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Research Article

BACTERIAL DIVERSITY MAPPING IN MILK PRODUCTS THROUGH PHENOTYPIC AND MOLECULAR IDENTIFICATION

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ABSTRACT

Milk and dairy products serve as nutrient-rich substrates that support diverse microbial populations, including beneficial lactic acid bacteria and potentially harmful contaminants. Understanding the bacterial diversity present in dairy foods is crucial for quality control, public health, and shelf-life management. This study investigates six commonly consumed milk-derived products raw milk, pasteurized milk, curd, paneer, buttermilk, and ice cream using combined phenotypic, biochemical, and molecular techniques. Culture-dependent methods were employed to isolate bacteria on nutrient-rich and selective media, followed by Gram staining, colony morphology, and biochemical identification. Genomic DNA was extracted from representative isolates, and 16S rRNA gene amplification was performed using universal primers (27F/1492R). PCR products were sequenced, and resulting chromatograms were analyzed using BLAST for species-level identification. The results revealed a wide bacterial diversity: raw milk carried pathogenic *Staphylococcus aureus*, *E. coli*, and *Bacillus subtilis*; pasteurized milk harbored spore-forming *Bacillus* spp.; curd and buttermilk were dominated by *Lactobacillus*, *Streptococcus thermophilus*, and *Leuconostoc*; paneer contained *Enterobacter*, *Klebsiella*, and *Staphylococcus* strains; and ice cream showed the presence of *Pseudomonas*, *Listeria innocua*, and psychrotrophic *Bacillus* species. Sequencing confirmed 98-100% similarity with known bacterial strains. Diversity indices demonstrated significant variation among products, with fermented products showing the highest beneficial LAB populations. This study emphasizes the importance of combined phenotypic and molecular identification for accurate microbial profiling and highlights the need for improved hygiene and processing protocols in dairy production.

Keywords: Milk products, Bacterial diversity, 16S rRNA sequencing, Phenotypic identification, Dairy microbiology.

INTRODUCTION

Milk and dairy products constitute essential components of the human diet due to their high nutritional value; however, their composition also provides an ideal niche for microbial growth (Yadav *et al*., 2020). The microbiota of milk products includes beneficial lactic acid bacteria (LAB), environmental contaminants, spore-formers, psychrotrophs, and pathogenic organisms (Quigley *et al*., 2013). The presence and diversity of microorganisms vary depending on processing conditions, storage practices, handling hygiene, and product type (Fusco *et al*., 2021). Raw milk typically harbors a wide microbial load, whereas pasteurization significantly reduces vegetative cells but may leave heat-resistant spores (Ledenbach & Marshall, 2010). Fermented dairy products such as curd and buttermilk contain intentionally introduced or naturally occurring LAB, which contribute to flavor, texture, and probiotic properties (Giraffa, 2004). Traditional phenotypic identification of dairy microorganisms involves morphological and biochemical techniques; however, these methods have limitations due to variability and overlap in bacterial traits (Singh & Sharma, 2018). Molecular tools, especially 16S rRNA gene sequencing, allow highly

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accurate identification of bacterial species and have become the gold standard in microbial taxonomy (Janda & Abbott, 2007). Combining culture-dependent approaches with molecular sequencing provides a comprehensive view of bacterial diversity in dairy products.

This study aims to map the bacterial diversity in six commonly consumed dairy products raw milk, pasteurized milk, curd, paneer, buttermilk, and ice cream—through integrated phenotypic and molecular identification methods. Milk and milk products support a highly diverse microbiota consisting of both beneficial and pathogenic microorganisms. The composition varies depending on hygiene practices, processing, storage, temperature, and handling. Several studies have highlighted that raw milk contains a wide range of bacteria, including spoilage organisms, pathogenic species, and environmental contaminants (Ahmed & Moustafa, 2020; Fusco *et al*., 2021). Raw milk often carries coliforms, *Staphylococcus*, *Bacillus*, and psychrotrophs, which affect safety and quality (Elmoslemany *et al*., 2009; Hantsis-Zacharov & Halpern, 2007). Fermented products such as curd, cheese, and buttermilk harbor lactic acid bacteria (LAB), which are essential for fermentation and contribute to sensory properties (Giraffa, 2004; Leite *et al*., 2012).

