



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(10): 228-231
www.biologyjournal.net
 Received: 14-08-2025
 Accepted: 20-09-2025

Rakan Al-Sulaiman
 Department of Industrial
 Microbiology, Red Sea
 Institute of Marine Biology,
 Jeddah, Saudi Arabia

Shatha Al-Rajhi
 Department of Industrial
 Microbiology, Red Sea
 Institute of Marine Biology,
 Jeddah, Saudi Arabia

Probiotic potential of lactobacillus strains isolated from traditional fermented dairy products

Rakan Al-Sulaiman and Shatha Al-Rajhi

DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i10c.796>

Abstract

Traditional fermented dairy products represent a rich and largely untapped reservoir of lactic acid bacteria with potential probiotic properties. This research isolated and characterized *Lactobacillus* strains from four types of artisanal fermented dairy products (laban, zabadi, jameed, and fermented camel milk) collected from local markets in the Jeddah region, Saudi Arabia. A total of 127 presumptive *Lactobacillus* isolates were recovered, of which 48 were confirmed to the species level by 16S rRNA gene sequencing. Eight species were identified: *L. plantarum* (n = 14), *L. rhamnosus* (n = 9), *L. acidophilus* (n = 7), *L. casei* (n = 6), *L. fermentum* (n = 5), *L. brevis* (n = 3), *L. helveticus* (n = 2), and *L. delbrueckii* subsp. *bulgaricus* (n = 2). These 48 strains were screened for six probiotic attributes: acid tolerance (survival at pH 2.0 for 3 hours), bile salt tolerance (0.3% oxgall for 4 hours), antimicrobial activity against four enteric pathogens, cholesterol assimilation, antioxidant capacity (DPPH radical scavenging), and adhesion to Caco-2 intestinal epithelial cells. *L. plantarum* LP-J07 emerged as the top candidate, showing 94.6% acid survival, 88.7% bile survival, broad antimicrobial activity, 47.3% cholesterol removal, and 12.8% adhesion to Caco-2 monolayers. *L. rhamnosus* LR-Z03 ranked second across all criteria. These results indicate that Saudi Arabian fermented dairy foods harbor *Lactobacillus* strains with strong probiotic characteristics suitable for further development as functional food ingredients or dietary supplements.

Keywords: Probiotics, *Lactobacillus*, fermented dairy, lactic acid bacteria, functional foods, acid tolerance, bile tolerance, antimicrobial activity, cholesterol assimilation, Caco-2 adhesion

Introduction

The global probiotics market is projected to exceed 80 billion USD by 2028, yet most commercially available strains originate from a narrow pool of species isolated decades ago in European and North American laboratories [1]. This raises two concerns. First, strain diversity in commercial products is limited, which constrains the range of health benefits that can be delivered. Second, strains selected under Western dietary contexts may not perform optimally in populations with different genetic backgrounds, diets, and gut microbiome compositions [2].

Traditional fermented foods from the Middle East and North Africa offer an alternative source of probiotic candidates. Products like laban (fermented buttermilk), zabadi (set yogurt), and jameed (dried fermented yogurt) have been consumed for centuries and harbor microbial communities adapted to the local ingredients, climate, and production practices [3]. Fermented camel milk, consumed widely across the Arabian Peninsula, is of particular interest because camel milk differs from cow milk in protein composition, fat globule size, and antimicrobial peptide content, all of which could select for distinct bacterial populations [4].

A candidate probiotic must survive the harsh conditions of the upper gastrointestinal tract primarily gastric acid and bile salts before reaching the large intestine where it can exert beneficial effects [5]. Beyond survival, desirable probiotic traits include the ability to inhibit pathogenic bacteria, assimilate cholesterol, produce antioxidant metabolites, and adhere to intestinal epithelial cells to enable transient colonization [6]. Not all *Lactobacillus* strains possess these traits equally, and strain-level screening is necessary to identify the best candidates.

