

Sequencing Analysis of Lactic Acid Bacteria as Probiotics from Ettawah Goat's Milk as Antimicrobial

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Abstract The utilization of fermented milk as probiotics to address nutritional concerns in Indonesia has been limited. Multiple studies have demonstrated that probiotics can mitigate dysbiosis by altering the gut microbiota composition. One reason is milk utilization from domesticated animals, attributed to the nutritional composition and presence of lactic acid bacteria (LAB) as probiotics in goat milk. This work aimed to evaluate the molecular potential of probiotic isolates derived from goat milk utilizing the 16S rRNA gene and their antimicrobial activities. This study employed the sequencing analysis of potential probiotic isolates from goat milk using the 16S rRNA gene. The analysis focused on examining the arrangement of lactic acid bacteria DNA in terms of acid-base length and evaluating their antimicrobial activity against *Escherichia coli*, *Salmonella* sp., *Klebsiella* sp., as well as their antifungal activity against *Candida albicans*. The research findings were obtained by analyzing the amplification of DNA sequences from probiotic isolates found in goat milk, namely in a 1391 bp region. Furthermore, the antibacterial activity was assessed by measuring the diameter of the clear Zone formed on the test bacteria *E. coli* (7.10 mm), *Salmonella* sp. (6.10 mm), *Klebsiella* sp. (9.13 mm), and the fungus *Candida albicans*

(15.19 mm). This study proves that LAB isolates derived from goat milk possess probiotic properties. This is supported by their amplification of the 16S rRNA gene, sequencing analysis showing similarity to *Lactiplantibacillus plantarum*, and their ability to inhibit the growth of pathogenic bacteria and exhibit antifungal activity.

Keywords Antimicrobial Activity, Gen 16S rRNA, Goat Milk, Probiotic, Lactic Acid Bacteria

1. Introduction

Goat milk has a reasonably high price, especially in the maintenance of Ettawah breeding goats, one of the reasons is that the Ettawah breeding goat business has a perfect business opportunity [1]. In Indonesia, the type of goat that is generally bred is the Ettawah breeding goat due to several advantages, such as its ability to produce milk, its ability to grow quickly, its ability to breed without requiring large areas of land, and its ability to adapt to the temperature and environment in Indonesia [2]. Ettawah goat milk is believed

to have many properties, such as nutrients utilized from milk very much, low allergenicity, and beneficial chemical composition, which are more similar to human milk than cow's milk. Etawah goat milk can be an alternative to cow's milk, which is currently commercial². In general, goat milk production is not as widely known as cow's milk even though goat milk has a pretty good chemical composition and provides good benefits for body health because milk contains many nutrients and bioactive components that play a role in maintaining body health [3].

The components of goat's milk contain nutrients needed for microbial growth. Microbes commonly found in milk are lactic acid bacteria. Lactic acid bacteria are facultative anaerobic bacteria that can live in various natural habitats, such as plants, the digestive tract, and various fermented food or beverage products. Lactic acid bacteria are a group of Gram-positive bacteria, non-spore-forming, cocci, and bacilli cell forms, producing lactic acid as the end product of carbohydrate fermentation. Lactic acid bacteria are grouped into several genera, namely *Streptococcus*, *Leuconostoc*, *Enterococcus*, *Pediococcus*, *Aerococcus*, *Alloicoccus*, *Weisella* and *Lactobacillus* [4].

Lactic acid bacteria (LAB) produce lactic acid as an end product of anaerobic metabolism and synthesize various beneficial metabolites [5,6]. Therefore, LABs are often isolated from multiple sources due to their potential uses, e.g., as starter cultures of various fermentation products. One type of LAB commonly isolated and used in the yogurt and cheese industry is *Lactobacillus* sp [7].

