



Profiling Bacteria on Goat Raw Milk from Smallholder Farms in Humid-Tropical Areas with a Metagenomics Approach

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ABSTRACT

Metagenomic analysis provides a cultivation-independent approach to comprehensively characterize microbial communities directly from their natural environments. This study aimed to profile the bacterial communities present in raw goat milk collected from two different goat breeds and farming systems in the Bogor region, West Java, Indonesia: Saanen goats (P1) and Peranakan Ettawah (PE) goats (P2). Raw milk samples were obtained from each farm and analyzed using full-length 16S rRNA gene sequencing based on the Nanopore platform. Taxonomic profiling was performed and visualized using taxonomic composition analysis, Venn diagrams, Krona plots, and phylogenetic tree reconstruction. Outcomes revealed distinct microbial compositions between the two samples. *Kocuria rhizophila* was identified as the dominant bacterial species in raw milk from Saanen goats (P1), whereas *Lactococcus garvieae* predominated in raw milk from PE goats (P2). These findings demonstrate that the microbiota of raw goat milk varies between farms and breeds, likely influenced by differences in management and environmental conditions. Full-length 16S Nanopore sequencing proved to be a useful tool for characterizing raw milk microbiota and provides valuable insights for improving raw milk safety and quality in smallholder goat farming systems.

Keywords: Goat milk, Metagenomics, Nanopore sequencing, Full-length 16S rRNA, Microbiota profiling, Smallholder farms, Food safety, Humid-tropical.

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INTRODUCTION

Dairy goat farming is perennially confronted with sanitation and foodborne contamination issues, ranging from pathogenic to non-pathogenic bacteria, worldwide (de Siqueira et al., 2021). These two categories of bacteria have specific roles respectively; some lactic acid bacteria (LAB) strains have demonstrated a strength in suppressing the prevalence of foodborne diseases (Fernandes et al., 2024). Currently, in Indonesia, this has developed into primarily urgent issues to engage (Tampubolon et al., 2024). Primarily, dairy goats in Indonesia are of the Saanen and Peranakan Ettawah (PE) genetic breeds, which are predominantly raised by smallholder farmers (Susilorini et al., 2022; Muwahhid et al., 2023).

Goat milk's rich nutrients (proteins, fats, sugars) and physicochemical properties provide an environment that

supports microbial survival and proliferation, including lactic acid bacteria and spoilage microbes (Clark & Gracia, 2017; Montesinos et al., 2025). Detection technology for raw milk-borne bacteria is rapidly developing. It started with the conventional method, like the methylene blue reduction test (MBRT) (Ahmadi, 2025), to the most up-to-date and sophisticated technology of artificial intelligence-based (Yang & Wei, 2021; Çelik, 2022). Each technology has advantages and disadvantages underlying the distinct viewpoints, respectively, and the situation between rapidity and accuracy is everlastingly coming across head-to-head and turning into a difficult choice to make in developing a raw milk-borne analysis instrument (Amiri et al., 2025). Nevertheless, current acquisition prioritizes rigorous, accurate milk-borne analysis over speed, and metagenomics was chosen to characterize the contaminating bacteria in raw goat milk from smallholder

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dairy goat farms in Indonesia. Metagenomics is a powerful tool for studying microbial diversity and their functions directly from samples without cultivation steps (You et al., 2022; Nam et al., 2023; Wang et al., 2025). This method provides a powerful way to understand the composition and functional capabilities of an entire microbial community directly from its natural environment (Galloway-Peña & Hanson, 2020). Microbial diversity in raw goat and sheep milk from Benin was investigated using metagenomic analysis (Adje et al., 2025). Metagenomics is a proven, reliable method to detect bacteria in milk (Beck et al., 2024; Mutmainna et al., 2025). Extrinsically, metagenomic milk-borne studies in humid tropical regions like Indonesia remain infrequent.

Regularly checking milk contaminants for pathogens, non-pathogens, and probiotic novel bacteria in the raw goat milk by a metagenomic approach could be built as an early detection system, which is the main reason for this research, and also to be considered as an initial microbiota data for smallholder dairy goat farms in Indonesia, especially in Bogor, West Java region. The objective of this research is to provide information about microbiota from Saanen and PE (Ettawah Descendent) goat milk. Therefore, this preliminary study addressed the following research question: Does raw goat milk collected from smallholder farms (Saanen vs. PE goats) in a humid-tropical area exhibit differences in bacterial community profiles based on full-length 16S rRNA Nanopore sequencing? The findings are expected to provide baseline information for future larger-scale studies and to support improvements in hygienic milking practices to ensure safer raw milk production.

