Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

Probiotic potential of lactic acid bacteria isolated from Kazakh fermented camel milk (shubat)

Akkenzhe Omarova[1]*, Assiya Serikbayeva[2], Aigerim Tuganbay[3], Talgat Ikombayev[4], Gulmira Kassenova[5], Sarzhan Sharipova[6], Elena Kukhar[1], Botagoz Kulzhanova[7], Ak Niyet Orazbek[8]

1. S.Seifullin Kazakh Agrotechnical Research University (KATRU), Astana, Kazakhstan
2. Kazakh National Agrarian Research University, Almaty, Kazakhstan
3. University of Helsinki, Helsinki, Finland
4. Astana IT University, Astana, Kazakhstan
5. Zhetysu State University named after I. Zhansugurov, Taldykorgan, Kazakhstan
6. Asfendiyarov Kazakh National Medical University (KazNMU), Kazakhstan
7. Research and Educational Center "Qazyna" LLP, Almaty, Kazakhstan
8. Almaty Branch of the Republican State Enterprise on the Right of Economic Management "Reference Center for Veterinary Medicine", Kazakhstan

* *Corresponding Author's Email: omarovaab.1989@gmail.com*

ABSTRACT

Camel milk and its fermented derivatives have attracted increasing attention due to their nutritional value and functional potential. However, information regarding the functional properties of lactic acid bacteria (LAB) indigenous to traditional Kazakh fermented camel milk (shubat) remains limited. This study aimed to identify LAB strains isolated from camel milk using molecular approaches and to evaluate selected probiotic-associated and technological properties. Four LAB isolates were identified as belonging to *Lactiplantibacillus plantarum* and the *Lacticaseibacillus casei/paracasei* group. The strains demonstrated high acidification activity in milk and substantial lactic acid production. All isolates exhibited antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*, with strains CM 4.2 and KAN SHNI 6 showing inhibition zones exceeding 40 mm. The isolates demonstrated variable cell surface-associated properties related to adhesion potential. Auto-aggregation increased during incubation, ranging from 51.84% to 79.06% after 24 h; however, no statistically significant differences were observed among strains. Co-aggregation with *E. coli* also increased over time, reaching 59–72% after 24 h. Strain CM 2A showed the highest cell surface hydrophobicity among the tested isolates ($p < 0.05$) and exhibited strong biofilm-forming ability under the evaluated conditions. Antibiotic susceptibility analysis revealed resistance patterns consistent with previously described species-specific characteristics, including reduced susceptibility to vancomycin and kanamycin. Differences in susceptibility to other clinically relevant antibiotics, including beta-lactams, macrolides, and tetracyclines, indicated strain-dependent variability. Overall, the investigated LAB strains isolated from camel milk demonstrated promising technological and functional properties for fermented dairy applications. Further genomic characterization and *in vivo* safety evaluations are required to confirm their suitability for probiotic use.

Keywords: Camel milk, Shubat, Fermentation, LAB, Probiotics, Antimicrobial activity.
Article type: Research Article.

INTRODUCTION

Shubat, a traditional fermented camel milk beverage, is deeply rooted in Central Asian culture, particularly among Kazakh communities in Kazakhstan, Turkmenistan, and Mongolia. As a naturally fermented product, shubat not

only preserves the nutritional qualities of camel milk but may also enhance its probiotic and functional properties, making it a subject of growing interest in food science and health research (Bilal *et al.* 2024). According to recent estimates, the global camel population is approximately 35–40 million animals (Faye 2020). Within this context, Kazakhstan is home to about 282,800 camels as of January 1, 2024, accounting for nearly 0.8% of the world's total camel population. Despite this relatively small share, camel husbandry plays an important role in the agricultural systems of Central Asia, particularly in arid and semi-arid regions. Camel milk contributes on average about 3.6% to the total milk production in Kazakhstan, and its share has increased by approximately 30% since independence of Kazakhstan. Over the past two decades, the production of camel milk–based products has increased fivefold (Akhmetsadykova *et al.* 2022). In Kazakhstan, camels are valued not only for their remarkable adaptability to harsh climatic conditions but also for their milk, which is traditionally consumed as shubat, a fermented camel milk beverage with cultural and nutritional significance. Growing interest in camel milk and its fermented products is driven by their unique biochemical composition, potential health benefits, and relevance to sustainable food systems in arid and semi-arid environments (Konuspayeva & Faye 2021). Camel milk is renowned for its high nutritional value and health-related properties. It contains significant amounts of vitamins, particularly vitamin C and B-group vitamins, essential minerals, and bioactive proteins. Camel milk has a relatively lower lactose content (approximately 4.84%), which may make it more suitable for individuals with lactose intolerance. Camel milk exhibits a slightly higher protein content compared to goat milk, both within the normal range for these species. The macronutrient composition of camel milk differs significantly from that of bovine milk, as it is characterized by lower fat and lactose contents and higher levels of vitamin C and protective proteins (Yadav *et al.* 2015; Konuspayeva 2020). Compared with cow's milk, camel milk is considered easier to digest and may be better tolerated by individuals with lactose intolerance (Khwaileh 2024). The therapeutic potential of camel milk has been associated with its bioactive components. These include vitamins (such as C, A, and B_2), as well as proteins and peptides, which have been linked to various health-promoting effects, including antihypertensive, antidiabetic, antiallergic, anticarcinogenic, antioxidant, and immunomod-ulatory properties (Mirmiran *et al.* 2017). In recent years, increasing scientific attention has been directed toward the technological applications and probiotic potential of LAB present in traditional Asian fermented milk products (Ismael *et al.* 2022). Numerous lactic acid bacteria, which are among the most used probiotic microorganisms, have been isolated from milk and assessed for safety. Victor *et al.* (2011) assessed the safety, cholesterol-lowering properties, and antimicrobial activity of 107 lactobacilli isolated from raw milk in the Western Highlands of Cameroon. Fifteen isolates were selected for bile and acid tolerance, and all showed the ability to assimilate cholesterol *in vitro* and bile salt hydrolase activity (Victor *et al.* 2011). Recent metagenomic studies have revealed that shubat hosts a complex microbial consortium predominantly composed of bacteria (≈96.6%), including members of the genera *Lactobacillus*, *Lactococcus*, and *Streptococcus*, along with yeasts and bacteriophages (Zhadyra *et al.* 2025). These microorganisms collectively contribute to amino acid and carbohydrate metabolism, vitamin biosynthesis, and the development of characteristic texture and flavor through various enzymatic activities. While traditional culture-based methods previously identified dominant LAB species, metagenomic approaches have uncovered additional low-abundance taxa, such as *Geotrichum candidum*, as well as phage populations that were not detected earlier. The lactic acid bacteria present in shubat are of particular interest, since some strains have been reported to exhibit probiotic-associated properties, including the ability to survive gastrointestinal conditions, adhere to intestinal mucosa, and exert antimicrobial, immunomodulatory, and anti-inflammatory effects. Studies on the composition and diversity of LAB in traditional dairy products have provided valuable data and strain resources that can be used for further probiotic strain selection and starter culture design (Wang *et al.* 2016). Recent studies have reported the isolation of several LAB strains from shubat with high tolerance to acidic environments and bile salts, strong antagonistic activity against pathogenic microorganisms, and the capacity to produce beneficial metabolites such as bacteriocins and exopolysaccharides. These probiotic cultures contribute not only to the safety and shelf life of shubat but also to its functional value by supporting gut health and enhancing host immunity (Akhmetsadykova *et al.* 2015). Understanding the probiotic-associated characteristics of LAB in shubat could facilitate the development of novel fermented dairy products with enhanced functional properties. Furthermore, characterization of these indigenous microbial communities is essential for optimizing and standardizing fermentation processes, thereby ensuring consistent product quality in both traditional and industrial production systems. Therefore, the present study aimed to isolate and molecularly-

identified lactic acid bacteria from traditionally produced shubat in Kazakhstan and to evaluate their probiotic potential and techno-functional characteristics for potential application in functional food production.

MATERIALS AND METHODS

Sample Collection and Microbiological Enumeration

Raw camel milk samples were collected from local farms in two regions of Kazakhstan: Almaty (southern region) and Astana (central region). The samples were aseptically collected in sterile containers and transported to the laboratory in insulated boxes with ice within 6 h after collection. For the isolation of LAB, a 5-mL aliquot of each sample was subjected to spontaneous fermentation at 37 °C for 24 h. Subsequently, serial 10-fold dilutions were prepared in sterile saline solution (8.5 g L^{-1} NaCl supplemented with 1 g L^{-1} peptone, pH 7.0). Enumeration and isolation of LAB were carried out using the pour-plate method. Total LAB counts were determined on MRS agar (Merck, USA) and M17 agar for the isolation of *Lactococci*. MRS agar supplemented with vancomycin (20 mg L^{-1}), bile esculin agar, and kanamycin-esculin agar were used to select vancomycin-resistant strains and Enterococci. The plates were incubated at 37 °C for 48–72 h under 5% CO_2 conditions. Following incubation, representative colonies were randomly selected and purified by subculturing in MRS broth for 24 h at 37 °C. Preliminary identification of the isolates was performed based on Gram staining, catalase activity and cell morphology. Cell morphology was examined using an Olympus DP74 microscope camera (Olympus Corporation, Japan). Only Gram-positive, catalase-negative, rod and cocci-shaped isolates were preserved in 20% (v/v) glycerol at −80 °C for further analysis.

Isolation and identification of LAB strains

Identification of LAB isolates using MALDI-TOF MS and API 50CHL

Identification of the LAB isolates was performed using a polyphasic approach. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) analysis was carried out using a Microflex LT mass spectrometer (Bruker Daltonics, Bremen, Germany). Fresh overnight pure cultures grown on MRS agar were prepared using the standard ethanol–formic acid extraction method according to the manufacturer's instructions. Mass spectra were acquired in linear positive mode within a mass range of 2–20 kDa. Species identification was performed using the Biotyper software (version 3.1). Identification scores ≥ 2.0 were considered reliable for species-level identification, while scores between 1.7 and 1.99 indicated genus-level identification. The carbohydrate fermentation profile of the isolates was further evaluated using the API 50 CHL system (bioMérieux, Marcy-l'Étoile, France). Bacterial suspensions were prepared in API 50 CHL medium adjusted to a turbidity of 2.0 McFarland. The strips were incubated at 37 °C under anaerobic conditions (sealed with sterile mineral oil), and the results were recorded after 24 and 48 h. Fermentation patterns were interpreted using the APIweb database.

DNA extraction and PCR conditions for molecular identification

Genomic DNA was extracted from pure cultures using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according the manufacturer's instructions for Gram-positive bacteria. The 16S rRNA gene was amplified by PCR using the universal primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). The PCR thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplicons were verified by electrophoresis on a 1.5% (w/v) agarose gel stained with ethidium bromide. Sequencing of the PCR products was performed at the Institute of Microbiology of the Czech Academy of Sciences (Prague, Czech Republic). The resulting sequences were trimmed and analyzed using the BLASTn tool against the NCBI database. Phylogenetic analysis was performed using MEGA 11.0 software. The evolutionary distances were calculated using the Neighbor-Joining method with 1,000 bootstrap replicates to assess tree reliability.

Determination of probiotic properties of selected LAB strains

Growth kinetics and acidification capacity in milk

The growth kinetics and acidification capacity of the LAB strains were evaluated using UHT-treated skimmed milk (0.3% fat; Pragolaktos, Prague) as a model medium. Glass tubes containing 5 mL milk were inoculated with 5% (v/v) active overnight culture and incubated at 37 °C for 24 h. Bacterial growth was monitored by viable cell counts (CFU mL^{-1}) and pH measurements at 0, 6, and 24 h of incubation. For microbial enumeration, serial tenfold

dilutions were prepared in sterile saline–peptone solution (8.5 g L^{-1} NaCl, 1 g L^{-1} peptone, pH 7.0). Aliquots of 0.1 mL were plated onto MRS agar (Merck, USA). The plates were incubated at 37 °C for 48 h under a 5% CO_2 atmosphere. The pH was measured using a calibrated digital pH meter pH-150MI. All experiments were performed in triplicate for statistical analysis.

Bile salt tolerance assay

Bile salt tolerance was evaluated in microplates using a Gen5 Microplate Reader (BioTek Instruments, Inc., USA). MRS supplemented with bile salts at concentrations of 0% (control), 0.3%, 0.5%, and 1.0% (w/v) was sterilized and then inoculated with 2% (v/v) overnight cultures. The 96-well microplates were incubated at 37 °C with shaking every 15 min for 48 h. Bacterial growth was monitored by measuring the optical density at 600 nm (OD600), with measurements recorded hourly. All experiments were performed in triplicate.

