μm filtration membrane. In order to determine the contribution of organic acids and hydrogen peroxide, the aliquots of the supernatant were neutralized to pH 6 with 1 N NaOH and catalase was added. Micro-plates of nutrient agar which had been inoculated with test pathogens were cut with wells (8 mm diameter), 100 μL of supernatant placed into the wells. Plates were left at 37°C and inhibition zones measured on millimeters. A negative control, such as the sterile culture medium, and a positive antimicrobial control was included (Hilmi *et al*., 2019).

Hemolytic Activity Assay

On blood agar plates, the bacterial strains hemolytic activity was evaluated. The strains were initially overnight cultured in MRS broth. A loopful of the actively growing culture was cultured onto blood agar plates. The inoculation plates were incubated for 24 hours at 37°C. After at this point, the plates were investigated in terms of hemolysis in relation to the colonies of bacteria. Clear zones positive around the colonies signified total red cell lysis (β-hemolysis). A greenish hemolysis (partial or incomplete) was suggested by discoloration around the colonies (α-hemolysis). Conversely, no observable change around the colonies in the medium indicated no hemolytic activity (γ -hemolysis). Positive and negative reference controls were used (Gajjar *et al*., 2019).

Gram Staining

Gram staining was used to determine the cell wall properties and the cell morphology of the strains. Fresh bacterial cultures were grown on clean glass slide with the help of smears. The smears were treated using crystal violet solution, iodine solution, alcohol as a decolorizing solution, and safranin as a counter-stain in that order after being allowed to dry in the air and on heat. The staining was done to the slides and the slides were rinsed in distilled water and then dried in air and put under a light microscope using oil immersion (100 x objective). Gram identification reaction was determined as either the retention or loss of the primary stain by microscopic examination of cells and morphology (Verhelst *et al*., 2005).

Biochemical Characterization of Strains

Catalase Activity

Freshly grown cultures were used in the determination of catalase activity. On MRS agar slants, strains were injected and incubated for 24 hours at 37 °C. A small fragment of an isolated colony was placed on a clean slide with glass surface, and 3% hydrogen peroxide added to it. Lack of the accumulation of the bubble was taken to be interpreted as negative catalase reaction (Yu *et al*., 2019).

Sugar Fermentation

Sugar fermentation tests were conducted in order to describe the patterns of carbohydrate use in 5 strains of lactic acid bacteria. The test was performed in carbohydrate-free basal culture medium (CHL basal medium), which is a carbohydrate-free base medium. Four ml of CHL medium were inoculated in four sterile test tubes and autoclaved. Sugar discs of individual sterile were aseptically placed on the tubes after being cooled. The bacterial cultures in each tube were inoculated with 0.1 mL of an active growing culture and left to incubate at 37°C. A control tube that was keeping the sterile water rather than inoculum was kept so as to observe any non-biological change of color. The change of color of the medium was used to record the

fermentation reactions. A shift to yellow was taken as a positive response that meant the presence of sugar fermentation, with a lack of change being noted as a negative response. The pattern of fermentations was compared and correlated with conventional descriptions of species-level characterization in Bergey Manual of Determinative Bacteriology (Almstahl *et al.*, 2013; Chathyushya *et al.*, 2021).

Citrate Utilization Test

Simmons citrate agar slants were utilized to determine the capacity of the strains to use citrate as the only form of carbon. The cultures were inoculated on the slants and allowed to incubate at 37°C not exceeding 48 h. Any shift in the color of the medium, where the medium changed color to become blue instead of green was regarded as a positive reaction (Aditi *et al.*, 2017).

Motility Test

The hanging drop method of Angmo (2016) was used to measure bacterial motility. Cultures that are not actively growing, were monitored under light microscope in order to observe directional movement (Cousin *et al.*, 2015).

Antioxidant Activity

Preparation of Cell-Free Extract

Antioxidant capacity of the lactic acid bacteria strains selected was tested using cell-free. extracts. Each strain was individually cultured by inoculating 1% (v/v) of overnight-grown cultures into MRS broth adjusted to pH 6.5 ± 0.2. The inoculation broths were shaken at 150 rpm for 24 hours at 37°C in an incubator. The cultured samples were centrifuged at 10,000 rpm for 5 minutes at 4°C to extract the bacterial cells after incubation. The cell-free extract (CFE) was obtained from the resultant supernatant and kept at 4°C until further examination. The extracts were used to assess non-enzymatic antioxidant activity (Debnath *et al.*, 2023).

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the cell-free extracts was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The study was conducted using a freshly made DPPH solution (5 mg per 100 mL ethanol). In short, 3,000 µL of the DPPH solution and 500 µL of cell-free extract were combined, and the mixture was allowed to sit at room temperature in the dark for 30 minutes. A control was done by substituting the extract with 500 µL of ethanol. Upon incubation, absorbance was measured at 517 nm using a spectrophotometer, with ascorbic acid serving as the reference standard. The antioxidant activity was reported as percentage DPPH radical scavenging and developed through the change of absorbance compared to the control (Al *et al.*, 2020).

Antibiotic Susceptibility Assay

The disc diffusion technique was used to determine the antibiotic sensitivity profile of the selected lactic acid bacteria strains. Four antibiotics were tested: tetracycline (10 µg/disc), ceftriaxone (10 µg/disc), erythromycin (10 µg/disc), and gentamicin (50 µg/disc). The bacterial inoculum was standardized to a turbidity equivalent to 0.5 McFarland, corresponding to approximately (1.5×10^8) CFU/mL. A 100-µL aliquot of each bacterial suspension was uniformly spread onto MRS agar plates under aseptic conditions. A reference control was used Antibiotic-impregnated discs were placed on the agar surface, and the plates were incubated at 37°C for 24 hours. Following incubation, the inhibition zones surrounding the antibiotic discs were measured in millimetres. The observed inhibition patterns were recorded for each strain. Susceptibility categories were interpreted only where appropriate, validated criteria were available for the organism and testing method. In the absence of validated organism-specific disc-diffusion breakpoints, the inhibition-zone measurements were treated as preliminary screening results rather than definitive susceptible or resistant classifications (Sharma *et al.*, 2017).

Survival in Artificial Gastric Fluid

The survivability of the chosen lactic acid bacteria under simulated conditions of the gastric environment was assessed with an artificial model of a gastric fluid. Overnight cultures were obtained using centrifugation, and sterile phosphate-buffered saline (pH 7.4) was used to wash them. The culture suspension was standardized to 10^8 - 10^9 CFU/mL. For the assay, 100 µL of the bacterial suspension was inoculated into 900 µL of synthetic gastric fluid composed of D-glucose (3.5 g/l) NaCl (2.05 g/l), KH_2PO_4 (0.6 g/l), $CaCl_2$ (0.11 g/l), KCl (0.37 g/l), bile salts (0.05 g/l), lysozyme (0.1 g/), and pepsin. Hydrochloric acid was

used to stabilize the pH of the solution to 2.5. Anaerobic incubation of the inoculated mixtures at 37°C was done as 0, 30 min and 2 h. Following every incubation period, viable total cell count was calculated, by plating relevant serial dilutions that had been prepared in PBS onto MRS agar plates and then incubation at 37°C was allowed to take place after plating on MRS agar plates (Kim *et al.*, 2007).

Statistical analysis:

All experiments were performed in triplicates. Data was reported as mean ± SD values.

