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Diversity of Lactic Acid Bacteria in Senduro Goat Milk

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ABSTRACT

Goat milk is currently widely consumed by Indonesian people. The Senduro breed of goats is one of the milk-producing goats widely cultivated by farmers in Senduro District, Lumajang Regency, East Java. Based on several studies, goat milk is a habitat for lactic acid bacteria, and research has been conducted on isolating lactic acid bacteria from goat milk. This study aims to isolate lactic acid bacteria from Senduro goat milk samples characterized morphologically and biochemically. The pour plate method was used to isolate lactic acid bacteria, followed by the purification of isolates using the streak plate method. Colony characterization was carried out by observing the colonies' shape, edge, elevation, surface, and color. Gram staining, endospore staining, catalase test, and motility test were carried out to confirm lactic acid bacteria isolates. This study has successfully isolated 50 isolates of lactic acid bacteria that have Gram-positive, non-endospore, catalase-negative, and non-motile characteristics. The morphological characteristics of lactic acid bacteria isolate colonies from milk samples from the three farms showed diversity. Several isolates from the three farms showed similar characteristics. Further potential testing and identification of each lactic acid bacteria isolate is necessary to provide information regarding its roles.

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INTRODUCTION

Lactic acid bacteria (LAB) are a group of bacteria that ferment carbohydrates in food ingredients to produce lactic acid as the main fermentation product (Bintsis, 2018). Lactic acid bacteria are Gram-positive, with negative catalase, non-endospores, and non-motile bacteria (Sandi et al., 2018). Based on the final fermentation product, LAB is grouped into homofermentative and heterofermentative (Sarwar et al, 2018). Homofermentative LAB ferments glucose to produce lactic acid, while heterofermentative LAB ferments glucose to produce lactic acid, ethanol, and CO_2 (Khalid, 2011). The taxonomic reorganization of lactic acid bacteria reclassified over 300 species in 7 genera and two families into a single family, Lactobacillaceae, which now includes 31 genera (Qiao et al., 2022).

The role of LAB is essential in the food and beverage industry. Lactic acid bacteria can play a role in food biopreservation and human health, known as probiotics(Ayivi et al., 2020), and cheese making (Coelho et al., 2022). Lactic acid bacteria can produce antimicrobial compounds that can improve food safety in the form of organic acids (lactic, acetic, and formic acids) and bacteriocins that can inhibit microbial growth (Ibrahim et al., 2021). Bacteriocins can fight antibiotic-resistant bacteria due to their narrow target activity, low toxicity, and high stability and specificity (Pircalabioru et al., 2021). Several studies have shown that LAB has antiviral (Ayivi et al., 2020), antibacterial (Azhar et al., 2022) properties such as *Lactobacillus plantarum* 2C12 (Arief et al., 2012) and antifungal (Yunilas & Mirwandhono, 2018). Some LABs can produce Gamma-Aminobutyric Acid (GABA) (Yogeswara et al., 2020; (Berliyanti et al., 2020), and GABA can act as a neurotransmitter (Indrowati et al., 2015). For instance, *Levilactobacillus brevis* F064A can produce GABA-

FMJ (Fermented Mulberry Juice) (Kanklai et al., 2021). Lactic acid bacteria also play a role in producing antioxidants (Berliyanti et al., 2020).

Animal milk contains high-quality nutrients due to its high micro and macronutrient content, making it good for consumption (Raza & Kim, 2018). Goat milk is one of the animals' milks consumed by people in Indonesia. One of the goat breeds that has the potential for milk production is the Senduro goat, which is widely cultivated by farmers in Senduro District, Lumajang Regency, East Java, Indonesia. Decree of the Minister of Agriculture Number: 1055/Kpts/ SR.120/10/2014 concerning the determination of the Senduro Goat Strain states that Senduro goats are a wealth of Indonesian genetic resources (Amam et al., 2022). The community generally uses Senduro goats for their milk and meat (Prasetyo & Nurkholis, 2018). Goat milk can be consumed fresh or as fermented milk.