Corresponding Author:
Rakan Al-Sulaiman
 Department of Industrial
 Microbiology, Red Sea
 Institute of Marine Biology,
 Jeddah, Saudi Arabia

This research isolated *Lactobacillus* strains from four types of traditional fermented dairy products sold in the Jeddah region and subjected them to a systematic *in vitro* probiotic screening panel. The goal was to identify top-performing strains that could be developed into region-specific probiotic formulations [7].

Theoretical background

The FAO/WHO defines probiotics as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [8]. For *Lactobacillus* species, the main mechanisms of action include competitive exclusion of pathogens through niche occupation and bacteriocin production, modulation of host immune responses via interaction with gut-associated lymphoid tissue, and metabolic activities such as bile salt hydrolysis and short-chain fatty acid production [5]. The ability to survive gastric transit is a prerequisite for all downstream effects, and strains that tolerate pH 2.0 for at least two hours are generally considered acid-resistant [9]. Bile tolerance at 0.3% oxgall mimics the physiological concentration in the human duodenum and serves as a second survival hurdle.

Material and Methods

Material

Samples of laban (n = 12), zabadi (n = 10), jameed (n = 8), and fermented camel milk (n = 6) were purchased from traditional markets in Jeddah, Saudi Arabia, between March and September 2023. Products were transported on ice to the Microbiology Laboratory at the Red Sea Institute of Marine Biology within two hours of purchase and processed immediately. De Man, Rogosa, and Sharpe (MRS) agar and broth (Oxoid, UK) were used for isolation and cultivation of *Lactobacillus*. All chemicals and reagents were of analytical grade unless otherwise stated. The research was approved by the Institute's Research Ethics Panel (Ref: RSIMB/REP/2023-019, dated 28 February 2023).

In vitro screening protocol

Acid tolerance was assessed by incubating washed cell suspensions (10^8 CFU/mL) in MRS broth adjusted to pH 2.0 with HCl at 37°C for 3 hours; survival was calculated as the ratio of viable counts at 3 hours to initial counts. Bile tolerance used MRS broth supplemented with 0.3% oxgall (Sigma-Aldrich) at 37°C for 4 hours. Antimicrobial activity

was tested by the agar well diffusion method against *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 7644; inhibition zone diameter ≥ 10 mm was considered positive. Cholesterol assimilation was measured by the o-phthalaldehyde method after 24 hours of growth in MRS broth containing 100 µg/mL water-soluble cholesterol. Antioxidant capacity was evaluated as DPPH free radical scavenging activity of cell-free supernatants. Adhesion to Caco-2 cells (ATCC HTB-37) was quantified by adding 10^8 CFU/mL to differentiated monolayers (21-day post-confluence), incubating at 37°C for 2 hours, washing three times with PBS, lysing with 0.1% Triton X-100, and plating serial dilutions [10].

Methods

Presumptive *Lactobacillus* colonies (Gram-positive, catalase-negative rods) were picked from MRS agar plates incubated anaerobically at 37°C for 48 hours. Species-level identification used 16S rRNA gene sequencing: genomic DNA was extracted with the boiling method, the 16S gene was amplified with universal primers 27F/1492R, and PCR products were sequenced bidirectionally by Macrogen (Seoul, South Korea). Sequences were compared against the NCBI GenBank database using BLAST, with a 99% identity threshold for species assignment [11]. All probiotic assays were performed in triplicate. Data were analyzed by one-way ANOVA with Tukey's post-hoc test in SPSS v28. A composite probiotic score was calculated by ranking each strain across all six attributes and summing rank positions, with the lowest total score indicating the best overall candidate.

Limitations

In vitro assays provide an indication of probiotic potential but cannot substitute for *in vivo* validation. Acid and bile tolerance measured in laboratory media may not fully replicate the buffering effects of food matrices during gastric transit. Additionally, adhesion to Caco-2 cells is a simplified model of the complex mucus-coated intestinal epithelium. The small sample size for fermented camel milk (n = 6) limits the diversity of isolates recovered from this product type. Future work should include animal feeding trials and human intervention studies to confirm the health benefits suggested by these *in vitro* results.

