Л.Н. Гумилев атындағы Еуразия ұлттық университетінің ХАБАРШЫСЫ.
BULLETIN of L.N. Gumilyov Eurasian National University.
ВЕСТНИК Евразийского национального университета имени Л.Н. Гумилева.
ISSN: 2616-7034. eISSN: 2663-130X

IRSTI 65.63.33; 62.09.39
Scientific article

https://doi.org/10.32523/2616-7034-2026-155-2-149-162

Isolation and characterization of autochthonous lactic acid bacteria from camel milk cheese for starter culture development

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Abstract. Camel milk represents a promising raw material for cheesemaking due to its high nutritional value; however, its processing is constrained by slow acidification kinetics and specific protein composition, which limit the effectiveness of conventional starter cultures developed for cow milk. This study aimed to isolate and characterize autochthonous lactic acid bacteria (LAB) from camel milk cheese and to evaluate their technological properties as potential candidates for starter culture development. Microorganisms were isolated by cultivation on selective media followed by purification. Preliminary characterization was performed based on morphological, gram-staining, and cultural characteristics, and taxonomic identification was subsequently confirmed by 16S rRNA gene sequence analysis. Acidification capacity was assessed through pH reduction and titratable acidity, while inhibitory activity of cell-free culture supernatants was evaluated against selected test cultures. Three LAB strains were isolated and identified as *Lactiplantibacillus sp.*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus*. All isolates were gram-positive and demonstrated pronounced acid-producing activity during 48 h of incubation. *Streptococcus thermophilus* exhibited the highest fermentative activity, reaching pH 3.9 and titratable acidity of 120°T. Selective inhibitory activity was observed against *Salmonella enteritidis*, whereas no inhibitory activity was detected toward *Staphylococcus aureus* and *Escherichia coli*. The results indicate that autochthonous LAB isolated from camel milk cheese possess significant technological potential and may represent promising candidates for the future development of specialized starter cultures. Further studies on their safety, functional characteristics, and technological performance are required before practical application in camel milk cheesemaking.

Keywords: camel milk cheese, lactic acid bacteria, autochthonous strains, morphology, antagonistic properties

Received: 22.02.2026. Accepted:18.06.2026. Available online: 30.06.2026

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Introduction

In recent years, there has been a steady increase in scientific and practical interest in camel milk as an alternative dairy product with distinctive physico-chemical and technological properties. Compared to cow's milk, camel milk is characterized by a different protein profile, higher buffering capacity, and slower acidification kinetics, which substantially affect coagulation processes and cheese structure formation [1-4]. These features limit the effectiveness of standard starter cultures developed for cow's milk and necessitate the development of specialized biotechnological solutions for camel milk processing.

A promising strategy involves the use of autochthonous lactic acid bacteria that constitute the natural microbiocenosis of camel milk and traditional fermented products derived from it. Previous studies have demonstrated that indigenous strains belonging to the genera *Lactiplantibacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, and *Streptococcus* exhibit high acidification activity, adaptation to the specific milk matrix, and antagonistic properties against undesirable microflora [5-8]. The application of such microorganisms as starter or adjunct cultures contributes to increased product yield, improved microbiological stability, and the development of characteristic flavor and aroma profiles in cheeses [9, 10].

This line of research is particularly relevant for the Republic of Kazakhstan, where camel husbandry and the production of traditional camel milk products are predominantly carried out within localized microbiological ecosystems. Despite the availability of isolated studies on the microflora of camel milk, systematic information on technologically significant autochthonous strains isolated from locally produced cheeses remains limited. Globally, commercial starter cultures adapted to cow's milk are still predominantly used in camel milk cheese production, which does not allow full exploitation of the regional microbiota potential [4, 11-13].

The investigation of autochthonous lactic acid bacteria is of considerable scientific and practical importance, as it expands current knowledge of LAB biodiversity in non-bovine dairy systems and facilitates the identification of strains with desirable technological properties for dairy fermentation. Such studies provide a scientific basis for the future development of specialized starter cultures adapted to the unique physicochemical characteristics of camel milk cheesemaking [7, 14].

Therefore, the aim of the present study was to isolate and characterize autochthonous lactic acid bacteria from camel milk cheese and to evaluate their technological properties, including acidification capacity and inhibitory activity, as a preliminary step toward the selection of potential starter culture candidates.

