2025, Scienceline Publication
World's Veterinary Journal
*World Vet J*, 15(2): 248-255.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2025.wvj27
PII: S232245682500027-15

# Identification and Characterization of Lactic Acid Bacteria Isolated from Fermented Camel Milk in Kenya

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ORIGINAL ARTICLE
Received: April 02, 2025
Revised: May 06, 2025
Accepted: May 29, 2025
Published: June 25, 2025

**ABSTRACT**

Somali camel milk is a vital dietary component for pastoral communities in Kenya's arid and semi-arid lands (ASAL) such as Baringo, Marsabit, Isiolo, Madera, Wajir, and Garissa, due to its high nutritional value. Camel milk provides approximately 50 kcal per 100 mL and contains nearly 2-3% protein, 3-4% fat, and 4-5% carbohydrates. It is also a source of essential minerals (calcium, magnesium, potassium, and iron) and vitamins (C, B, and A). Traditional fermenting of camel milk is practiced by pastoral communities to enhance its nutritional content, digestibility, and shelf life. The purpose of this study was to isolate and identify the antimicrobial potential of *lactic acid* bacteria (LAB) from the Somali breed of camel milk sourced from Mombasa, Isiolo, and Kajiado counties in Kenya. LAB from the milk samples was cultured on de Man, Rogosa, and Sharpe (MRS) agar. Based on morphological and biochemical characteristics, twenty-three cocci-shaped LAB isolates were selected for further analysis. All of the LAB isolates were positive for Gram staining and Triple Sugar Iron tests but negative for the catalase test. The LAB isolates showed no hemolytic activity and inhibited the growth of *Staphylococcus aureus* and *Escherichia coli.* One isolate also inhibited the growth of *Candida albicans*. Molecular analysis targeting *16S rRNA* identified *Enterococcus faecium, Enterococcus durans, Leuconostoc mesenteroides and leuconostoc pseudomesentroides* among the isolates. The findings revealed fermenting and antimicrobial properties of LAB isolated from fermented milk derived from the Somali breed of camel. These LAB isolates were found to be potentially useful for the development of starter cultures in the production of probiotics.

**Keywords:** Antimicrobial activity, Camel milk, *Enterococcus* fermentation, Lactic acid bacteria, *Leuconostoc*

## INTRODUCTION

More than 11 million camels inhabit the Arid and Semi-Arid Lands (ASAL) of East Africa (Muuo, 2019). In Kenya, pastoral communities, including the Somali, Gabbra, Rendille, and Turkana, raise camels primarily for milk and meat consumption. Turkana, Baringo, Marsabit, Wajir, Mandera, Isiolo, Garissa, and Tana River counties are home to pastoral communities such as the Somali, Gabbra, Rendille, and Turkana (Isako and Kimindu, 2019). Pastoralists in Kenya's ASAL regions manage a large population (70%) of livestock camels, cattle, goats, and sheep, leading to a continuous rise in livestock production, such as milk and meat (Muuo, 2019). Kenya is the world's top producer of camel milk, producing approximately 1.165 million liters annually (Oselu et al., 2022).

The increase in camel milk production is attributed to improved camel-rearing practices, and better production methods supported by different organizations such as the Ewaso Nyiro Development Authority, FARM lands, Lands Resource Management, Arid Lands, CARE Kenya, Kenya Agricultural and Livestock Research Organization (KALRO), USAID, and Food for the Hungry (FH). These organizations promote camel rearing among Kenyan pastoralist communities, alongside the rising demand for camel milk in Kenya (Oselu et al., 2022).

Camel milk is valued for its high nutritional content, comprising approximately 88.1% water, 3.1% protein, 3.5% fat, 4.4% lactose, and 0.79% ash. It is considered superior to bovine milk and comparable to human milk in terms of its nutritional profile (Isako and Kimindu, 2019). Notably, camel milk is rich in hydrolyzed β-caseins, lacks β-lactoglobulin (a common allergen), and contains an optimal balance of essential amino acids such as leucine, isoleucine, valine, and lysine for human diets (Ho et al., 2022). It also has lower levels of sugar and unhealthy fats such as cholesterol. Camel milk in all breeds has a high concentration of insulin and vitamin C, which are beneficial to human health (Ho et al., 2022). Camel milk is further enriched with high levels of minerals such as calcium, magnesium, potassium, Phosphorus, as well as proteins with anti-bacterial, anti-diabetic, and anti-cancer properties, including immunoglobulins, lactoferrin, lysozyme, and Lactoperoxidase (Ho et al., 2022).