Determination of organic acids

Determination of organic acids (lactic, acetic, citric and succinic acids) produced by isolated LAB were determined by high-performance liquid chromatography (HPLC). Aliquots of UHT skimmed milk (40 mL; 0.3% fat; Pragolaktos, Prague) were inoculated with 5% (v/v) active LAB culture and incubated at 37 °C for 24 h.
Sample preparation was performed according to a modified deproteinization protocol. Briefly, 140 mg of the fermented milk was weighed into sterile microcentrifuge tubes and mixed with 1 mL of 96% ethanol (v/v). The mixture was vortexed thoroughly and incubated at room temperature for 30 min to ensure complete protein precipitation. The samples were then centrifuged at 12 000 × g for 10 min at 22 °C. The resulting cell-free supernatant was filtered through a 0.22 μm membrane filter (Millipore, USA) and transferred into HPLC vials for analysis.

Antimicrobial properties

The isolates of LAB were cultured in de Man, Rogosa and Sharpe (MRS) broth (Merck, USA) and incubated at 37 °C for 24 h. Indicator pathogens, including *Staphylococcus aureus* (*S. aureus*) *CCM 4516, Escherichia coli (E. coli) CCM 4517, Bacillus cereus* (*B. cereus*) *1410A* and *Pseudomonas aeruginosa* (*P. aeruginosa*) *CCM 1961*, were obtained from the Culture Collection of Microorganisms, Department of Dairy, Fat and Cosmetic Science, University of Chemistry and Technology, Prague. The strains were cultured in Brain Heart Infusion (BHI) broth and incubated at 37 °C for 18 h. Antagonistic activity was evaluated using 10 mL of BHI soft agar inoculated with 100 μL of pathogen suspension, to obtain a final concentration of approximately 10^4–10^5 CFU mL^{-1} (pH 6.5). After solidification of the agar, 5 μL of fresh LAB cultures were spotted onto the surface of the agar plates. The plates were incubated at 37 °C under 5% CO_2 for 24 h. Antimicrobial activity was quantified by measuring the diameter of the inhibition zones (mm) surrounding the LAB spots using a digital caliper. All assays were performed in triplicate.

Antibiotic resistance profile

The antibiotic susceptibility of the LAB isolates was determined using a combination method, including the disk diffusion method, the E-test (gradient strips), and the broth microdilution method (Microlatest MIC G+). In the disk diffusion assay, 100 μL of bacterial suspension adjusted to 1 McFarland was spread onto MRS agar supplemented with cysteine and allowed to dry. Subsequently, antibiotic-impregnated disks were placed on the agar surface. Fourteen antibiotic disks (Oxoid, UK) were tested including: ampicillin (10 μg), clindamycin (2 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), chloramphenicol (30 μg), kanamycin (30 μg), linezolid (10 μg), quinupristin (15 μg), rifampicin (5 μg), streptomycin (10 μg), tetracycline (30 μg), trimethoprim (5 μg), and vancomycin (5 μg and 30 μg). The E-test was performed using a plastic strip with a predefined antibiotic gradient to determine the minimum inhibitory concentration (MIC) for one antibiotic per plate. A 100-μL strain suspension was distributed on MRS agar, and the E-test strip was placed before incubation. Resistance rates for nine antibiotics (gentamycin, streptomycin, chloramphenicol, tetracycline, erythromycin, clindamycin, ampicillin, vancomycin, kanamycin) were calculated according to European Food Safety Authority (EFSA) standards. For MIC G+ testing, LAB strain suspensions were added to wells in a labeled plate. After 24 hours of incubation at 37 °C, wells were analyzed using natural dispersed light. Testing of 12 agents showed varying MIC ranges: gentamycin (0.25-128 mg L^{-1}), ampicillin (0.25-16 mg L^{-1}), erythromycin (0.06-8 mg L^{-1}), clindamycin (0.12-16 mg L^{-1}), tetracycline (0.25-12 mg L^{-1}), vancomycin (0.12-16 mg L^{-1}), and chloramphenicol (0.25-32 mg L^{-1}).

The cell surface hydrophobicity (CSH)

The Microbial Adhesion to Hydrocarbons (MATH) assay was performed according to the method described by Zoueki *et al.* (2010) with minor modifications to evaluate the cell surface hydrophobicity of the LAB isolates. LAB cultures were grown in MRS broth at 37 °C under 5% CO_2 for 18 h. The cells were harvested by centrifugation at 6000 × g at 4 °C for 10 min and washed twice with phosphate-buffered saline (PBS). The optical density of bacteria was adjusted as A600 nm = 0.5 ± 0.05. A 3-mL bacterial suspension was mixed with 1 mL xylene, n-hexane and hexadecane (Sigma-Aldrich, USA). The two-phase system was thoroughly mixed by vortexing for 30 seconds. The two phases were separated for 1 h at 37 °C, 1 mL of the aqueous phase was carefully withdrawn and measured at A600 nm. The decrease in absorbance of the aqueous phase was considered as a measure of cell surface hydrophobicity (H%), which was calculated using the equation:

$$\mathrm{H\%} = \left[\frac{\mathrm{A0} - \mathrm{A}}{\mathrm{A0}}\right] \times 100$$

where A_0 is the absorbance of the bacterial suspension before extraction and A is the absorbance after extraction with xylene, n-hexane, or hexadecane. All experiments were performed in triplicate to ensure reproducibility.

Auto-aggregation assay

The auto-aggregation ability of the LAB isolates was determined according to the method described by Zawistowska-Rojek *et al.* with minor modifications. The assay was performed as previously described (Zawistowska-Rojek *et al.* 2022), with adaptations to suit the experimental conditions. LAB isolates were cultured in MRS broth for 18 hours at 37 °C. The bacterial cells were collected through centrifugation at 6000 × g and 4 °C for 10 minutes, followed by two washes with PBS before being resuspended in PBS buffer. The optical density of the bacterial suspension was adjusted to A600 nm = 0.5 ± 0.05. A 20-mL sample of this suspension was shaken for 30 seconds and then allowed to rest without agitation. Optical density measurements were taken by carefully withdrawing 1 mL from the supernatant of the suspension at different time points (0, 6, and 24 hours). The percentage of auto-aggregation was calculated using the formula:

$$\mathrm{Auto-aggregation(\%)} = (1 - At/A0) \times 100,$$

where A_t is the absorbance at the indicated time points of 6 and 24 hours, and A_0 is the absorbance at time t = 0 hours. The auto-aggregation percentage for the isolates was determined after 6 and 24 hours of incubation.

Biofilm formation assay

Biofilm formation was evaluated using a modified method from Shokri *et al.* (2018). LAB strains were cultured in MRS broth for 24 h. Subsequently, 200 µL of a 0.5 McFarland bacterial suspension was added to the wells of sterile microtiter plates. One plate was incubated for 24 h, and a duplicate plate was incubated for 48 h at 37 °C without shaking. After incubation, the medium was removed, and the wells were washed three times with 200 µL PBS, pH 7.2, to remove planktonic cells. Plates were air-dried for 30 min and then stained with 200 µL of 0.1% crystal violet solution for 30 min. Following staining, the wells were washed and dried again for 30 min.

For quantitative analysis, 200 µL of destaining solution (96% ethanol, 80:20 acetone) was added to each well. Subsequently, 100 µL of the destained solution was transferred to a new plate, and optical density (OD) was measured at 590 nm using a microtiter plate reader. The average OD from control wells was subtracted from the test wells, and the assay was repeated three times for both strains.

Co-aggregation assay

The co-aggregation assay was performed using a modified auto-aggregation method as previously described (Barzegar *et al.* 2023). LAB and *E. coli* CCM 4517 suspensions were harvested by centrifugation at 6000 × g for 10 min at 4 °C, washed twice with PBS, and resuspended in PBS to an optical density of A600 nm = 0.5 ± 0.05. Equal volumes of the lactobacilli and *E. coli* suspensions (20 mL each) were mixed by vortexing for 10 s and incubated at room temperature without agitation. At 0, 6, and 24 h, 0.5 mL of the upper suspension layer was carefully collected, and optical density was measured at 600 nm.

The percentage of co-aggregation was calculated using the following formula:

$$\mathrm{Co-aggregation(\%)} = [(Apat + Alacto)/2 - Amix]/(Apat + Alacto)/2 \times 100$$

where A_{pat} and A_{lacto} are the OD values of the separate bacterial suspensions, and A_{mix} is the OD of the mixed suspension after 6 and 24 hours.

Statistical analysis

The statistical analysis was carried out using SPSS software [Version 31.0.2.0 (126), IBM, NYC, United States]. The data were analyzed using ANOVA of variance under the General Linear Model procedure and were then reported as mean ± standard deviations. Tukey's test was used to compare the means, and differences were considered as significant based on a $p < 0.05$, with higher levels of significance ($p < 0.001$) noted where applicable based on F-test results.

RESULTS

Comparative analysis of microbial loads in raw and fermented camel milk

The microbial counts of raw and spontaneously fermented camel milk samples are presented in Table 1 and Fig. 1. No statistically significant differences in microbial counts on MRS, MRS supplemented with vancomycin, or M17 media were observed between raw and fermented camel milk samples from the Almaty region ($p > 0.05$). On MRS agar, viable counts changed from 10.18 ± 0.10 to 10.13 ± 0.18 log CFU mL^{-1}, while on M17 agar they changed from 10.01 ± 0.27 to 9.78 ± 0.03 log CFU mL^{-1}.

Table 1. The number of microorganisms in fresh camel milk and after spontaneous fermentation on various nutrient media.

Samples	MRS+ VAN	Bile esc	MRS	M17	Kan + esculin
Camel milk, Almaty (CMA)	10.34± 0.88^{a}	8.57 ± 0.44^{b}	10.18 ± 0.10^{a}	10.01 ± 0.27^{a}	9.60 ± 0.42^{a}
Camel milk, Astana (CMAs)	10.12 ± 0.16^{a}	7.64 ± 0.19^{c}	10.26 ± 0.08^{a}	9.94 ± 0.05^{a}	6.26 ± 0.24^{c}
Fermented camel milk, Almaty (FCMA)	10.07 ± 0.11^{a}	8.31 ± 0.03^{b}	10.13 ± 0.18^{a}	9.78 ± 0.03^{a}	9.31 ± 0.05^{a}
Fermented camel milk, Astana (FCMAs)	10.10 ± 0.13^{a}	8.68 ± 0.03^{a}	10.11 ± 0.14^{a}	NG^{b}	5.30 ± 0.30^{d}

Note: NG – no detectable growth. Values are expressed as mean ± standard deviation (SD; n = 3). Different superscript letters within the same column indicate significant differences among samples according to Tukey's HSD test ($p < 0.05$). Two-way repeated-measures ANOVA revealed significant effects of media, samples, and their interaction ($p < 0.001$).

In samples collected from the Astana region, microbial counts on bile esculin agar increased from 7.64 ± 0.19 to 8.68 ± 0.03 log CFU mL^{-1} after spontaneous fermentation. In contrast, no detectable growth was observed on M17 agar in fermented camel milk from Astana (FCMAs). Counts on kanamycin-esculin agar were higher in samples from the Almaty region (9.60 ± 0.42 and 9.31 ± 0.05 log CFU mL^{-1}) than in samples from the Astana region (6.26 ± 0.24 and 5.30 ± 0.30 log CFU mL^{-1}). Two-way repeated-measures ANOVA revealed significant effects of sample type, culture medium, and their interaction on microbial counts ($p < 0.001$).

Isolation and identification of LAB strains in samples

Identification of LAB isolates using MALDI-TOF MS, API 50 CHL and 16S rRNA gene sequencing

The identification of LAB isolates was performed using a polyphasic taxonomic approach, combining matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), carbohydrate fermentation profiling (API 50 CHL), and partial 16S rRNA gene sequencing. According to MALDI-TOF MS analysis, strains CM 2A and CM 4.1 were assigned to *Lactiplantibacillus plantarum*, whereas CM 4.2 and KAN SHNI 6 were assigned to *Lacticaseibacillus paracasei*. API 50 CHL analysis showed similar carbohydrate utilization patterns among the isolates (Table 3). API 50 CHL analysis revealed similar carbohydrate fermentation profiles among the isolates. All strains fermented glucose, fructose, mannose, maltose, lactose, and sucrose, whereas variability was observed for dulcitol, turanose, melibiose, inulin, gentiobiose, and D-tagatose. Partial 16S rRNA gene sequencing assigned strain CM 2A to *L. plantarum*, CM 4.1 to *L. paracasei*, CM 4.2 to *L. casei*, and KAN SHNI 6 to *L. plantarum* (Table 4). The obtained partial 16S rRNA gene sequences were deposited in the GenBank database under accession numbers PZ065656 (CM 2A), PZ065657 (CM 4.1), PZ065658 (CM 4.2), and PZ065659 (KAN SHNI 6).