RESULT AND DISCUSSION

Culture revival and growth characteristics

All five selected strains, were successfully revived, with visible growth observed after overnight incubation at 37°C. The growth obtained after revival indicated that the strains remained viable during storage and could be used for further probiotic characterization. This also ensured that all strains were evaluated from a comparable starting point in the subsequent experiments. The growth of the selected strains in MRS broth and soy milk is presented in Figure 1(a) and Figure 1(b), respectively

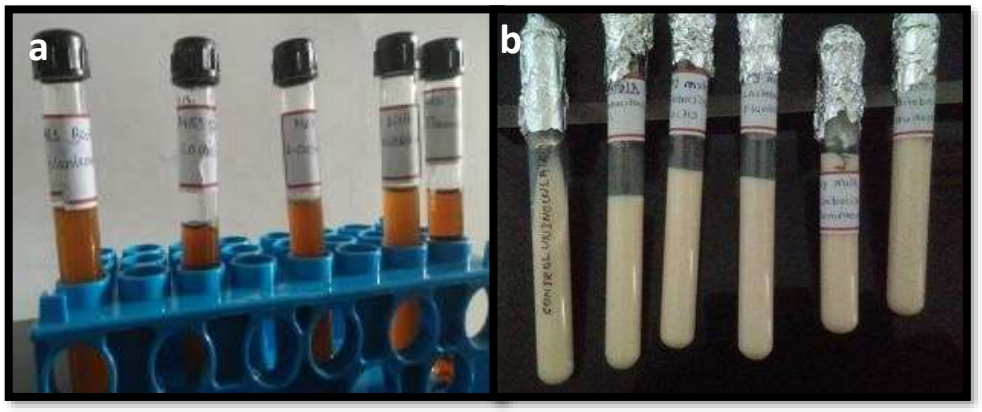


Figure 1: Growth of used lactic acid bacterial strains in different media. (a) MRS broth, (b) Soy milk

Growth of the strains was also accompanied by visible coagulation of the soy milk after fermentation. The coagulation observed in the fermented soy milk may have resulted mainly from organic acid production, particularly lactic acid. As fermentation progressed, the increasing acid concentration lowered the pH of the medium, which destabilized the soy proteins and led to their aggregation and visible coagulation. The observed change was consistent with active fermentation and indicated that the LAB strains were able to grow in the soy milk matrix. Similar protein coagulation has been reported during the fermentation of soy-based products, although the extent of coagulation may differ depending on the bacterial strain and fermentation conditions (Piazentin *et al.*, 2020; Khairani *et al.*, 2024).

Acid tolerance

All five strains grew across the tested pH range, with maximum growth observed at approximately pH 6.0. Growth gradually decreased as the medium became more acidic; however, viable cells were still detected at pH 2.0. The acid tolerance pattern of the five strains at the different pH levels is presented in Figure 2 This indicates that the strains retained a degree of tolerance to acidic conditions. Such tolerance is an important characteristic during probiotic screening, as probiotic cells encounter the highly acidic environment of the stomach during gastrointestinal transit.

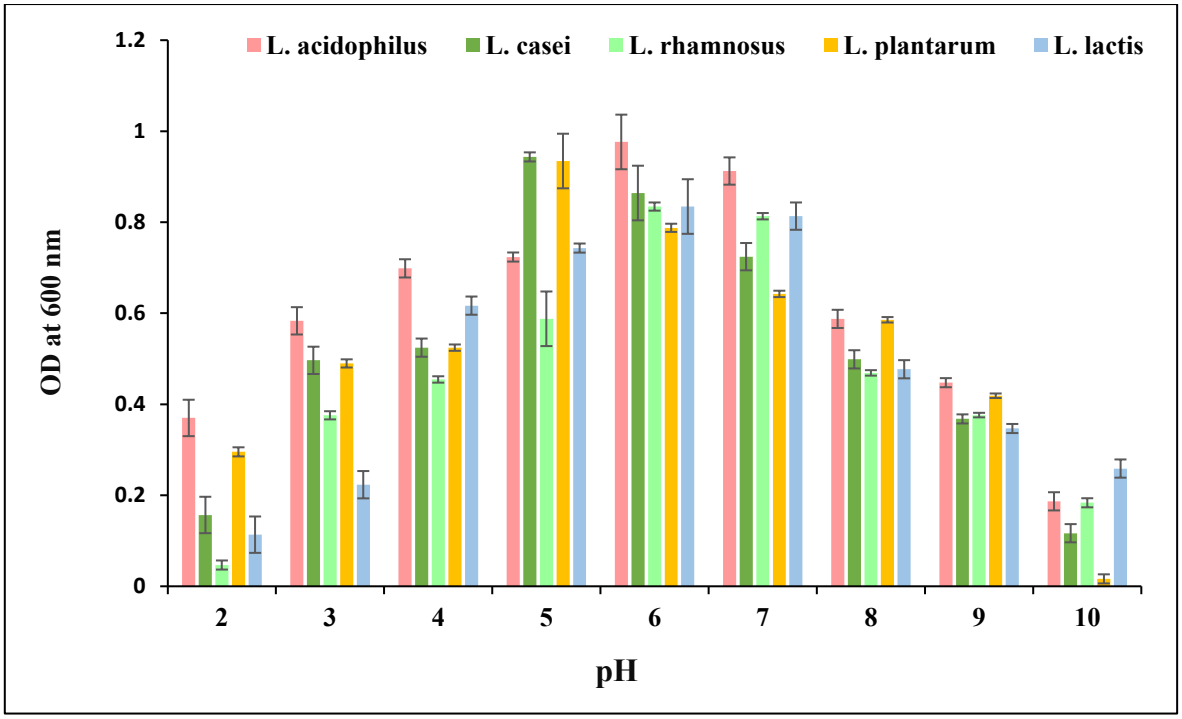


Figure 2: Acid tolerance of *Lactic acid bacteria* strains at different pH levels.

The reduced growth observed at acidic pH may be associated with increased proton entry and accumulation within the bacterial cells. This can disrupt intracellular pH balance and interfere with membrane function, protein stability, and enzyme activity. LAB can respond to acid stress through several mechanisms, including regulation of intracellular pH, removal of excess protons, modification of membrane composition, and activation of stress-response pathways. The ability of all five strains to remain viable under low-pH conditions suggests that they can withstand acidic stress to some extent, although their level of tolerance may vary among strains.

The acid tolerance exhibited in the study is in agreement with previous reports on LAB isolated from different food sources. Khushboo et al. (2023) investigated LAB from dietary sources and assessed gastrointestinal tolerance as one of the probiotic characteristics. Some of their strains were able to withstand conditions relevant to gastrointestinal passage. Similarly, Yumnam et al. (2025) found differences in gastrointestinal stress tolerance among LAB isolated from rice-based fermented foods. These findings are consistent with the strain-dependent response observed in the present study and indicate that acid tolerance should be evaluated at the strain level rather than generalized across LAB. Differences in acid resistance have also been reported in recent in-vitro studies using simulated gastric conditions. Ozluk and Krausova (2025) evaluated LAB present in commercially available probiotic products under simulated gastric conditions and found variation in their ability to survive gastric stress. Their findings further support the importance of assessing acid resistance when evaluating the probiotic potential of individual LAB strains.

Bile salt tolerance

All five strains-maintained growth when exposed to bile salts, although growth gradually declined as the bile concentration increased. Growth was highest in the absence of bile salts and at 0.1%, while a reduction in growth was observed at 0.3-0.5%. The growth responses of the selected strains at the different bile salt concentrations, based on the scoring system, are presented in Table 1. Despite this decline, all strains showed detectable growth at 1.0% bile salt concentration, indicating that they retained a relatively high level of bile tolerance. The progressive decrease in growth with increasing bile concentration suggests that higher bile levels placed greater physiological stress on the tested strains.