Materials and research methods

Study object and sample preparation

Cheese produced from raw camel milk (*Camelus dromedarius*) collected in the Turkestan region of the Republic of Kazakhstan was used as the study material. Fresh milk was processed without thermal treatment using a traditional cheesemaking procedure without the addition of starter cultures. The obtained cheese samples were stored at 12 °C and 95% relative humidity for 3 days prior to microbiological analysis.

Isolation and preliminary characterization of lactic acid bacteria

Lactic acid bacteria (LAB) were isolated using the standard serial dilution plating technique on de Man-Rogosa-Sharpe (MRS) agar (Condalab, Spain). Cheese samples (10 g) were homogenized in sterile distilled water, serially diluted up to 10^{-7}, and 0.1 mL aliquots were spread onto

MRS agar plates. Incubation was carried out aerobically at 37 °C for 48 h. Colonies exhibiting typical LAB morphology were counted and selected. All experiments were performed in duplicate.

Presumptive LAB isolates were identified based on Gram staining and catalase activity. Gram-positive, catalase-negative colonies were purified by repeated streaking on MRS agar and incubated at 37 °C for 48 h. Cell morphology was examined microscopically after methylene blue staining.

16S rRNA

The taxonomic classification of the bacterial strains was determined based on 16S rRNA gene sequencing using the Sanger method. Sequencing of bacterial 16S rRNA gene fragments was performed on a 3500 DNA Analyzer (Applied Biosystems, USA) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) in accordance with the manufacturer's protocol [BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, Applied Biosystems, USA].

Phylogenetic analysis was performed using MEGA 6 software.

Assessment of milk coagulation and acidification activity

Milk coagulation ability was evaluated by inoculating purified LAB isolates into commercially available sterile cow milk "Lactel" (1.5% fat content), followed by incubation at 37 °C for 48 h. Coagulum formation was visually assessed.

Acidifying ability of the strains during cultivation in growth medium.The pH and titrated acidity (°T) values were determined in bacterial cultures grown in MRS broth (Condalab, Spain) at 0, 24, and 48 h of incubation.

Evaluation of inhibitory activity

Inhibitory activity of cell-free supernatants of lactic acid bacteria was assessed using the agar well diffusion method against *Salmonella enteritidis*, *Staphylococcus aureus*, and *Escherichia coli*. Indicator strains were cultured on meat-peptone agar. Cell-free supernatants of LAB cultures were introduced into agar wells, and plates were incubated under appropriate conditions. Inhibition zones were measured in millimeters to quantify antimicrobial activity.

Statistical analysis

All experiments were performed in duplicate, and results are presented as mean ± standard deviation. Data processing was carried out using standard descriptive statistical methods.

Results

Microbial growth was observed in the first four decimal dilutions following incubation at 37 °C (Figure 1), indicating high viability of the microflora in the analyzed sample. Confluent and semi-confluent growth was recorded in the 10^{-1}-10^{-2} dilutions, reflecting a high concentration of microbial cells in the original product. Clearly isolated colonies suitable for subsequent isolation and identification were formed at the 10^{-3}-10^{-4} dilutions, whereas at higher dilutions (10^{-5}-10^{-7}) microbial growth was markedly reduced or absent.