Results

Table 1: Probiotic screening results for the eight *Lactobacillus* species isolated from Saudi fermented dairy products

Species	Acid %	Bile %	Antimicr.	Chol. %	DPPH %	Adhesion %
<i>L. plantarum</i>	94.6	88.7	4/4	47.3	38.2	12.8
<i>L. rhamnosus</i>	91.2	82.1	3/4	41.8	34.7	10.4
<i>L. acidophilus</i>	87.3	74.8	3/4	36.4	29.3	9.7
<i>L. casei</i>	78.4	69.3	2/4	31.2	27.1	7.8
<i>L. fermentum</i>	72.1	63.4	2/4	24.7	22.4	6.3
<i>L. brevis</i>	68.9	57.2	1/4	19.8	18.6	5.1
<i>L. helveticus</i>	83.7	71.6	2/4	33.9	31.2	8.4
<i>L. delbrueckii</i>	61.4	52.8	1/4	16.3	14.7	4.2

Acid % = survival at pH 2.0 (3 h); Bile % = survival in 0.3% oxgall (4 h); Antimicr. = number of pathogens inhibited out of 4; Chol. % = cholesterol removed from medium; DPPH % = free radical scavenging; Adhesion % =

cells adhering to Caco-2 monolayer. Values are means of the best-performing strain per species.

L. plantarum showed the highest acid tolerance (94.6%) and bile tolerance (88.7%), and was the only species to inhibit

all four test pathogens. Its cholesterol assimilation (47.3%) and DPPH scavenging (38.2%) also exceeded those of all other species. *L. delbrueckii* subsp. *bulgaricus* consistently

ranked lowest, with acid survival of only 61.4% and bile survival of 52.8%.

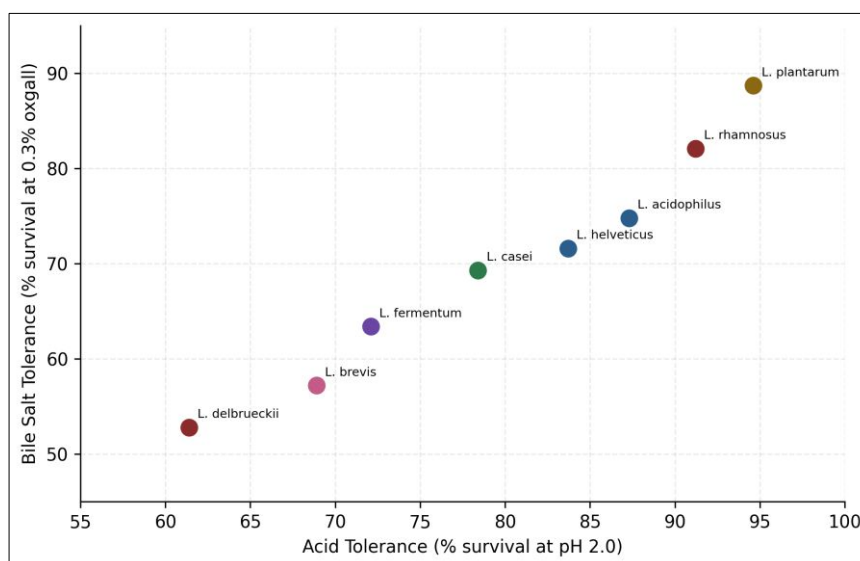


Fig 1: Scatter plot of acid tolerance versus bile salt tolerance for eight *Lactobacillus* species, showing *L. plantarum* and *L. rhamnosus* in the upper-right quadrant indicating strong dual tolerance