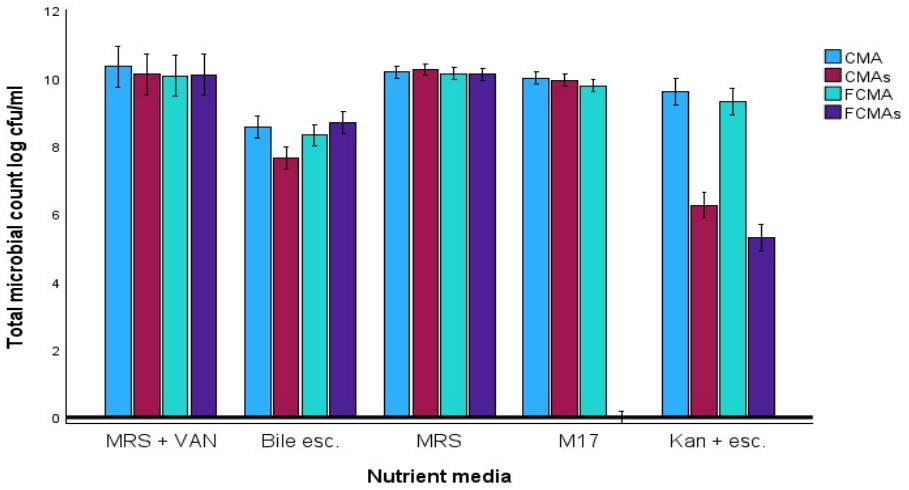

Fig. 1. Total microbial counts (log CFU mL^{-1}) of CMA, CMAs, FCMA, and FCMAs grown on different nutrient media (MRS + vancomycin, bile esculin agar, MRS, M17, and kanamycin–esculin agar). Values represent mean ± standard deviation (SD; n = 3). Significant effects of media type, sample, and their interaction were detected by two-way repeated-measures ANOVA ($p < 0.001$). No detectable growth was observed for FCMAs on M17 medium.

Table 2. Classification results of Bruker Daltonik MALDI Biotyper.

Strain	Best Match	Score Range	Mean ± SD
CM 2A	*Lactiplantibacillus plantarum*	2.24–2.35	2.30 ± 0.04
CM 4.1	*Lactiplantibacillus plantarum*	2.18–2.32	2.27 ± 0.05
CM 4.2	*Lacticaseibacillus paracasei*	2.43–2.46	2.45 ± 0.01
KAN SHNI 6	*Lacticaseibacillus paracasei*	2.28–2.43	2.36 ± 0.06

Table 3. Comparative results of carbohydrates fermentation of strains.

Carbohydrates	Test	CM 2A	CM 4.1	CM 4.2	KAN SHNI 6
L-arabinose	LARA	±	-	±	-
D-Ribose	RIB	+	+	+	+
D-Galactose	GAL	+	+	+	+
D-Glucose	GLU	+	+	+	+
D-Fructose	FRU	+	+	+	+
D-Mannose	MNE	+	+	+	+
Dulcitol	DUL	-	-	-	+
L-sorbose	SBE	+	-	-	-
Cellobiose	CEL	+	+	+	±
L-rhamnose	RHA	±	-	-	-
D-mannitol	MAN	+	+	+	+
D-sorbitol	SOR	+	+	+	+
N-acetylglucosamine	NAG	+	+	+	+
Amygdalin	AMY	+	+	-	±
Arbutin	ARB	+	+	+	±
Salicin	SAL	+	+	+	±
Melezitose	MLZ	+	+	+	+
Amidon (starch)	AMD	-	±	-	-
D-Turanose	TUR	-	+	+	+
Maltose	MAL	+	+	+	+
Lactose	LAC	+	+	+	+
Melibiose	MEL	+	+	-	-
Saccharose	SAC	+	+	+	+
Inulin	INU	-	±	-	+

Methyl-D-Glucopyranoside	MDG	-	-	±	-
Gentiobiose	GEN	+	+	±	-
D-trehalose	TRE	+	+	+	+
D-tagatose	TAG	+	±	±	+
Potassium gluconate	GNT	+	±	±	±
D-xylose	DXYL	-	-	-	-
Identification results		*Lb.rhamnosus*	*Lb.plantarum*	*Lb.paracasei*	*Lb.paracasei*

Note: '+' = Positive; '–' = Negative; '±' = Variable.

Table 4. Identification of LAB isolates using MALDI-TOF MS, API 50 CHL and 16S rRNA gene sequencing.

Strain	Origin	API 50 CHL	MALDI-TOF MS	16S rRNA Sequencing
CM 2A	Camel milk (Astana)	*L. rhamnosus* (96.1%)	*L. plantarum*	*L. plantarum*
CM 4.1	Camel milk (Almaty)	*L. plantarum* (98.9%)	*L. plantarum*	*L. paracasei*
CM 4.2	Camel milk (Almaty)	*L. paracasei* (79.9%)	*L. paracasei*	*L. casei*
KAN SHNI 6	Camel milk (Astana)	*L. paracasei* (96.4%)	*L. paracasei*	*L. plantarum*

The phylogenetic relationships among the isolates based on partial 16S rRNA gene sequences are shown in Fig. 2. Strains CM 2A and KAN SHNI 6 clustered with *L. plantarum* reference sequences, whereas CM 4.1 clustered with the *L. paracasei* lineage. Strain CM 4.2 clustered between *L. casei* and *L. paracasei* reference sequences.

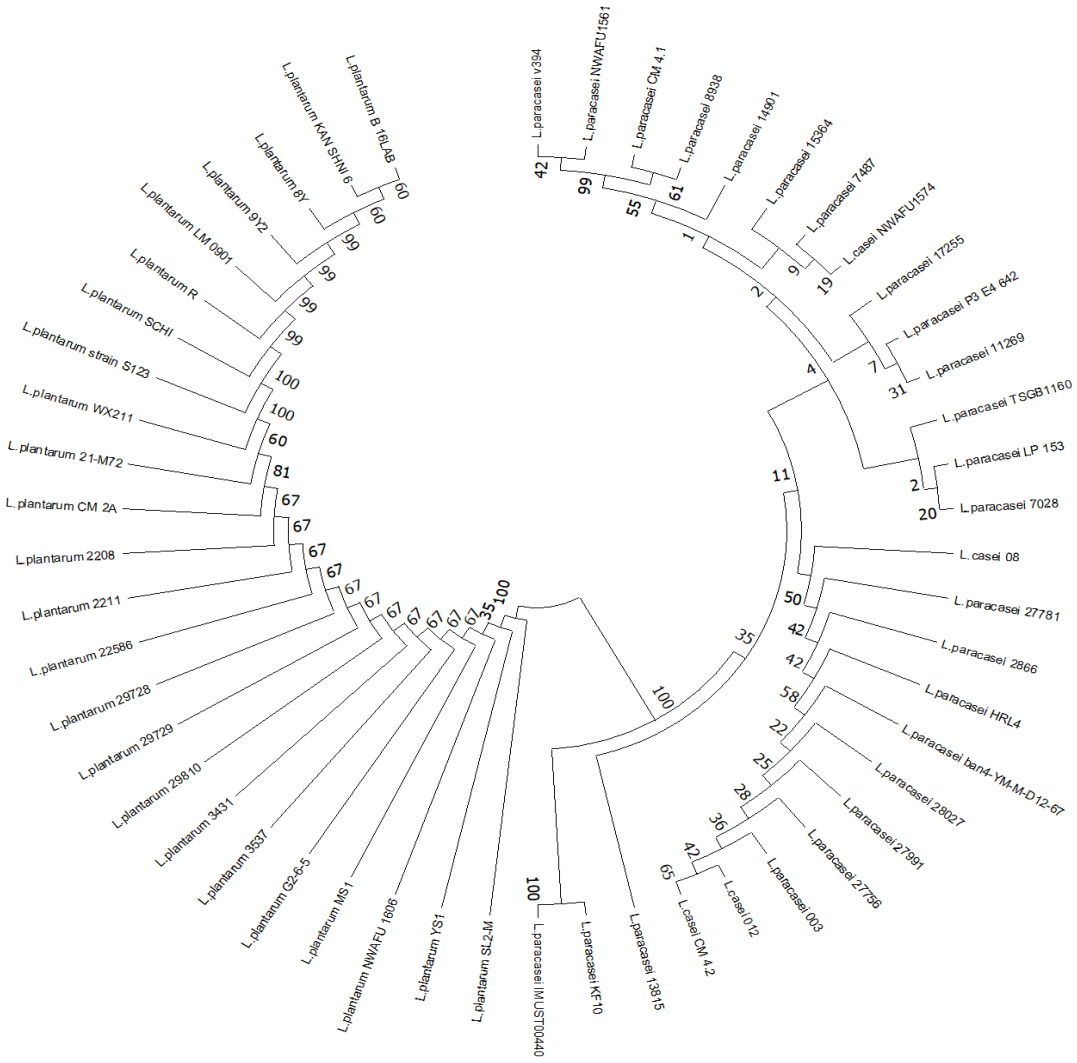

Fig. 2. Phylogenetic dendrograms of the strains based on 16S rRNA gene sequencing.

Determination of probiotic properties of selected LAB strains

Capacity for growth in milk

The growth performance and acidification activity of the selected LAB strains during incubation in skimmed UHT-treated milk at 37 °C for 24 h are presented in Table 5 and Figs. 3-4. All tested strains were able to grow in

milk and caused a decrease in pH after 24 h of incubation. A significant effect of incubation time was observed for both pH values and viable cell counts ($p < 0.001$). Significant differences among strains were detected for pH ($p = 0.008$) and viable cell counts ($p < 0.001$). The interaction between incubation time and strain was not significant for pH or viable cell counts ($p > 0.05$).

Table 5. pH levels and viable cell counts of LAB strains cultivated in skimmed UHT-treated milk at 37 °C.

Variable	Time	CM 2A	CM 4.1	CM 4.2	KAN SHNI 6
pH	0	6.36 ± 0.15^{a}	6.40 ± 0.20^{a}	6.30 ± 0.10^{a}	6.40 ± 0.40^{a}
	24	4.81 ± 0.29^{b}	4.13 ± 0.06^{a}	4.00 ± 0.40^{a}	4.90 ± 0.20^{b}
Log CFU mL^{-1}	0	8.09 ± 0.06^{a}	7.77 ± 0.41^{a}	8.534 ± 0.12^{a}	8.55 ± 0.61^{a}
	24	10.11 ± 0.05^{b}	9.14 ± 0.09^{a}	10.43 ± 0.02^{c}	10.23 ± 0.01^{bc}

The initial pH values of milk samples ranged from 6.30 to 6.40. After 24 h of fermentation, pH values decreased to 4.00–4.90 depending on the strain. The lowest final pH values were observed for CM 4.1 and CM 4.2 (4.13 and 4.00, respectively), whereas CM 2A and KAN SHNI 6 showed final pH values of 4.81 and 4.90, respectively.
The viable cell counts increased for all strains after 24 h incubation. Initial counts ranged from 7.77 to 8.55 log CFU mL^{-1}, while final counts reached 9.14–10.43 log CFU mL^{-1}. The highest viable cell count after fermentation was observed for CM 4.2 (10.43 ± 0.02 log CFU mL^{-1}), followed by KAN SHNI 6 (10.23 ± 0.01 log CFU mL^{-1}) and CM 2A (10.11 ± 0.05 log CFU mL^{-1}).

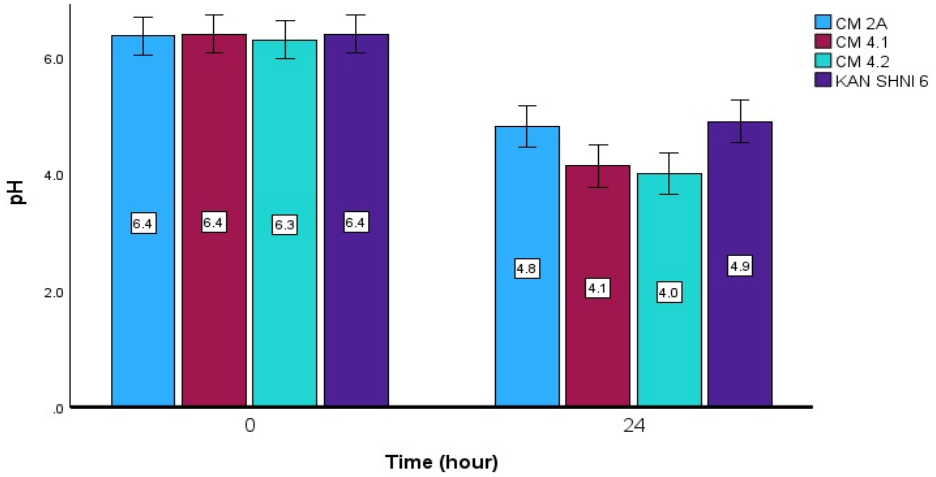

Fig. 3. Changes in pH values of milk fermented with LAB strains CM 2A, CM 4.1, CM 4.2, and KAN SHNI 6 during incubation. Values represent mean ± SD (n = 3). Statistical analysis was performed using One-Way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Bile tolerance

The growth of four LAB strains was evaluated in MRS broth containing 0.0%, 0.3%, 0.5%, and 1.0% bile salts. Growth was assessed by measuring optical density (OD600) at 0 h and after 24 h of incubation (Table 6).
At the initial time point (0 h), all strains exhibited similar optical density values across the tested bile concentrations, ranging from approximately 0.186 to 0.321. These values reflect the baseline cell density prior to incubation, indicating that the starting bacterial populations were relatively comparable among treatments. After 24 h incubation, a marked increase in OD values was observed in most strains, indicating active growth. Strains CM 2A, CM 4.1, and KAN SHNI 6 in plain MRS (0.0% bile) demonstrated strong growth, reaching OD values of 1.696, 1.711 and 1.698 respectively. Strain CM 4.2 exhibited a lower OD value (1.092) and increased sensitivity to bile salts. At 0.3% bile concentration, its OD decreased drastically to 0.177, and only slight increases were observed at 0.5% and 1.0% bile (0.243 and 0.358, respectively). Post hoc comparisons using Tukey's HSD test further confirmed that strain CM 4.2 exhibited significantly lower growth than other strains. Meanwhile, strains CM 2A, CM 4.1 and KAN SHNI 6 did not differ significantly from each other, suggesting similar bile tolerance capabilities (Table 6). Growth of lactic acid bacteria (LAB) strains in MRS broth containing different bile concentrations (0.0%, 0.3%, 0.5%, and 1.0%) measured as optical density at 600 nm (OD600) at 0 h and 24 h. Values represent mean ± standard deviation (n = 3). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$).