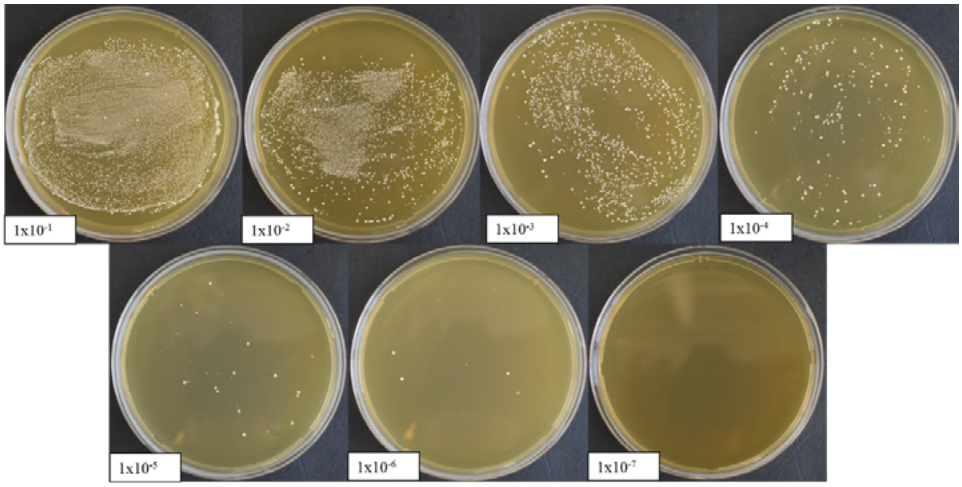


Figure 1. Microbial growth of camel milk cheese samples at 10^{-4} dilution after incubation at 37 °C for 48 h.

The colonies were predominantly circular in shape, with smooth margins and a matte or slightly glossy surface (Figure 2), which is characteristic of lactic acid bacteria.

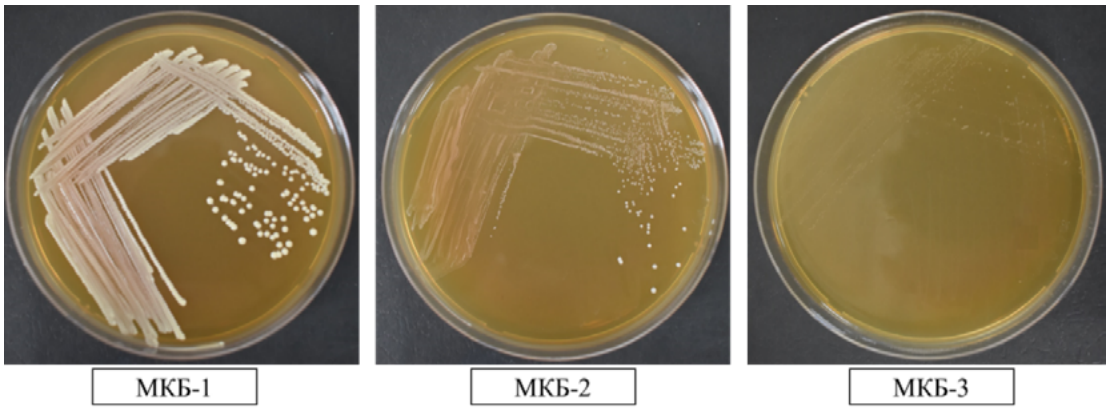
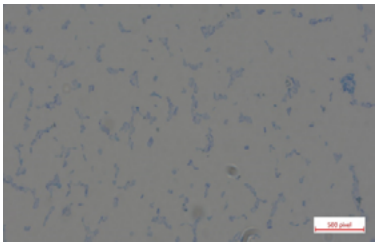
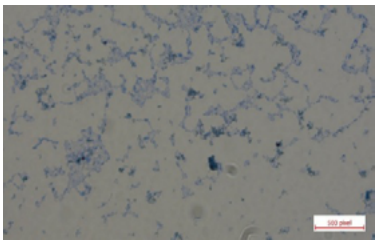
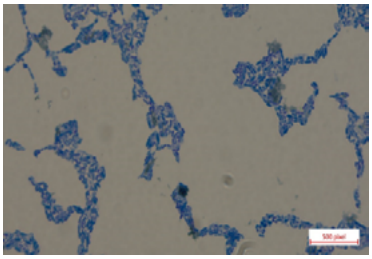


Figure 2. Colony morphology of the isolated lactic acid bacteria

Three bacterial isolates were obtained through microbiological analysis, all of which were Gram-positive and catalase-negative. Microscopic observation revealed cells with both rod-shaped and coccoid morphologies. Based on preliminary identification, the isolates were assigned to *Lactiplantibacillus sp.*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus* (Table 1).