Table 1: Growth of selected bacterial strains at different bile salt concentration scoring system.

Bile salt concentration (%)	*L. acidophilus*	*L. casei*	*L. rhamnosus*	*L. plantarum*	*L. lactis*
0.0	+++	+++	+++	+++	+++
0.1	+++	+++	+++	+++	+++
0.3	++	++	+++	++	++
0.5	+	+	++	+	+
1.0	+	+	+	+	+

+++ = maximum growth; ++ = moderate growth; + = poor growth; – = no growth

Bile tolerance is an important property in the selection of probiotic microorganisms because bile salts can interact with bacterial cell membranes, alter membrane permeability, and interfere with cellular homeostasis. In the present study, all tested strains-maintained growth at bile salt concentrations of 0.5% and 1.0%, indicating their ability to withstand relatively high levels of bile-associated stress. This tolerance may contribute to their survival during gastrointestinal passage, where exposure to bile is an important physiological challenge. This ability may contribute to their survival during passage through the gastrointestinal tract. Such adaptations may include changes in membrane composition, maintenance of membrane integrity, efflux mechanisms and enzymatic transformation of bile salts, including bile salt hydrolase (BSH)-mediated deconjugation. However, the specific mechanisms responsible for the observed tolerance were not investigated in the present study and require further biochemical or molecular confirmation.

The present results align with previous reports from several recent studies reporting bile tolerance among potentially probiotic LAB. Khushboo et al. (2023) evaluated LAB obtained from different dietary sources and reported that several strains were able to tolerate bile salt concentrations ranging from 0.5 to 2%, although the degree of tolerance varied considerably among strains. Their findings further demonstrated that bile tolerance was strain-dependent, supporting the progressive reduction in growth observed in the present study. Similarly, Abdel Tawab et al. (2023) reported good bile salt tolerance among selected LAB isolated from Egyptian fermented foods, with the selected strains maintaining viability in the presence of bile salts and demonstrating other characteristics associated with probiotic potential.

More recently, Almeida *et al.* (2024) reported that LAB isolated from cow's milk survived exposure to 0.5% bile salts, with most strains showing survival rates above 82% after 4 h of exposure. Notably, *Lactobacillus plantarum*, *L. rhamnosus*, *L. paracasei* and *Lactococcus lactis* strains demonstrated comparatively good tolerance. These findings are particularly relevant to the present study because *L. rhamnosus, L. plantarum* and *Lactococcus lactis* were also included among the strains evaluated here. Similarly, Bentahar et al. (2024) reported high bile salt tolerance and good survival under simulated gastrointestinal conditions in LAB isolated from traditional Algerian goat milk butter. Their findings further support the use of bile tolerance as an important criterion when screening LAB for potential probiotic applications.

The bile tolerance observed in the present study is consistent with the findings of Boricha et al. (2019), who reported that selected *Lactic acid bacteria* strains isolated from food and human sources were able to tolerate bile salts. Bile salt hydrolase activity was also detected in some of their strains and was suggested as one possible factor contributing to bile tolerance. Similarly, Umeh and Achi (2024) reported good acid and bile tolerance among LAB isolated from indigenous fermented foods, with BSH activity detected in some strains. Together, these findings support the strain-dependent nature of bile tolerance in LAB. Bile salt modification through BSH activity may contribute to resistance in some strains, although this mechanism would require direct assessment to establish its role in the strains examined in the present study.

All five strains tolerated bile salts, with detectable growth maintained even at 0.5-1.0%. These results indicate that the strains can withstand bile exposure to some extent and therefore show potential to tolerate gastrointestinal conditions. Since bile tolerance can vary with both the strain and the conditions of exposure, these findings should be further evaluated using simulated gastrointestinal digestion along with other functional and safety assessments required for probiotic characterization.

NaCl tolerance and osmotic stress resistance

All five strains tolerated the tested NaCl concentrations, although growth generally declined with increasing salt concentration. Some strains continued to grow even at 15% NaCl, indicating their ability to withstand relatively high salt levels. The growth response of the tested strains across the NaCl concentration range of 3-15% is shown in Figure 3. The decrease in optical density at higher NaCl concentrations may be associated with osmotic stress, as elevated salt levels reduce water availability to bacterial cells and can disrupt normal cellular processes. The persistence of growth at these concentrations suggests that the tested strains were able to adapt to, or withstand to some extent, the osmotic stress imposed by high salt levels.

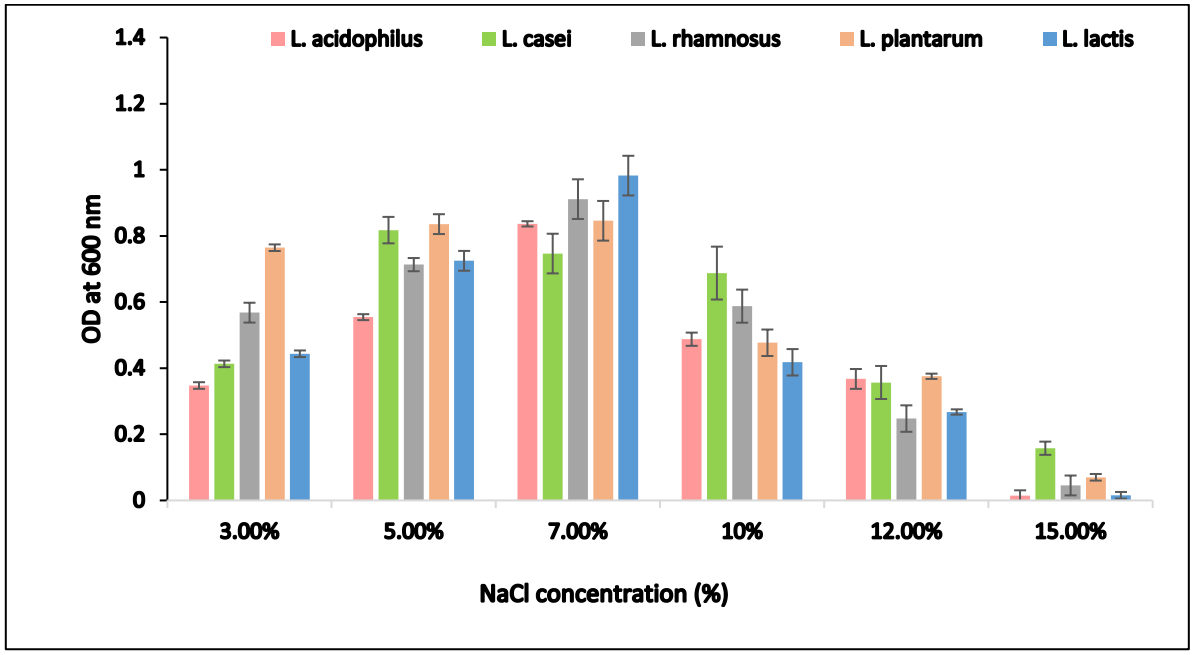


Figure 3: Growth response of *Lactic acid bacteria* strains at different NaCl concentrations (3-15%).

The ability of the tested LAB strains to grow at elevated NaCl concentrations suggests that they possess mechanisms that help maintain cellular osmotic balance under salt stress. These mechanisms may include the accumulation of compatible solutes, changes in cell envelope properties, and regulation of intracellular water balance. Palomino et al. (2013) reported that increased osmotic pressure can cause structural and physiological changes in LAB that contribute to stress tolerance. Differences in salt tolerance among LAB strains have also been reported by Abdel Tawab *et al.* (2023) in strains obtained from fermented foods. Similarly, Bentahar *et al.* (2024) found variation in NaCl tolerance among LAB obtained from traditional fermented dairy products. The findings of the present study follow a similar pattern and indicate that salt tolerance is a strain-dependent characteristic that may be relevant to the performance of LAB during fermented food production.

