Diversity of bacteria isolated from raw milk of Senduro goats

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Abstract. Goat's milk is widely consumed because of its good nutrition. However, goat's milk is also a good habitat for several types of bacteria that may affect the milk quality. Therefore, information concerning the bacteria's total number and diversity is crucial. This research aimed to reveal the total number and diversity of bacteria in Senduro goat's milk from three farms. Samples of raw goat's milk were collected from each farm in a sterile container. Then, the enumeration of the bacteria's number was done using the Total Plate Count (TPC) method. The total number of bacteria obtained from goat's milk on farms 1, 2, and 3 were 1.5 x 10^2 CFU/mL, 8.8 x 10^1 CFU/mL, and 1.3 x 10^2 CFU/mL, respectively. The morphological characteristics of the colonies showed the diversity of bacterial isolates in raw goat milk from the three farms. The three samples also shared several bacterial isolates with the same characteristics. It also can be concluded that the bacteria's total number in goat's milk from three farms is still acceptable and meets the standard. Identification of the group of each bacterial isolate is needed shortly to deepen our understanding of their role.

1 Introduction

Microbes are ubiquitous, meaning they are present almost everywhere, making it possible for microbes to be isolated from various natural habitats in nature. Microbes, one of which is bacteria, can be isolated from various sources because they have a wide habitat so their potential can be identified and utilized further. Goat's milk is rich in nutrients so it can be a habitat for microbes.

Commercial milk based on goat, cow, and sheep milk is produced and consumed worldwide [1]. Goat milk is a very nutritious product that has advantages over other milk [2]. Goat's milk can treat people who suffer from cow's milk allergy and gastro-intestinal disorders [3]. Increasing consumer interest in functional foods has led to goat's milk becoming popular due to its high digestibility and nutritional quality, potential nutraceutical, and low allergenicity properties [3].

Fresh or fermented milk from cows and goats is consumed in many parts of the world. Milk and dairy products are generally considered the main food source for probiotic Lactic Acid Bacteria (LAB), which are microbiota that are advantageous to human health [4].

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Aquabacterium fontiphilum, Methyllibium petroleiphilum, Piscinobacter aquaticus and *Staphylococcus xylosus* are successfully isolated from goat's milk [1], as well as mastitis-causing bacteria from Etawa cross-breed goat milk samples identified as *Staphylococcus* sp., *Neisseria* sp., *Pseudomonas* sp., *Escherichia coli, Listeria* sp., and *Corynebacterium* sp. [5].

One type of goat that is cultivated in Indonesia is the Senduro goat which is widely cultivated by breeders in Senduro District, Lumajang Regency. Senduro Goat is a wealth of Indonesian genetic resources as stipulated by the Decree of the Minister of Agriculture Number: 1055/Kpts/SR.120/10/2014 concerning the determination of Senduro Goat Lines [6]. The Senduro goat is filial of crossing Indian Etawah goats with Kacang goats and Jawarandu goats which has been going on for a century [7]. Based on the pairwise distance alignment, it shows that there are differences in nucleotide bases in Senduro and Etawa breed goats. In addition, Senduro and Etawa breed goats when compared to the NCBI Capra hircus database: D84201.1 have a high variation in nucleotide base arrangement [8].

Goat milk has various indigenous microbiota. Information on goat milk microbiota is essential for product diversification. The microbiota is responsible for the distinctive characteristics of the product and should be considered for use by the dairy industry [9]. Therefore, it is necessary to study raw Senduro goat milk to determine the number and diversity of bacteria. This research is preliminary research that aims to isolate bacteria from raw Senduro goat milk so that their diversity and dominance can be determined.

2 Methods

This exploratory research was carried out to isolate bacteria from raw Senduro goat milk. The research was carried out in several stages at the Genetics and Molecular Biology Laboratory, Faculty of Mathematics and Natural Sciences (FMNS), Universitas Negeri Surabaya.

2.1 Sample collection

Raw goat milk samples were taken from three different farms in Senduro District, Lumajang Regency. Sampling of goat milk was carried out by directly milking goats and collected using a sterile Erlenmeyer flask. Three milk samples were taken from each farm which were then combined (composite sample). Samples of goat milk that have been taken are stored in a cool box for further transport to the laboratory.

2.2 Isolation and enumeration of Bacteria

Raw goat milk samples were diluted using a series of dilutions up to a 10^{-6} dilution. One mL of milk sample was transferred into a test tube containing 9 mL of sterile distilled water as a 10^{-1} dilution, then 1 ml of the dilution was taken and transferred to the next test tube as a 10^{-2} dilution, and so on until the 10^{-6} dilution. Each time the suspension is transferred to the next test tube, it is mixed using a vortex until the suspension becomes homogeneous. One mL of the sample suspension was taken from each dilution to be poured into a Petri dish, then Plate Count Agar (PCA) media was poured in a liquid state into the Petri dish and homogenized (pour plate method). Bacterial colonies formed after incubation at 37°C for 24 hours were counted based on the Total Plate Count (TPC).