Several studies have shown that milk and its processed products can be a source of LAB isolates. LAB has been successfully isolated from fermented milk from Etawah goats (Febrina et al., 2019). Rahmawati et al. (2021) isolated LAB from the fresh milk of Etawa goats from Kopelma Village, Banda Aceh. Isolation of LAB from Etawah goat milk and testing its ability to produce bacteriocin has also been carried out (Fitria & Ardyati, 2014). Isolation of LAB from goat milk yogurt and testing of its antibacterial activity has been done by Saravanan et al. (2011), and isolation of LAB from Saanen goat milk kefir by (Rumaisha et al., 2020). Perin & Nero (2014) stated that isolated LAB from goat milk from a farm in Vicosa, Brazil, and tested its antimicrobial potential.

Based on the research results, goat milk can be a source for isolating LAB for further research on its potential. One of the goats cultivated in Indonesia is the Senduro goat, which produces milk. Isolating bacteria from Senduro goat milk will obtain potential LAB isolates. This research is an initial study as an effort to isolate, purify, and characterize the phenotypic LAB isolates, including colony morphology, Gram properties, catalase production, endospore formation, and motility in Senduro goat milk samples so that they can be studied further to determine their potential.

MATERIALS AND METHODS

This research is an exploratory study to isolate LAB from Senduro goat milk samples. Milk samples were taken directly from the Senduro goat farm in Senduro District, Lumajang Regency, East Java, Indonesia. Isolation and characterization of LAB were conducted at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya. The research was conducted in the following stages.

Sampling

Milk samples were taken from three goat farms, which had been collected and then put into sterile containers. The three farms used different feeds. The first farm used green fodder with additional concentrate (complete feed) and cassava dregs (gamblong). The second farm used green fodder with additional concentrate (complete feed), tofu dregs, and corn bran (tumpi). The third farm used green fodder, corn flour, and cassava.

LAB Isolation

LAB was isolated using MRS broth and Glucose Yeast Peptone (GYP) (20 g/L glucose, 10 g/L yeast extract, 10 g/L peptone, 10 g/L Na-acetate, 5 ml/L salt solution, 1000 mL aquadest) medium. Milk samples were taken as much in as 10 mL each and put into 90 mL of MRS broth medium. Milk samples in the medium were then incubated at 37°C for 24 hours. Salt solution 0.85% was used for the dilution and made by dissolving 4 g/L $MgSO_4.7 H_2O$, 2 g/L $MnSO_4.4 H_2O$, 2 g/L $FeSO_4.7 H_2O$, 2 g/L NaCl, and 1000 mL aquadest. Each sample was diluted at 10^{-1} – 10^{-7} using 0.85% NaCl solution, then poured onto a plate using MRS and GYP agar (15 g/L agar) containing 1% $CaCO_3$ and 1% goat milk in duplicate. Incubation was carried out at 37°C for 24 – 48 hours. Observations were made on bacterial colonies that formed a clear zone around the colony (Utama et al., 2018).

Purification of LAB isolates

Bacterial isolates forming clear zones around colonies and show different characteristics was purified using the streak plate method on MRS agar and GYP agar medium. Incubation was carried out at 37°C for 24 hours. The purified isolates were transferred into MRS and GYP agar slant medium using the streak method for stock culture.

Colony observation

The characteristics of bacterial colonies included colony shape, elevation, edge, surface, and colony color (Ismail et al., 2018).

Gram staining

The slide was cleaned using 70% alcohol and air-dried. The bacterial preparation was made by taking bacteria using an inoculating loop aseptically and placing them on the slide. The preparation was then fixed over a spirit burner flame. The preparation was dripped with crystal violet solution and left for 1 minute. After that, the preparation was washed with running water and dried. The preparation was then dripped with iodine solution and left for 1 minute, then washed with running water and dried. The preparation was dripped with 95% alcohol until the purple color disappeared, then washed with running water and dried. The preparation was then dripped with safranin and left for 30 seconds. The preparation was then washed with running water and dried. Bacteria are observed using a microscope. Gram-positive bacteria if the bacterial cells are purple, while Gram-negative if the bacterial cells are red (Ismail et al., 2018).

KOH test

The KOH test was performed by mixing bacterial cells with a 3% KOH solution on a glass slide. The bacterial and KOH suspensions are continuously homogenized on the glass slide. Gram-positive bacteria do not form a viscous gel or stringouts (negative-KOH reaction), while Gram-negative bacteria form a viscous gel or stringouts (Powers, 1995).