Goat milk also has a population of LAB, so the isolation and characterization of LAB, especially *Lactobacillus* spp., from goat milk is interesting [8]. According to Badis et al. [9], the LAB composition of goat milk is dominated by *Lactobacillus* spp. However, the microbiota of goat milk can vary. In addition, *Lactobacillus* spp. has probiotic properties that can provide health benefits, especially in balancing the microbiota in the digestive tract [10]. According to research, the health benefits of *Lactobacillus* spp. as probiotic bacteria candidates are that they can modulate the immune system, protect the epithelial lining, and have anti-tumor effects. Studies by Dempsey & Corr [11] also indicated that *Lactobacillus* spp. could treat several diseases, such as respiratory infections, bacterial vaginosis, and atopic dermatitis. In addition, Amelia et al. [12] research stated that LAB is vital in lowering blood sugar. It stated that LAB, as a probiotic from Dadiah, can be antidiabetic [13].

Several researchers have widely tested the potential of lactic acid bacteria as antimicrobials. LAB isolates from fermented book fish have antibacterial properties against *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* [14]. Besides, *Lactobacillus fermentum* isolates are also able to inhibit the growth of bacteria

commonly found on the skin surface, such as *Staphylococcus aureus*, *Propionibacterium acne*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* [15].

Based on the description above, the authors are interested in knowing the ability of lactic acid bacteria from Ettawa goat milk to be antimicrobials by knowing the genus of the bacteria through sequencing analysis.

2. Materials and Methods

2.1. Materials

The goat milk used in the study was taken from a dairy goat farm in Gunung Pangilun, Padang City, Indonesia. After milking, it is stored in sterile containers and carried in a cool box in a cold state. Analysis was conducted at the Biomedical Laboratory and Microbiology Laboratory of Baiturrahmah University.

2.2. Isolation of lactic Acid Bacteria

Each sample was weighed and placed into a 9 mL MRS broth test tube. The test tube was sealed and mixed thoroughly, then transferred into an anaerobic jar and kept at 37 °C for 24 hours. This process was done as a 10-1 dilution. The 10-1 results are extracted in a volume of 1 mL and added to a test tube containing 9 mL of MRS broth. The mixture is then sealed and homogenized. Next, it is transferred to an anaerobic jar and incubated at 37 °C for 24 hours, representing a 10-2 dilution. This process is repeated until a dilution of 10-6 is achieved. A volume of 100 microliters was extracted and inoculated onto a Petri plate containing MRS medium using the spread method. The inoculum was obtained from 10-6 and 10-7 dilutions. The hockey stick-like tool was sterilized with alcohol and used to spread the inoculum on the plate evenly. The plate was then subjected to heat from a Bunsen burner and aerated. Every Petri plate was labeled, placed within an anaerobic jar, and then incubated for 48 hours at a temperature of 37 °C. The Petri plate containing the incubated sample was retrieved and examined. The colony appeared singular, exhibiting a round shape, smooth texture, and a yellowish-white tint.

Furthermore, a well-defined and evident area encircled the colony, which signifies the existence of lactic acid bacteria. Moreover, the streak technique accomplished the purification of a solitary colony. This process entailed meticulously choosing a solitary colony by employing a sterile ose syringe and spreading it onto a petri dish containing MRS media. The dish was subsequently incubated for 48 hours at 37 °C [16].

The bacterial culture was collected and spread evenly on a slide. It was then treated with crystal violet and left undisturbed for 60 seconds. Subsequently, the object was rinsed with distilled water, and a complicated iodine

2.3. Gram Stain

solution was applied. The solution was allowed to sit for 60 seconds. Subsequently, the substance was rinsed with flowing distilled water, followed by a wash with 96% alcohol and another with flowing distilled water. The sample was stained with safranin and left for 30 seconds before being rinsed with distilled water. It was then dried and observed under a microscope at a magnification of 1000× using immersion oil [17].

2.4. The Catalase Test

I accidentally dropped a glass thing when I was 24 years old, and then I added two drops of 3% hydrogen peroxide. The bubbles indicated a positive catalase reaction, signifying oxygen production by the catalase enzyme in the bacterial culture. The laboratory experiment involved catalase-negative bacteria, which did not form air bubbles [16].