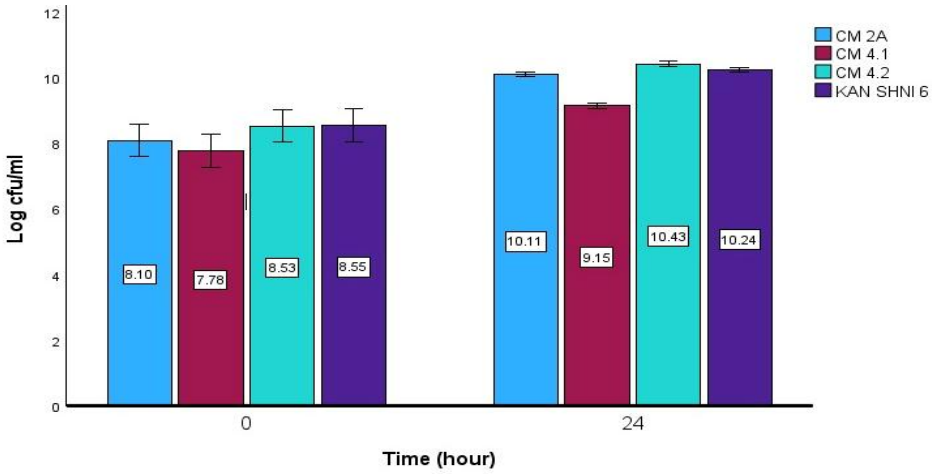

Fig. 4. Changes in viable cell counts (log CFU mL^{-1}) of LAB strains during incubation in skimmed UHT milk. Values represent mean ± SD (n = 3). Statistical differences were evaluated using One-Way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Table 6. Growth (OD600) of LAB strains at 0 h and 24 h under different bile concentrations.

Time	Strain	Bile concentration (%)			
		0.0	**0.3**	**0.5**	**1.0**
0 h	CM 2A	0.191 ± 0.008[a]	0.200 ± 0.00[a]	0.241 ± 0.011[a]	0.292 ± 0.017[a]
	CM 4.1	0.195 ± 0.007[a]	0.202 ± 0.002[a]	0.237 ± 0.003[a]	0.284 ± 0.006[a]
	CM 4.2	0.186 ± 0.013[a]	0.264 ± 0.026[a]	0.284 ± 0.059[a]	0.321 ± 0.006[a]
	KAN SHNI 6	0.210 ± 0.014[a]	0.215 ± 0.003[a]	0.284 ± 0.009[a]	0.283 ± 0.011[a]
24 h	CM 2A	1.696 ± 0.020[a]	1.217 ± 0.019[b]	1.298 ± 0.041[b]	1.319 ± 0.119[b]
	CM 4.1	1.711 ± 0.013[a]	1.240 ± 0.033[b]	1.245 ± 0.010[b]	1.193 ± 0.057[b]
	CM 4.2	1.092 ± 0.041[c]	0.177 ± 0.009[d]	0.243 ± 0.071[d]	0.358 ± 0.025[c]
	KAN SHNI 6	1.698 ± 0.046[a]	1.290 ± 0.013[b]	1.320 ± 0.054[b]	1.241 ± 0.047[b]

Determination of organic acids

The results of the HPLC analysis of camel milk samples fermented with strains CM 2A, CM 4.1, CM 4.2, and KAN SHNI 6 are presented in Table 7. In general, lactic acid was found at substantially higher concentrations compared to citric, succinic, and acetic acids ($p < 0.05$).

Strain CM 2A produced 6.58–6.87 mg g^{-1} lactic acid, whereas citric and succinic acids were much lower. Similarly, strain KAN SHNI 6 exhibited lactic acid concentrations of 4.86–5.62 mg g^{-1}, with relatively low citric and succinic acids. Strains CM 4.1 and CM 4.2 showed the same trend, with lactic acid predominating over the other organic acids measured. Acetic acid levels remained comparatively low across all samples, ranging from 0.31 to 0.53 mg g^{-1}.

Table 7. Organic acid concentrations across LAB strains.

Strains	Citric acid	Succinic acid	Lactic acid	Acetic acid
CM 2A	0.054 ± 0.012[a]	0.036 ± 0.001[a]	6.708 ± 0.348[a]	0.358 ± 0.012[c]
CM 4.1	0.060 ± 0.012[a]	0.025 ± 0.001[b]	4.545 ± 0.348[c]	0.419 ± 0.012[b]
CM 4.2	0.000 ± 0.012[c]	0.012 ± 0.001[c]	5.095 ± 0.348[b]	0.528 ± 0.012[a]
KAN SHNI 6	0.030 ± 0.012[b]	0.012 ± 0.001[c]	5.273 ± 0.348[b]	0.325 ± 0.012[c]

Values are expressed as mean ± standard deviation, means of triplicate experiments. Different superscript letters within the same column indicate significant differences according to the Bonferroni-adjusted pairwise comparisons, $p < 0.05$. Two-way repeated ANOVA measures showed significant effects of organic acid type, treatment, and their interactions, $p < 0.001$.

Antimicrobial properties

The antimicrobial activity of the four LAB strains against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* is presented in Table 8. All strains inhibited the growth of at least three of the tested indicator microorganisms, although the size of the inhibition zones varied among strains. Strain CM 4.2 exhibited the strongest activity against *B. cereus* (32.78 mm) and produced inhibition zones ≥ 40 mm against *S. aureus* and *E. coli*. Strain KAN SHNI 6 also showed strong antimicrobial activity, producing inhibition zones ≥

40 mm against *B. cereus*, 29.0 mm against *P. aeruginosa*, 24.65 mm against *S. aureus*, and 17.9 mm against *E. coli*. Strains CM 2A and CM 4.1 showed lower inhibitory activity. CM 2A produced inhibition zones of 19.78 mm against *B. cereus*, 20.2 mm against *S. aureus*, and 18.0 mm against *E. coli*, whereas CM 4.1 showed inhibition zones of 14.6 mm against *B. cereus*, 18.36 mm against *S. aureus*, and ≥ 40 mm against *E. coli*. No inhibitory activity against *P. aeruginosa* was observed for strains CM 2A, CM 4.1, or CM 4.2.

Table 8. The antagonistic test results.

Strains	***B.cereus 1410 A***	***S.aureus CCM 4516***	***E.coli CCM 4517***	***P.aeruginosa CCM 1961***
CM 2A	19.78	20.2	18.0	-
CM 4.1	14.6	18.36	++++	-
CM 4.2	32.78	++++	++++	-
KAN SHNI 6	++++	24.65	17.9	29.0

Note: ≥ 40 mm indicates that the inhibition zone exceeded the measurable diameter of the assay plate.

Antibiotic resistance profile

The antibiotic susceptibility profiles of strains CM 2A, CM 4.1, CM 4.2, and KAN SHNI 6 were evaluated using the disk diffusion assay, E-test, and minimum inhibitory concentration (MIC) determination (Tables 9–11). Overall, the three methods revealed comparable susceptibility patterns, although some differences were observed depending on the assay. In the disk diffusion assay (Table 9), all isolates were resistant to vancomycin and kanamycin. All isolates were resistant to streptomycin, whereas resistance to gentamicin was observed in three strains and intermediate susceptibility in strain KAN SHNI 6. In contrast, all isolates were susceptible to linezolid, rifampicin, and tetracycline, although CM 2A showed intermediate susceptibility to ampicillin, trimethoprim, and quinupristin/dalfopristin, while CM 4.1 and CM 4.2 exhibited intermediate susceptibility to clindamycin.

Table 9. Antibiotic disk diffusion zone diameter range.

Antibiotics	**Abb.**	**Disk content (μg)**	**Diameter of the growth retardation zone (mm)**			
			CM 2A	**CM 4.1**	**CM 4.2**	**KAN SHNI 6**
Ampiciliin	AMP	10	19 (I)	24 (S)	14 (I)	25(S)
Vancomycin	VAN	5	-(R)	-(R)	-(R)	-(R)
Vancomycin	VAN	30	-(R)	-(R)	-(R)	-(R)
Gentamicin	GN	10	-(R)	-(R)	-(R)	11(I)
Kanamycin	K	30	-(R)	-(R)	-(R)	-(R)
Streptomycin	S	10	-(R)	-(R)	-(R)	-(R)
Erythromycin	ERY	15	29 (S)	26 (S)	33 (S)	30(S)
Clindamycin	CLI	2	9 (R)	12 (I)	17 (I)	40(S)
Tetracycline	TE	30	25 (S)	21(S)	34(S)	27(S)
Chloramphenicol	C	30	-(R)	28(S)	33 (S)	38(S)
Trimethoph	TEM	5	15(I)	-(R)	-(R)	32(S)
Quinupristin and dalf	QD	15	12(I)	15(I)	20(S)	25(S)
Linezolid	LZD	10	36 (S)	31(S)	38(S)	35(S)
Ciprofloxacin	CIP	5	-(R)	-(R)	15	-(R)
Rifampicin	RIF	5	25 (S)	27(S)	31(S)	29(S)

Note: S- sensitive; I – intermediate; R -resistant.

The E-test results (Table 10) confirmed resistance of all strains to vancomycin, gentamicin, kanamycin, and ciprofloxacin. All isolates were susceptible to ampicillin, whereas susceptibility to erythromycin, clindamycin, and tetracycline varied among strains. CM 2A was resistant to erythromycin and clindamycin, whereas CM 4.1, CM 4.2, and KAN SHNI 6 remained susceptible to clindamycin. MIC determination (Table 11) further confirmed resistance of all isolates to vancomycin and teicoplanin (MIC ≥16 μg mL^{-1}). All strains were susceptible to ampicillin, erythromycin, clindamycin, linezolid, and chloramphenicol, whereas intermediate susceptibility to tetracycline was observed for CM 2A, CM 4.1, and KAN SHNI 6. Penicillin susceptibility differed among strains, with CM 2A and CM 4.1 classified as resistant and CM 4.2 and KAN SHNI 6 as susceptible. Overall, the antibiotic susceptibility profiles differed among the investigated strains, although resistance to glycopeptide antibiotics was consistently observed in all isolates.

Table 10. The antibiotic sensitivity profile using the E-test.

Antibiotics	µg mL^{-1}	Zone diameter			
		CM 2A	CM 4.1	CM 4.2	KAN SHNI 6
AMP	256-0.016	0.047 (S)	0.064(S)	0.125(S)	0.032(S)
VAN	256-0.016	-(R)	-(R)	-(R)	-(R)
GN	256-0.016	16(R)	24(R)	24(R)	12(R)
K	256-0.016	-(R)	-(R)	-(R)	-(R)
S	1024-0.064	256 (S)	48(S)	64(S)	96(S)
ERY	256-0.016	1.5 (R)	0.75(S)	0.75(S)	0.75(R)
CLI	256-0.016	1.5(R)	0.75(S)	0.75(S)	0.016(S)
TE	256-0.016	3(R)	0.50(R)	0.50(R)	3(R)
CIP	256-0.016	3(R)	4(R)	3(R)	1.5(R)

Note: Susceptible (S): a bacterial strain is defined as susceptible when it is inhibited at a concentration of a specific antimicrobial equal or lower than the established cut-off value (S ≤ x mg L^{-1}); Resistant (R): a bacterial strain is defined as resistant when it is not inhibited at a concentration of a specific antimicrobial higher than the established cut-off value (R > x mg L^{-1}).

Table 11. MIC values (µg mL^{-1}) of different antibiotic to the tested strains.

Antibiotics	CM 2A	CM 4.1	CM 4.2	KAN SHNI 6
PEN	≤0.5(R)	≤0.5(R)	≤0.25(S)	≤1(S)
AMP	≤0.25(S)	≤0.25(S)	≤0.25(S)	≤0.5(S)
ERY	0.06(S)	0,06(S)	-(S)	0.06(S)
CLI	-(S)	-(S)	-(S)	-(S)
LZD	≤0.5(S)	≤1(S)	≤0.25(S)	≤0.5(S)
CMP	≤1(S)	≤2(S)	≤0.5(S)	≤1(S)
TET	≤4(I)	≤4(I)	≤0.5(S)	≤4(I)
T/S	-(S)	0.03/0.6(S)	-(S)	0.03/0.6(S)
GEN	-(S)	0.25(S)	-(S)	-(S)
VAN	≥16(R)	≥16(R)	≥16(R)	≥16 (R)
TEC	≥16(R)	≥16(R)	≥16(R)	≥16 (R)
NFT	-(S)	2(S)	-(S)	-(S)

Note: S- sensitive; I – moderately resistant; R -resistant.