Fermentation has long been practiced to improve the nutritional quality, digestibility, and shelf life of foods (Motarjemi, 2002). In pastoral societies, fermented camel milk is a primary source of food consumed. Lactic Acid bacteria (LAB) play a crucial role in preparing the diet of fermented camel milk, among many other dairy products, for instance, cheese, yogurt, and Kefir. Lactic Acid bacteria are Generally Recognized as Safe (GRAS) bacteria that play a crucial role in the fermentation and preservation of food and feed, whether they are introduced as starter cultures under controlled conditions or as part of the natural microbiota (Yang et al., 2012).



To cite this paper: Gulled MA, Kariuki DW, and Omolo KM (2025). Identification and Characterization of *Lactic Acid* Bacteria Isolated from Fermented Camel Milk in Kenya. *World Vet. J.*, 15(2): 248-255. DOI: https://dx.doi.org/10.54203/scil.2025.wvj27

A range of food sources, such as milk, dairy products, meat, meat products, cereals, and plants, contain Lactic Acid Bacteria (Daouadji et al., 2020). Lactic acid bacteria metabolism generates a number of substances, some of which have been shown to have antimicrobial properties, including organic acids, di-acetyl, hydrogen peroxide, acetoin, 2,3-butanediol, acetaldehyde, benzoate, bacteriocin, reuterin, and others (Daouadji et al., 2020). Some LAB strains possess narrow and broad spectrums and can inhibit bacteria of the same or different species (Yang et al., 2012). Lactic acid bacteria are known to express bacteriocins that contribute to food preservation. Therefore, LABs have received a lot of attention as biological preservatives (Yang et al., 2012). This study aimed to isolate and characterize LAB from fermented milk of the Somali breed of camels sourced from three counties of Mombasa, Isiolo, and Kajiado in Kenya.

## MATERIALS AND METHODS

### Ethical approval

All procedures in the present study were conducted in accordance with standard microbiological practices.

### Sampling of fermented camel milk

Fermented milk from Somali breed camels was collected from Mombasa, Isiolo, and Kajiado counties in Kenya. A total of seven samples (three from Isiolo, two from Mombasa, and two from Kajaido) were aseptically collected using sterile Falcon tubes and then stored at 4°C in the PAUSTI laboratory (Kenya).

### Isolation of Lactic acid bacteria

To isolate LAB from fermented camel milk, nine mL of sterile normal saline was mixed with one mL of fermented camel milk. The contents were serially diluted from $10^{-1}$ to $10^{-7}$ using pipettes and culture bottles (Ismail et al., 2018). Thereafter, 0.1 ml of the subsequent diluent was applied onto the de Man, Rogosa, and Sharpe (MRS) agar surface (HiMedia Ltd., Mumbai, India, Cat. M641) under normal room temperature (25°C) by using the sterile microbiological hood (Nyamaifofe et al., 2024). Following inoculation, the plates were incubated for 48 hours at 37°C under anaerobic conditions (Mulaw et al., 2019). The colonies formed were streaked onto fresh MRS agar (HiMedia Ltd., Mumbai, India, Cat. M641) using a sterile inoculating needle for further purification. The isolates were then incubated (Thermo Fisher Scientific, USA) for 24 to 48 hours at 37°C (Ismail et al., 2018). These colonies were characterized morphologically and biochemically, and only the catalase-negative and Gram-positive bacteria were selected. The selected isolates were maintained in 20% v/v glycerol at -20°C awaiting further analysis (Abdel Tawab et al., 2023).

### Morphological identification by gram staining

Once a pure culture was established on the Petri dish, observations of colony morphology, such as shape and color, were among the visual elements recorded under the light microscope (Dragonfly 200, UK). A morphological and cultural analysis was conducted using the Gram staining technique described by Goa et al. (2022).

### Biochemical identification

#### *Catalase test*

Catalase production was monitored using a clean microscopic slide. On the slide, a loopful of bacterial culture was mixed with a 3% H2O2 solution (Aditya Birla Chemicals, India) (Royal, 2012).