2.5. Test Type of Fermentation

A 1 mL aliquot of LAB strain was introduced into a test tube containing 9 mL MRS Broth. Subsequently, the Durham tube was put into the test tube in an inverted orientation. The sample was placed in an incubator for 48 hours and examined for the presence or absence of air bubbles in the Durham tube [16].

2.6. Acid Resistance Test

Implemented according to the methodology outlined by Islam et al. [18], 1 mL bacterial culture sample was added to 9 mL MRS broth media during this experiment. The mixture was then incubated at a temperature of 37 °C. Periodically, a 1 mL bacterial culture sample was taken from the MRS broth and added to a test tube containing 9 mL of MRS broth solution with a pH of 3. The pH was adjusted to 3 by adding 5N HCl. Subsequently, the concoction was subjected to incubation for 90 minutes. Subsequently, the fluid was diluted to a concentration of 10⁻⁶. The sample was subsequently introduced onto MRS agar media using the spread technique. The specimen was incubated at a precise temperature of 37 °C for 48 hours. The Colony Forming Unit (CFU) plate count methodology quantified the viable bacterial colonies. According to Chua et al. [19], LAB-goat's milk isolates showed a resistance percentage of 40% and survived at a pH of 2. These isolates can undergo additional testing to determine their susceptibility to bile salts.

2.7. Antimicrobial Activity

The antibacterial activities of the LAB isolate's supernatant were evaluated against a range of pathogens, including *Staphylococcus aureus*, *Salmonella* spp., *Klebsiella* spp., and the fungus species *Candida albicans*. The LAB cultures were cultivated in MRS broth for 18-22 hours. Following incubation, the liquid portion (supernatant) was isolated from the bacterial cells using

centrifugation at 10,000 revolutions per minute for 5 minutes at a temperature of 37 °C. This process was carried out to obtain an extract free from any cells.

Afterward, 20 milliliters of nutritional agar (NA) were put into Petri dishes and infected with a test bacterium at a concentration of 0.2% (volume/volume medium). After the agar had set, precise wells with a diameter of 6 mm were painstakingly created by punching into the agar. Subsequently, each well was filled with 50 microliters of the LAB supernatant. The plates were placed without oxygen and kept at 37 °C for 24 hours. This was done to facilitate the interaction between the liquid portion of the sample and the microorganisms being tested. After the incubation period, the size of the areas where microbial growth was inhibited around each well was evaluated to assess the effectiveness of the antimicrobial substance. This method was used significantly to achieve dependable and consistent outcomes [20].

2.8. Extraction of Genomic DNA

Ten milliliters of bacterial culture were harvested into 1.5 mL microtubes through centrifugation. The supernatant, or liquid phase, was carefully removed, leaving the compacted cell pellet behind. A volume of 200 µL of lysis buffer was added to each pellet-containing microtube, followed by vortexing for 10 seconds to resuspend the cells. Next, 20 microliters of proteinase K were added to the suspension and thoroughly mixed to ensure a homogeneous cell lysate. The microtubes were then incubated at 56 °C for 30 minutes, with intermittent vortexing for 5 seconds every 10 minutes to enhance the lysis process. After incubation, 20 microliters of RNase solution were added to each tube and mixed by vortexing for 5 seconds. The tubes were subsequently incubated at room temperature for 10 minutes.

To precipitate nucleic acids, 400 microliters of ice-cold 50% ethanol were added to each microtube, and the contents were mixed by vortexing for 5 seconds. The mixture was then transferred to a purification column, placed in a collection tube, and centrifuged at 10,000 rpm for 1 minute. After centrifugation, the flow-through was discarded, and the purification column was transferred to a new collection tube. Then, 500 µL of Wash Buffer I was added to the column, followed by centrifugation at 10,000 rpm for 1 minute. The wash solution was discarded, and the column was placed back into the collection tube. A subsequent wash step with 500 µL of Wash Buffer II was performed, followed by centrifugation at 10,000 rpm for 3 minutes. The column was then transferred to a new, sterile 1.5 mL microtube.