Endospore staining

The slide was cleaned using 70% alcohol and dried. The bacterial preparation was made by taking bacteria using an inoculating loop aseptically and placing them on the slide. The preparation was fixed over a spirit burner flame. The preparation was covered using filter paper, dripped with malachite green and cooled. The preparation was placed on a wire heated over boiling water for 5 minutes. The preparation was washed carefully with running water. The preparation was dripped with safranin, left for 60 seconds, then washed with running water and dried. The preparation was observed under a microscope at 1000x magnification. The test result is positive if the bacterial cells are red and the endospores are green (Ismail et al., 2018).

Catalase test

The catalase test was carried out by adding a drop of 3% hydrogen peroxide (H_2O_2) solution to the bacterial culture placed on a glass object. Observations were made on the appearance or absence of gas produced by the bacterial cells shortly after being dripped with H_2O_2 (Goa et al., 2022).

Motility test

The motility test of bacterial isolates was carried out using a Sulfide Indole and Motility (SIM) medium in a test tube. Bacterial isolates were taken using an inoculating loop, inserted perpendicularly into the SIM medium, and then incubated at 37°C for 24-48 hours (Ismail et al., 2018). Observation of the motility test was the growth of bacteria on the medium. Bacteria that only grew around the inserted location showed negative results, while bacteria that grew on the surface of the medium or spread in the medium showed positive results.

RESULTS AND DISCUSSION

The research has successfully isolated 50 bacterial isolates that are suspected to be lactic acid bacteria, characterized by being gram-positive, catalase-negative, non-motile, and non-endospore-forming. The isolation process was carried out using MRS agar and GYP agar medium to obtain as many LABs as possible from Senduro goat milk samples. Milk sampling was carried out at three farm locations. The first farm is a Senduro goat farm that uses green fodder with additional concentrate (complete feed) and cassava dregs (*gamblong*), and the second farm uses green fodder with additional concentrate (complete feed), tofu dregs, and corn bran (*tumpi*). Third farm uses green fodder with additional corn flour and cassava. Early detection of the presence of LAB can be seen from the clear zone around the bacterial colonies growing on MRS and GYP agar media supplemented with 1% $CaCO_3$ (Figure 1).

In this study, 33 LAB isolates were isolated from MRS agar medium, and 17 were isolated from GYP agar medium. Colony characterization was carried out on 50 LAB isolates by observing the shape, edge, elevation, surface, and color varied. The observations showed that all isolates had colonies with smooth surfaces, while the shape, edge, elevation, and color of the colonies varied (Tables 1-3). The shape of LAB colonies is punctiform (16 isolates), circular (17 isolates), spindle (6 isolates), and irregular (11 isolates). The edges of LAB colonies are entire (39 isolates), undulate (10 isolates), and lobate (1 isolate). The elevation of LAB colonies is flat (29 isolates), raised

Table 1 Characteristics of LAB colonies in milk samples from first farm

Code	Shape	Edge	Elevation	Surface	Colour
MRS agar					
SP1B1	Punctiform	Entire	Flat	Smooth	Yellowish white
SP1B2	Punctiform	Entire	Raised	Smooth	White
SP1B3	Punctiform	Entire	Flat	Smooth	White
SP1B4	Circular	Entire	Convex	Smooth	Yellowish white
SP1B5	Circular	Entire	Flat	Smooth	Transparent white
SP1B6	Circular	Entire	Convex	Smooth	Milky white
SP1B7	Spindle	Entire	Flat	Smooth	Milky white
SP1B8	Irregular	Entire	Flat	Smooth	Transparent white
SP1B9	Punctiform	Undulate	Flat	Smooth	White
GYP agar					
SP1C4	Circular	Entire	Flat	Smooth	Transparent white
SP1C5	Circular	Entire	Convex	Smooth	White