MATERIALS & METHODS

Milk Collecting Procedure

Raw milk samples were aseptically collected from lactating goats at two smallholder farms in Bogor, West Java, Indonesia. Samples were grouped as P1 (Saanen goats) and P2 (PE goats) and collected into sterile 100mL bottles. For this preliminary study, milk samples were pooled to obtain one representative composite sample per group by combining equal volumes of raw milk from individual animals within each group. A total volume of 50mL from each composite sample (P1 and P2) was used for DNA extraction and subsequent metagenomic analysis.

Profiling of Microbiota

According to the Genetika Science protocol (2025), microbiota profiling using metagenomics consists of three stages: first, sample preparation and sequencing; second, post-sequencing and third, analysis.

a. Sample Preparation & Sequencing

All steps in sample preparation and sequencing were done according to Genetika Science protocol (2025). DNA was extracted from a raw goat milk sample using the Quick-DNA Magbead Plus Kit (D40842), and the concentration was determined using a NanoDrop spectrophotometer and a Qubit fluorometer. After that, DNA was amplified with 16S primers (27F-1492R) using Phusion Plus Green PCR Master Mix (Thermo Scientific, F632L). Then, library preparation was

performed using the Nanopore Ligation sequencing amplicons - Native Barcoding Kit 96 V14 (SQK-NBD114.96), and the final library was sequenced on the Nanopore GridIon platform. To minimize contamination, all procedures were conducted using sterile consumables in a clean working area, and sample handling was separated between pre-PCR and post-PCR workflows. A negative control (blank extraction/PCR control) was included to monitor potential contamination. Sequencing depth was recorded as the total number of reads generated per sample and the number of reads retained after quality filtering, which were used for downstream taxonomic classification and visualization.

b. Post Sequencing

All post-sequencing steps were performed according to the Genetika Science protocol. Nanopore sequencing was operated by using MinKNOW software version 25.03.7. Eventually, base calling was performed using Dorado version 7.8.3 with a high-accuracy model.

c. Bioinformatics Analysis

Bioinformatics analysis was performed in accordance with the Genetika Science protocol (2025). Adapter and primer sequences were trimmed using Dorado version 7.8.3, and reads were filtered for quality and length using Nanofilt, then visualized with NanoPlot. Filtered reads were classified using the Centrifuge classifier with bacterial and fungal indices that were built using the NCBI bacteria TargetedLoci database. Finally, downstream analysis and visualizations were performed with Pavian, Krona Tools, and RStudio (R version 4.3). For analysing diversity, Taxonomic composition, and Differential abundance.

Statistical Analysis

The present study was observational because it was a preliminary research project. Thus, the statistical approach applied in this study was primarily descriptive. Samples were collected in three replicates; however, they were subsequently composited prior to metagenomic analysis. Therefore, the resulting data and visualizations present aggregated outcomes interpreted descriptively.

RESULTS

Taxonomic Profile of Goat Milk

Taxonomic profiling based on full-length 16S rRNA nanopore sequencing revealed distinct bacterial community structures between raw goat milk samples from the P1 (Saanen) and P2 (Peranakan Ettawah, PE) farms (Fig. 1). The top 10 relative abundances in the taxonomic profile are defined as the proportion of microbial sequences relative to the total reads successfully annotated in each sample. The number of reads includes not only those that can be classified at a specific taxonomic level, but also reads assigned within the same taxonomic group. As shown in Fig. 1, *Korucia rhizophila* was the most dominant species in P1 (Saanen), whereas *Lactococcus garvieae* was the most dominant species in P2 (PE). The top 10 microbial species shown in Fig. 1 had the highest relative abundance based on total reads across all samples. These taxa indicated the