Table 1

Colony and cell morphology

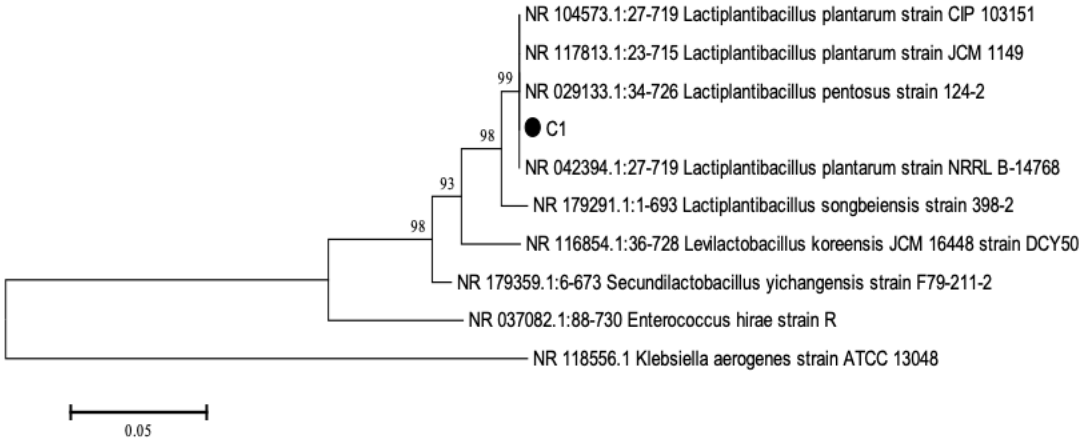
Strain designation	Isolates under oil immersion, 10×100 magnification	Gram reaction	Catalase	Colony morphology	Species name
LAB-1		Gram-positive	Negative	White, convex, 2–3 mm in diameter. Caused milk coagulation. 3×10^7 CFU / mL.	*Lactiplantibacillus sp.*
LAB-2		Gram-positive	Negative	White, small, 1 mm in diameter. Caused milk coagulation. 1×10^7 CFU / mL.	*Leuconostoc mesenteroides*
LAB-3		Gram-positive	Negative	Beige to translucent, pinpoint. Caused milk coagulation. 7×10^7 CFU /mL	*Streptococcus thermophilus*

All isolated strains were capable of fermenting milk, with viable cell counts ranging from 1×10^7 to 7×10^7 CFU/mL.

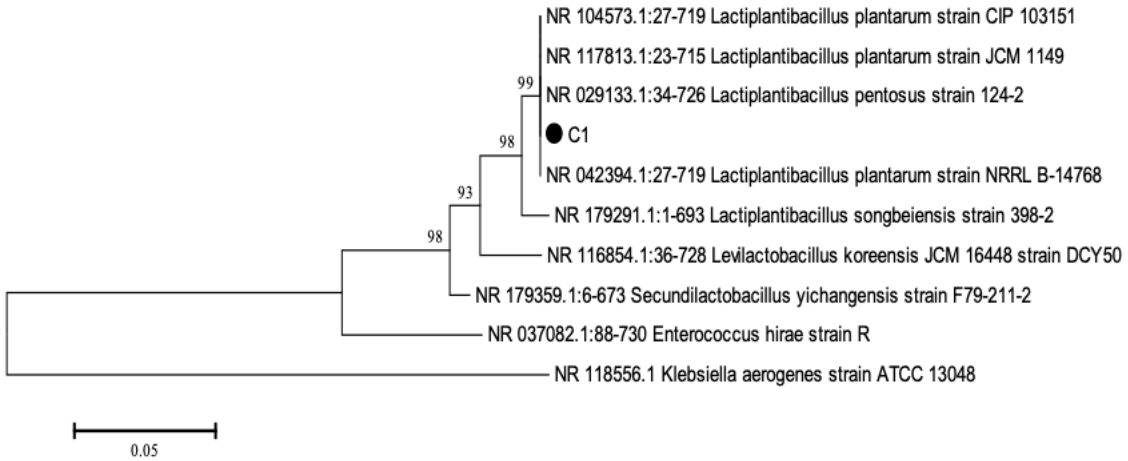
The results of the acidifying ability assessment are presented in Table 2. All strains demonstrated a significant decrease in pH and an increase in titratable acidity over 48 incubation in cow milk.