Antimicrobial activity against pathogenic bacteria

Growth inhibition was observed for both *Escherichia coli* and *Staphylococcus aureus* following treatment with cell-free supernatants from all five LAB strains, as indicated by the formation of inhibition zones ranging from 8.0 to 11.0 mm. The antimicrobial activity of the cell-free supernatants against *E. coli* and *S. aureus* is shown in Figure 4, and the corresponding inhibition-zone measurements are presented in Table 2. Among the tested strains, *L. rhamnosus* produced the largest inhibition zones, measuring 10.1 ± 0.6 mm against *E. coli* and 11.0 ± 0.3 mm against *S. aureus*. The other strains also produced clear zones of inhibition, but these were smaller than those observed for *L. rhamnosus*. Under the conditions used in this assay, *L. rhamnosus* therefore exhibited the strongest antimicrobial effect relative to the other strains.

Table 2: Antimicrobial activity of *Lactic acid bacteria* strains against selected pathogens.

S. No.	Strain Name	Zone of inhibition (mm) against *E. coli*	Zone of inhibition (mm) against *S. aureus*
1.	*Lactobacillus acidophilus*	9.1 ± 0.5	10.0 ± 0.5
2.	*Lactobacillus casei*	8.1 ± 0.4	9.0 ± 0.5
3.	*Lactobacillus rhamnosus*	10.1 ± 0.6	11.0 ± 0.3
4.	*Lactobacillus plantarum*	8.0 ± 0.4	8.1 ± 0.3
5.	*Lactococcus lactis*	8.0 ± 0.3	8.0 ± 0.3

Values are expressed as mean ± SD.

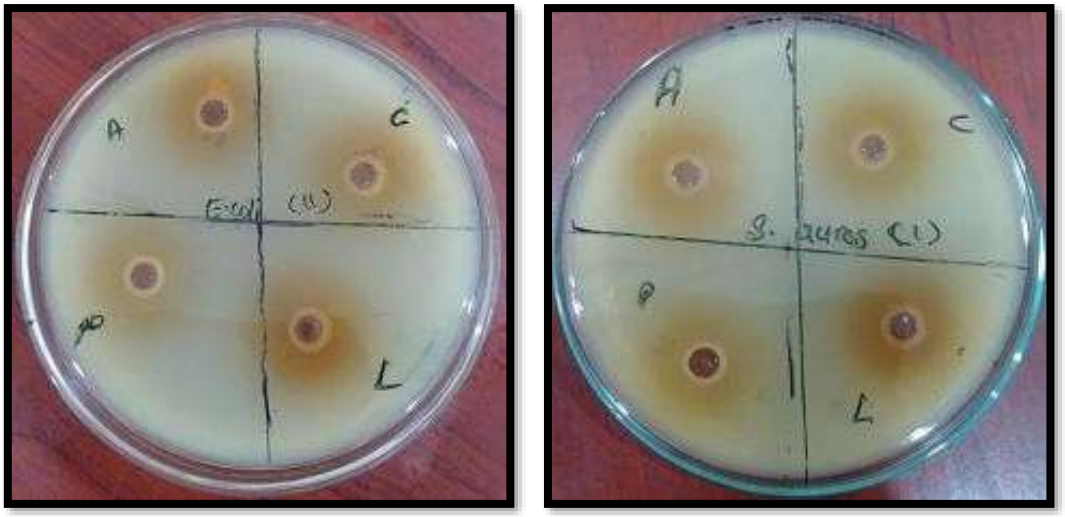


Figure 4: Antimicrobial test of strains against *E. coli* and *S. aureus*.

The tested LAB strains inhibited both Gram-negative and Gram-positive bacteria, indicating that their antimicrobial activity was effective against bacteria with different cell envelope structures. Several mechanisms may contribute to this activity. Organic acids produced during LAB metabolism, particularly lactic acid, can reduce the pH in the surrounding environment and create conditions that inhibit the growth of susceptible bacteria. Depending on the strain, hydrogen peroxide, bacteriocins, and other antimicrobial metabolites may also be involved. The relative contribution of these compounds, however, cannot be determined from the present assay alone.

The higher antimicrobial activity of *L. rhamnosus* indicates strain-dependent variation, possibly due to differences in organic acids, bacteriocins and other antimicrobial metabolites. Similar strain-dependent activity has been reported in probiotic LAB Bentahar *et al.* (2024), supporting *L. rhamnosus* as a promising candidate for further probiotic evaluation. Further evaluation using standardized gastrointestinal models and appropriate viability measurements would help clarify the gastrointestinal robustness of the investigated strain.

Hemolytic activity and preliminary safety assessment

All five bacterial species evaluated in this study exhibited a non-hemolytic phenotype on blood agar after incubation at 37°C for 24 h. The hemolytic activity profile of the tested lactic acid bacteria strains is presented in Table 3. No visible hemolysis was observed around the bacterial growth. This finding indicates that the tested cultures did not demonstrate detectable red blood cell lysis under the experimental conditions employed. The absence of hemolysis represents a preliminary safety-related observation; however, it does not independently establish the overall safety of the strains. Further strain-level characterization and molecular safety assessment are required before these strains can be considered for potential probiotic applications.

Table 3: Hemolytic Activity of *Lactic acid bacteria* Strains.

Strains	Hemolytic activity		
	A	β	γ
Lactobacillus acidophilus	-	-	+
Lactobacillus casei	-	-	+
Lactobacillus rhamnosus	-	-	+
Lactobacillus plantarum	-	-	+
Lactococcus lactis	-	-	+

α = alpha hemolysis (partial hemolysis), β = beta hemolysis (complete hemolysis), γ = gamma hemolysis (no hemolysis / non-hemolytic activity).

The present findings are consistent with previous reports describing the absence of hemolytic activity among potentially probiotic LAB. Qian et al. (2018) reported that selected probiotic LAB strains showed no detectable hemolytic activity, supporting the use of non-hemolytic behavior as an initial safety criterion. More recently, Abdel Tawab et al. (2023) reported that selected LAB obtained from fermented foods were non-hemolytic and exhibited several other desirable probiotic characteristics. Similarly, Bentahar et al. (2024) observed non-hemolytic activity among selected lactic acid bacteria strains obtained from traditional Algerian goat milk butter, further supporting the importance of this characteristic during preliminary probiotic screening.

Morphological and biochemical characteristics

Microscopic examination showed that all tested strains were Gram-positive and displayed morphological characteristics consistent with LAB. The Gram-staining characteristics of the selected strains are shown in Figure 5. The *Lactic acid bacteria* strains appeared as rod-shaped cells, whereas *Lactococcus lactis* exhibited a coccoid morphology. All strains were catalase-negative and non-motile. These phenotypic characteristics are in agreement with the typical features of LAB and, together with the observed cell morphology, provide additional support for the preliminary identification and purity of the cultures. The differences in carbohydrate fermentation among the strains, particularly the broader utilization pattern of *L. plantarum* and the more limited profile of *Lactococcus lactis*, are presented in Table 4. These differences indicate variation in their metabolic capabilities. They may affect their fermentation performance and determine how well individual strains adapt to different food matrices (Das et al., 2019).

Table 4: Sugar fermentation pattern of selected *Lactic acid bacteria* strains.