#### *CO2 gas production from glucose fermentation*

Gas production from glucose was determined in a modified MRS broth to determine the fermentative characteristics of LAB isolates. A total of 9 ml of MRS broth containing 1% w/v glucose was prepared and inoculated with an overnight colony culture of LAB. The contents were incubated in inverted Durham tubes at 37°C for one week, and the production of acid and gas bubbles in Durham tubes was monitored (Mulaw et al., 2019).

#### *Test for triple sugar iron agar*

The pure culture of each isolate was introduced into Triple Sugar Iron (TSI) agar using an inoculation loop, with a deep and slanted surface. The tubes were incubated at 37°C, and color changes in the medium were monitored during the incubation period of seven days (Goa et al., 2022).

#### *Hemolytic activity test*

Blood agar base (HiMedia Laboratories, India) containing 5% (w/v) sheep blood was used to assess the hemolytic activity of the isolates. The positive control in this test was *Staphylococcus aureus* (*S. aureus*). Hemolytic activity was assessed after 48 hours of incubation at 37°C (Asadi et al., 2022).

### Screening of antimicrobial activity

#### *The indicator strains*

The antimicrobial activity of LAB isolates was evaluated using *S. aureus*, *Escherichia coli* (*E. coli*), and *Candida albicans* (*C. albicans*) indicator strains obtained from the Institute for Biotechnology Research (IBR) at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja, Kenya.

***Preparation of cell-free supernatant***

An aliquot of the LAB colony was inoculated into 10 ml of the MRS broth, and the contents were incubated at 37°C for 48 hours. After incubation, the bacterial culture was centrifuged for 5 minutes at 13000 rpm to produce a Cell-Free Supernatant (CFS), which was then filtered through 0.20 µm hole size filters (Thermo Fisher Scientific, UK) (Goa et al., 2022).

***Antimicrobial activity of the cell-free supernatant***

Antibacterial activity of CFS was determined using the agar-well diffusion method (Balouiri et al., 2016). Normal saline was used to suspend indicator strains of microbes, and a 0.5% McFarland standard was used to regulate the cell density. The contents were seeded on pre-dried nutrient agar plates (HiMedia Laboratories, India). Wells of 6 mm diameter were made using a core borer, and 100 µL of CFS from each LAB strain was separately placed into the wells. The plates were prepared in triplicate and incubated at 37$^{o}$C for 24 hours. Antimicrobial activity was determined by measuring the diameter of the inhibition zone (Balouiri et al., 2016).

**Molecular identification of isolated lactic acid bacteria strains**

***Amplification and sequencing of the 16S rRNA gene***

Following the manufacturer's instructions, the Zymo Bacterial Genomic DNA extraction Kit (USA) was used to extract genomic bacterial DNA from LAB grown overnight in MRS broth at 37°C. To confirm DNA integrity, the sample was run in a 1% agarose gel electrophoresis (Qiagen, Germany) (Nyamaifofe et al., 2024). A Nano Drop spectrophotometer (PCR Max Lambda, Staffordshire, UK) was used to determine the concentration and purity of the DNA. To amplify LAB *16S rRNA*, bacterial universal primers 1492 R: 5-GGT TAC CTT GTT ACG ACT T-3 and 27 F: 5-AGA GTT TGA TCC TGG CTC AG-3 were used (Aboubacar et al., 2021). Polymerase Chain Reaction (PCR) was carried out using a 50 µL reaction that contained 25 µL AccurisTM Taq DNA Polymerase Master Mix and Master Mix Red, 2 µL of forward primer, 2 µL of reverse primer, and 19 µL of RNase-free water. Before amplification, the contents were mixed and subjected to the following PCR conditions, including primer annealing at 57°C for 30 seconds, primer extension at 72°C for 1.5 minutes, 35 cycles at 95°C for 30 seconds, and initial denaturation of the target DNA at 95°C for 5 minutes. After 5 minutes of elongation at 72°C and a four-minute cooling period, the PCR was stopped. A ProFlex PCR system thermo-cycler was used for the reactions. A 2% (w/v) agarose gel stained with Gel Purple (Inqaba Biotech, South Africa) was used for the electrophoresis, and the results were viewed using the Uvitec Cambridge gel documentation system (Uvitec, UK) to confirm the size and presence of the *16S rRNA* gene. The 1490 bp PCR products were found using a 1 kb DNA ladder. Sanger sequencing was conducted on the purified amplified products by Human Genomics, Macrogen, Singapore (Aboubacar et al., 2021).