Cell surface hydrophobicity (CSH)

The cell surface hydrophobicity (CSH) of LAB strains CM 2A, CM 4.1, CM 4.2, and KAN SHNI 6 toward xylene, n-hexane, and hexadecane is presented in Table 12 and Fig. 5. Significant differences in CSH were observed among the tested strains ($p < 0.05$). Strain CM 2A exhibited the highest hydrophobicity, with values of 55.33%, 43.67%, and 59.67% toward xylene, hexane, and hexadecane, respectively.

Strain CM 4.1 showed intermediate hydrophobicity toward all three solvents, whereas strain CM 4.2 exhibited the lowest values (13.33–16.67%). Strain KAN SHNI 6 demonstrated moderate hydrophobicity toward xylene (47.33%) but lower affinity for hexane (19.00%) and hexadecane (24.67%). Overall, strain CM 2A exhibited the highest cell surface hydrophobicity, whereas strain CM 4.2 showed the lowest values. Two-way repeated-measures ANOVA revealed a significant effect of strain ($p < 0.05$), whereas neither solvent type nor the interaction between strain and solvent had a significant effect on CSH ($p > 0.05$).

Table 12. Cell surface hydrophobicity (%) of LAB strains toward xylene, hexane and hexadecane (mean ± SD).

Strains	Solvent		
	Xylene	Hexane	Hexadecane
CM 2A	55.33 ± 11.02^{a}	43.67 ± 7.37^{a}	59.67 ± 29.20^{a}
CM 4.1	39.33 ± 25.78^{ab}	37.00 ± 24.58^{ab}	49.33 ± 14.22^{a}
CM 4.2	13.33 ± 5.03^{c}	16.67 ± 1.53^{c}	14.33 ± 4.04^{b}
KAN SHNI 6	47.33 ± 21.94^{ab}	19.00 ± 4.58^{bc}	24.67 ± 10.97^{b}

Values are presented as mean ± standard deviation (SD), n = 3. Different superscript letters within the same column indicate significant differences among treatments according to Tukey's HSD test ($p < 0.05$). A two-way repeated measures ANOVA revealed a significant effect of treatment, while solvent type and the strains interaction were not statistically significant ($p > 0.05$).

Auto-aggregation assay

The auto-aggregation ability of LAB strains CM 2A, CM 4.1, CM 4.2, and KAN SHNI 6 isolated from spontaneously fermented camel milk (shubat) is presented in Table 13. All strains exhibited higher auto-aggregation after 24 h than after 6 h of incubation. After 6 h, strain CM 2A showed the highest auto-aggregation value (37.69 ± 12.97%), whereas CM 4.1, CM 4.2, and KAN SHNI 6 exhibited lower values ranging from 20.52

± 1.87% to 23.12 ± 3.45%. After 24 h, auto-aggregation increased to 79.06 ± 3.83% for CM 2A, 51.90 ± 30.20% for CM 4.1, 61.01 ± 33.64% for CM 4.2, and 51.84 ± 26.62% for KAN SHNI 6. Although strain CM 2A showed the highest mean auto-aggregation values at both time points, no statistically significant differences were detected among the strains ($p > 0.05$), as indicated by identical superscript letters (Table 13).

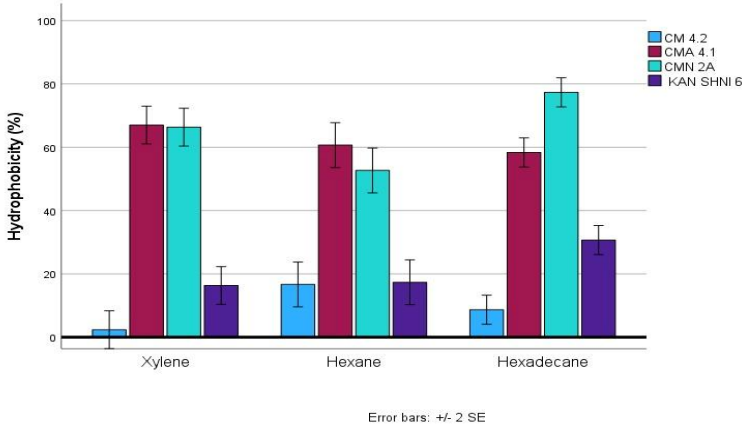

Fig. 5. Cell surface hydrophobicity (%) of LAB strains toward xylene, hexane, and hexadecane. Values are presented as mean ± standard deviation (SD; n = 3). Different superscript letters within the same column indicate significant differences among strains according to Tukey's HSD test ($p < 0.05$). Two-way repeated-measures ANOVA revealed a significant effect of strain, whereas solvent type and the strain × solvent interaction were not statistically significant ($p > 0.05$).

Table 13. The results of determination auto-aggregation (%) of LAB strains.

Time	Auto-aggregation (%)			
	CM 2A	**CM 4.1**	**CM 4.2**	**KAN SHNI 6**
After 6 hours	37.69 ± 12.97^{a}	20.52 ± 1.87^{a}	23.12 ± 3.45^{a}	21.11 ± 5.27^{a}
After 24 hours	79.06 ± 3.83^{a}	51.90 ± 30.20^{a}	61.01 ± 33.64^{a}	51.84 ± 26.62^{a}

Values are mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$). All groups share the same letter, no significant differences between groups.

Biofilm formation assay

The biofilm-forming ability of LAB strains isolated from spontaneously fermented camel milk (shubat) was evaluated after 24 and 48 h of incubation using the crystal violet staining method (Table 14). Fig. 6 shows the relationship between the measured optical density (OD590) of stained biofilm biomass and LAB strains after 24 h (A) and 48 h (B) of incubation. Each value represents the mean of three independent replicates. After 24 h of incubation, significant differences in biofilm formation were observed among the tested strains ($p < 0.05$). Strains CM 4.2 and CM 4.1 demonstrated the highest biofilm biomass values (OD590 = 0.179 ± 0.014 and 0.162 ± 0.015, respectively), indicating stronger adhesion ability compared with the other isolates. Strains CM 2A and KAN SHNI 6 showed lower biofilm formation capacity. After 48 h of incubation, all LAB strains demonstrated an increase in biofilm biomass compared with 24 h, indicating a time-dependent development of biofilm formation. The highest numerical value was observed for strain CM 4.2 (OD590 = 0.252 ± 0.043), followed by CM 2A, CM 4.1, and KAN SHNI 6. However, statistical analysis revealed no significant differences among the tested strains after 48 h ($p > 0.05$). Table 14 presents the biofilm formation capacity of LAB strains at 24 and 48 h. Values are expressed as mean ± standard deviation (SD) of three independent replicates (n = 3). Different superscript letters within the same column indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$).

Table 14 – Biofilm formation at 24 h and 48 h (mean ± SD)

Treatment	24 h	48 h
blank MRS	0.073 ± 0.004^{c}	0.619 ± 0.461^{a}
CM 2A	0.115 ± 0.013^{b}	0.216 ± 0.013^{b}
CM 4.1	0.162 ± 0.015^{a}	0.209 ± 0.013^{b}
CM 4.2	0.179 ± 0.014^{a}	0.252 ± 0.043^{b}
KAN SHNI 6	0.107 ± 0.014^{bc}	0.198 ± 0.030^{b}

Values are presented as mean ± standard deviation (SD), n = 3. Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p < 0.05$). No significant differences among treatment were observed at 48 h.

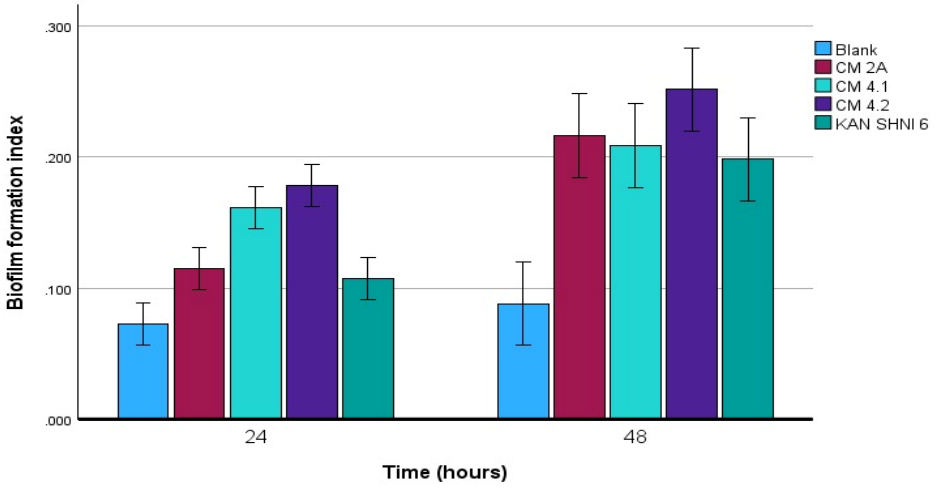

Fig. 6. Biofilm formation capacity of LAB strains isolated from fermented camel milk after 24 h (A) and 48 h (B) of incubation measured by crystal violet staining (OD590).

Co-aggregation assay

The co-aggregation ability of LAB isolates with *Escherichia coli* CCM 4517 was evaluated after 6 and 24 h of incubation (Table 15). The tested strains demonstrated different levels of co-aggregation capacity, with an increase in co-aggregation percentage observed during prolonged incubation. After 6 h of incubation, strain CM 2A showed the highest co-aggregation value (37 ± 9.9%) compared with the other tested isolates. Strains CM 4.1 and CM 4.2 exhibited moderate co-aggregation abilities with values of 24 ± 3.0% and 20 ± 23.4%, respectively, while KAN SHNI 6 demonstrated the lowest co-aggregation percentage (17 ± 10.5%). Following 24 h of incubation, all LAB isolates showed increased co-aggregation with *E. coli* CCM 4517. The highest co-aggregation percentage was observed for strain KAN SHNI 6 (72 ± 15.3%), followed by CM 4.1 (63 ± 15.1%), whereas CM 2A and CM 4.2 showed similar values (59 ± 20.5% and 59 ± 16.0%, respectively).

Table 15. The results of co-aggregation (%) assay between *E. coli* CCM 4517 with bacterial isolates after 6h and 24 h.

Time	Co-Agg, %			
	CM 2A	CM 4.1	CM 4.2	KAN SHNI 6
After 6 hours	37 ± 9.9*	24 ± 3.0	20 ± 23.4	17 ± 10.5
After 24 hours	59 ± 20.5*	63 ± 15.1	59 ± 16.0	72 ± 15.3

These results indicate a time-dependent enhancement of co-aggregation ability, suggesting that prolonged incubation promotes stronger interactions between LAB cells and the indicator pathogenic strain.

Table 15 presents the co-aggregation percentages of LAB isolates with *E. coli* CCM 4517 after 6 and 24 h of incubation. Values are expressed as mean ± standard deviation (SD) of three independent measurements.

DISCUSSION

Comparative analysis of microbial loads in raw and fermented camel milk

The high microbial counts observed in both raw and fermented camel milk indicate that spontaneous fermentation maintains a dense microbial community dominated by lactic acid bacteria. Similar microbial loads have been reported in raw camel milk from Central Asia and North Africa, where diverse LAB populations contribute to spontaneous fermentation (Rahmeh *et al.* 2022). In samples from the Almaty region, spontaneous fermentation resulted in no statistically significant changes in viable counts on most media according to Tukey's HSD test ($p < 0.05$). Only minor changes in viable counts were observed after fermentation, suggesting that spontaneous fermentation did not markedly affect the overall LAB population in samples from Almaty region. Although these differences were not statistically significant, the observed tendency toward reduced counts may reflect the selective pressure during fermentation caused by progressive acidification. Similar patterns have been described during traditional camel milk fermentation, where decreasing pH suppresses less acid-tolerant microorganisms

while maintaining high populations of LAB. A different pattern was observed in the Astana samples. In this case, microbial counts on bile esculin agar increased from 7.64 to 8.68 log CFU mL^{-1} after fermentation, indicating the proliferation of bile-esculin-positive bacteria. Such increases may reflect the proliferation of bile-esculin-positive bacteria, including *Enterococcus* spp. and other esculin-hydrolyzing LAB. Similar dynamics during traditional shubat fermentation have been reported by Akhmetsadykova *et al.* (2022) and Bilal *et al.* (2024), who observed expansion of *Enterococcus* and *Lactobacillus* populations during the intermediate stages of spontaneous fermentation. Interestingly, no detectable growth (NG) was observed for fermented camel milk from Astana on M17 medium. This result may indicate suppression or low abundance of streptococcal populations in the later stages of fermentation, potentially due to competitive interactions with other LAB or increased acidity of the fermented product. The consistently high counts observed on MRS-based media suggest that culturable LAB represented a major fraction of the microbial community under the cultivation conditions used in this study. Final LAB concentrations exceeded the microbial levels generally considered desirable for fermented dairy products containing viable lactic acid bacteria. Comparable LAB levels have been reported in fermented camel milk products from Kazakhstan, Mongolia and China (Li *et al.* 2024; Sheikh *et al.* 2024). The interregional variability observed between Almaty and Astana samples likely reflects differences in environmental conditions, animal feeding practices, hygiene parameters during milking, and the baseline microbiota of raw milk. Recent comparative microbiome studies emphasize that the diversity and structure of bacterial communities in raw milk and spontaneously fermented dairy products are significantly influenced by geographical origin, as well as by season, storage conditions, and pasture management, due to region-specific microbial reservoirs and climatic factors (Zhong *et al.* 2016; Ryu *et al.* 2021). Overall, the microbial counts obtained in this study are consistent with contemporary literature describing spontaneous camel milk fermentation as a process largely driven by lactic acid bacteria, accompanied by selective acidification and regional variability in microbial composition. These findings indicate that traditionally fermented camel milk represents a rich source of indigenous LAB with potential for further selection as starter or candidate probiotic cultures.