The taxonomic classification of the isolated lactic acid bacteria was confirmed using a molecular genetic method based on the analysis of 16S rRNA gene nucleotide sequences. For isolate C1, comparative analysis of the 16S rRNA gene sequence revealed 100.00% homology with representatives of the genus *Lactiplantibacillus* sp. Isolate C2 was identified as *Leuconostoc mesenteroides*. Comparative analysis of the 16S rRNA gene nucleotide sequence revealed 100.00% similarity with the reference strain *Leuconostoc mesenteroide*s ATCC 8293 (NR_074957.1). For isolate C3F, analysis of the 16S rRNA gene sequence showed the highest similarity to the reference strain *Streptococcus thermophilus* DSM 20617 (NR_118998.1). The homology level was 99.31%.

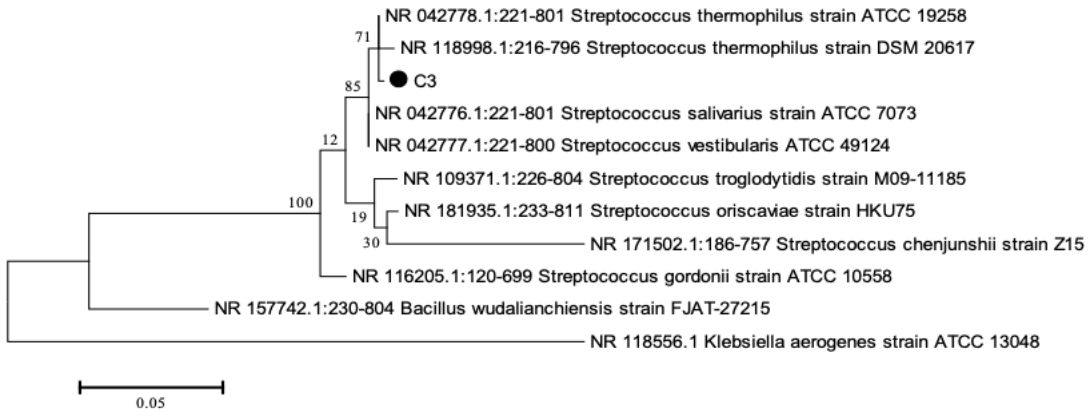
Phylogenetic analysis based on 16S rRNA gene sequences confirmed the taxonomic position of the isolates (Figure 3).



C1 - The degree of homology with the closest Lactiplantibacillus sp. strains was 100.00%.



C2 - The degree of homology with the closest strain, NR 074957.1:63-798 *Leuconostoc mesenteroides* strain ATCC 8293, was 100.00%.



C3 - The degree of homology with the closest relative, *Streptococcus thermophilus* strain DSM 20617 (NR 118998.1:216-796), was 99.31%.

Figure 3 Phylogenetic tree based on 16S rRNA gene sequences

Phylogenetic analysis revealed that isolates C1, C2, and C3 clustered within distinct and well-supported clades corresponding to the genera *Lactiplantibacillus, Leuconostoc*, and *Streptococcus,* respectively. The constructed phylogenetic tree confirmed their taxonomic placement and demonstrated clear separation from related reference strains, with bootstrap support values indicating reliable clustering.

Table 2

Acidifying ability of the strains during cultivation in growth medium

No.	Isolates	pH			Titratable acidity (°T)		
		0 h	24 h	48 h	0 h	24 h	48 h
1	*Lactiplantibacillus sp.*	5.10±0.05	4.40±0.04	4.00±0.03	58.00±0.05	70.00±0.50	85.00±0.50
2	*Leuconostoc mesenteroides*	4.91±0.06	4.20±0.05	3.90±0.04	67.00±0.05	80.00±0.60	100.00±0.50
3	*Streptococcus thermophilus*	4.83±0.04	4.10±0.03	3.90±0.03	91.00±0.04	105.00±0.90	120.00±0.50

Lactiplantibacillus sp. decreased the pH from 5.10 ± 0.05 to 4.00 ± 0.03, accompanied by an increase in titratable acidity from 58.00 ± 0.05 to 85.00 ± 0.50 °T. *Leuconostoc mesenteroides* reached a final pH of 3.90 ± 0.04 with a titratable acidity of 100.00 ± 0.50 °T. The highest acidifying ability was observed in *Streptococcus thermophilus*, which after 48 hours exhibited a pH of 3.90 ± 0.03 and titratable acidity of 120.00 ± 0.50 °T.

The results of the inhibitory activity of cell-free supernatants of lactic acid bacteria are presented in Table 3 and Figure 4.