***Phylogenetic analysis of lactic acid bacteria***

The quality of the *16S rRNA* gene sequences from LAB isolates was assessed using the SNAP GENE software. To detect closely similar bacterial *16S rRNA* gene sequences, test sequences were compared with applicable sequence data from the National Centre for Biotechnology Information (NCBI), applying nucleotide BLAST.

**Statistical analysis**

Triplicate data on inhibition zone diameters (antimicrobial activity) were analyzed using Microsoft Excel. The mean and standard deviation were computed using Microsoft Excel's built-in formulas for descriptive statistics.

## RESULTS

**Morphological and biochemical characteristics of the isolates**

A total of 23 LAB were obtained from fermented milk sourced from the Somali breed of camels. The colonies appeared creamy to white. All the 23 bacterial isolates were Gram-positive, cocci-shaped, and catalase-negative. They all produced acid during fermentation, as indicated by the medium turning yellow on TSI agar. The obtained results are summarized in Table 1.

**Hemolytic activity**

Unlike the positive control *Staphylococcus aureus*, which exhibited hemolytic activity, all LAB isolates tested negative for hemolytic activity.

**Screening of antimicrobial activity**

The cell-free supernatants (CFS) from the 23 LAB isolates were assessed for antimicrobial activity against three indicator strains: C. *albicans, S. aureus*, and *E. coli* in an agar well diffusion assay. All isolates exhibited inhibitory activity against *S. aureus* and *E. coli*. One isolate (1.3.6) was active against *C. albicans* (Table 2).

**Molecular identification of bacterial isolates**

The *16S rRNA* gene was sequenced, and the phylogenetic analysis was carried out. Two groups of bacteria, namely *Enterococcus and Leuconostoc*, were identified in the present study. Among the isolates, 20 clustered with *L. mesenteroides* and *L. pseudomesentroide*, while the remaining 3 isolates showed a sequence similar to *E. faecium* and *E. durans*. *Escherichia coli* was included as an outgroup to root the tree. This helped to determine the root of a phylogenetic tree and establish the direction of evolution. These isolates represented a distinct evolutionary lineage within the tree, as shown in Figure 1.

**Table 1.** Morphological and biochemical characteristics of the lactic acid bacteria isolates

| SN | Isolate code | Gram staining | Microscopic morphology | Catalase | TSI | $CO_2$ production |
|---|---|---|---|---|---|---|
| 1 | I.1.1 | + | Cocci | - | + | + |
| 2 | I.1.5 | + | Cocci | - | + | + |
| 3 | I.2.1 | + | Cocci | - | + | - |
| 4 | I.2.3 | + | Cocci | - | + | + |
| 5 | I.2.5 | + | Cocci | - | + | - |
| 6 | I.3.2 | + | Cocci | - | + | + |
| 7 | I.3.3 | + | Cocci | - | + | + |
| 8 | I.3.5 | + | Cocci | - | + | + |
| 9 | 1.3.6 | + | Cocci | - | + | + |
| 10 | M.4.1 | + | Cocci | - | + | + |
| 11 | M.4.2 | + | Cocci | - | + | + |
| 12 | M.4.3 | + | Cocci | - | + | + |
| 13 | M.5.1 | + | Cocci | - | + | + |
| 14 | M.5.2 | + | Cocci | - | + | + |
| 15 | M.5.3 | + | Cocci | - | + | - |
| 16 | M.5.4 | + | Cocci | - | + | + |
| 17 | M.5.5 | + | Cocci | - | + | + |
| 18 | K.6.1 | + | Cocci | - | + | + |
| 19 | K.6.4 | + | Cocci | - | + | + |
| 20 | K.6.5 | + | Cocci | - | + | + |
| 21 | K.7.2 | + | Cocci | - | + | + |
| 22 | K.7.4 | + | Cocci | - | + | + |
| 23 | K.7.5 | + | Cocci | - | + | + |

SN: Serial number, I: Isiolo, M: Mombasa, K: Kajiado, TSI: Triple sugar iron agar, $CO_2$: Carbon dioxide, +: Positive test, -: Negative test