To elute the DNA, 50 µL of elution buffer was carefully added to the center of the purification column. The column was left at room temperature for 2 minutes to allow DNA to dissolve into the elution buffer and then centrifuged at 15,000 rpm for 1 minute. The purification column was discarded, and the resulting DNA solution was stored at -20 °C for future use [21].

2.9. Electrophoresis of Genomic DNA

A 1% agarose gel was prepared by measuring 0.5 grams of agarose powder and transferring it to a suitable container. Subsequently, 50 mL of $0.5\times$ TBE buffer was added to the container. The mixture was then heated in a microwave on medium power for about 3 minutes or until the agarose was wholly dissolved, yielding a clear and homogenous solution. Following this, 5 μ L of ethidium bromide was carefully added along the inner wall of the container near the agarose solution, and the contents were thoroughly mixed to ensure even distribution of the stain. The gel casting tray and comb were then positioned appropriately in the mold to accommodate the number of samples to be analyzed. The molten agarose solution was gently poured into the gel tray and left undisturbed for 20-30 minutes to solidify.

For the electrophoresis run, a mixture was prepared by combining genomic DNA with 1 μ L of $10\times$ BPB and 7 μ L of $1\times$ TE buffer, resulting in a final volume of 10 μ L. Similarly, a DNA ladder with a 50 ng/ μ L concentration was prepared to serve as a molecular weight marker. Once the agarose gel had fully solidified, the comb was carefully removed, and the gel tray was removed from the mold. The electrophoresis apparatus was assembled by placing the gel tray inside the chamber and pouring $0.5\times$ TBE buffer until the gel was completely submerged. The prepared samples were then carefully loaded into the wells, starting with the leftmost well for the DNA ladder, followed by the genomic DNA samples. Each well was packed with a single sample to avoid cross-contamination.

The electrophoresis apparatus was securely closed with its cover, and the power supply was set to 100 volts for 30 minutes. The electrophoresis was initiated to separate the DNA fragments. After the run, the power source was disconnected, the chamber lid was removed, and the gel tray was carefully retrieved. The gel was placed in an appropriate viewing apparatus or transported to the designated storage container [22].

2.10. Quantification of Genomic DNA

The UV-Vis Spectrophotometer Biodrop machine was activated by hitting the power button and turning on the monitor screen. The "Life Sciences" menu was then selected, followed by "Nucleotides" and finally "DNA". Choose the pathlength detector option to be "0.5 mm lite", then select the concentration unit as "ng/ μ L," set the sample name/code, and enable the internal storing function. I initially used tissue Kimwipes to clean the detector, followed by performing the blanking procedure. Dispense one microliter of solvent and position the tip precisely between the detector wires on the Biodrop. Next, I selected

the grey cuvette icon on the monitor screen and patiently waited for the numerical value "0.00" to be displayed. The detector was once again cleaned using Kimwipes tissue. Subsequently, 1 μ L of the DNA sample was transferred using a pipette. The orange cuvette symbol on the monitor screen was then tapped, and the user waited for the measurement figures to be shown.

2.11. Amplification of the 16S rRNA Gene

Prepare a sterile 0.2 mL tube and place it in an ice-filled container. Add two μ L each of forward primer 16S rRNA_F (10 ng/ μ L) and reverse 16S rRNA_R (10 ng/ μ L) to the bottom of the tube. A total of 2 μ L of genomic DNA, with a concentration of 10 ng/ μ L, was introduced into the solution in the tube. A 25 μ L aliquot of OD Blue Mastermix was introduced into the fluid within the tube. Additional 19 microliters of water containing no nuclease were added to the solution in the tube. The PCR was conducted at specific temperatures and durations. The solution was subsequently homogenized. The overall volume of the reaction was 50 microliters. Ensure that the tube is labeled correctly and that the exterior of the tube is dry. The tube was inserted into the PCR machine and operated using the 16S rRNA primer schedule outlined in Table 1. Following the completion of the PCR cycle, the PCR reaction mixture was subjected to electrophoresis on a 1% agarose gel. The leftmost well was filled with two μ L of a 1 kb marker, while the next well was filled with five μ L of each PCR reaction cocktail. The PCR reaction cocktail was subsequently kept in a tube at a temperature of -20°C . Following the completion of electrophoresis, the agarose gel was observed using the gel-doc system.