Table 3

Inhibition zones (mm) produced by cell-free supernatants of LAB isolates (agar well diffusion assay)

No.	Isolates	*Salmonella enteritidis* NT, mm	*Staphylococcus aureus* 16, mm	*Escherichia coli NT,* mm
1	*Lactiplantibacillus sp.*	21.0±2.0	<1	<1
2	*Leuconostoc mesenteroides*	17.3±0.7	<1	<1
3	*Streptococcus thermophilus*	20.5±0.5	<1	<1

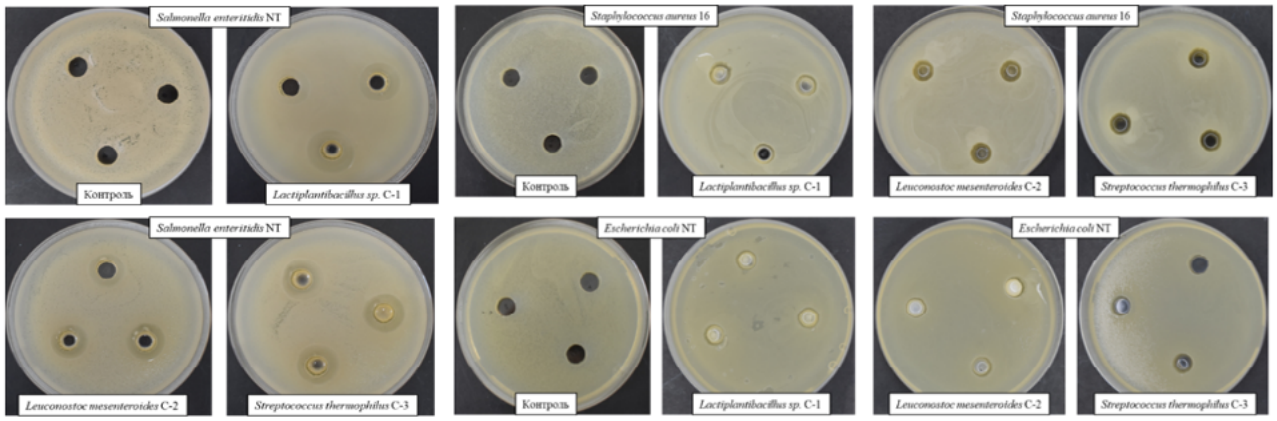


Figure 4. Inhibition zones produced by cell-free supernatants of isolated lactic acid bacteria against indicator test cultures using agar well diffusion assay.

All tested cell-free supernatants showed inhibitory activity against *Salmonella enteritidis* NT, with inhibition zones ranging from 17 to 21 mm depending on the isolate. No consistent inhibition was observed against *Staphylococcus aureus* or *Escherichia coli* under the experimental conditions used.

No further interpretation regarding the mechanism of inhibition can be made in the absence of neutralized, heat-treated, or enzyme-treated supernatant controls; therefore, the observed effects are reported strictly as inhibitory activity of crude culture supernatants.

Discussion

The results of this study confirm the dominance of lactic acid bacteria (LAB) in the microbial community of camel milk cheese, in agreement with current concepts of spontaneous fermentation in traditional dairy products. Previous metagenomic and culture-dependent studies have reported the frequent occurrence of LAB belonging to the genera *Lactiplantibacillus sp.*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus* in fermented dairy matrices, including camel milk and other animal-derived products [15, 16]. These findings align with previous reports identifying these species as dominant members of LAB communities during dairy fermentation [17, 18].

It should be noted that the diversity of LAB in camel milk products is highly variable and depends on geographic origin, raw material quality, and fermentation conditions. In addition to the dominant genera identified in this study, other LAB such as *Lentilactobacillus kefiri, Lactobacillus kefiranofaciens*, and *Bifidobacterium* spp. have been reported in fermented camel milk products (shubat), highlighting the complexity of these microbial ecosystems [19]. This emphasizes the need for in-depth strain-level and molecular approaches for comprehensive characterization of camel milk microbiota.[20].