Sugar	*L. acidophilus*	*L. casei*	*L. rhamnosus*	*L. plantarum*	*Lactococcus lactis*
Arabinose	-	-	-	+	-
Cellobiose	+	+	-	+	-
Fructose	+	+	+	+	+
Galactose	+	+	+	+	+
Lactose	+	+	-	-	+
Maltose	+	+	+	+	+
Mannitol	-	-	-	+	-
Mannose	+	+	+	+	+
Melibiose	-	-	-	-	-
Raffinose	-	-	-	+	-
Rhamnose	-	-	+	-	-
Salicin	+	+	+	+	-
Sorbitol	-	-	-	+	-

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Sucrose	+	+	+	+	+
Trehalose	+	+	+	+	-
Xylose	-	-	-	+	-

Note: + = Acid production, - = no acid production

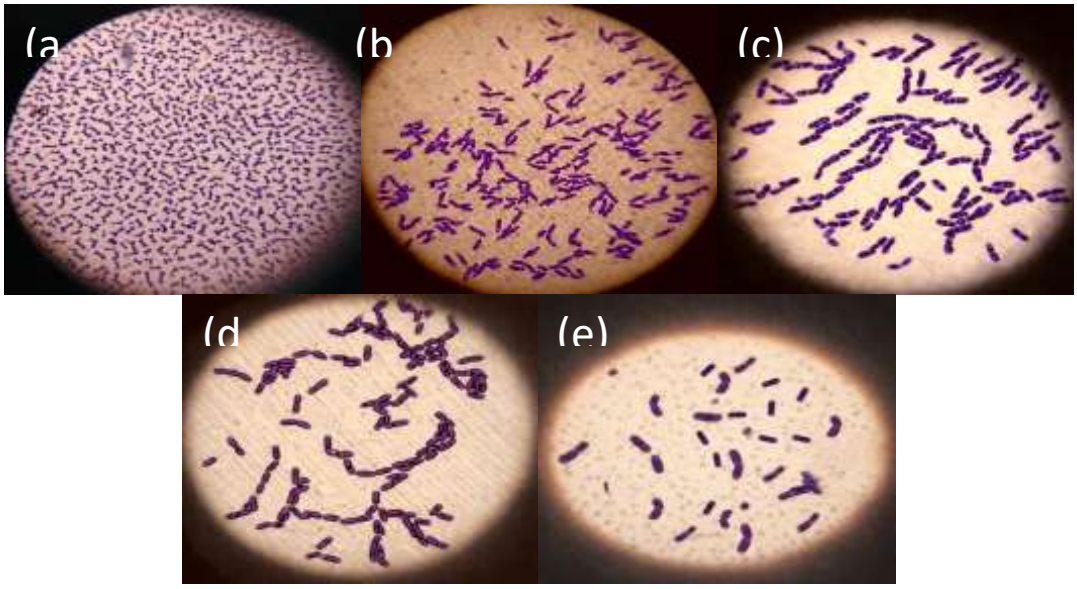


Figure 5: Gram staining of selected lactic acid bacterial strains: (a) *Lactobacillus casei*, (b) *Lactobacillus rhamnosus*, (c) *Lactobacillus plantarum*, (d) *Lactococcus lactis*, and (e) *Lactobacillus acidophilus*.

Antioxidant activity

All five strains exhibited measurable DPPH radical-scavenging activity, with values ranging from approximately 54.6% to 72.3%. The DPPH free radical-scavenging activity of the cell-free extracts from the tested strains is shown in Figure 6. Among the tested strains, *L. plantarum* showed the highest scavenging activity, followed by *L. acidophilus* and *L. rhamnosus*. In comparison, *Lactococcus lactis* showed lower antioxidant activity under the conditions tested.

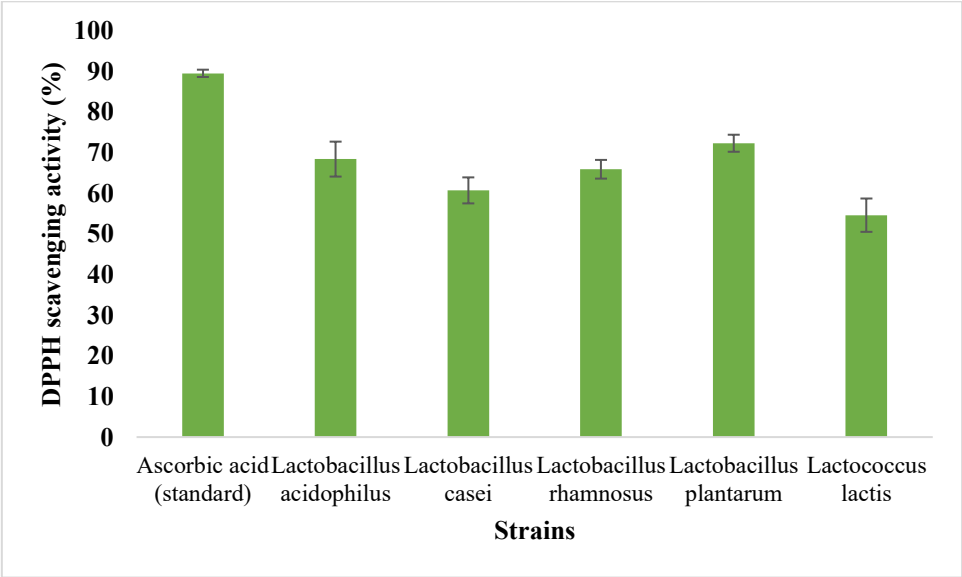


Figure 6: DPPH free radical scavenging activity of cell-free extracts of *Lactic acid bacteria* strains.

The DPPH assay showed that the cell-free extracts contained compounds with free-radical-scavenging capacity. LAB can contribute to antioxidant activity through the production or release of several metabolites, including peptides, organic acids, and other low-molecular-weight compounds. The differences recorded among the strains may be related to variations in their metabolic activity and in the types or amounts of compounds released into the cell-free extracts. However, the assay does not provide information on the individual compounds underlying the observed activity.

The relatively higher DPPH-scavenging activity of *L. plantarum* shows that the functional profile of a probiotic strain cannot be judged from one characteristic alone. In the present study, the strains differed in their responses to gastrointestinal stress, antimicrobial activity, and antioxidant assays. A strain that performs well in one test may not show the same level of activity in another. Similar strain-to-strain differences in antioxidant activity have been reported among *Lactic acid bacteria* strains, supporting the need to assess this property individually for each strain rather than treating it as a common feature of the species (Mu *et al.*, 2018; Debnath *et al.*, 2023).

Antibiotic susceptibility profile and safety considerations

The antibiotic susceptibility assay showed clear differences on the response of the LAB strains. *L. acidophilus*, *L. casei*, and *L. rhamnosus* were susceptible to tetracycline and erythromycin but resistant to gentamicin and ceftriaxone. In contrast, *L. plantarum* was resistant to all four antibiotics tested, while *Lactococcus lactis* remained susceptible to tetracycline, erythromycin, and ceftriaxone but showed resistance to gentamicin. The broader resistance pattern observed in *L. plantarum* warrants further investigation, particularly to determine whether the resistance is intrinsic to the strain or associated with acquired and potentially transferable resistance determinants. Molecular analysis would be required to distinguish between these possibilities. (Georgieva *et al.*, 2015).