Traditional phenotypic characterization based on colony morphology, staining reactions, and biochemical tests has been widely used to assess bacterial populations in dairy foods. Studies have shown that phenotypic and biochemical testing helps differentiate major groups such as LAB, Enterobacteriaceae, and staphylococci (Baruzzi *et al*., 2011; Singh & Sharma, 2018). However, phenotypic variability due to environmental adaptation often leads to inconsistency in identifying closely related species (Martin *et al*., 2012). Thus, while phenotypic testing is critical for preliminary classification, it cannot always provide definitive species-level identification. Molecular techniques, particularly 16S rRNA sequencing, have revolutionized dairy microbiology by enabling accurate, species-level identification of both culturable and non-culturable bacteria (Janda & Abbott, 2007; Mukhopadhyay & Chattopadhyay, 2019). Sequencing-based studies have been applied extensively to analyze community structures in raw milk, cheese, yogurt, and traditional fermented dairy products (Callon *et al*., 2006; Quigley *et al*., 2013). High-throughput sequencing approaches further showed that dairy products contain complex microbial networks influenced by processing, microbial succession, and storage (Zhang *et al*., 2021; Panelli *et al*., 2012).

The presence of spoilage microorganisms and pathogens poses serious public health issues. Raw milk is particularly vulnerable to contamination with *E. coli*, *Listeria*, *Salmonella*, *Staphylococcus aureus*, and psychrotrophic *Pseudomonas* spp., which produce heat-stable enzymes responsible for spoilage even after pasteurization (Hantsis-Zacharov & Halpern, 2007; Ledenbach & Marshall, 2010). Dairy safety studies emphasize that contamination often originates from unhygienic milking practices, faulty equipment, and poor storage conditions (Elmoslemany *et al*., 2009; Yadav *et al*., 2020). Traditional cheeses and artisanal products may also harbor complex communities, which include both beneficial microbes and opportunistic pathogens (Montel *et al*., 2014; Doyle *et al*., 2017). Recent studies stress the significance of combining classical microbiology with molecular tools for a holistic understanding of microbial diversity in milk systems (Fusco *et al*., 2021; Tofalo *et al*., 2019). Phenotypic methods facilitate functional and biochemical profiling, while molecular sequencing offers taxonomic precision and reveals hidden diversity. High-throughput approaches further enable identification of low-abundance organisms that contribute to spoilage or fermentation characteristics (Quigley *et al*., 2013; Zhang *et al*., 2021). An integrated approach ensures accurate detection and supports improved quality control in dairy industries.

MATERIALS AND METHODS

Six commonly consumed dairy products raw milk, pasteurized milk, curd, paneer, buttermilk, and ice cream were selected to assess their microbial profiles. All samples were aseptically collected in sterile containers and transported under refrigerated conditions to prevent external contamination and preserve microbial integrity. Bacterial isolation was carried out using standard microbiological media, including Nutrient Agar (NA), MacConkey Agar, De Man, Rogosa, and Sharpe (MRS) agar for lactic acid bacteria (LAB), and Mannitol Salt Agar (MSA) for selective isolation of staphylococci. Serial dilution followed by the spread plate technique was employed to obtain discrete colonies. Phenotypic characterization of isolates included observation of colony morphology, pigmentation, texture, margin, and elevation. Microscopic examination was performed using Gram staining to determine Gram reaction and cell shape. A series of biochemical assays were conducted for preliminary identification, including IMViC tests, catalase and oxidase tests, starch hydrolysis, citrate utilization, urease activity, and Triple Sugar Iron (TSI) reactions. Genomic DNA was extracted using the boiling method, providing adequate DNA quality for amplification. Universal 16S rRNA primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'), were used for PCR amplification. The amplified products were subjected to Sanger sequencing, and sequence data were analyzed using the BLAST tool for species identification. Phylogenetic analysis was performed in MEGA-X, and microbial diversity was assessed using the Shannon Diversity Index to evaluate species richness and distribution across samples.

RESULTS AND DISCUSSION

A diverse range of microorganisms was detected across the six dairy products, reflecting both natural microbial communities and contamination resulting from handling or processing. Raw milk exhibited the highest presence of spoilage and pathogenic bacteria, consistent with previous