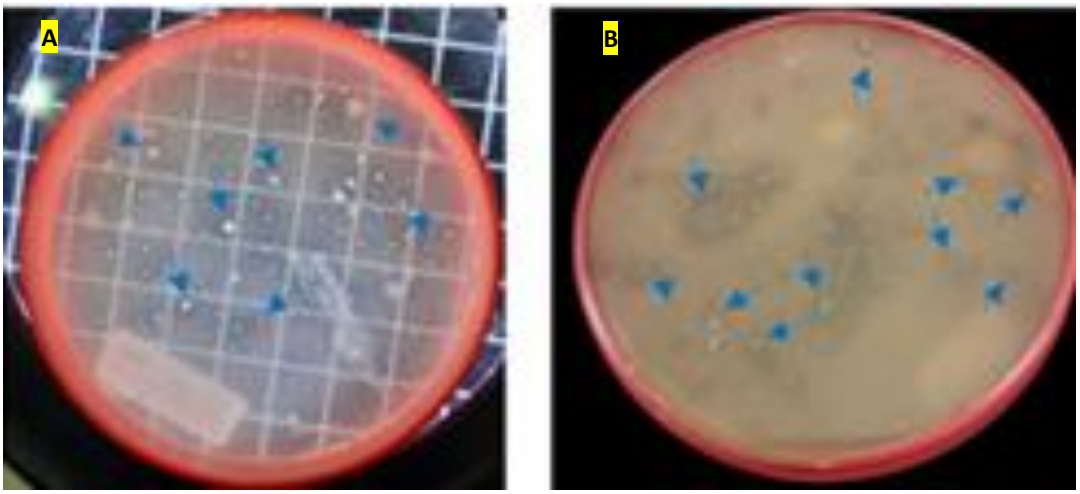

Figure 1. Pour plate results of goat milk samples in MRS agar (A) and GYP agar (B)

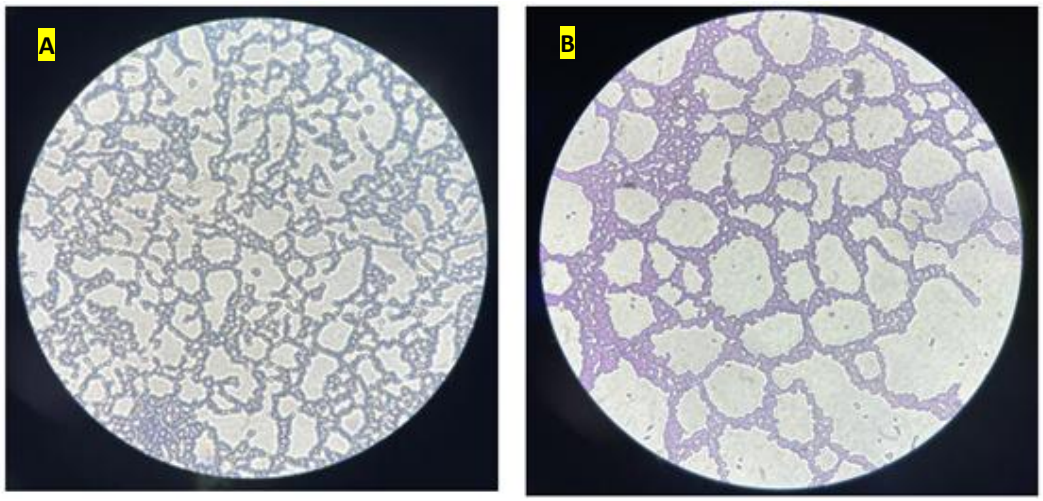

Figure 2. Gram staining of isolate SP2C7 (A) and SP1B7 (B) (Magnification: 1000x)

(16 isolates), and convex (5 isolates). The color of LAB colonies is yellowish (17 isolates), white (10 isolates), milky white (18 isolates), transparent white (12 isolates), and creamy white (3 isolates). LAB isolates isolated from Rape Pakatikng have the same characteristics, namely cream-colored, circular, intact edges, convex elevation, and smooth surfaces (Rahayu & Setiadi, 2023). LAB isolated from Mandai has irregular, punctiform, and circular colony shapes with undulate and entire edges, while the elevation of the colonies is raised (Siregar et al., 2014). LAB from Dengke Naniura has an oval-shaped colony, rounded-edge shape, flat surface, and white colony color (Nasri et al., 2021). The results of the characterization of LAB colonies in milk samples from the first farm are presented in Table 1.