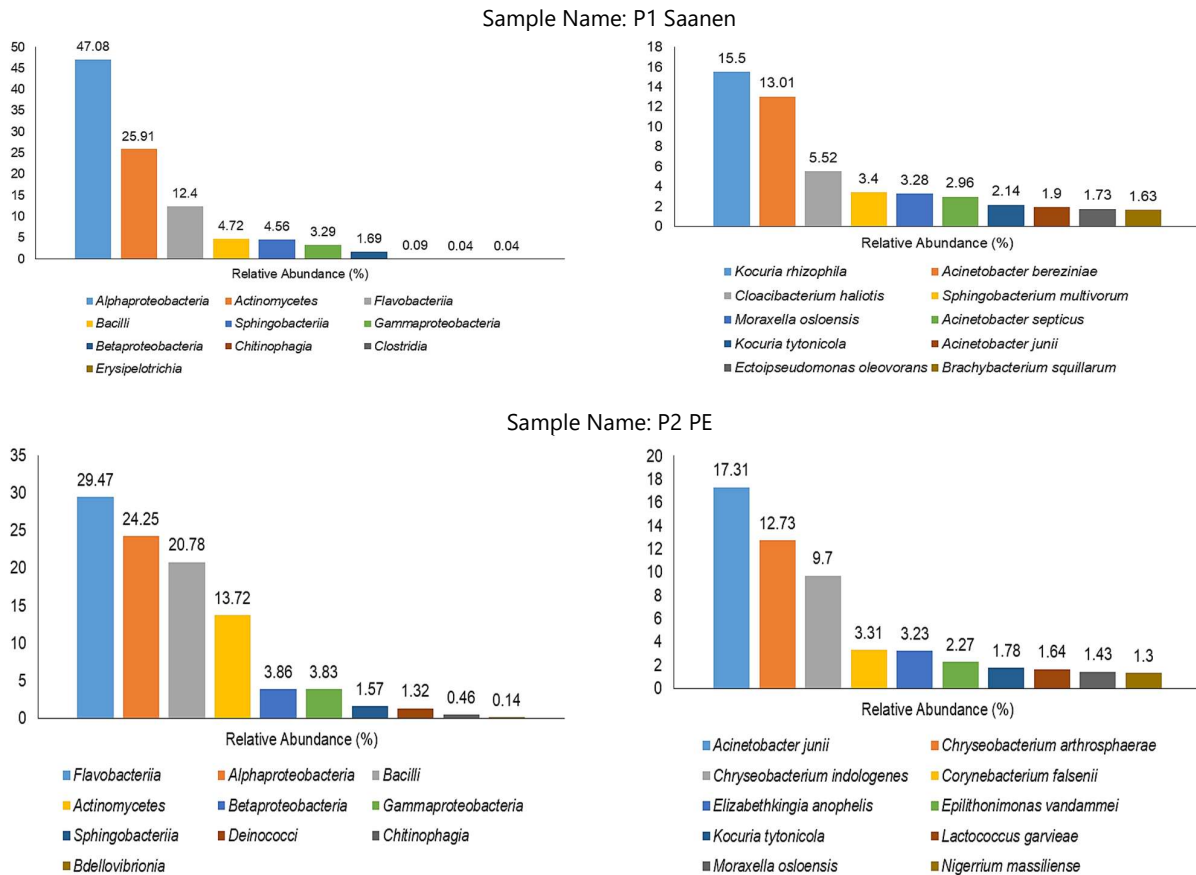


Fig. 1: Taxonomic profiling, in group P1 Saanen, and group P2 PE.

core microbiota present in goat milk from both breeds. When the relative abundance profiles of P1 and P2 were compared descriptively (Fig. 1), clear differences in dominant species composition were observed. P1 (Saanen) was characterized by the predominance of *Korucia rhizophila*, while P2 (PE) showed higher dominance of *Lactococcus garvieae*. Other species in the top 10 also showed varying relative proportions between the two groups, indicating that breed differences might influence microbial community structure.

Venn Diagram

The Venn diagram (Fig. 2) illustrates the shared and unique bacterial taxa detected in P1 and P2. Several taxa were common to both samples, suggesting a potential core raw-milk microbiota in both groups. However, each sample also contained unique taxa, reflecting differences in bacterial community composition between Saanen (P1) and PE (P2) raw milk samples. A total of 431 taxa were shared between both groups, indicating a substantial core microbiota common to goat milk regardless of breed. In contrast, 995 taxa were unique to PE samples, and 701 to Saanen samples, highlighting clear breed-specific differences in microbial composition. The higher number of unique taxa in PE milk suggested greater microbial diversity or stronger environmental influence, potentially related to differences in management practices, local environment, or host factors. The diagram consisted of overlapping circles, where each circle represents a specific sample. The overlapping area

indicated the taxa shared by the two samples. Overall, this pattern indicated that while both goat breeds shared a common microbial foundation, each breed harbored a distinct set of bacteria that might contribute differently to milk quality, stability, and functional properties.