Isolation and identification of LAB strains in samples

Identification of LAB isolates using MALDI-TOF MS, API 50 CHL and 16S rRNA gene sequencing

Accurate identification of LAB isolated from fermented dairy products is essential because closely related species often exhibit similar phenotypic characteristics but may differ in their technological and functional properties. Therefore, a polyphasic approach combining MALDI-TOF MS, API 50 CHL, and partial 16S rRNA gene sequencing was used in the present study. MALDI-TOF MS protein fingerprinting using the Bruker MALDI Biotyper system provided reliable species-level identification for all isolates, with score values ≥ 2.0. According to the manufacturer, scores between 2.0 and 2.29 indicate secure genus identification and probable species identification, while scores ≥ 2.30 indicate highly probable species identification. Several studies have demonstrated that MALDI-TOF MS provides reliable identification of LAB isolated from fermented dairy products when appropriate reference spectra are available (Bouchibane *et al.* 2022). API 50 CHL profiles showed substantial overlap among the isolates, limiting species discrimination based on carbohydrate utilization alone. Similar limitations have been reported for closely related members of the *Lactiplantibacillus* and *Lacticaseibacillus* genera, which frequently exhibit highly comparable biochemical characteristics (Majhenič *et al.* 2017; Lee *et al.* 2023). Partial 16S rRNA gene sequencing was used as an additional molecular identification tool. However, due to the high sequence similarity among members of the *Lactiplantibacillus*/*Lacticaseibacillus* complex, partial 16S rRNA sequences alone do not always provide unambiguous species-level discrimination. Therefore, taxonomic assignments were interpreted using a polyphasic approach that combined MALDI-TOF MS, API 50 CHL, and 16S rRNA sequencing. Where discrepancies occurred, isolates were conservatively referred to as members of the *Lacticaseibacillus casei/paracasei* group or *L. plantarum* group. Discrepancies between MALDI-TOF MS/API 50 CHL and partial 16S rRNA sequencing were observed for several isolates. Such inconsistencies have been widely reported for members of the former *Lactobacillus* complex since closely related species share highly similar phenotypic characteristics and nearly identical 16S rRNA gene sequences. These discrepancies reflect the high sequence similarity (> 99%) among closely related species within the former *Lactobacillus* complex and the limited resolution of single-method identification approaches. The present findings highlight an important limitation of partial 16S rRNA gene sequencing for resolving species within the *L. casei/paracasei* group. Consequently, the taxonomic assignments in this study should be interpreted cautiously and in conjunction with MALDI-TOF MS and phenotypic characterization. Future studies employing whole-

genome sequencing or multilocus sequence analysis would provide higher taxonomic resolution and allow unequivocal species identification.

Determination of probiotic properties of selected LAB strains

Capacity for growth in milk

The ability of LAB to rapidly acidify milk is one of the most important technological characteristics of starter cultures, as it promotes milk coagulation, inhibits undesirable microorganisms, and contributes to the development of the texture and sensory properties of fermented dairy products. In the present study, all tested isolates efficiently acidified skimmed milk, indicating their ability to utilize lactose and produce organic acids under the experimental conditions. Similar acidification characteristics have been reported for *L. plantarum* and *L. paracasei* isolated from traditional fermented dairy products (Leroy & De Vuyst 2004). Although all isolates showed good acidification performance, strains CM 4.1 and CM 4.2 produced a greater decrease in pH than the remaining strains. Such strain-dependent variation has previously been reported among LAB and is generally attributed to differences in lactose metabolism, growth kinetics, and organic acid production (Terpou *et al.* 2019). Rapid acidification is considered an important technological attribute, since it shortens fermentation time and contributes to microbiological stability by lowering the pH of the product. Therefore, the acidification performance observed in the present study suggests that the investigated isolates may be suitable for further evaluation as starter or adjunct cultures for fermented camel milk.

Bile tolerance

Bile tolerance is a crucial property for probiotic bacteria, as it determines their survival in the small intestine and functional activity. Although intrinsic bile tolerance appears to be strain dependent, both lactobacilli and bifidobacteria can progressively adapt to the presence of bile salts, and resistant derivatives can be obtained from sensitive wild type strains by subculturing in gradually increasing concentrations of bile (Noriega *et al.* 2006; Guglielmotti *et al.* 2007). On some occasions, bile salt-resistant strains can also be obtained by selection toward other stress factors (Chou & Weimer 1999) and bile-adapted strains usually display cross-resistances to other stress factors (Margolles *et al.* 2003). In the present study, the growth of the four LAB strains was evaluated in MRS broth supplemented with different bile concentrations (0.0%, 0.3%, 0.5%, and 1.0%). Optical density (OD600) was measured at 0 h and 24 h to assess the ability of the strains to tolerate bile salts. At the initial time point (0 h), all strains exhibited similar optical density values across the tested bile concentrations, ranging from approximately 0.186 to 0.321. These values reflect the baseline cell density prior to incubation, indicating that the starting bacterial populations were relatively comparable among treatments. After 24 h incubation, a marked increase in OD values was observed in most strains, indicating active growth. Strains CM 2A, CM 4.1, and KAN SHNI 6 in plain MRS (0.0% bile) demonstrated strong growth, reaching OD values of 1.696, 1.711 and 1.698 respectively. Strain CM 4.2 exhibited a lower OD value (1.092) and increased sensitivity to bile salts. At 0.3% bile concentration, its OD decreased drastically to 0.177, and only slight increases were observed at 0.5% and 1.0% bile (0.243 and 0.358, respectively). This indicated that CM 4.2 had lower tolerance to bile compared with the other strains. Post hoc comparisons using Tukey's HSD test further confirmed that strain CM 4.2 exhibited significantly lower growth than other strains. Meanwhile, strains CM 2A, CM 4.1 and KAN SHNI 6 did not differ significantly from each other, suggesting similar bile tolerance capabilities (Table 6). Several studies have reported that LAB isolated from camel milk exhibit considerable bile salt tolerance (Ruiz *et al.* 2013). For example, LAB strains survived in 0.3% Oxgal, approximating physiological bile levels in the human small intestine (Abushelaibi *et al.* 2017). Another study evaluating the probiotic properties of LAB isolated from camel milk reported high survival rates under bile salt exposure, reaching 81.35 ± 3.64%, along with acid tolerance up to 92.06 ± 1.82%, demonstrating strong resistance to gastrointestinal conditions (Begley *et al.* 2002). Overall, the results indicate that strains CM 2A, CM 4.1 and KAN SHNI 6 demonstrate strong bile tolerance and promising probiotic potential. In contrast, strain CM 4.2 appears to be more sensitive to bile salts and may be less suitable for applications requiring high bile resistance.

Determination of organic acids

The investigated LAB strains exhibited a similar organic acid profile, with lactic acid being the predominant fermentation product in all samples. The organic acid profiles obtained in this study are consistent with previous reports by Szczerbiec *et al.* (2022), who demonstrated that *Lactobacillus* spp. predominantly produce lactic acid, whereas citric, succinic, and other organic acids are present at considerably lower concentrations. A similar pattern

was observed for all strains investigated in the present study. Although the absolute concentrations reported by Szczerbiec *et al.* (2022) differed from those observed in the present study because of differences in experimental conditions and units of measurement, both studies demonstrated that lactic acid is the major end product of carbohydrate fermentation by LAB (Nuryana *et al.* 2019). Comparable findings have been reported for lactic acid bacteria (LAB), including *Lactobacillus* spp., where lactic acid is the predominant metabolite, produced at significantly higher concentrations than acetic, citric, and succinic acids (González de Llano *et al.* 1996; Nuryana *et al.* 2019). This pattern, characteristic of homofermentative LAB (Vinderola *et al.* 2019), reflects their metabolic efficiency and strain-specific fermentation kinetics (Li *et al.* 2022). Lactic acid contributes to acidification of the fermentation medium and is considered one of the factors involved in the inhibition of undesirable microorganisms (Stanojević-Nikolić *et al.* 2016). Other organic acids such as acetic, propionic, and butyric acids contribute to antifungal activity against spoilage fungi like *Penicillium* and *Mucor* (Garnier *et al.* 2020). Overall, the predominance of lactic acid indicates favorable fermentation characteristics of the investigated LAB strains and supports their further evaluation as potential starter or adjunct cultures for fermented camel milk products.

Antimicrobial properties

The antagonistic activity of lactic acid bacteria (LAB) against foodborne and opportunistic pathogens is considered an important technological characteristic, since it may contribute to the microbial stability and safety of fermented dairy products (Goa *et al.* 2022). All tested strains exhibited antagonistic activity against most of the studied pathogens, though the size of inhibition zones varied. Among the investigated isolates, strains CM 4.2 and KAN SHNI 6 exhibited broader and stronger antagonistic activity than CM 2A and CM 4.1, indicating considerable strain-dependent variability in antimicrobial properties. In contrast, strains CM 2A and CM 4.1 displayed moderate inhibition (14.6–20.2 mm) and lacked activity against *Pseudomonas aeruginosa*. The absence of inhibitory activity against *P. aeruginosa* in some isolates may reflect differences in the production of antimicrobial metabolites, such as organic acids, bacteriocins, or other strain-specific inhibitory compounds (Stanojević-Nikolić *et al.* 2016). Similar selective antagonistic patterns have been documented in LAB isolates from fermented milks and traditional dairy products, where inhibitory spectra vary depending on strain origin and target organism (Muñoz-Atienza *et al.* 2013). Consistent with these reports, strains CM 4.2 and KAN SHNI 6 exhibited broader inhibitory spectra than CM 2A and CM 4.1, indicating substantial strain-dependent variability in antagonistic activity. Overall, the observed antimicrobial activity indicates that these camel milk-derived LAB strains warrant further evaluation as adjunct cultures with bioprotective properties in fermented dairy products. The marked strain-dependent differences observed in this study emphasize the importance of selecting individual isolates according to the desired technological and antimicrobial characteristics.

Antibiotic resistance profile

All isolates indicated resistance to kanamycin and most to gentamicin, which is consistent with previous studies indicating that LAB frequently carry intrinsic resistance to aminoglycosides due to the absence of transport mechanisms required for antibiotic uptake (Dec *et al.* 2025; Mikołajczuk-Szczyrba *et al.* 2025). All isolates exhibited resistance to kanamycin and vancomycin, whereas resistance to gentamicin was observed in most strains. Susceptibility to the remaining antibiotics varied among isolates, indicating strain-dependent resistance profiles. Strains CM 4.1 and C M 4.2 displayed intermediate or strain-dependent responses for tetracycline, clindamycin, and quinupristin–dalfopristin. These differences may reflect strain-specific genetic determinants, including intrinsic resistance mechanisms and, potentially, the presence of acquired resistance genes. Previous studies have reported the occurrence of tetracycline resistance genes, such as tet(M) and tet(W), in some *Lactiplantibacillus* and *Lacticaseibacillus* strains (Zheng *et al.* 2020). E-test results were generally consistent with the disk diffusion and MIC assays. Notably, all strains exhibited intrinsic resistance to glycopeptide antibiotics (vancomycin, teicoplanin; MIC $\geq$ 16 μg mL^{-1}), consistent with the presence of modified peptidoglycan termini (D-Ala-D-Lac), a feature common among *Lactiplantibacillus* and related species. Variations in MICs for erythromycin, clindamycin, and tetracycline further emphasize the need for careful evaluation of each LAB strain before considering their potential application as starter or adjunct cultures. Overall, LAB isolated from shubat indicated diverse antibiotic susceptibility profiles, including predictable intrinsic resistance patterns and strain-specific sensitivity to clinically relevant antibiotics. These observations align with Horáčková *et al.* (2022), who reported that *L. plantarum* strains from fermented dairy and non-dairy products display intrinsic resistance to glycopeptides and variable resistance to macrolides, tetracyclines, and lincosamides. Such information is

important for evaluating the technological suitability and safety of camel milk-derived LAB intended for application in fermented dairy products (Horáčková *et al.* 2022; Carboni *et al.* 2025). However, the present study evaluated only phenotypic antibiotic susceptibility. Further studies should include genomic screening for transferable antibiotic resistance determinants to better assess the safety of these isolates for potential food applications.