Survival under simulated gastric conditions

The artificial gastric fluid assay provided a more direct measure of the ability of the tested strains to withstand gastric stress. Viable counts decreased as the exposure time at pH 2.5 increased; however, viable cells were recovered from all five strains after 2 h. The survival of the tested *Lactic acid bacteria* strains in synthetic gastric juice at pH 2.5 is presented in Table 5. Among the strains, *L. rhamnosus* showed the highest survival, with approximately 5.4×10^5 CFU/mL remaining at the end of the 2-h exposure period. This was followed by *L. plantarum* (3.9×10^5 CFU/mL) and *L. acidophilus* (2.8×10^5 CFU/mL). Although *L. casei* and *Lactococcus lactis* showed lower viable counts, both strains were still detectable after 2 h of exposure to the simulated gastric conditions.

Table 5: Survival of Lactic acid bacteria strains in synthetic gastric juice (pH 2.5)

Strains	**0 min CFU mL^{-1} (% survival)**	**30 min CFU mL^{-1} (% survival)**	**2 h CFU mL^{-1} (% survival)**
Lactobacillus acidophilus	2.8×10^8 (100)	1.7×10^6 (0.60)	2.8×10^5 (0.10)
Lactobacillus casei	2.9×10^8 (100)	1.2×10^6 (0.41)	2.0×10^5 (0.07)
Lactobacillus rhamnosus	3.1×10^8 (100)	2.6×10^6 (0.84)	5.4×10^5 (0.17)
Lactobacillus plantarum	3.0×10^8 (100)	2.1×10^6 (0.70)	3.9×10^5 (0.13)
Lactococcus lactis	2.7×10^8 (100)	8.4×10^5 (0.31)	1.3×10^5 (0.05)

The decline in viable counts with prolonged exposure to the simulated gastric conditions indicates increasing acid and pepsin stress on the bacterial cells. Among the tested strains, *L. rhamnosus* showed comparatively higher survival during gastric exposure and also exhibited the strongest antimicrobial activity. These combined characteristics indicate that *L. rhamnosus* may be a promising candidate for further probiotic evaluation. (Stasiak *et al.*, 2021).

Safety-Related Phenotypic Characteristics

The investigated strains exhibited Gram-positive, non-motile, catalase-negative, and non-hemolytic phenotypes under the conditions tested. The absence of observed hemolysis represents a favorable preliminary characteristic during the assessment

of potential probiotic candidates. However, phenotypic observations alone are insufficient to establish the overall safety of a bacterial strain. The antibiotic susceptibility results also indicated variation among the investigated strains, highlighting the importance of strain-specific safety evaluation. In particular, apparent resistance to certain antibiotics requires further investigation to distinguish intrinsic resistance from potentially acquired resistance determinants. Molecular identification and genomic screening for virulence-associated genes, transferable antimicrobial resistance determinants, and other undesirable genetic characteristics would provide additional evidence for safety assessment. Consequently, the present phenotypic findings should be considered preliminary rather than definitive evidence of probiotic safety.

Significance and implications of the study

The combined assessment of acid and bile tolerance, salt stress resistance, antimicrobial and antioxidant activities, hemolytic behavior, antibiotic susceptibility, and gastric survival revealed clear differences among the strains. *L. rhamnosus* showed stronger antimicrobial activity and better survival under simulated gastric conditions, while *L. plantarum* exhibited the highest antioxidant activity. These differences indicate that probiotic characteristics can vary considerably between strains and highlight the value of assessing multiple functional and safety-related properties during probiotic screening.

Overall, the tested LAB demonstrated promising in vitro probiotic characteristics and potential for functional food applications. However, further molecular, genomic, stability and further in vivo studies are needed to confirm their probiotic effectiveness and suitability for safe use before practical application.

CONCLUSION

The five tested lactic acid bacterial cultures showed differences in acid and bile tolerance, salt tolerance, antimicrobial activity, DPPH radical-scavenging activity and survival under simulated gastric conditions. L. rhamnosus showed the highest viable count after 2 h in simulated gastric fluid at pH 2.5 (approximately 5.4×10^5 CFU/mL) and produced the largest inhibition zones against E. coli (10.1 ± 0.6 mm) and S. aureus (11.0 ± 0.3 mm). L. plantarum showed the highest DPPH radical-scavenging activity (72.3%). All five cultures were non-hemolytic under the conditions tested, catalase-negative and non-motile. These findings indicate strain-dependent differences in selected in vitro characteristics; however, they do not establish clinical efficacy or comprehensive safety. Molecular confirmation of identity, investigation of antimicrobial-resistance determinants, and validation in relevant food systems and appropriate in vivo models are needed before suitability for probiotic applications can be concluded.

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Ethical Statement

Not applicable

Use of Artificial Intelligence (AI) Tools

AI tools have been used for paraphrasing.

Conflict of Interest

There is no conflict of interest.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

[1] Abdel Tawab, F. I., Abd Elkadr, M. H., Sultan, A. M., Hamed, E. O., El-Zayat, A. S., & Ahmed, M. N. (2023). Probiotic potentials of lactic acid bacteria isolated from Egyptian fermented food. *Scientific Reports, 13*, 16601. https://doi.org/10.1038/s41598-023-43752-0.

[2] Abdul Hakim, B. N., Xuan, N. J., & Oslan, S. N. H. (2023). A comprehensive review of bioactive compounds from lactic acid bacteria: Potential functions as functional food in dietetics and the food industry. *Foods*, *12*(15), 2850. https://doi.org/10.3390/foods12152850

[3] Aditi, F. Y., Rahman, S. S., & Hossain, M. M. (2017). A study on the microbiological status of mineral drinking water. *The open microbiology journal*, *11*, 31. https://doi.org/10.2174/1874285801711010031

[4] Al-Dhabi, N. A., Valan Arasu, M., Vijayaraghavan, P., Esmail, G. A., Duraipandiyan, V., Kim, Y. O., & Kim, H. J. (2020). Probiotic and antioxidant potential of Lactobacillus reuteri LR12 and Lactobacillus lactis LL10 isolated from pineapple puree and quality analysis of pineapple-flavored goat milk yoghurt during storage. *Microorganisms*, *8*(10), 1461. https://doi.org/10.3390/microorganisms8101461

[5] Al-Kharousi, Z. S. (2025). Highlighting lactic acid bacteria in beverages: Diversity, fermentation, challenges, and future perspectives. *Foods*, *14*(12), 2043. https://doi.org/10.3390/foods14122043

[6] Almeida, J. M., Maffei, J. T., Gebara, C., Minafra, C., Toledo-Silva, B., Gonçalves, M. C., Langoni, H., Thaler Neto, A., Souza, F. N., & Silva, N. C. C. (2024). Exploring probiotic potential and antimicrobial properties of lactic acid bacteria from cow's milk. *Applied Food Research, 4*, 100461. https://doi.org/10.1016/j.afres.2024.100461.

[7] Almståhl, A., Lingström, P., Eliasson, L., & Carlén, A. (2013). Fermentation of sugars and sugar alcohols by plaque Lactobacillus strains. *Clinical Oral Investigations*, *17*(6), 1465-1470. https://doi.org/10.1007/s00784-012-0832-z

[8] Bangar, S. P., Suri, S., Trif, M., & Ozogul, F. (2022). Organic acids production from lactic acid bacteria: A preservation approach. *Food bioscience*, *46*, 101615. https://doi.org/10.1016/j.fbio.2022.101615

[9] Bentahar, M. C., Benabdelmoumene, D., Robert, V., Dahmouni, S., Qadi, W. S., Bengharbi, Z., & Mediani, A. (2024). Evaluation of probiotic potential and functional properties of Lactobacillus strains isolated from Dhan, traditional Algerian goat milk butter. *Foods*, *13*(23), 3781. https://doi.org/10.3390/foods13233781

[10] Bentahar, M. C., Benabdelmoumene, D., Robert, V., et al. (2024). Evaluation of probiotic potential and functional properties of *Lactobacillus* strains isolated from Dhan, traditional Algerian goat milk butter. *Foods, 13*, 3781. https://doi.org/10.3390/foods13233781.