Fig. 2: Venn diagram showing the shared and unique bacterial taxa detected in P1 and P2.

Krona Plot

Krona plots are used to visualize metagenomic data based on hierarchical taxonomic levels. In the Krona Plot of sample P1 Saanen, the Actinomycetota microbiota group had the highest percentage at 15% with the species *Kocuria rhizophila*, while in the Krona Plot of sample P2 PE, the Bacillota microbiota group had the highest percentage at 17% with the species *Lactococcus garvieae* (Fig. 3 and 4).

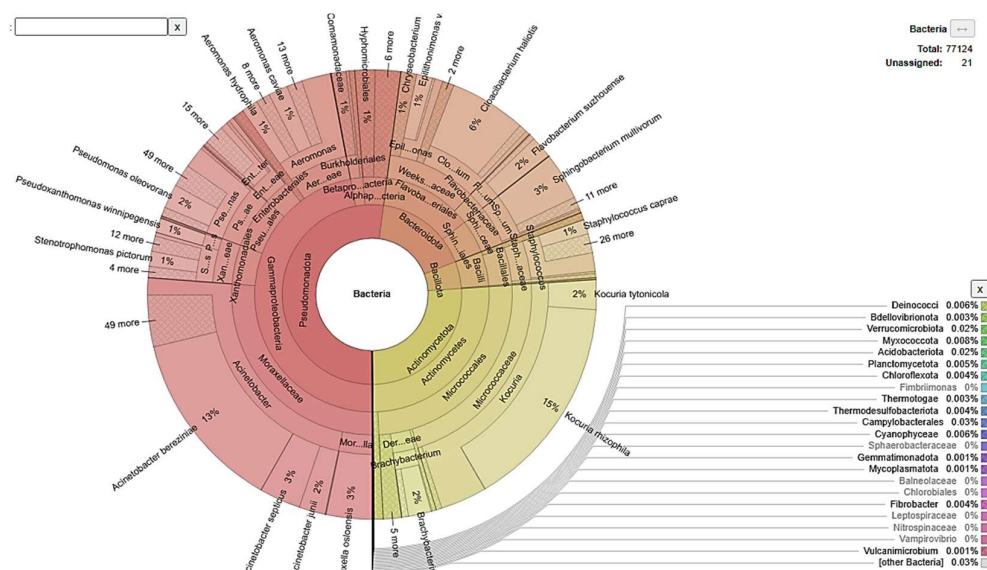


Fig. 3: Taxonomic composition of P1 Saanen.