**Table 2.** Antimicrobial activity of lactic acid bacteria against pathogenic microbes

| Isolates | *E. coli ATCC 25922* | *S. aureus ATCC 43300* | *C. albicans ATCC 64124* |
|---|---|---|---|
| I.1.1 | 15.5 ± 0.707 | 20.05 ± 0.070 | - |
| I.1.5 | 18 ± 0.00 | 19.25 ± 0.35 | - |
| I.2.1 | 20.05 ± 0.070 | 20.15 ± 0.07 | - |
| I.2.3 | 20.25 ± 0.070 | 16.25 ± 0.35 | - |
| I.2.5 | 15.5 ± 0.35 | 20.1 ± 0.00 | - |
| I.3.2 | 20.05 ± 0.07 | 19 ± 0.00 | - |
| I.3.3 | 20.05 ± 0.35 | 20.05 ± 0.07 | - |
| I.3.5 | 20 ± 0.00 | 20.1 ± 0.00 | - |
| 1.3.6 | 22 ± 0.00 | 21 ± 0.00 | 17 ± 0.00 |
| M.4.1 | 20.2 ± 0.14 | 18.25 ± 0.35 | - |
| M.4.2 | 20 ± 0.0.00 | 19.25 ± 0.35 | - |
| M.4.3 | 20.25 ± 0.07 | 20 ± 0.00 | - |
| M.5.1 | 15.3 ± 0.42 | 20 ± 0.00 | - |
| M.5.2 | 20 ± 0.00 | 20.05 ± 0.07 | - |
| M.5.3 | 15.25 ± 0.35 | 20.25 ± 0.07 | - |
| M.5.4 | 20.5 ± 0.14 | 20 ± 0.00 | - |
| M.5.5 | 14.5 ± 0.70 | 19.25 ± 0.035 | - |
| K.6.1 | 20 ± 0.00 | 20.15 ± 0.07 | - |
| K.6.4 | 15.5 ± 0.70 | 20.1 ± 0.14 | - |
| K.6.5 | 12.3 ± 0.42 | 20.25 ± 0.07 | - |
| K.7.2 | 12 ± 0.00 | 20.5 ± 0.00 | - |
| K.7.4 | 19.25 ± 0.35 | 20.25 ± 0.07 | - |
| K.7.5 | 15 ± 0.00 | 20.1 ± 0.00 | - |

Values are Mean ± Standard Deviation. Each value represents the average of three readings. *E. coli*: *Escherichia coli*, and *C. albicans*: *Candida albicans, S. aureus*: *Staphylococcus aureus*