Table 1. Characteristics of goat milk LAB

Isolate	Catalase	Type Fermentation	Gram- Staining
SKE1	Negative	Homo Fermentative	Gram Positive
SKE2	Negative	Homo Fermentative	Gram Positive
SKE3	Negative	Homo Fermentative	Gram Positive
SKE4	Negative	Homo Fermentative	Gram Positive
SKE5	Negative	Homo Fermentative	Gram Positive
SKE6	Negative	Homo Fermentative	Gram Positive
SKE7	Negative	Homo Fermentative	Gram Positive

2.12. Sequencing

The 16S rRNA gene PCR product from bacterial samples was sequenced in both directions (bi-directional) using the Sanger sequencing method. The sequencing was conducted at Genetika Lab Science in Jakarta.

After incubation for 24 hours and then cultivation of LAB culture from goat's milk, 13 isolates were obtained. Then, 7 uniform LAB isolates were selected for further testing starting from macroscopic observations with intact

3. Results

3.1. Characteristics of LAB from Goat Milk

colonies and yellowish-white and shiny colors. Furthermore, the seven isolates were tested for characteristics of probiotic candidates in Table 1.

3.2. Acid Resistance

Furthermore, one of the requirements of LAB can be said to be a probiotic candidate if it has resistance to gastric acid (low pH) with a pH range of 2-4 under normal gastric acid conditions in Table 2.

Table 2. Viability of LAB Acid Resistance

Isolate	Viabilities (%)
KE1	12,20
SKE2	16,37
SKE3	48,28
SKE4	57,78
SKE5	51,4
SKE6	30,43
SKE7	57,89

3.3. Antimicrobial Activity

Isolates with the two highest viability ratings were tested for antimicrobial activity against pathogenic bacteria, as shown in Table 3.

Table 3. Antimicrobial Activity

Table 4. SKE7 Nucleotide BLAST Analysis Results

Description	Max Score	Total Score	Query Cover	E Value	Per. Ident	Acession
Lactiplantibacillus plantarum strain S034-52w2	2569	12788	100%	0.0	100%	CP140142.1
Lactiplantibacillus plantarum strain V4861_16S ribosomal RNA gene	2569	2569	100%	0.0	100%	OR945935.1
Lactiplantibacillus plantarum strain V4857_16S ribosomal RNA gene	2569	2569	100%	0.0	100%	OR945934.1
Lactiplantibacillus plantarum strain J50	2569	12804	100%	0.0	100%	CP140096.1
Lactiplantibacillus plantarum strain YW2189	2569	12788	100%	0.0	100%	CP139818.1
Lactiplantibacillus plantarum strain GXZWU1_16S ribosomal RNA gene	2569	2569	100%	0.0	100%	OR807408.1
Lactiplantibacillus plantarum strain SN21-2	2569	12802	100%	0.0	100%	CP138486.1
Lactiplantibacillus plantarum strain GA_C_14	2569	12804	100%	0.0	100%	CP138486.1
Lactiplantibacillus plantarum strain BC17_16S ribosomal RNA gene	2569	2569	100%	0.0	100%	OR793168.1
Lactiplantibacillus plantarum strain A7_16S ribosomal RNA gene	2569	2569	100%	0.0	100%	OR763289.1

4. Discussion

4.1. Characteristics of LAB from Goat Milk

Isolate	Clear Zone (mm)			
	<i>E. coli</i>	<i>Salmonella sp.</i>	<i>Klebsiella sp.</i>	<i>Candida Albicans</i>
SKE4	6,87	6,43	4,87	7,8
SKE7	7,10	6,10	9,13	15,19

3.4. Molecular Analysis

The best isolate of all tests carried out sequencing analysis by looking at the DNA base length arrangement of SKE7 isolates.