2.3 Characterization of Bacterial Colonies

Bacterial colony characterization was carried out on bacterial isolates growing on PCA media. The characteristics of bacterial colonies observed include colony shape, elevation, edges, surface, and color. The dominance of bacterial isolates is seen from the number of each bacterial isolate.

2.4 Purification of Bacterial Isolates

Bacterial colonies that differ based on their colony characteristics are then subcultured on Nutrient Agar (NA) media using the streak plate method to obtain pure isolates. Incubation was carried out at 37°C for 24 hours. Pure isolates were inoculated using the streak method on slanted NA media for culture stocks.

3 Results and Discussion

In this study, the sample used as a source of bacterial isolates was raw Senduro goat milk taken directly from the farm. Previous studies reveal that 57% of 100 milk samples and 51.66% of 60 milk products showed growth of *E. coli* [10]. Total bacteria in raw goat milk from different regions of Sardinia ranged between 10^5 and 10^7 CFU/mL; the average number of Enterobacteriaceae did not exceed 4 log CFU/mL while *E. coli* and coagulase-positive Staphylococci were less than 1.5 and 2 log CFU/mL, respectively; Total LAB counts ranged from 10^4 to 10^7 CFU/mL, and yeast counts averaged between 10^3 and 10^5 CFU/mL [9].

In this study, the TPC method was used to count the number of bacteria in raw goat milk samples. Table 1 shows the number of bacteria in raw goat milk samples. The results of counting the number of bacteria show that the highest number of bacteria was found in the goat's milk samples from farm 1, followed by samples from farms 3 and 2.

Table 1. Enumeration of bacterial counts in goat milk samples.

Milk Sample	Number of bacteria (CFU/mL)
Farm 1	1.5×10^2
Farm 2	8.8×10^1
Farm 3	1.3×10^2

Bacterial number data (Table 1) indicated that the number of bacteria in goat milk samples on all farms is below the maximum standard limit for microbial contamination based on SNI 01-6366-2000. The maximum threshold for microbial contamination in fresh milk based on SNI 01-6366-2000 is 1×10^6 CFU/ml [11]. An example of the pour plate results for the goat's milk sample is presented in Fig 1.

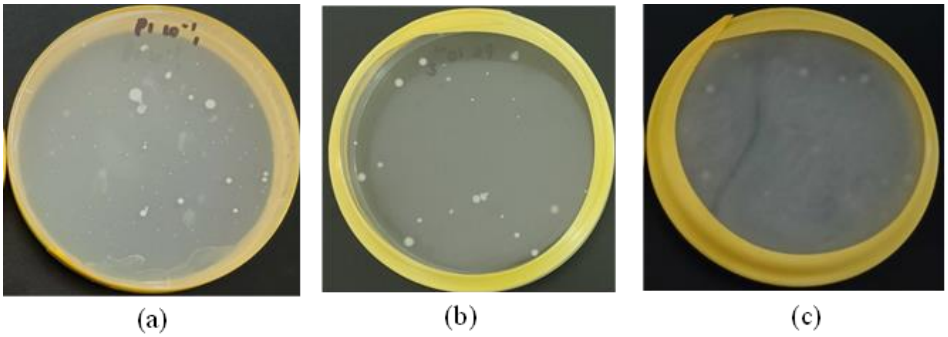

Fig. 1. Bacterial colonies of milk samples: (a) farm 1; (b) farm 2; (c) farm 3.

The pour plate results for each goat's milk sample obtained bacterial isolates that had different colony characteristics. Based on differences in colony characteristics, six bacterial isolates were obtained from goat milk samples from farm 1, six bacterial isolates from goat milk samples from farm 2, and six bacterial isolates from goat milk samples from farm 3, resulting in a total of 18 bacterial isolates. The characteristics of each bacterial isolate in goat milk samples from farm 1 are presented in Table 2.

Table 2. Characteristics of bacterial colonies in goat milk samples from farm 1

Isolate	Form	Elevation	Edge	Surface	Color
P1A1	Circular	convex	entire	smooth	white
P1A2	punctiform	flat	entire	smooth	white
P1A3	Irregular	flat	undulate	smooth	white
P1A4	Spindle	flat	entire	smooth	white
P1A5	Circular	raised	entire	smooth	white
P1A6	Irregular	flat	lobate	smooth	white

In goat milk samples from farm 2, bacterial isolates with distinct characteristics were obtained. Table 3 shows the characteristics of bacterial isolate colonies in samples from farm 2.