L. plantarum displayed high adaptability to non-standard dairy matrices and contributed to flavor and texture development through proteolytic and aroma-forming activities [21, 22]. In the present study, all isolates demonstrated acidification ability in a MRS broth. The observed increase in titratable acidity from 58 ± 0.05°T to 85 ± 0.5°T over 48 h, which is comparable to reported values (~75-90°T) in milk fermented with *L. plantarum* and related LAB, reflecting efficient lactose metabolism and acid production [23,24]. This proteolytic activity supports the generation of bioactive peptides, including potential ACE-inhibitory compounds, indicating the functional food potential of camel milk cheeses [25, 26]. However, due to the use of cow milk as a model substrate, direct extrapolation of these results to camel milk fermentation systems is not possible.

The acidifying capacity of *S. thermophilus* (final pH 3.9 ± 0.03 and titratable acidity 120 ± 0.5°T after 48 h) is consistent with previous studies reporting pH values of 4.2-4.6 and titratable acidity between 77 and 104°T within 24 h of fermentation in similar dairy systems [17,27]. These results demonstrate robust metabolic activity of *S. thermophilus* in camel milk, despite the inhibitory components present in this matrix.

The roles of *L. mesenteroides* and *L. plantarum* as adjunct cultures enhancing flavor and bioactive compound formation are well documented. These species contribute to diacetyl, organic acid, and volatile metabolite production [19,25]. However, functional profiles vary considerably between strains, affecting flavor metabolite synthesis, exopolysaccharide production, and antioxidant capacity [19].

All LAB isolates inhibited *Salmonella enteritidis*, forming growth inhibition zones of 17–23 mm, comparable to previously reported ranges of 7-22 mm in LAB from camel milk [28]. In contrast, no inhibition was observed against *Staphylococcus aureus* and *Escherichia coli*, reflecting strain- and species-specific antimicrobial properties and medium-dependent effects, as reported in prior studies [21,23,28]. The antimicrobial activity of LAB is generally associated with the production of organic acids, hydrogen peroxide, bacteriocins, and other antimicrobial metabolites, although the contribution of individual factors varies among strains and target microorganisms [17,27]. Since neutralized, heat-treated, and enzyme-treated supernatants were not included in the present study, the observed inhibitory activity cannot be attributed to any specific antimicrobial mechanism or metabolite. The selective inhibition pattern observed may reflect differences in the susceptibility of the indicator microorganisms as well as the physicochemical properties of crude culture supernatants and the limitations of agar diffusion assays.

Recent research also emphasizes that functional properties of LAB – including antioxidant activity, stress tolerance, and probiotic potential – differ significantly among strains, which is crucial when selecting starter cultures for targeted technological and functional traits in fermented products [17,19]. Overall, these findings support the use of autochthonous LAB for developing starter cultures adapted to the protein composition and physicochemical properties of camel milk [29]. Industrial applications should involve further strain-level characterization, including evaluation of probiotic properties, bacteriocin production, and bioactive peptide synthesis, to ensure product safety and fermentation stability [23,25,30].

Conclusion

In this study, autochthonous isolates of lactic acid bacteria were isolated and characterized from cheese produced from camel milk. Based on 16S rRNA gene sequence analysis, the *Lactiplantibacillus sp.*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus*. All strains demonstrated the ability to coagulate milk and exhibited pronounced acidification activity, with *S. thermophilus* showing the highest acidification capacity under the conditions tested. In addition, the cell-free culture supernatants inhibited the growth of *Salmonella enteritidis,* while no inhibitory activity was detected against *Staphylococcus aureus* or *Escherichia coli.*

The obtained results indicate that the isolated autochthonous LAB possess technological properties relevant to dairy fermentation and are promising candidates for the future development of specialized starter cultures for traditional and artisanal camel milk cheese production. Nevertheless, further investigations, including comprehensive safety assessment, detailed technological characterization, and validation in camel milk fermentation systems, are necessary before their industrial application.

Author Contributions

G.E.A., A.Zh., U.A.A. – conceptualization; G.E.A., A.Zh.A. – data curation; G.E.A., U.A.A., A.A.A. – formal analysis; G.E.A. – writing-original draft preparation; G.E.A., U.A.A. – writing-review & editing; G.E.A., U.A.A. – supervision. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant number AP26199549).

Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Түйе сүтінен жасалған ірімшіктен автохтонды сүтқышқылды бактерияларды бөліп алу және оларды арнайы ашытқы дақылдарын әзірлеуде қолдану мүмкіндіктерін сипаттау

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Аңдатпа. Түйе сүті өзінің жоғары тағамдық құндылығына байланысты ірімшік өндіруге перспективалы шикізат болып табылады. Алайда оны өңдеу сүтқышқылдық реакцияның баяу жүрісі мен ерекше ақуыз құрамымен шектеледі, бұл сиыр сүтіне арналған дәстүрлі ашытқы дақылдарының тиімділігін төмендетеді. Осы зерттеудің мақсаты – түйе сүтінен жасалған ірімшіктен автохтонды сүтқышқылды бактерияларды (СҚБ) бөліп алу және оларды арнайы ашытқы дақылдарында қолдану үшін технологиялық әлеуетін бағалау болды. Микроорганизмдерді селективті ортада өсіру арқылы бөліп алып, кейін тазарту жүргізілді, бастапқы идентификация морфологиялық, Грам бойынша бояу және культуралық сипаттамаларға негізделді. Қышқылдық қабілет pH төмендеуі және титрленетін қышқылдық арқылы бағаланды, ал антагонистік белсенділік тест-дақылдарға қатысты

анықталды. Үш СҚБ штаммы бөлініп, олар *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides* және *Streptococcus thermophilus* ретінде анықталды. Барлық изоляттар грампозитивті болып, 48 сағаттық инкубация барысында айқын қышқыл түзуші белсенділік көрсетті. *Streptococcus thermophilus* ең жоғары ферментативті белсенділікке ие болып, pH мәні 3,9 және титрленетін қышқылдық 120°Т жетті. *Salmonella enteritidis*-ке қатысты селективті антагонистік әсерлер анықталды, ал *Staphylococcus aureus* және *Escherichia coli*-ге қарсы ингибирлеуші белсенділік байқалған жоқ. Алынған нәтижелер түйе сүтіне бейімделген автохтонды сүтқышқылды бактериялардың елеулі технологиялық әлеуетке ие екенін және оларды түйе сүті ірімшігін өндіру үшін аймақтық бағытталған ашытқы дақылдарын әзірлеуде перспективалы кандидат ретінде қарастыруға болатынын көрсетеді.

Түйін сөздер: түйе сүтінен жасалған ірімшік, сүтқышқылды бактериялар, автохтонды штаммдар, морфология, антагонистік қасиеттер

Выделение и характеристика автохтонных молочнокислых бактерий из сыра из верблюжьего молока для разработки заквасочных культур

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Аннотация. Верблюжье молоко представляет собой перспективное сырьё для сыроделия благодаря высокой пищевой ценности; однако его переработка ограничивается замедленной кинетикой кислотообразования и специфическим белковым составом, что снижает эффективность традиционных заквасочных культур, разработанных для коровьего молока. Целью настоящего исследования являлось выделение автохтонных молочнокислых бактерий (МКБ) из сыра из верблюжьего молока и оценка их технологического потенциала для применения в специализированных заквасочных культурах. Микроорганизмы выделяли путём культивирования на селективных средах с последующей очисткой, предварительную идентификацию проводили на основе морфологических, грам-окрашивающих и культуральных характеристик. Кислотообразующую способность оценивали по снижению pH и титруемой кислотности, антагонистическую активность – по отношению к тест-культурам. Были выделены три штамма МКБ, идентифицированные как *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides* и *Streptococcus thermophilus*. Все изоляты являлись грамположительными и проявляли выраженную кислотообразующую активность в течение 48 ч инкубации. *Streptococcus thermophilus* характеризовался наибольшей ферментативной активностью, достигая значения pH 3,9 и титруемой кислотности 120°Т. Отмечены селективные антагонистические эффекты в отношении *Salmonella enteritidis*, при этом ингибирующая активность в отношении *Staphylococcus aureus* и *Escherichia coli* не выявлена. Полученные результаты свидетельствуют о том, что автохтонные молочнокислые бактерии, адаптированные к верблюжьему молоку, обладают значительным технологическим потенциалом и могут рассматриваться как перспективные кандидаты для разработки регионально ориентированных заквасочных культур для производства сыра из верблюжьего молока.

Ключевые слова: сыр из верблюжьего молока, молочнокислые бактерии, автохтонные штаммы, морфология, антагонистические свойства

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