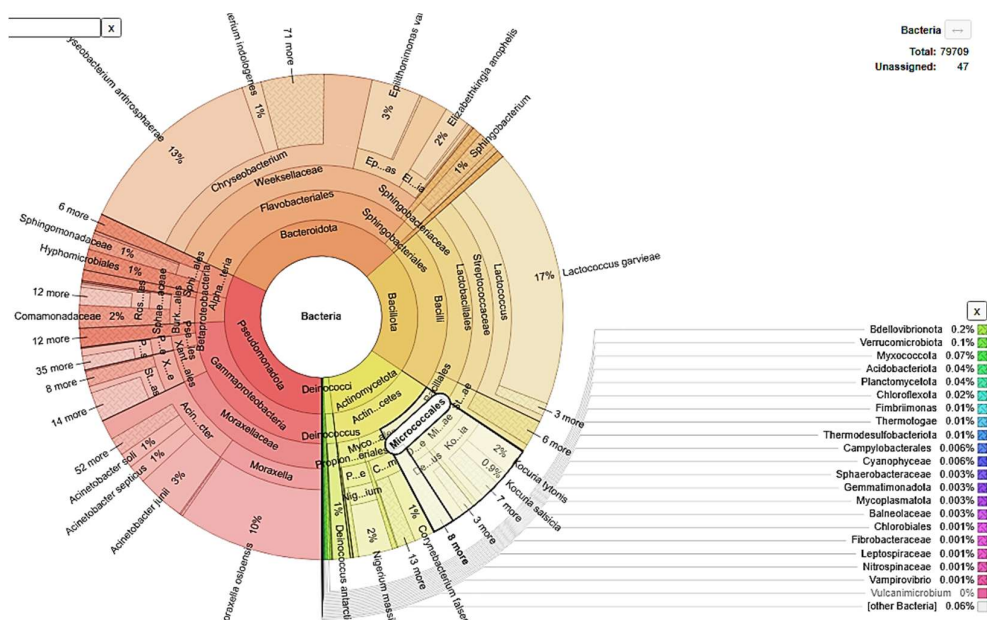


Fig. 4: Taxonomic composition of P2 PE.

The Taxonomic composition of the Saanen goat indicated that the bacterial community was predominantly composed of the phyla *Proteobacteria* and *Actinobacteria*, with *Gammaproteobacteria* as the most abundant class and *Kocuria rhizophila* as the dominant species (approximately 15%). The predominance of *K. rhizophila* suggested a strong influence of environmental and teat-skin-associated microbiota, which is characteristic of tropical goat milk production systems and reflects the fresh condition of the milk prior to fermentation. The presence of psychrotrophic bacteria in the genera *Pseudomonas*, *Acinetobacter*, and *Stenotrophomonas* indicated a potential risk of milk quality deterioration during cold storage due to enzymatic activity, although no dominant pathogenic bacteria were found. The relatively low proportion of *Firmicutes*, including lactic acid bacteria, further confirmed that spontaneous fermentation

had not occurred. Overall, this taxonomic composition reflected a balance between environmental microbiota and opportunistic bacteria, with important implications for milking hygiene management and the quality of Saanen goat milk.

The taxonomic composition of PE goat milk showed that the bacterial community was dominated by *Proteobacteria* and *Firmicutes*, with *Gammaproteobacteria* the most abundant class and *Actinomycetota* the dominant species (around 17%). The high presence of *L. garvieae* suggested that lactic acid bacteria played an important role in this milk, indicating early microbial activity and good adaptation to the milk environment, although its potential as an opportunistic pathogen should still be considered. In addition, the presence of environmental and psychrotrophic bacteria such as *Pseudomonas*, *Acinetobacter*, and *Moraxella* reflects the influence of farm environment and milking practices on the

milk microbiota. Other bacterial groups, including *Actinobacteria* and *Bacteroidota*, were detected in lower proportions, showing a smaller contribution from skin- and environment-associated microbes. Overall, this microbial profile suggested breed-related and management-driven differences in milk microbiota and highlights their potential impact on milk quality and stability in PE goat milk.

Phylogenetic Tree

Phylogenetic trees were used to visualize the relationships between taxonomic levels within a sample. In this diagram (Fig. 5 and 6), the width of each node is proportional to the number of identification reads associated with that taxonomy. In sample P1, Saanen belongs to the phylum *Actinomycetota*, whereas in sample P2, PE belongs to the phylum *Firmicutes* (Firmicutes-Bacillota).

The phylogenetic tree of the Bacteriome from Saanen raw milk, obtained from a smallholder farmer in a humid tropical region, as shown in Fig. 5, successfully isolated various bacteria, including both pathogenic and non-pathogenic strains. A total of 10 bacterial phyla were found, predominantly *Pseudomonadota* and *Actinomycota*. The family was also classified into 10 orders, with roles for *Moraxellaceae* derived from *Pseudomonadota* and for *Micrococcaceae* from the *Actinomycota* phylum. At the genus level, *Kocuria* and *Acinetobacter* were dominant, with *Kocuria rhizophila* and *Acinetobacter bereziniae* as the specific strains. The current discovery was close to another investigator in Benin, which has a climate similar to that of Indonesia, where the *Pseudomonadota* and *Actinomycota* phyla were among the largest contaminants in raw dairy goat milk (Adje et al., 2025).