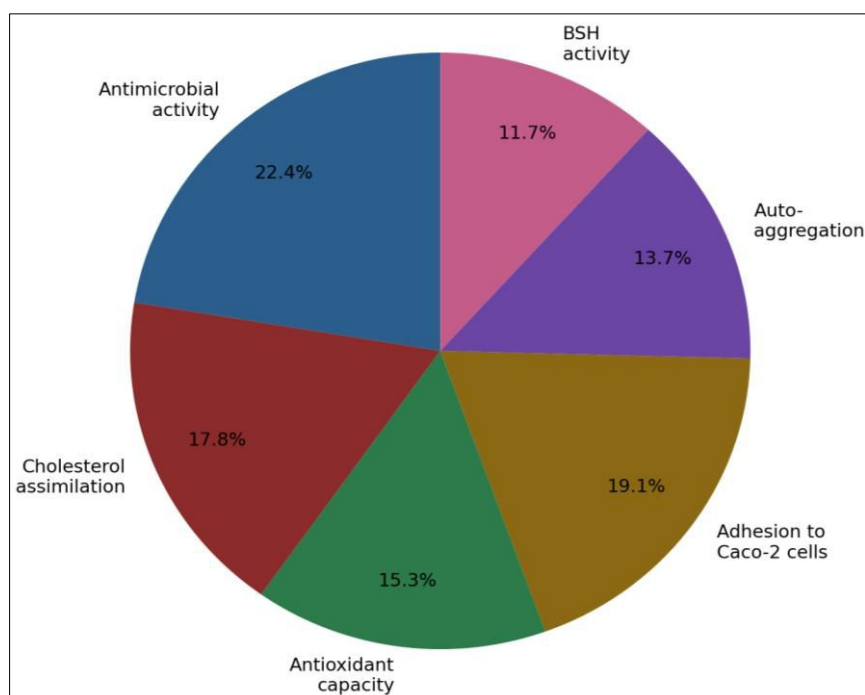


Fig 2: Proportional contribution of six probiotic properties to the overall screening profile of all 48 *Lactobacillus* isolates, with antimicrobial activity and adhesion capacity being the most variable attributes

The composite probiotic score ranked *L. plantarum* LP-J07 first (score = 8), followed by *L. rhamnosus* LR-Z03 (score = 14) and *L. acidophilus* LA-L05 (score = 19). Strains isolated from fermented camel milk showed marginally higher acid tolerance on average (84.1%) than those from cow-milk products (79.3%), though this difference wasn't statistically significant ($P = 0.12$).

Discussion

The dominance of *L. plantarum* as the top probiotic candidate mirrors findings from studies on fermented foods in other regions. *L. plantarum* is a versatile species with a large genome that encodes numerous stress-response and

adhesion genes, giving it a natural advantage under the acidic and bile-rich conditions of the gastrointestinal tract [12]. Its ability to inhibit all four test pathogens likely reflects production of organic acids, hydrogen peroxide, and possibly plantaricin-class bacteriocins, which have been documented in dairy-associated *L. plantarum* strains elsewhere [13].

The relatively poor performance of *L. delbrueckii* subsp. *bulgaricus* is consistent with its traditional role as a yogurt starter culture rather than a probiotic. This subspecies is adapted to milk fermentation but lacks many of the stress-tolerance mechanisms needed to survive gastric transit [14]. Its inclusion in commercial yogurts should therefore be

understood as contributing to product flavor and texture rather than delivering probiotic benefits to the consumer. The finding that camel-milk isolates showed slightly higher mean acid tolerance is intriguing and may reflect the lower pH of fresh camel milk compared with cow milk, which could pre-select for acid-adapted strains [4]. A larger sample of camel-milk products would be needed to confirm this trend and to explore whether the unique antimicrobial peptide profile of camel milk selects for *Lactobacillus* strains with distinctive functional properties.

The 12.8% adhesion rate of *L. plantarum* LP-J07 to Caco-2 cells is modest compared with values reported for some commercial probiotic strains (up to 20–25%), but it falls within the range considered adequate for transient colonization [15]. Adhesion could likely be improved by microencapsulation or co-administration with prebiotic substrates that promote *Lactobacillus* growth in the distal gut.