Table 2. Characteristics of LAB colonies in milk samples from second farm

Code	Shape	Edge	Elevation	Surface	Colour
MRS agar					
SP2B1	Punctiform	Entire	Flat	Smooth	Yellowish white
SP2B2	Punctiform	Entire	Raised	Smooth	Milky white
SP2B3	Circular	Entire	Raised	Smooth	Milky white
SP2B4	Spindle	Entire	Flat	Smooth	Yellowish white
SP2B5	Irregular	Entire	Raised	Smooth	Milky white
SP2B6	Irregular	Entire	Raised	Smooth	Transparent white
SP2B7	Circular	Undulate	Flat	Smooth	Transparent white
SP2B8	Punctiform	Undulate	Flat	Smooth	White
GYP agar					
SP2C1	Punctiform	Entire	Flat	Smooth	Yellowish white
SP2C2	Punctiform	Entire	Flat	Smooth	White
SP2C3	Circular	Entire	Convex	Smooth	Milky white
SP2C4	Circular	Entire	Flat	Smooth	Transparent white
SP2C5	Circular	Entire	Flat	Smooth	White transparent
SP2C6	Circular	Entire	Convex	Smooth	Creamy white
SP2C8	Spindle	Entire	Flat	Smooth	White
SP2C9	Spindle	Entire	Flat	Smooth	Milky white

GYP medium was used by (Azizah et al., 2021) to isolate LAB from tempeh and tape samples as probiotic candidates. GYP medium is also for physiological testing of the probiotic *Lactobacillus* sp. Mar 8 has been encapsulated using a spray dryer to lower cholesterol (Yulinery et al., 2007). MRS medium is a medium used to grow LAB. Several studies have succeeded in isolating LAB using an MRS medium, including the isolation of LAB from the traditional food Sarobuong (Saryono et al., 2023). MRS medium was utilized to isolate LAB from 23 local dairy product samples, including cow milk, buffalo milk, cheese, and yogurt, collected from various areas of Ahwaz city (Karami et al., 2017).

The bacterial isolates obtained were confirmed based on the LAB characters, namely Gram-positive, catalase-negative, non-motile, and non-endospores (Rumaisha et al., 2020). Based on Gram staining (Figure 2), endospore staining (Figure 3), catalase test, and motility test (Figure 4) showed that the bacterial isolates that were LAB characters were Gram-positive, catalase-negative, non-motile, and non-endospores as presented in Table 4-6.

Gram staining was carried out to determine the characteristics of the LAB isolates isolated from Senduro goat milk samples. Gram staining is an

important characterization tool for distinguishing bacterial isolates based on their cell wall structure. The Gram staining results showed that all bacterial cells were purple (Figure 2), indicating that the bacterial isolates tested were Gram-positive (Paray et al., 2023). The KOH test confirms the characteristics of Gram properties. The results of the KOH test also showed that the bacterial isolates were Gram-positive because they did not form gels or string outs (Powers, 1995). LAB isolated from Senduro goat milk samples are Gram-positive based on Gram staining and KOH tests.

Endospore staining carried out in this study aims to detect the ability of bacterial isolates obtained to form endospores. Endospore staining uses Malachite Green and Safranin as dyes so that endospores are colored green and bacterial cells are red (Oktari et al., 2016). The study showed that bacterial cells observed after endospore staining showed a red color so it can be concluded that the bacterial isolates were non-endospores (Figure 3). These results are to the characteristics of LAB, namely non-endospores (Nurhikmayani et al., 2019).

The motility test conducted in the study aims to determine whether the bacterial isolates obtained are motile. A motility test was conducted using a SIM medium to observe bacterial cell movement. The results of the motility test, it shows that the LAB isolate has non-motile properties. This is indicated by observations of bacterial growth, which is only in the loop inoculation puncture area, not spreading throughout the medium. The characteristics of LAB are non-motile (Nurhikmayani et al., 2019).

The catalase test was conducted to determine the ability of bacterial isolates to produce catalase (Rahayu & Setiadi, 2023). The results showed that bacterial isolates could not form catalase because no O2 bubble indicator appeared when bacterial cells were dripped with H2O2. These results in line with the statement that LAB does not produce the catalase enzyme, which converts hydrogen peroxide into water and oxygen (Ismail et al., 2018). Overall results of the catalase test, Gram staining, motility test, and endospore staining are presented in Table 4-6. Table 4 shows the results of the microscopic and biochemical characterization of LAB isolates in Senduro goat milk from first farm. The results of microscopic and biochemical characterization of Senduro goat milk bacterial isolates at farm 2 are presented in Table 5.