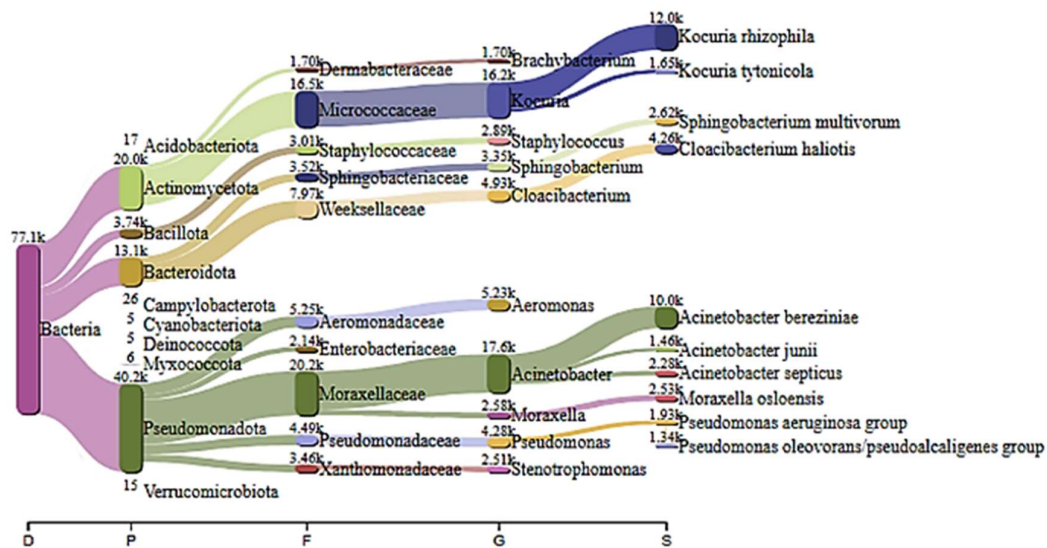


Fig. 5: Phylogenetic tree of bacteria on Saanen raw milk.

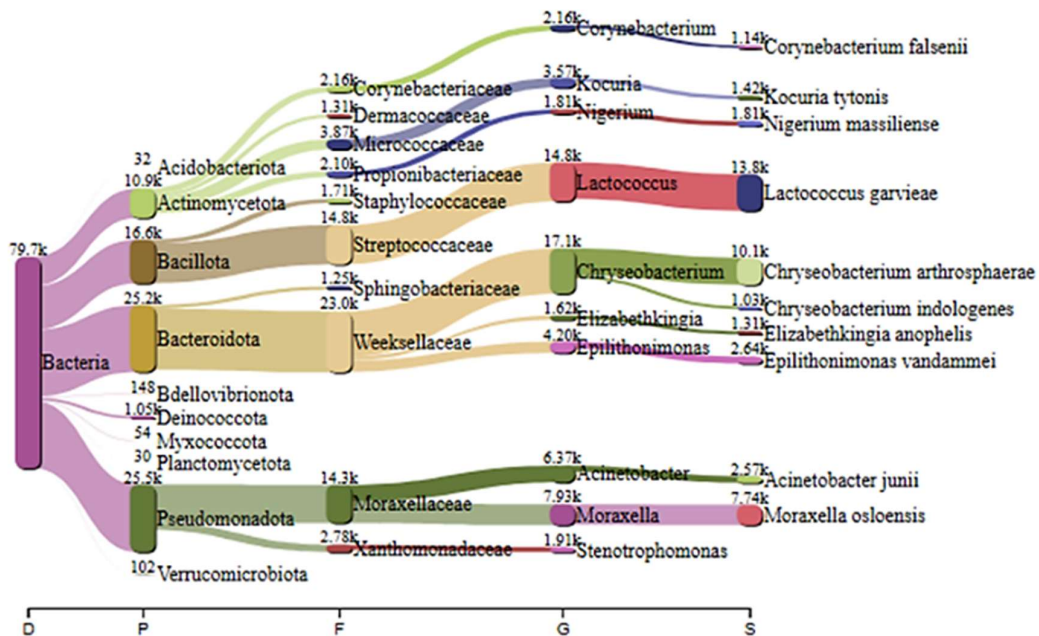


Fig. 6: Phylogenetic tree of bacteria on PE raw milk.

The phylogenetic tree of the Bacteriome from PE raw milk that was obtained from a smallholder farmer in a humid tropical region, as shown in Fig. 6, successfully isolated various bacteria, including pathogenic and non-pathogenic strains. A total of 10 bacterial phyla were found, predominantly *Bacillota*, *Bacteriodota*, and *Pseudomonadota*. The family was also classified into 10 orders, with roles for *Streptococaceae* derived from *Bacillota*, *Weeksellaceae* derived from *Bacteriodota*, and for *Moraxellaceae* from the *Pseudomonadota* phylum. At the genus level, *Lactococcus*, *Chryseobacterium*, and *Moraxella* were dominant, while the specific strains were *Lactococcus garviae* and *Moraxella osloensis*.