[11] Boricha, A. A., Shekh, S. L., Pithva, S. P., Ambalam, P. S., & Vyas, B. R. M. (2019). In vitro evaluation of probiotic properties of Lactobacillus species of food and human origin. *Lwt*, *106*, 201-208. https://doi.org/10.1016/j.lwt.2019.02.021

[12] Bustos, A. Y., Taranto, M. P., Gerez, C. L., Agriopoulou, S., Smaoui, S., Varzakas, T., & Enshasy, H. A. E. (2025). Recent advances in the understanding of stress resistance mechanisms in probiotics: Relevance for the design of functional food systems. *Probiotics and antimicrobial proteins*, *17*(1), 138-158. https://doi.org/10.1007/s12602-024-10273-9

[13] Chathyushya, K. B., Prakash, M. S., Kodandapani, Y., Bheema, B., Babu, J. J. G., & Hemalatha, R. (2021). Studies on the isolation and molecular characterization of Lactobacillus spp. from human breast milk and assessment of their probiotic potential. *Environmental & Experimental Biology*, *19*(4). https://doi.org/10.22364/eeb.19.20

[14] Chauhan, K., & Rao, A. (2024). Clean-label alternatives for food preservation: An emerging trend. *Heliyon*, *10*(16). https://doi.org/10.1016/j.heliyon.2024.e35815

[15] Chen, K. H., & McFeeters, R. F. (1986). Utilization of electron acceptors for anaerobic metabolism by Lactobacillus plantarum. Enzymes and intermediates in the utilization of citrate. *Food microbiology*, *3*(1), 83-92.

[16] Costa, S., Summa, D., Radice, M., Vertuani, S., Manfredini, S., & Tamburini, E. (2024). Lactic acid production by Lactobacillus casei using a sequence of seasonally available fruit wastes as sustainable carbon sources. *Frontiers in Bioengineering and Biotechnology*, *12*, 1447278. https://doi.org/10.3389/fbioe.2024.1447278

[17] Cousin, F. J., Lynch, S. M., Harris, H. M., McCann, A., Lynch, D. B., Neville, B. A., & O'Toole, P. W. (2015). Detection and genomic characterization of motility in Lactobacillus curvatus: confirmation of motility in a species outside the Lactobacillus salivarius clade. *Applied and environmental microbiology*, *81*(4), 1297-1308. https://doi.org/10.1128/aem.03594-14

[18] Dacre, J. C., & Sharpe, M. E. (1956). Catalase production by lactobacilli. *Nature*, *178*(4535), 700-700. https://doi.org/10.1038/178700a0

[19] Dahou, A. A., Doukani, K., Tahlaiti, H., Bouhenni, H., Bekihal, A., & Doubbi-Bounouaa, L. (2024). Assessment of the Functional Potential of Two Autochthonous Lactic Strains of Lactobacilli Isolated from Algerian Dairy Products. *Asian Journal of Dairy & Food Research*, *43*(4). https://doi.org/10.18805/ajdfr.drf-335

[20] Das, A. J., Das, M. J., Miyaji, T., & Deka, S. C. (2019). Growth and metabolic characterization of four lactic acid bacteria species isolated from rice beer prepared in Assam, India. Access Microbiology, 1(4), e000028. https://doi.org/10.1099/acmi.0.000028

[21] Debnath, N., Yadav, P., & Yadav, A. K. (2023). Assessment of probiotic and antioxidant potential of indigenous Lactobacillus strains isolated from human faecal samples. *Indian Journal of Microbiology*, *63*(4), 677-692. https://doi.org/10.1007/s12088-023-01129-x

[22] Dinev, T., Beev, G., Tzanova, M., Denev, S., Dermendzhieva, D., & Stoyanova, A. (2018). Antimicrobial activity of Lactobacillus plantarum against pathogenic and food spoilage microorganisms: a review. *Bulgarian Journal of Veterinary Medicine*, *21*(3). https://doi.org/10.15547/bjvm.1084

[23] Fijan, S. (2014). Microorganisms with claimed probiotic properties: an overview of recent literature. *International journal of environmental research and public health*, *11*(5), 4745-4767. https://doi.org/10.3390/ijerph110504745

[24] Gajjar, P., Patel, P., Rathod, Z., & Patel, B. (2019). 2. Isolation, characterization and identification of probiotic bacteria from fruits and vegetables by priyanka gajjar, priyanka patel, zalak rathod and baldev patel. *LIFE SCIENCES LEAFLETS*, *110*, 11-to. https://doi.org/10.1109/icoei56765.2023.10125958

[25] Georgieva, R., Yocheva, L., Tserovska, L., Zhelezova, G., Stefanova, N., Atanasova, A., & Karaivanova, E. (2015). Antimicrobial activity and antibiotic susceptibility of Lactobacillus and Bifidobacterium spp. intended for use as starter and probiotic cultures. Biotechnology & Biotechnological Equipment, 29(1), 84-91. https://doi.org/10.1080/13102818.2014.987450

[26] Gökmen, G. G., Sarıyıldız, S., Cholakov, R., Nalbantsoy, A., Baler, B., Aslan, E., & Kışla, D. (2024). A novel Lactiplantibacillus plantarum strain: Probiotic properties and optimization of the growth conditions by response surface methodology. *World Journal of Microbiology and Biotechnology*, *40*(2), 66. https://doi.org/10.1007/s11274-023-03862-3

[27] Gupta, A., & Tiwari, S. K. (2015). Probiotic potential of bacteriocin-producing Enterococcus hirae strain LD3 isolated from dosa batter. *Annals of Microbiology*, *65*(4), 2333-2342. https://doi.org/10.1007/s13213-015-1075-4

[28] Hernandez-Martinez, J. M., Gonzalez-del Rosario, I., Bolanos-Reynoso, E., & Mendez-Contreras, J. M. (2024). Anaerobic Fermentation with Lactobacillus acidophilus LA-3 for the Production of Lactic Acid and Protein-Rich Biomass from Waste of the Non-centrifugal Cane Sugar Agroindustry. *Sugar Tech*, *26*(4), 1124-1133. https://doi.org/10.1007/s12355-024-01453-6

[29] Hilmi, B., Bustami, Y., Trongsatitkul, T., & Hamid, Z. A. A. (2019). The effect of natural antimicrobial agents on Staphylococcus aureus and Escherichia coli growth. *Journal of Physical Science*, *30*, 55-63. https://doi.org/10.21315/jps2019.30.s2.5

[30] https://doi.org/10.1016/s0740-0020(86)80030-2

[31] Ibrahim, S. A., Ayivi, R. D., Zimmerman, T., Siddiqui, S. A., Altemimi, A. B., Fidan, H., & Bakhshayesh, R. V. (2021). Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. *Foods*, *10*(12), 3131. https://doi.org/10.3390/foods10123131

[32] Kamaliya, D. B., Javia, B. M., Gadhvi, M. S., & Dudhagara, D. R. (2023). Isolation and characterization of probiotic bacteria from fruit pulp, screening of probiotic properties and production of bacteriocin and probiotic curd. *Open J. Biol. Sci*, *8*, 033-042. https://doi.org/10.17352/ojbs.000036

[33] Khairani, A. F., Lantika, U. A., Ramadhanti, J., Bashari, M. H., Shalannandia, W. A., Wikayani, T. P., ... & Ritonga, M. A. (2024). Soyghurt potentially controls the level of sFlt1 and PLGF in preeclampsia maternal serum-induced placental trophoblast cell in vitro. *Journal of Experimental Pharmacology*, 111-122. https://doi.org/10.2147/jep.s446961

[34] Khushboo, K., Karnwal, A. K., & Malik, T. M. (2023). Characterization and selection of probiotic lactic acid bacteria from different dietary sources for development of functional foods. *Frontiers in Microbiology, 14*, 1170725. https://doi.org/10.3389/fmicb.2023.1170725.