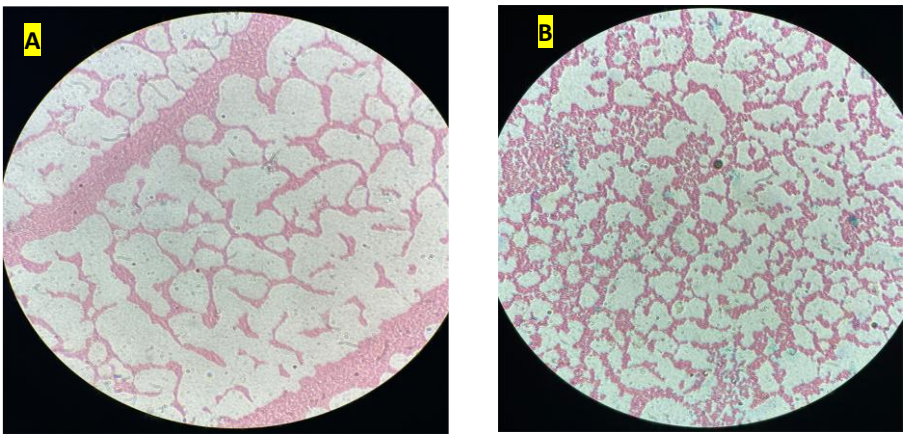

Figure 3 Endospore staining of isolate SP1C5 (A) and SP1B2 (B) (showing non-endospore results) (magnification: 1000x)

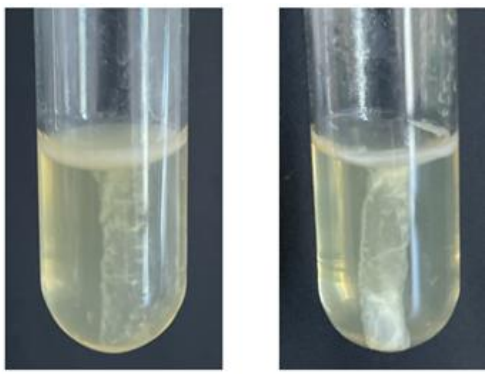

Figure 4. Motility test of isolate SP3B1 (A) and SP2C6 (B)

Table 3. Characteristics of LAB colonies in milk samples from third farm

Code	Shape	Edge	Elevation	Surface	Color
MRS agar					
SP3B1	Punctiform	Entire	Flat	Smooth	Milky white
SP3B2	Circular	Entire	Flat	Smooth	Transparent white
SP3B3	Circular	Entire	Raised	Smooth	Transparent white
SP3B4	Circular	Entire	Raised	Smooth	Milky white
SP3B5	Irregular	Undulate	Raised	Smooth	Milky white
SP3B6	Circular	Entire	Flat	Smooth	Milky white
SP3B7	Circular	Entire	Flat	Smooth	White
SP3B8	Punctiform	Entire	Flat	Smooth	Creamy white
SP3B9	Punctiform	Entire	Flat	Smooth	White
SP3B10	Punctiform	Entire	Raised	Smooth	White
SP3B11	Punctiform	Entire	Flat	Smooth	Transparent white
SP3B12	Spindle	Entire	Flat	Smooth	Creamy white
SP3B13	Irregular	Undulate	Raised	Smooth	Transparent white
SP3B14	Irregular	Undulate	Flat	Smooth	Transparent white
SP3B15	Irregular	Undulate	Raised	Smooth	Milky white
SP3B16	Irregular	Undulate	Flat	Smooth	Milky white
GYP agar					
SP3C1	Punctiform	Entire	Flat	Smooth	Milky white
SP3C2	Punctiform	Entire	Raised	Smooth	Milky white
SP3C3	Circular	Entire	Raised	Smooth	Milky white
SP3C5	Spindle	Entire	Flat	Smooth	Milky white
SP3C6	Irregular	Undulate	Raised	Smooth	Milky white
SP3C9	Irregular	Undulate	Raised	Smooth	Yellowish white
SP3C10	Irregular	Lobate	Raised	Smooth	Yellowish white