Table 3. Characteristics of bacterial colonies in goat milk samples from farm 2

Isolate	Form	Elevation	Edge	Surface	Color
P2A1	Circular	convex	entire	smooth	white
P2A2	Circular	flat	entire	smooth	transparent white
P2A3	Spindle	flat	entire	smooth	white
P2A4	Irregular	flat	lobate	smooth	white
P2A5	Irregular	raised	undulate	smooth	white
P2A6	Irregular	flat	Erose	smooth	white

The characteristics of the bacterial isolates obtained from goat milk samples at farm 3 are shown in Table 4.

Table 4. Characteristics of bacterial colonies in goat milk samples from farm 3

Isolate	Form	Elevation	Edge	Surface	Color
P3A1	Punctiform	flat	Entire	smooth	white
P3A2	Circular	flat	Entire	smooth	white
P3A3	Irregular	raised	Entire	rough	white
P3A4	Irregular	flat	Filamentous	smooth	white
P3A5	Circular	raised	Erose	smooth	white
P3A6	rhizoid	raised	Lobate	smooth	white

Several bacterial isolates isolated from raw Senduro goat milk samples had the same colony characteristics (Table 2-4). Isolate P1A1 has the same colony characteristics as isolate P2A1, namely circular shape, white color, entire edge, convex elevation, and smooth surface. The characteristics of the colonies which are punctiform in shape, white in color, entire edges, flat elevations, and smooth surfaces are the characteristics of P1A2 and P3A1 isolates. Isolates P1A4 and P2A3 have the same colony characteristics, namely spindle shape, white color, entire edge, flat elevation, and smooth surface, while isolates P1A6 and P2A4 have an irregular colony shape, white color, lobate edges, flat elevation, and smooth surface.

Counting the number of colonies for each bacterial isolate was carried out on bacterial isolates growing from raw goat milk samples on each farm to determine their dominance. The dominant bacterial isolate in goat milk samples from farm 1 was the P1A2 isolate (326 colonies) (Table 5). The dominant bacterial isolate in the goat's milk sample from farm 2 was the P2A3 isolate (229 colonies) (Table 6) while in the sample of farm 3, the dominant was the P3A1 isolate with a total of 201 colonies (Table 7).

Table 5. Number of Bacterial Colonies in Goat Milk Samples from Farm 1

Isolate	Number of Colonies
P1A1	120
P1A2	326
P1A3	3
P1A4	81
P1A5	11
P1A6	1

Table 6. Number of Bacterial Colonies in Goat Milk Samples from Farm 2

Isolate	Number of Colonies
P2A1	90
P2A2	37
P2A3	229
P2A4	1
P2A5	1
P2A6	1

Table 7. Number of Bacterial Colonies in Goat Milk Samples from Farm 3

Isolate	Number of Colonies
P3A1	201
P3A2	65
P3A3	2
P3A4	1
P3A5	8
P3A6	6

Milk and its processed products are a source of bacterial isolation. A previous study has successfully isolated LAB from raw and fermented milk of Etawah goats [12]. Prevalence of *Lactobacillus* sp. isolated from Etawah goat milk at Senduro, Lumajang farms by 14/24 (58.3%) and Tempurejo, Jember by 15/24 (62.5%) with the diversity of *Lactobacillus plantarum* (26/29; 89.7%) and *Lactobacillus brevis* (3/29); 10.3%) [13]. Several studies related to the microbiota of goat milk have been done, such as the isolation of LAB from fresh milk of Etawah goats from Kopelma Village, Banda Aceh [14], isolation and potential to produce bacteriocins of microbiota from Etawah goat milk [15], isolation and potential as antimicrobial of microbiota from goat milk farmed in Vicosa, Brazil [16]. Lactic acid bacteria have also been isolated from dairy products such as goat's milk yogurt which was also tested for its antibacterial activity [17] and from Saanen goat's milk kefir [18]. A total of 158 LAB strains isolated from raw goat milk in Algeria belong to 5 genera, namely: *Lactobacillus* (50.63%), *Lactococcus* (25.94%), *Streptococcus* (14.56%), *Leuconostoc* (7.59%) and *Pediococcus* (1.26%) [19].

The most common pathogenic bacteria isolated from raw milk samples are *E. coli*, *Staphylococcus aureus*, *Shigella*, *Klebsiella*, and spore-forming *Bacillus* species which can cause serious enteric diseases if they are consumed with contaminated milk [21]. Nuhriawangsa et al. [21] stated that raw goat milk had a high total plate count (7.8×10^6 CFU/ml) with Coliform and *E. coli* detected in the samples studied. Information on bacterial diversity is important in order to know the role and potential of each bacterial isolate.

4 Conclusion

The research carried out obtained 18 bacterial isolates from raw Senduro goat milk samples taken from three different farms. Based on the number of bacterial colonies, shows that certain isolates are dominant in milk samples on each farm. The dominant isolates in farms 1, 2, and 3 were isolates P1A2, P2A3, and P3A1, respectively. The potential of these dominant bacteria needs to be studied further to determine their potential.

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