Cell surface hydrophobicity (CSH)

Cell surface hydrophobicity (CSH) is considered an important physicochemical characteristic of bacterial cells that may contribute to their initial interactions with host surfaces. This parameter is commonly used as an indirect indicator of the potential adhesion ability of probiotic candidates; however, CSH alone does not confirm intestinal colonization or probiotic functionality, as adhesion is a complex process involving multiple bacterial and host factors (Qureshi *et al.* 2020; Farid *et al.* 2021). In the present study, LAB isolates demonstrated strain-dependent differences in cell surface hydrophobicity. Among the tested strains, CM 2A exhibited the highest hydrophobicity values, suggesting stronger interactions with hydrophobic solvents and potentially greater surface affinity compared with the other isolates. Strain KAN SHNI 6 showed intermediate hydrophobicity, whereas CM 4.2 demonstrated lower values, indicating differences in surface properties among the investigated LAB strains. The variable response of CM 4.1 depending on the solvent used further confirms that bacterial surface characteristics are influenced by the type of hydrophobic interaction assessed. Similar strain-specific variations in CSH have been reported among lactic acid bacteria from different ecological niches. For example, *L. paracasei* strains demonstrated higher hydrophobicity values than *L. casei*, reaching 38.18% in the n-hexadecane assay compared with 14.7% for *L. casei* (Dehghani Champiri *et al.* 2023). Zhang *et al.* (2025) also reported higher surface hydrophobicity of *L. paracasei* E compared with the well-characterized probiotic strain *L. rhamnosus* GG. In another study, *L. plantarum* strains isolated from camel milk exhibited high hydrophobicity values, reaching up to 99% when evaluated using xylene (Sharma *et al.* 2021). Furthermore, LAB isolates have been reported to show considerable affinity toward organic solvents, reflecting differences in their cell surface composition and physicochemical properties (Dlamini *et al.* 2019). The obtained results are also consistent with the findings of Bindu & Lakshmidevi (2021), who reported variable adhesion-related properties of LAB strains, with hydrophobicity values ranging from 41–53% for n-hexane and 58–66% for hexadecane assays. Differences in CSH among bacterial strains are mainly associated with variations in cell surface structures, including membrane proteins, glycolipids, teichoic and lipoteichoic acids, and extracellular polymeric substances, which contribute to hydrophobic interactions and bacterial adhesion characteristics (El Oirdi *et al.* 2023). Previous studies have demonstrated that LAB isolated from dairy environments exhibit strain-specific hydrophobicity profiles. Ikombayev *et al.* (2025) reported similar differences among LAB strains obtained from raw goat milk and cottage cheese, emphasizing the influence of bacterial origin on surface properties. Recent investigations of indigenous lactobacilli isolated from fermented foods, infant feces, and human milk also revealed considerable variability in hydrophobicity, adhesion to intestinal cell models, and immunomodulatory responses, indicating that surface hydrophobicity represents one of several factors involved in host–microbe interactions (Duche *et al.* 2025). Therefore, the CSH results obtained in this study suggest that the investigated LAB strains possess distinct surface characteristics that may contribute to their adhesion-related properties. Further investigations, including direct adhesion assays using intestinal epithelial cell models and genomic characterization of adhesion-associated factors, are required to confirm their functional potential.

Auto-aggregation assay

Auto-aggregation is considered an important surface-associated property of lactic acid bacteria that may contribute to cell–cell interactions, adhesion processes, and biofilm development. Although high auto-aggregation ability is often associated with improved adhesion potential, this parameter alone cannot confirm intestinal colonization or probiotic efficacy, as these processes depend on multiple bacterial and host-related factors (Lau & Quek 2024). In the present study, all investigated LAB strains demonstrated a time-dependent increase in auto-aggregation capacity, indicating progressive cell–cell interactions during incubation. The observed differences among strains suggest that auto-aggregation is a strain-specific characteristic influenced by bacterial surface properties. However, the absence of statistically significant differences indicates that none of the tested isolates demonstrated a superior auto-aggregation ability under the evaluated conditions. Similar strain-dependent variations in auto-aggregation have been reported for LAB isolated from different fermented dairy environments.

For example, LAB strains obtained from traditional goat milk products exhibited high auto-aggregation levels, ranging from approximately 84% to 93% after 24 h of incubation, which was associated with their potential adhesion capacity and interactions with intestinal epithelial cells (da Silva *et al.* 2019). In another study, Yang *et al.* (2023) reported auto-aggregation values ranging from 30% to 55% among LAB isolates, together with high co-aggregation ability against *Listeria monocytogenes* and *Escherichia coli*. These findings highlight the variability of aggregation-related properties among LAB strains and emphasize the importance of evaluating multiple functional characteristics when selecting potential probiotic candidates. The auto-aggregation results obtained in this study, together with other evaluated surface-associated properties, indicate that the investigated LAB strains possess different interaction capacities that may contribute to their functional potential. Further studies involving direct adhesion assays with intestinal epithelial cell models and *in vivo* validation are required to confirm their biological relevance.

Biofilm formation assay

Biofilm formation is considered an important functional characteristic of lactic acid bacteria (LAB), as it reflects their ability to establish cell–cell interactions, produce extracellular matrix components, and maintain attachment to surfaces under *in vitro* conditions. Although biofilm-forming ability may be associated with enhanced adhesion potential and competitive interactions with other microorganisms, it does not directly confirm intestinal colonization or probiotic effectiveness *in vivo* (Fonseca *et al.* 2021). In the present study, LAB isolates demonstrated strain-dependent differences in biofilm formation capacity, with an overall increase observed after prolonged incubation. The variation among strains may be related to differences in genetic background, cell surface properties, and metabolic activity, including the production of extracellular polymeric substances (EPS), surface-associated proteins, and other factors involved in biofilm development (Jurášková *et al.* 2022). Among the tested isolates, strain CM 4.2 showed the highest numerical biofilm formation values at both incubation periods, indicating a greater ability to develop biofilm under the evaluated conditions. This property may be associated with differences in surface characteristics and EPS production; however, further molecular and biochemical analyses are required to confirm the specific mechanisms involved. Strain CM 4.1 also demonstrated relatively high biofilm-forming capacity, suggesting potential functional relevance for further investigation. Previous studies have demonstrated that biofilm formation among LAB strains is highly variable and depends on the bacterial species, strain origin, and environmental conditions. LAB isolated from fermented dairy products often exhibit different biofilm-forming capacities, reflecting adaptation to specific ecological niches and differences in cell surface composition. Overall, the obtained results indicate that LAB strains isolated from fermented camel milk possess distinct biofilm-forming abilities that increase over time under *in vitro* conditions. Strains CM 4.2 and CM 4.1 showed promising biofilm-associated characteristics and may be considered for further evaluation together with other probiotic-related properties, including adhesion assays, safety assessment, and genomic characterization.

Co-aggregation assay

Co-aggregation is an important surface-associated property of LAB that reflects their ability to interact with other bacterial cells. However, this *in vitro* characteristic alone does not confirm intestinal colonization or pathogen exclusion. In the present study, all LAB strains showed increased co-aggregation after 24 h of incubation, indicating time-dependent enhancement of cell–cell interactions. KAN SHNI 6 demonstrated the highest co-aggregation value after 24 h, followed by CM 4.1, while CM 2A and CM 4.2 showed comparable levels. These differences indicate that co-aggregation ability is strain-dependent and may be influenced by variations in cell surface properties. Similar variability among LAB strains has been reported in previous studies. LAB isolated from raw Nigerian goat milk showed co-aggregation values ranging from 34% to 94% against different pathogens (Akinyemi *et al.* 2024). Other studies have also suggested that aggregation-related properties may contribute to microbial interactions and should be considered together with other functional characteristics (Abushelaibi *et al.* 2017; Nami *et al.* 2019). Overall, the tested LAB strains demonstrated promising co-aggregation ability under *in vitro* conditions. Further studies, including adhesion assays, safety evaluation, and genomic characterization, are required to confirm their functional potential.

CONCLUSION

This study demonstrates that indigenous lactic acid bacteria (LAB) isolated from traditional Kazakh shubat and identified as *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* possess valuable technological and

functional characteristics relevant for fermented dairy applications. All tested isolates exhibited rapid acidification capacity and lactic acid production, with the highest level observed for strain CM 2A (6.87 mg g^{-1}), indicating their potential suitability as starter or adjunct cultures. The investigated LAB strains showed strong antimicrobial activity against important foodborne and technological contaminants, including *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus*, supporting their potential application in natural biopreservation strategies. In addition, strain-dependent differences were observed in surface-associated properties, including auto-aggregation, co-aggregation, cell surface hydrophobicity, and biofilm formation. Among the tested isolates, CM 2A demonstrated a favorable combination of functional characteristics, suggesting its potential for further investigation. The antibiotic susceptibility profiles were generally consistent with previously reported species-specific patterns; however, comprehensive safety evaluation, including whole-genome analysis and additional functional assessments, is required before potential probiotic application.

Overall, camel milk-derived LAB represents a promising microbial resource for the development of functional fermented dairy products. Further studies, including *in vivo* validation, are necessary to confirm their safety, technological performance, and biological relevance.

Funding

This research was funded by Ministry of Science and Higher Education of the Republic of Kazakhstan [grant number AP22685499] within the framework of grant funding for research by young scientists under the "Zhas Galym" project for 2024–2026, titled "Potential application of probiotic lactic acid bacteria exopolysaccharides in food biotechnology".

REFERENCES

Abushelaibi, A, Al-Mahadin, S, El-Tarabily, K, Shah, NP & Ayyash, M 2017, Characterization of potential probiotic lactic acid bacteria isolated from camel milk. *LWT-Food Science and Technology*, 79: 316–325.

Akhmetsadykova, S, Baubekova, A, Konuspayeva, G, Akhmetsadykov, N, Faye, B & Loiseau, G 2015, Lactic acid bacteria biodiversity in raw and fermented camel milk. *African Journal of Food Science and Technology*, 6: 84–88.

Akhmetsadykova, SH, Konuspayeva, G & Akhmetsadykov, N 2022, Camel breeding in Kazakhstan and future perspectives. *Animal Frontiers*, 12: 71–77.

Akinyemi, MO, Ogunremi, OR, Adeleke, RA & Ezekiel, CN 2024, Probiotic potentials of lactic acid bacteria and yeasts from raw goat milk in Nigeria. *Probiotics and Antimicrobial Proteins*, 16: 163–180.

Barzegar, H, Behbahani, BA, Mirzaei, A & Sheikhjan, MG 2023, Assessing the protection mechanisms against *Enterobacter aerogenes* by analyzing aggregation, adherence, antagonistic activity, and safety properties of potentially probiotic strain *Lactobacillus brevis* G145. *Microbial Pathogenesis*, 181: 106175.

Begley, M, Gahan, CG & Hill, C 2002, Bile stress response in *Listeria monocytogenes* LO28: Adaptation, cross-protection, and identification of genetic loci involved in bile resistance. *Applied and Environmental Microbiology*, 68: 6005–6012.

Bilal, Z, Akhmetsadykova, S, Baubekova, A, Tormo, H, Faye, B & Konuspayeva, G 2024, The main features and microbiota diversity of fermented camel milk. *Foods*, 13: 1985.

Bindu, A & Lakshmidevi, N 2021, Identification and in vitro evaluation of probiotic attributes of lactic acid bacteria isolated from fermented food sources. *Archives of Microbiology*, 203: 579–595.

Bouchibane, M, Zabouri, Y, Cheriguene, A, Kaced, A, Chougrani, F & Saada, DA 2022, Application of MALDI-TOF mass spectrometry to identify lactic acid bacteria isolated from artisanal dairy products in Algeria. *Asian Journal of Dairy and Food Research*, 41: 377–383.

Carboni, G, Mura, ME, Chessa, M, Blaiotta, G, Nudda, A & Mangia, NP 2025, Occurrence of antibiotic resistance in *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum* strains isolated from traditional Sardinian fermented food. *Antibiotics (Basel)*, 15.

Chou, LS & Weimer, B 1999, Isolation and characterization of acid- and bile-tolerant isolates from strains of *Lactobacillus acidophilus*. *Journal of Dairy Science*, 82: 23–31.

Da Silva, LA, Lopes Neto, JHP & Cardarelli, HR 2019, Safety and probiotic functionality of isolated goat milk lactic acid bacteria. *Annals of Microbiology*, 69: 1497–1505.

Dec, M, Herman-Ostrzyżek, K, Zomer, A & Urban-Chmiel, R 2025, Susceptibility of Lactobacillaceae strains to aminoglycoside antibiotics in the light of EFSA guidelines. *Life (Basel)*, 15.

Dehghani Champiri, I, Bamzadeh, Z, Rahimi, E & Rouhi, L 2023, *Lacticaseibacillus paracasei* LB12, a potential probiotic isolated from traditional Iranian fermented milk (Doogh). *Current Microbiology*, 80.