DISCUSSION

The present study revealed clear differences in the bacterial community composition of raw goat milk obtained from smallholder farms raising Saanen (P1) and Peranakan Ettawah (PE; P2) goats in a humid-tropical environment. The predominance of *Kocuria rhizophila* in Saanen milk and *Lactococcus garviae* in PE milk highlights distinct microbial signatures that likely reflect a combination of breed characteristics, farm management practices, milking hygiene, and environmental exposure.

Members of the genus *Kocuria* are Gram-positive, catalase-positive, and coagulase-negative coccoid bacteria that belong to the family Micrococcaceae, order Actinomycetales and class Actinobacteria (Savini et al., 2010). These bacteria are ubiquitous in nature and are commonly found in soil, water, on skin, and on the mucosal surfaces of animals. Among the 18 recognized *Kocuria* species, several—including *K. rhizophila*, *K. kristinae*, *K. rosae*, *K. marina* and *K. varians*—have been reported as opportunistic pathogens, particularly in immunocompromised individuals or patients with underlying conditions (Savini et al., 2010; Sohn et al., 2015). In contrast, PE goat milk was dominated by *Lactococcus garviae*, a Gram-positive, catalase-negative, facultatively anaerobic coccus classified within the lactic acid bacteria (LAB) group (Russo et al., 2012; Mawardi et al., 2024). Species of the genus *Lactococcus* are widely recognized for their role in dairy fermentation and for producing bacteriocins that inhibit foodborne pathogens (Mawardi et al., 2024). The high relative abundance of *L. garviae* in PE milk may indicate early microbial adaptation to the milk environment and potential functional relevance for fermentation processes. Despite its association with beneficial LAB functions, *L. garviae* has also been identified as an opportunistic pathogen in humans and animals, underscoring the need for careful monitoring when raw milk is consumed without pasteurization (Russo et al., 2012; Lee et al., 2025).

The dominance of *Kocuria rhizophila* in P1 and *Lactococcus garviae* in P2 indicated distinct microbial signatures between the two raw milk samples. Differences in microbial composition may be influenced by farm hygiene, milking environment, equipment sanitation, animal health status, and local climatic conditions. These findings underscore the importance of improving hygienic milking

procedures and implementing microbiological monitoring to ensure the safety and quality of raw milk in humid-tropical smallholder dairy goat systems. Raw milk from dairy goats produced by Indonesian smallholder farmers is commonly classified as low quality due to the abundance of microbial contaminants, and it often exceeds the national maximum standards, particularly for *E. coli* and Coliform (Nuhriawangsa et al., 2019). It was a factor in increasing the prevalence of mastitis (Suwito et al., 2023; Lestari et al., 2025) and it has also been scientifically proven that developing countries struggle more to address this issue (Idamokoro, 2023). In particular, the developed country performs better (Tomáška et al., 2024). As a key factor, well-managed dairy goat farming, notably the implementation of good hygiene milking practices, is essential (Isidro-Requejo et al., 2024).

Beyond safety concerns, raw goat milk can be a source of beneficial microorganisms. Lately, findings have revealed contamination of dairy goat raw milk with Spotted Fever Group Rickettsiae (SFGR) bacteria (Cisak et al., 2017). Likewise, in the Japanese Saanen breed, investigators identified potential probiotic bacterial strains for cultivation in the dairy industry (Tanaka et al., 2022). Another group of safety probiotics, grouped as lactic acid bacteria (LAB), was also successfully characterized from another breed of dairy goats (da Silva et al., 2019). Probiotics improve consumers' health status, including anticancer, hypocholesterolemic, and antihypertensive effects (Arief et al., 2023; Adiyoga et al., 2024). Lactic acid bacteria can also improve the quality of fermented goat milk (Setyawardani et al., 2020; Yang et al., 2022; Arief et al., 2026). A novel probiotic LAB strain was successfully isolated from a Nigerian dairy goat and differed from previously reported strains and from strains from another breed and location (Akinyemi et al., 2024). The quality of fermented milk is also influenced by the bacterial profile and the type of milk used (Elcheninov et al., 2023; Jankowska et al., 2026). The metagenomic profile of goat milk kefir has also been investigated, revealing the presence of several lactic acid bacteria (Sumarmono et al., 2023). Thus, a specific country with specific farming management, ambient conditions, and breed types would alter the types of pathogenic and probiotic bacteria strains and characteristics contaminated in the raw milk of dairy goats.