The electrophoresis results after the analysis using PCR from the SKE7 isolate can be seen in Figure 1. The PCR results were then subjected to sequencing analysis, followed by BLAST analysis with the results in Table 4.

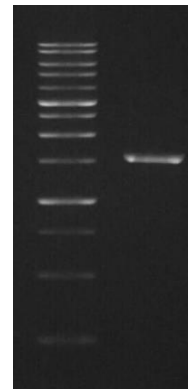


Figure 1. PCR amplification results in SKE7

Showed in Table 1, out of the seven samples taken from goat's milk, none showed air bubbles in the catalase test, suggesting that the catalase activity was harmful. This occurs because LAB cannot manufacture the catalase

enzyme that converts hydrogen peroxide into water and oxygen [23]. It is asserted that LAB cannot synthesize a catalase enzyme capable of decomposing hydrogen peroxide into water and oxygen. Likewise, the fermentative type test of all isolates is included in the homo fermentative type. The positive result of the fermentative test using Durham tubes suggested that the fermentation process was hetero-fermentative, as evidenced by the development of air bubbles in the Durham tube.

The presence of the (-) symbol indicates that the fermentation process was homofermentative, as seen by the lack of air bubbles in the Durham tube. The fermentative type test was undertaken to categorize LAB into either homofermentative or heterofermentative groupings. All samples exhibited homofermentative fermentation, indicating the presence of only lactic acid [24].

This is certainly in line with previous research where the characteristics of LAB are catalase-negative and belong to the homo fermentative type [25]. The Gram staining results of the growing bacterial colonies showed that the bacteria were Gram-positive. Several other studies have also reported the identification of lactic acid bacteria with Gram-positive characteristics [26,27,28,29]. The cell shape of lactic acid bacteria can help in knowing the genus of lactic acid bacteria. The cell shape of lactic acid bacteria is grouped into two families, *Streptococcaceae* and *Lactobacillaceae*. The *Lactobacillaceae* family has a bacillus shape and consists of the genus *Lactobacillus*. The *Streptococcaceae* family is round and consists of the genera *Streptococcus*, *Leuconostoc*, and *Pediococcus* [30].

4.2. Acid Resistance

The viability of acid resistance of LAB from goat's milk can be seen in Table 2. Under pH 3 conditions, it is known that the viability of goat's milk isolates ranged from 12.20 - 57.89%. SKE4 followed the highest viability of isolate SKE7 at 57.78%. This is in line with research conducted by Ramadhanti [31], where for further tests, only the highest viability (>40%) is taken for further characteristic analysis. A probiotic strain must withstand the main environmental factors to benefit the host: the stomach's high acidity and the concentration of bile components in the proximal intestine [32]. Probiotics must withstand the stomach's very acidic conditions to reach the intestines and create a proper environment [33]. Therefore, evaluating the tolerance of lactic acid bacteria isolates is necessary to promote gastric juice. Studies have indicated that probiotic bacteria can survive for 2.5 hours in an acidic pH three environment. Because the stomach's instability during transit makes it difficult for bacteria to thrive, probiotic strains must resist low pH [34].

4.3. Antimicrobial Activity

In Table 3, both isolates produce clear zones to inhibit the growth of pathogenic bacteria and fungi. The best

results were obtained by SKE7, which inhibited *E. coli* 7.19 mm, *Salmonella* sp. 6.10 mm, *Klebsiella* sp. 9.13 mm, and the fungus *Candida Albicans* 15.19 mm. Where it can be seen that isolate SKE7 is better as an antifungal than an antibacterial, this certainly adds new insights not only as antibacterial, it turns out that LAB is also capable of antifungal.