Dlamini, ZC, Langa, RL, Aiyegoro, OA & Okoh, AI 2019, Safety evaluation and colonization abilities of four lactic acid bacteria as future probiotics. *Probiotics and Antimicrobial Proteins*, 11: 397–402.

Duche, RT, Kaur, J, Singh, A, Sangwan, V, Sihag, MK, Nwagu, TN, Ezeogu, LI & Panwar, H 2025, Surface properties and potential immunomodulatory effects of candidate probiotic lactobacilli for gastrointestinal applications. *Advanced Gut & Microbiome Research*, 2025: 7768975.

El Oirdi, S, Lakhlifi, T, Fraberger, V, D'Amico, S, Kaddouri, A, Bouymajane, A, Yatim, M & Belhaj, A 2023, Probiotic features of three lactic acid bacteria strains isolated from foods and characterization of their bioactive molecules. *Microbiology*, 92: 881–891.

Farid, W, Masud, T, Sohail, A, Ahmad, N, Naqvi, SMS, Khan, S, Ali, A, Khalifa, SA, Hussain, A, Ali, S, Saghir, M, Siddeeg, A & Manzoor, MF 2021, Gastrointestinal transit tolerance, cell surface hydrophobicity, and functional attributes of *Lactobacillus acidophilus* strains isolated from indigenous Dahi. *Food Science & Nutrition*, 9: 5092–5102.

Faye, B 2020, How many large camelids in the world? A synthetic analysis of the world camel demographic changes. *Pastoralism*, 10: 25.

Fonseca, HC, De Sousa Melo, D, Ramos, CL, Dias, DR & Schwan, RF 2021, Probiotic properties of lactobacilli and their ability to inhibit the adhesion of enteropathogenic bacteria to Caco-2 and HT-29 cells. *Probiotics and Antimicrobial Proteins*, 13: 102–112.

Garnier, L, Penland, M, Thierry, A, Maillard, MB, Jardin, J, Coton, M, Salas, ML, Coton, E, Valence, F & Mounier, J 2020, Antifungal activity of fermented dairy ingredients: Identification of antifungal compounds. *International Journal of Food Microbiology*, 322: 108574.

Goa, T, Beyene, G, Mekonnen, M & Gorems, K 2022, Isolation and characterization of lactic acid bacteria from fermented milk produced in Jimma Town, Southwest Ethiopia, and evaluation of their antimicrobial activity against selected pathogenic bacteria. *International Journal of Food Science*, 2022: 2076021.

González De Llano, D, Rodriguez, A & Cuesta, P 1996, Effect of lactic starter cultures on the organic acid composition of milk and cheese during ripening—analysis by HPLC. *Journal of Applied Bacteriology*, 80: 570–276.

Guglielmotti, D, Marcó, MB, Vinderola, C, De Los Reyes Gavilán, C, Reinheimer, J & Quiberoni, A 2007, Spontaneous *Lactobacillus delbrueckii* phage-resistant mutants with acquired bile tolerance. *International Journal of Food Microbiology*, 119: 236–242.

Horáčková, Š, Vrchotová, B, Koval, D, Omarova, A, Sluková, M & Štětina, J 2022, Use of *Lactiplantibacillus plantarum* for dairy and non-dairy fermented products. *Czech Journal of Food Sciences*, 40: 392–399.

Ikombayev, T, Ospanova, A, Omarova, A, Horáčková, Š, Tuganbay, A, Kassenova, G, Zhamurova, V, Sharipova, S & Suleimenova, Z 2025, Functional properties of lactic acid bacteria isolated from raw goat milk and cottage cheese. *Journal of Agriculture and Food Research*, 21: 101822.

Ismael, M, Gu, Y, Cui, Y, Wang, T, Yue, F, Yantin, Q & Lü, X 2022, Lactic acid bacteria isolated from Chinese traditional fermented milk as novel probiotic strains and their potential therapeutic applications. *3 Biotech*, 12: 337.

Jurášková, D, Ribeiro, SC & Silva, CC 2022, Exopolysaccharides produced by lactic acid bacteria: From biosynthesis to health-promoting properties. *Foods*, 11: 156.

Khwaileh, HAA 2024, Table of content. *Journal of College of Sharia and Islamic Studies*, 42.

Konuspayeva, G & Faye, B 2021, Recent advances in camel milk processing. *Animals*, 11: 1045.

Konuspayeva, GS 2020, Camel milk composition and nutritional value, in *Handbook of Research on Health and Environmental Benefits of Camel Products*. IGI Global Scientific Publishing.

Lau, LYJ & Quek, SY 2024, Probiotics: Health benefits, food application, and colonization in the human gastrointestinal tract. *Food Bioengineering*, 3: 41–64.

Lee, HB, Bang, WY, Shin, GR, Jeon, HJ, Jung, YH & Yang, J 2023, Isolation, characterization, and safety evaluation of the novel probiotic strain *Lacticaseibacillus paracasei* IDCC 3401 via genomic and phenotypic approaches. *Microorganisms*, 12: 85.

Leroy, F & De Vuyst, L 2004, Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, 15: 67–78.

Li, H, Gao, J, Chen, W, Qian, C, Wang, Y, Wang, J & Chen, L 2022, Lactic acid bacteria isolated from Kazakh traditional fermented milk products affect the fermentation characteristics and sensory qualities of yogurt. *Food Science & Nutrition*, 10: 1451–1460.

Li, N, Xu, W, Meng, L, Zhao, Y, Zhao, X, Zheng, N, Zhang, Y & Wang, J 2024, Metagenomics reveals differences in spore-forming bacterial diversity in raw milk in different regions and seasons in China. *Food Research International*, 198: 115317.

Majhenič, A, Lorbeg, P & Treven, P 2017, Enumeration and identification of mixed probiotic and lactic acid bacteria starter cultures, in *Probiotic Dairy Products*. 207–251.

Margolles, A, GarcíA, L, Sánchez, B, Gueimonde, M & De Los Reyes-Gavilán, CG 2003, Characterization of a *Bifidobacterium* strain with acquired resistance to cholate—a preliminary study. *International Journal of Food Microbiology*, 82: 191–198.

Mikołajczuk-Szczyrba, A, Wnęk-Auguścik, K, Średnicka, P, Shymialevich, D, Jaroszewska, E, Wojtczak, A, Zapaśnik, A, Bucka-Kolendo, J, Cieślak, H & Nasiłowska, J 2025, Genomic and phenotypic landscape of antibiotic resistance in gut lactic acid bacteria from livestock environments. *Genes*, 16: 1518.

Mirmiran, P, Ejtahed, HS, Angoorani, P, Eslami, F & Azizi, F 2017, Camel milk has beneficial effects on diabetes mellitus: A systematic review. *International Journal of Endocrinology and Metabolism*, 15: e42150.

Muñoz-Atienza, E, Gómez-Sala, B, Araújo, C, Campanero, C, Del Campo, R, Hernández, PE, Herranz, C & Cintas, LM 2013, Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiology*, 13: 15.

Nami, Y, Vaseghi Bakhshayesh, R, Mohammadzadeh Jalaly, H, Lotfi, H, Eslami, S & Hejazi, MA 2019, Probiotic properties of *Enterococcus* isolated from artisanal dairy products. *Frontiers in Microbiology*, 10: 300.

Noriega, L, Cuevas, I, Margolles, A & De Los Reyes-Gavilan, CG 2006, Deconjugation and bile salts hydrolase activity by *Bifidobacterium* strains with acquired resistance to bile. *International Dairy Journal*, 16: 850–855.

Nuryana, I, Andriani, A, Lisdiyanti, P & Yopi 2019, Analysis of organic acids produced by lactic acid bacteria. *IOP Conference Series: Earth and Environmental Science*, 012054.

Qureshi, N, Gu, Q & Li, P 2020, Whole genome sequence analysis and in vitro probiotic characteristics of a *Lactobacillus* strain *Lactobacillus paracasei* ZFM54. *Journal of Applied Microbiology*, 129: 422–433.

Rahmeh, R, Akbar, A, Alomirah, H, Kishk, M, Al-Ateeqi, A, Al-Milhm, S, Shajan, A, Akbar, B, Al-Merri, S & Alotaibi, M 2022, Camel milk microbiota: A culture-independent assessment. *Food Research International*, 159: 111629.

Ruiz, L, Margolles, A & Sánchez, B 2013, Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Frontiers in Microbiology*, 4: 396.

Ryu, S, Park, WS, Yun, B, Shin, M, Go, G-W, Kim, JN, Oh, S & Kim, Y 2021, Diversity and characteristics of raw milk microbiota from Korean dairy farms using metagenomic and culturomic analysis. *Food Control*, 127: 108160.

Sharma, A, Lavania, M, Singh, R & Lal, B 2021, Identification and probiotic potential of lactic acid bacteria from camel milk. *Saudi Journal of Biological Sciences*, 28: 1622–1632.

Sheikh, A, Ibrahim, HIM, Almathen, F, Alfattah, M & Khalifa, A 2024, Beneficial probiotic bacteria prevalence in different lactating dromedary camel milk of Saudi Arabia. *Saudi Journal of Biological Sciences*, 31: 103879.

Shokri, D, Khorasgani, MR, Mohkam, M, Fatemi, SM, Ghasemi, Y & Taheri-Kafrani, A 2018, The inhibition effect of lactobacilli against growth and biofilm formation of *Pseudomonas aeruginosa*. *Probiotics and Antimicrobial Proteins*, 10: 34–42.

Stanojević-Nikolić, S, Dimić, G, Mojović, L, Pejin, J, Djukić-Vuković, A & Kocić-Tanackov, S 2016, Antimicrobial activity of lactic acid against pathogen and spoilage microorganisms. *Journal of Food Processing and Preservation*, 40: 990–998.

Szczerbiec, D, Piechocka, J, Głowacki, R & Torzewska, A 2022, Organic acids secreted by *Lactobacillus* spp. isolated from urine and their antimicrobial activity against uropathogenic *Proteus mirabilis*. *Molecules*, 27: 5557.

Terpou, A, Papadaki, A, Lappa, IK, Kachrimanidou, V, Bosnea, LA & Kopsahelis, N 2019, Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, 11: 1591.

Victor, SD, François, ZN, Marie, KP, Alberto, C & Florence, F 2011, Probiotic properties of lactobacilli strains isolated from raw cow milk in the western highlands of Cameroon. *Innovative Romanian Food Biotechnology*, 12–28.

Vinderola, G, Ouwehand, A, Salminen, S & Von Wright, A 2019, *Lactic acid bacteria: Microbiological and functional aspects*. CRC Press.

Wang, D, Liu, W, Ren, Y, De, L, Zhang, D, Yang, Y, Bao, Q, Zhang, H & Menghe, B 2016, Isolation and identification of lactic acid bacteria from traditional dairy products in Baotou and Bayannur of Midwestern Inner Mongolia and q-PCR analysis of predominant species. *Korean Journal for Food Science of Animal Resources*, 36: 499–507.

Yadav, AK, Kumar, R, Priyadarshini, L & Singh, J 2015, Composition and medicinal properties of camel milk: A review. *Asian Journal of Dairy and Food Research*, 34: 83–91.

Yang, Z, Ni, L, Tian, W & Chi, H 2023, Screening and identification of goat-milk-derived lactic acid bacteria with bacteriocin-like activity and probiotic potentials. *Microorganisms*, 11: 849.

Zawistowska-Rojek, A, Kośmider, A, Stępień, K & Tyski, S 2022, Adhesion and aggregation properties of *Lactobacillaceae* strains as protection ways against enteropathogenic bacteria. *Archives of Microbiology*, 204: 285.

Zhadyra, S, Tao, F & Xu, P 2025, Exploring the microbiome and functional metabolism of fermented camel milk (Shubat) using metagenomics. *Foods*, 14: 1102.

Zhang, N, Karkman, A, Hiippala, K, Satokari, R, Takala, TM & Saris, PEJ 2025, Comparative genomics and characterization of highly adhesive and stress resistant *Lacticaseibacillus paracasei* strain E. *Current Research in Food Science*, 11: 101245.

Zheng, J, Wittouck, S, Salvetti, E, Franz, C, Harris, HMB, Mattarelli, P, O'Toole, PW, Pot, B, Vandamme, P, Walter, J, Watanabe, K, Wuyts, S, Felis, GE, Gänzle, MG & Lebeer, S 2020, A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *International Journal of Systematic and Evolutionary Microbiology*, 70: 2782–2858.

Zhong, Z, Hou, Q, Kwok, L, Yu, Z, Zheng, Y, Sun, Z, Menghe, B & Zhang, H 2016, Bacterial microbiota compositions of naturally fermented milk are shaped by both geographic origin and sample type. *Journal of Dairy Science*, 99: 7832–7841.

Zoueki, CW *et al.* 2010, Bacterial adhesion to hydrocarbons: Role of asphaltenes and resins. *Colloids and Surfaces B: Biointerfaces*, 79(1) 219-226, 10.1016/j.colsurfb.2010.03.054.