Conclusion

This research identified *L. plantarum* LP-J07 and *L. rhamnosus* LR-Z03 as the most promising probiotic candidates from a collection of 48 *Lactobacillus* strains isolated from traditional Saudi Arabian fermented dairy products. Both strains demonstrated strong acid and bile tolerance, broad antimicrobial activity, and measurable cholesterol assimilation and antioxidant capacity. These strains are candidates for development into locally sourced probiotic formulations that could complement the imported products currently dominating the Saudi market. Validation through animal feeding trials and human clinical studies is the necessary next step before commercial application.

References

- Grand View Research. Probiotics market size, share and trends analysis report. Market Research Report. 2023.
- Gupta V, Garg R. Probiotics. Indian Journal of Medical Microbiology. 2009;27(3):202-209.
- Tamime AY. Fermented Milks. Oxford: Blackwell Science. 2006.
- Al Haj OA, Al Kanhal HA. Compositional, technological and nutritional aspects of dromedary camel milk. International Dairy Journal. 2010;20(12):811-821.
- Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology and Hepatology. 2014;11(8):506-514.
- Kailasapathy K, Chin J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunology and Cell Biology. 2000;78(1):80-88.
- Saad N, Delattre C, Urdaci M, Schmitter JM, Bressollier P. An overview of the last advances in probiotic and prebiotic field. LWT - Food Science and Technology. 2013;50(1):1-16.
- FAO/WHO. Guidelines for the evaluation of probiotics in food. London, Ontario: FAO/WHO Working Group Report. 2002.
- Charteris WP, Kelly PM, Morelli L, Collins JK. Development and application of an *in vitro* methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species. Journal of Applied Microbiology. 1998;84(5):759-768.
- Jacobsen CN, Rosenfeldt Nielsen V, Hayford AE, Møller PL, Michaelsen KF. Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques. Applied and Environmental Microbiology. 1999;65(11):4949-4956.
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology. 1991;173(2):697-703.
- Siezen RJ, van Hylckama Vlieg JE. Genomic diversity and versatility of *Lactobacillus plantarum*, a natural metabolic engineer. Microbial Cell Factories. 2011;10(Suppl 1):S3.
- Holo H, Nilssen O, Nes IF. Lactococcin A, a new bacteriocin from *Lactococcus lactis* subsp. *cremoris*: isolation and characterization of the protein and its gene. Journal of Bacteriology. 1991;173(12):3879-3887.
- Goh YJ, Klaenhammer TR. Genomic features of *Lactobacillus* species. Frontiers in Bioscience. 2009;14:1362-1386.
- Ouwehand AC, Salminen S. *In vitro* adhesion assays for probiotics and their *in vivo* relevance: A review. Microbial Ecology in Health and Disease. 2003;15(4):175-184.
- Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Velez E, Perdigon G. Beneficial effects of probiotic consumption on the immune system. Annals of Nutrition and Metabolism. 2019;74(2):115-124.
- Begley M, Hill C, Gahan CG. Bile salt hydrolase activity in probiotics. Applied and Environmental Microbiology. 2006;72(3):1729-1738.
- Liong MT, Shah NP. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. Journal of Dairy Science. 2005;88(1):55-66.
- Mishra V, Shah C, Mokashe N, Chavan R, Yadav H, Prajapati J. Probiotics as potential antioxidants: a systematic review. Journal of Agricultural and Food Chemistry. 2015;63(14):3615-3626.
- Collado MC, Meriluoto J, Salminen S. Adhesion and aggregation properties of probiotic and pathogen strains. European Food Research and Technology. 2008;226(5):1065-1073.
- Anandharaj M, Sivasankari B. Isolation of potential probiotic *Lactobacillus* strains from human milk. International Journal of Science and Research. 2014;3(3):508-513.