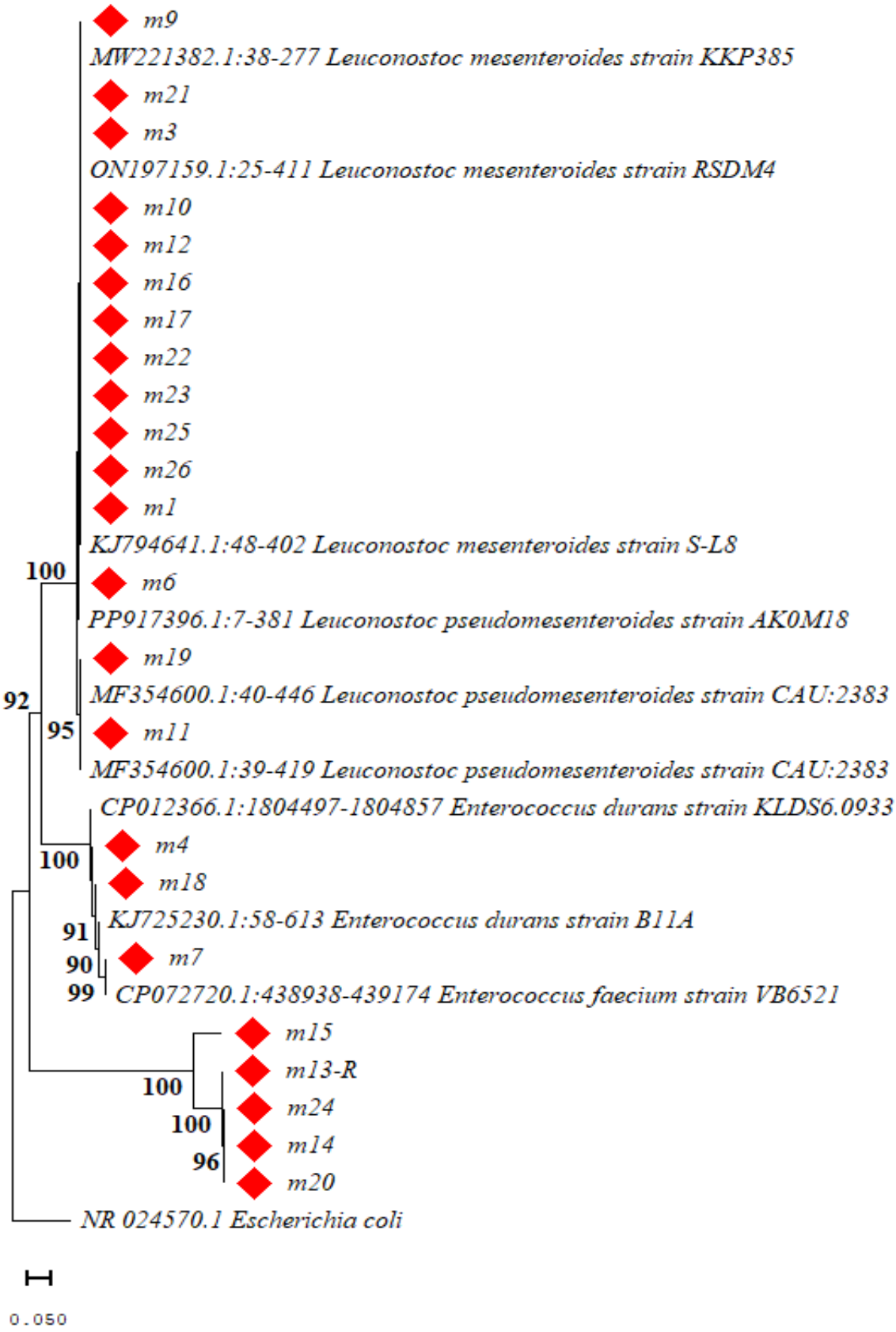


**Figure 1.** The phylogenetic tree demonstrated the relatedness of *Lactic acid* bacteria isolates. Red diamonds represent *Lactic acid* bacteria isolates, primarily identified as *Leuconostoc* and *Enterococcus* species. *Escherichia coli* (NR_024570.1) was included as an outgroup to root the tree. Numbers (100,99,96,95,92,91 and 90) represent bootstrap values, which indicate the confidence level of each branch; higher values (high bootstrap value, e.g., above 70, show strong support for the grouping of the isolates).

## DISCUSSION

In the present study, 23 bacterial isolates from fermented milk sourced from the Somali breed of camels were identified and characterized. Morphological analysis revealed cocci and creamy to white colonies, consistent with Lactic acid bacteria traits commonly resident in dairy products (Haro et al., 2020). Biochemical analysis showed the presence of gram-positive, catalase-negative, and triple sugar iron fermentation, revealing the presence of bacteria with the ability to ferment milk and other dairy products. A total of 23 LAB isolates were tested for blood hemolytic activity to evaluate their safety for potential use in probiotic and starter culture development. The bacteria that exhibit hemolytic activity pose health risks related to bacteremia or anemia in consuming communities. In the present study, none of the isolates displayed hemolytic activity, indicating their potential safety for use as probiotic or starter culture candidates. This finding aligns with previous reports by Karnwal and Malik (2020) and Asadi et al. (2022), who also observed an absence of hemolytic activity in LAB strains isolated from fermented products. The absence of hemolysis confirmed the safety of the bacteria isolated from fermented milk sourced from the Somali breed of camels, and this supports the suitability of the bacterial isolates for potential use in the development of starter cultures and probiotics (Nyamaifofe et al., 2024). Lactic acid bacteria from camel milk generate different antimicrobial compounds, such as hydrogen peroxide, organic acids, and bacteriocins, which inhibit the growth of several pathogenic bacteria, as stated in a former study (Nawaz et al., 2024). The Lactic acid bacteria strains improve digestion, strengthen the immune system, and provide resistance to many ailments by balancing the intestinal microbial community and preventing harmful bacteria from adhering to the intestinal epithelium (Nawaz et al., 2024).