The dominance of certain bacteria in raw milk directly affected food safety. The high prevalence of *Kocuria rhizophila* and *Lactococcus garviae* indicated strong environmental influence and differences in microbiota among animal breeds. Although *Lactococcus* species are generally beneficial, *L. garviae* is known as an opportunistic pathogen in humans and animals, so its presence in raw milk must be carefully monitored, especially if the milk is consumed without pasteurization. For instance, while *Lactococcus* species are common in the microbiota of raw milk, certain strains, such as *Lactococcus garviae*, can be opportunistic pathogens, especially in immunocompromised individuals, and should be monitored in raw products intended for consumption without heat treatment (Lee et al., 2025). The use of natural flavonoid extracts has been investigated to enhance milk production and increase rumen microbial diversity, as revealed by metagenomic

analysis. These changes improve overall goat milk quality (Lu et al., 2025). Metagenomic studies have shown that *Lactococcus* can be abundant in fresh raw milk but may decrease relative to spoilage bacteria during storage. The presence of psychrotrophic spoilage bacteria such as *Pseudomonas* spp. and *Acinetobacter* spp. also posed risks to both milk quality and safety. *Pseudomonas* was frequently the dominant genus in refrigerated raw milk and contributed to spoilage through heat-resistant proteases and lipases, which remained active even after pasteurization and shortened shelf life (Lee et al., 2025). Additionally, psychrotrophic *Acinetobacter* and other Gram-negative bacteria were common in raw milk and can carry antibiotic resistance genes, potentially acting as reservoirs for antimicrobial resistance transmission along the cold chain (Wang et al., 2024). These findings highlight the need for rigorous pasteurization, effective cold-chain management, and rapid detection systems. Sequence-based metagenomic profiling provided comprehensive and sensitive detection of both spoilage and potentially pathogenic bacteria, allowing earlier risk assessment than traditional culture-based methods.

Metagenomic profiling using full-length 16S rRNA Nanopore sequencing proved effective in capturing a broad overview of the raw milk microbiota, including both dominant and low-abundance taxa. However, this approach has inherent limitations in species-level resolution and taxonomic assignment confidence, particularly for closely related bacteria (Angell et al., 2020; Oikonomou et al., 2020; Biada et al., 2025). Despite these limitations, the approach offers significant advantages over culture-based methods by enabling rapid, comprehensive detection of spoilage organisms and potentially pathogenic bacteria, supporting earlier risk assessment and targeted interventions (Beck et al., 2024; Gyax et al., 2025).

Overall, the dominance of *Kocuria rhizophila* and *Lactococcus garvieae* reflects strong environmental and management influences on raw goat milk microbiota in humid-tropical smallholder systems. These findings highlight the need for improved hygienic milking practices, routine microbiological surveillance and the use of advanced sequencing-based monitoring tools to enhance the safety and quality of raw milk while preserving its functional and probiotic potential.

Conclusion

This preliminary study profiled bacterial communities in raw goat milk from two smallholder farms in Bogor, West Java, Indonesia, using full-length 16S rRNA Nanopore sequencing. The dominant bacterial species detected were *Kocuria rhizophila* in Saanen goat milk (P1) and *Lactococcus garvieae* in PE goat milk (P2), indicating differences in bacterial community composition between the two samples. These baseline results may support early microbial monitoring, hygiene improvement programs, and food safety risk assessment in smallholder dairy goat production. Future studies should include more individual milk samples, additional farms, negative controls, and statistical analysis to validate microbiota differences and strengthen the interpretation of the findings.

DECLARATIONS

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Data Availability: All data has been presented in the manuscript.

Ethics Statement: This research was evaluated and approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, IPB University (Approval No. 380/KEH/SKE/IX/2025).

Author's Contribution: N.N, MSS, IIA, and CBD as designers of the research; NN, MSS conducted the research and collected data; IIA and CBD conducted data interpretation; SP and MA provided supporting data. All authors wrote the manuscript and approved the final manuscript.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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