Table 4 Microscopic and biochemical characteristics of LAB isolates in goat milk samples from first farm

Code	Catalase	Gram	Motility	Endospore
SP1B1	-	+	-	-
SP1B2	-	+	-	-
SP1B3	-	+	-	-
SP1B4	-	+	-	-
SP1B5	-	+	-	-
SP1B6	-	+	-	-
SP1B7	-	+	-	-
SP1B8	-	+	-	-
SP1B9	-	+	-	-
SP1C4	-	+	-	-
SP1C5	-	+	-	-

Table 5. Microscopic and biochemical characteristics of LAB isolates in goat milk samples from second farm

Code	Catalase	Gram	Motility	Endospore
SP2B1	-	+	-	-
SP2B2	-	+	-	-
SP2B3	-	+	-	-
SP2B4	-	+	-	-
SP2B5	-	+	-	-
SP2B6	-	+	-	-
SP2B7	-	+	-	-
SP2B8	-	+	-	-
SP2C1	-	+	-	-
SP2C2	-	+	-	-
SP2C3	-	+	-	-
SP2C4	-	+	-	-
SP2C5	-	+	-	-
SP2C6	-	+	-	-
SP2C8	-	+	-	-
SP2C9	-	+	-	-

Table 6. Microscopic and biochemical characteristics of LAB isolates in goat milk samples from third farm

Code	Catalase	Gram	Motility	Endospore
SP3B1	-	+	-	-
SP3B2	-	+	-	-
SP3B3	-	+	-	-
SP3B4	-	+	-	-
SP3B5	-	+	-	-
SP3B6	-	+	-	-
SP3B7	-	+	-	-
SP3B8	-	+	-	-
SP3B9	-	+	-	-
SP3B10	-	+	-	-
SP3B11	-	+	-	-
SP3B12	-	+	-	-
SP3B13	-	+	-	-
SP3B14	-	+	-	-
SP3B15	-	+	-	-
SP3B16	-	+	-	-
SP3C1	-	+	-	-
SP3C2	-	+	-	-
SP3C3	-	+	-	-
SP3C5	-	+	-	-
SP3C6	-	+	-	-
SP3C9	-	+	-	-
SP3C10	-	+	-	-

Microscopic and biochemical characterization of bacterial isolates from Senduro goat milk from farm 3 in Table 6. Research shows that Senduro goat milk is a habitat and source of nutrition for LAB. This is proven by obtaining LAB isolates with the characteristics of Gram-positive bacteria, catalase-negative, do not form endospores, and are not motile. Several studies have also succeeded in isolating LAB from goat milk samples. Research conducted by Islam et al. (2021) isolated and identified 50 LAB strains from 18 goat milk samples based on morphological, physiological, biochemical, and genotypic characteristics. *Enterococcus faecium* with antimicrobial and probiotic properties (Azhar et al., 2022). *Leuconostoc*, *Enterococcus*, and *Lactobacillus* have been isolated from Etawa crossbred goat milk with antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* (Khairan et al., 2019).

Based on the results of LAB isolation, the number of LAB isolates in the first, second, and third farms differed. Several factors, including environmental factors and feeds, may have influenced this. Likewise, the number of LAB growing on GYP and MRS agar media varies greatly. In milk from the first farm, the number of LAB was 11 isolates consisting of 9 isolates obtained from MRS medium and 2 isolates growing on GYP agar medium. The number of LAB from milk from the second farm was 16, consisting of 8 isolates growing on MRS medium and eight others growing on GYP agar medium. For milk samples from the third farm, the number of LAB was the highest, namely 23 isolates consisting of 16 isolates growing on MRS agar medium and seven isolates in the medium. The research that has been conducted provides information on the diversity of LAB isolates isolated from Senduro goat milk.

CONCLUSION

The study obtained 50 isolates of LAB from Senduro goat milk from three different farms. LAB isolates have gram-positive, non-endospore, catalase-negative, and non-motile characteristics with diverse colony characteristics in shape, edge, elevation, and color. The LAB isolates obtained need to be further tested for their potential. It is important to identify beneficial LAB isolates and to be widely utilized.

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