The antimicrobial activity shows variability among LAB isolates against harmful microorganisms. *Lactobacilli* are bacteria classified as Gram-positive, meaning they retain a purple stain when subjected to a specific staining technique. They do not create spores and can ferment different types of carbohydrates, converting them into organic acids. Certain strains of *Lactobacilli* generate lactic acid. Acidic pH levels impact other microorganisms' growth, as Zhang et al. stated [35]. Based on a previous study, the generation of H₂O₂ during metabolic processes significantly inhibits the proliferation of harmful microbes [36]. According to Hamidah et al. [37], LAB produces various antibacterial compounds such as hydrogen peroxide (H₂O₂), carbon dioxide (CO₂), diacetyl (2,3-butanedione), and bacteriocins. These compounds are antagonistic to the growth of several pathogenic and food spoilage bacteria. Fauziah et al. [38] stated that bacteriocins inhibit energy production and protein biosynthesis of pathogenic bacteria. Specific strains of *Lactobacilli* can synthesize bacteriocins, which are chemicals that inhibit the growth of pathogenic bacteria. These bacteriocins are extensively employed in the food preservation business [39].

4.4. Molecular Analysis

It can be seen in Figure 1; it is known that the results of PCR amplification of isolate SKE7 obtained the length of the DNA base acid array of 1391 bp. The 16S rRNA gene has about 1550 base pairs and about 500 bases at the end of the sequence, a hypervariable region [40]. This area is a part that distinguishes between organisms.

Based on molecular identification with 16S rRNA gene sequencing and analyzed in genebank through nucleotide analysis on the blat website (<https://blast.ncbi.nlm.nih.gov/Blast>), it is known that isolate SKE7 has a similarity Query Cover 100% with the bacterium *Lactiplantibacillus plantarum*. The equation of the 16S rDNA sequence with a similarity range of 93% to 97% can indicate similarity at the genus level. However, there are differences at the level of species. However, the similarity is less than 93%. The organism probably belongs to a new species, as its nitrogenous base sequence has yet to be incorporated into the gene bank database. According to Haas et al. [41], lower 16S rDNA sequence similarity (<96%) is most likely caused by overlapping electropherograms and the possibility of forming different "chimeric sequences." In contrast, identities with ≥98% similarity in database entries can be obtained because overlapping electropherograms have been discarded [42].

Other literature shows that in Algerian goat milk,

Lactobacillus species commonly found are *L. paracasei*, *L. casei*, *L. plantarum*, and *L. rhamnosus* [43]. This is not similar to previous research that found *Lacticaseibacillus paracasei* and *Limosilactibacillus fermentum* isolated from goat milk in the Jakarta area [44]. This aligns with the results of research conducted by Pisano et al. [45] obtained *Lc. lactis* subsp. *Lactis*, *E. faecium* and *Lb. paracasei* isolated from Sardinian goat milk. This is in line with research conducted by Pisano et al. (2019), the LAB genus was dominated by *Lactococcus* and *Lactobacillus* spp., where *Lactococcus lactis* (15.6%) and *Lacticaseibacillus paracasei* (14.5%) were found to be the LAB species that dominated goat milk.

5. Conclusions

Ettawah goat milk is one of the animal products that is a source of probiotics because it contains lactic acid bacteria. After determining the characteristics of LAB, seven isolates were obtained that have the potential to be probiotic candidates. Of the seven isolates, all produce negative catalysts and are included in homo fermentative and gram-positive bacteria. Viability to acidic conditions is known with a range of 12.20 - 57.89% and the best results in isolate SKE7 after a molecular approach with sequencing techniques are known to have a kinship with *Lactiplantibacillus plantarum*. Further analysis needs to be done related to other complete characteristics so that it meets as a probiotic, and phylogenetic analysis needs to be done to ensure the level of similarity.

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