In the current study, all the bacterial isolates showed antibacterial activity against *S. aureus* and *E. coli*, whereas only one isolate (1.3.6) inhibited the growth of *C. albicans*. The observed antibacterial activity of the tested isolates against *E. coli* and *S. aureus* aligns with previous findings, which supports the suitability of isolates for enhancing food safety and preservation (Rezaei et al., 2020). The apparent lack of antifungal activity against *C. albicans* by the majority of the Lactic acid bacteria isolates (22 isolates) suggests that most of the tested LAB strains exhibit more potent activity against bacterial rather than fungal pathogens (Nafissa et al., 2022).

Molecular characterization revealed that the majority of the isolates (20 out of 23) belonged to *Leuconostoc*, particularly the subspecies *Leuconostoc mesenteroides* and Leuconoctoc *pseudomesenteroides*. Three isolates were found to belong to *Enterococcus durans* and *Enterococcus facecium* spp. These findings are consistent with previous studies reporting *Leuconostoc* dominance in camel milk and other dairy products (Tan et al., 2010; Mokdad et al., 2020). *Leuconostoc mesenteroides* is particularly important due to its role in dairy fermentation, where it produces bacteriocins and converts citrate into diacetyl and acetoin, enhancing the flavor and texture of fermented products (Lore et al., 2005). The limited presence of *Enterococcus* may reflect the unique microbial ecology of camel milk, which favors *Leuconostoc* (Khedid et al., 2009; Rezaei et al., 2020). The findings from the present study support the presence of LAB in fermented camel milk products, such as susac in Kenya, which has been associated with improved safety, extended shelf life, and enhanced sensory qualities of the products (Lore et al., 2005). This finding demonstrates the unique antimicrobial and fermentative characteristics of the Lactic acid bacteria (Lore et al., 2005).

The antimicrobial properties, safety profile, and functional characteristics of *Leuconostoc mesenteroides* make it a useful candidate in the fermentation of camel milk. Because of its ability to inhibit foodborne pathogens, generate bacteriocins, and improve the organoleptic qualities of fermented camel milk, LAB plays a crucial role in enhancing the safety and quality of dairy products. Although less dominant, the presence of *Enterococcus* species contributes to microbial diversity and may also support pathogen inhibition, reinforcing the potential use of LAB from camel milk in the development of probiotics and starter cultures for fermented dairy products (Lore et al., 2005).

## CONCLUSION

The present study identified *Leuconostoc* spp. and *Enterococcus* spp. (duran and feacium) from fermented milk of the Somali breed of camels. Morphological and biochemical characterization of these strains of bacteria showed that they were gram-positive cocci, catalase negative, and generally capable of fermenting sugars to produce acid. Additionally, they exhibited antimicrobial activity. These characteristics confirmed that the identified bacterial isolates are lactic acid bacteria with antimicrobial activity. The strains of Lactic acid bacteria, namely *Leuconostoc* spp. and *Enterococcus* spp., are qualified for potential use in the development of probiotics and starter cultures. Based on the results, this study recommends the use of the identified Lactic acid bacteria isolates in the production of fermented milk and other dairy products.

## DECLARATIONS

### Acknowledgments

The authors extend their sincere gratitude to the Pan African University Institute for Basic Sciences, Technology, and Innovation (PAUSTI) for providing the opportunity and support to conduct the present study. The authors would also like to acknowledge special thanks to supervisors, lecturers, and the entire faculty for their invaluable guidance and mentorship throughout the study.

**Funding**

The present study was financially supported by the African Union through the Pan African University Institute for Basic Sciences, Technology, and Innovation (PAUSTI). The authors sincerely appreciate this generous funding, which made the study possible.

**Author's contributions**

Maryan Abdirahman Gulled conceptualized the study's idea, designed the study, conducted the laboratory experiments, analyzed the data, and wrote the manuscript. Daniel Wainaina Kariuki provided overall study guidance, ensured the study followed the correct research pathway, and contributed to manuscript corrections and improvements. Kevin Mbogo Omolo conducted the data analysis, provided guidance on the appropriate methods and materials, and made critical revisions to the manuscript. All authors approved the final edition of the manuscript.

**Competing interests**

The authors have not declared any conflicts of interest.

**Ethical considerations**

All authors have thoroughly examined the manuscript for any ethical concerns, including plagiarism, research integrity, data manipulation or misrepresentation, and duplicate submission.

**Availability of data and materials**

All data obtained in the present study are relevant and have been incorporated into the published manuscript. Additional details or inquiries will be available upon reasonable request from the corresponding author.

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