[35] Kim, J. A., Bayo, J., Cha, J., Choi, Y. J., Jung, M. Y., Kim, D. H., & Kim, Y. (2019). Investigating the probiotic characteristics of four microbial strains with potential application in feed industry. *PloS one*, *14*(6), e0218922. https://doi.org/10.1371/journal.pone.0218922

[36] Kim, P. I., Jung, M. Y., Chang, Y. H., Kim, S., Kim, S. J., & Park, Y. H. (2007). Probiotic properties of Lactobacillus and Bifidobacterium strains isolated from porcine gastrointestinal tract. *Applied microbiology and biotechnology*, *74*(5), 1103-1111. https://doi.org/10.1007/s00253-006-0741-7

[37] Kowalczyk, M., Radziwill-Bienkowska, J. M., Marć, M. A., Jastrząb, R., Mytych, J., Siedlecki, P., & Szczepankowska, A. K. (2024). Screening for probiotic properties and potential immunogenic effects of lactobacilli strains isolated from various food products. *Frontiers in Microbiology, 15*, 1430582. https://doi.org/10.3389/fmicb.2024.1430582.

[38] Kristoffersen, S. M., Ravnum, S., Tourasse, N. J., Økstad, O. A., Kolstø, A. B., & Davies, W. (2007). Low concentrations of bile salts induce stress responses and reduce motility in Bacillus cereus ATCC 14570. *Journal of bacteriology*, *189*(14), 5302-5313. https://doi.org/10.1128/jb.00239-07

[39] Maftei, N. M., Raileanu, C. R., Balta, A. A., Ambrose, L., Boev, M., Marin, D. B., & Lisa, E. L. (2024). The potential impact of probiotics on human health: an update on their health-promoting properties. *Microorganisms*, *12*(2), 234. https://doi.org/10.3390/microorganisms12020234

[40] Mokoena, M. P. (2017). Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. *Molecules*, *22*(8), 1255. https://doi.org/10.3390/molecules22081255

[41] Mu, G., Gao, Y., Tuo, Y., Li, H., Zhang, Y., Qian, F., & Jiang, S. (2018). Assessing and comparing antioxidant activities of lactobacilli strains by using different chemical and cellular antioxidant methods. *Journal of dairy science*, *101*(12), 10792-10806. https://doi.org/10.3168/jds.2018-14989

[42] Neville, B. A., Forde, B. M., Claesson, M. J., Darby, T., Coghlan, A., Nally, K., & O'Toole, P. W. (2012). Characterization of pro-inflammatory flagellin proteins produced by Lactobacillus ruminis and related motile Lactobacilli. *PloS one*, *7*(7), e40592. https://doi.org/10.1371/journal.pone.0040592

[43] Özlük, G., & Krausová, G. (2025). Gastric survival of lactic acid bacteria in probiotic-labelled products from the Turkish market: An in vitro study. *Czech Journal of Food Sciences, 43*(5), 344–351. https://doi.org/10.17221/36/2025-CJFS.

[44] Palomino, M. M., Allievi, M. C., Gründling, A., Sanchez-Rivas, C., & Ruzal, S. M. (2013). Osmotic stress adaptation in Lactobacillus casei BL23 leads to structural changes in the cell wall polymer lipoteichoic acid. *Microbiology*, *159*(Pt_11), 2416-2426. https://doi.org/10.1099/mic.0.070607-0

[45] Piazentin, A. C. M., Da Silva, T. M. S., Florence-Franco, A. C., Bedani, R., Converti, A., & de Souza Oliveira, R. P. (2020). Soymilk fermentation: effect of cooling protocol on cell viability during storage and in vitro gastrointestinal stress. *Brazilian Journal of Microbiology*, *51*(4), 1645-1654. https://doi.org/10.1007/s42770-020-00369-z

[46] Qian, B., Yin, L., Yao, X., Zhong, Y., Gui, J., Lu, F., & Zhang, J. (2018). Effects of fermentation on the hemolytic activity and degradation of Camellia oleifera saponins by Lactobacillus crustorum and Bacillus subtilis. *FEMS microbiology letters*, *365*(7), fny014. https://doi.org/10.1093/femsle/fny014

[47] Sharma, C., Gulati, S., Thakur, N., Singh, B. P., Gupta, S., Kaur, S., & Panwar, H. (2017). Antibiotic sensitivity pattern of indigenous lactobacilli isolated from curd and human milk samples. *3 Biotech*, *7*(1), 53. https://doi.org/10.1007/s13205-017-0682-0

[48] Staniszewski, A., & Kordowska-Wiater, M. (2021). Probiotic and potentially probiotic yeasts. Characteristics and food application. *Foods*, *10*(6), 1306. https://doi.org/10.3390/foods10061306

[49] Stasiak-Różańska, L., Berthold-Pluta, A., Pluta, A. S., Dasiewicz, K., & Garbowska, M. (2021). Effect of simulated gastrointestinal tract conditions on survivability of probiotic bacteria present in commercial preparations. International journal of environmental research and public health, 18(3), 1108. https://doi.org/10.3390/ijerph18031108

[50] Umeh, N. M., & Achi, O. K. (2024). Bile salt hydrolase activity of cholesterol-lowering probiotic lactic acid bacteria isolated from indigenous fermented foods. *Path of Science, 10*(8).

[51] Verhelst, R., Verstraelen, H., Claeys, G., Verschraegen, G., Van Simaey, L., De Ganck, C., & Vaneechoutte, M. (2005). Comparison between Gram stain and culture for the characterization of vaginal microflora: definition of a distinct grade that resembles grade I microflora and revised categorization of grade I microflora. *BMC microbiology*, *5*(1), 61. https://doi.org/10.1186/1471-2180-5-61

[52] Yang, D., Zhang, Y., Zhao, L., Wang, Y., Rao, L., & Liao, X. (2021). Pressure-resistant acclimation of lactic acid bacteria from a natural fermentation product using high pressure. *Innovative Food Science & Emerging Technologies*, *69*, 102660. https://doi.org/10.1016/j.ifset.2021.102660

[53] Yu, T., Kong, J., Zhang, L., Gu, X., Wang, M., & Guo, T. (2019). New crosstalk between probiotics Lactobacillus plantarum and Bacillus subtilis. *Scientific Reports*, *9*(1), 13151. https://doi.org/10.1038/s41598-019-49688-8

[54] Yumnam, H., Nath, S., Chakraborty, P., & Sharma, I. (2025). Assessment of potential probiotic lactic acid bacteria in rice-based fermented products of Southern Assam, Northeast India. *Frontiers in Microbiology, 16*, 1536593. https://doi.org/10.3389/fmicb.2025.1536593.

[55] Zawistowska-Rojek, A., Zaręba, T., & Tyski, S. (2022). Microbiological testing of probiotic preparations. *International Journal of Environmental Research and Public Health*, *19*(9), 5701. https://doi.org/10.3390/molecules22081255

[56] Ziarno, M. (2007). Survival of lactic acid bacteria in simulated duodenal fluid depending on cholesterol presence. *Polish Journal of Food and Nutrition Sciences*, *57*(4C), 625-631. https://doi.org/10.1515/pjfns-2017-0027