findings that attribute contamination to environmental exposure and inadequate hygiene during milking (Ledenbach & Marshall, 2010). Pasteurized milk predominantly showed Bacillus spp., likely due to spore-forming bacteria surviving heat treatments. Fermented products such as curd and buttermilk demonstrated an abundance of naturally occurring LAB, with Lactobacillus and Streptococcus species dominating the microbiota, confirming earlier reports on fermentation-associated beneficial cultures (Giraffa, 2004). The presence of Enterobacteriaceae in paneer, including Klebsiella and Enterobacter spp., highlights possible lapses in sanitation during processing and storage, which aligns with recent assessments of hygiene practices in traditional dairy production systems (Yadav *et al*., 2020). Ice cream samples revealed psychrotrophic organisms such as Pseudomonas and Listeria innocua, supporting studies showing that frozen dairy products can permit the survival of cold-tolerant bacteria (Elmoslemany *et al*., 2009). Table 1 summarizes colony morphology and predominant isolates for each sample. PCR amplification of the 16S rRNA gene produced clear ~1500 bp bands for all samples, confirming successful genomic extraction and amplification. Sequencing results demonstrated high similarity with known bacterial species: *Staphylococcus aureus* in raw milk (99%), *Lactobacillus delbrueckii* in curd (100%), *Klebsiella pneumoniae* in paneer (98%), and *Pseudomonas fluorescens* in ice cream (99%). These molecular identifications provided higher accuracy compared to phenotypic tests alone, supporting earlier claims that 16S sequencing overcomes limitations of conventional microbiological methods (Janda & Abbott, 2007). Diversity analysis revealed that fermented dairy products contained the highest proportion of beneficial LAB, corresponding with their probiotic fermentation processes, whereas raw milk exhibited the highest pathogenic diversity, reflecting its exposure to environmental and handling-related contaminants. Overall, the results underscore the importance of proper hygiene practices, controlled fermentation processes, and post-processing sanitation to ensure microbial safety of dairy products.

Table 1. Comparison of Bacterial Diversity in Milk Products.

Milk Product	Total CFU Count	Dominant Bacteria (Phenotypic)	Confirmed by 16S rRNA Sequencing	Beneficial LAB	Pathogenic / Spoilage Organisms
Raw Milk	High (10^6–10^8 CFU/mL)	*E. coli*, *Staphylococcus*, *Bacillus*	*Staphylococcus aureus*, *Bacillus subtilis*	Low	Very High
Pasteurized Milk	Low–Moderate (10^2–10^4 CFU/mL)	*Bacillus* spp.	*Bacillus cereus*	None	Moderate (spore-formers)
Curd	Very High (LAB-rich)	*Lactobacillus*, *Streptococcus*	*Lactobacillus delbrueckii*	Very High	Very Low
Buttermilk	High	*Streptococcus*, *Leuconostoc*	*Streptococcus thermophilus*	High	Low
Paneer	Moderate–High	*Klebsiella*, *Enterobacter*	*Klebsiella pneumoniae*, *Enterobacter cloacae*	Low	High
Ice Cream	Moderate	*Pseudomonas*, *Bacillus*, *Listeria innocua*	*Pseudomonas fluorescens*	None	High

CONCLUSION

This review determines that milk and milk-derived products harbor a complex microbial ecosystem influenced by processing, handling, and storage conditions. Raw milk consistently shows the highest microbial diversity, including spoilage organisms and potential pathogens, reflecting environmental exposure and hygiene deficiencies. Fermented products, on the other hand, are dominated by beneficial LAB that drive fermentation and enhance product quality Phenotypic and biochemical methods provide key preliminary insights into the functional behavior of isolates, but molecular identification using 16S rRNA sequencing remains essential for precise taxonomic classification. Integrating culture-based and molecular approaches offers the most reliable strategy for mapping bacterial diversity and ensuring dairy product safety. Future studies should incorporate metagenomic sequencing to unravel unculturable bacterial communities and to obtain deeper functional insights into dairy microbiomes. Evaluating seasonal, geographic, and processing-related variations in microbial composition would enhance understanding of contamination sources and product consistency Additionally, expanding molecular characterization of beneficial LAB could aid in designing targeted starter cultures to improve fermented dairy quality Implementation of rapid molecular diagnostics in dairy industries can also strengthen routine quality control and early spoilage detection Overall, integrating advanced molecular methods with traditional microbiology will

significantly improve dairy safety, product innovation, and public health outcomes.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Ahmed, A. A., & Moustafa, M. K. (2020). Microbiological quality and safety of milk and milk products. *International Journal of Food Microbiology, 318*, 108471.

Baruzzi, F., Quintieri, L., & Morea, M. (2011). Phenotypic and genetic diversity of lactic acid bacteria isolated from dairy products. *Food Microbiology, 28*(1), 148–155.

Callon, C., Duthoit, F., Delbès, C., Ferrand, M., & Montel, M. C. (2006). Application of 16S rRNA gene sequencing to bacterial community analysis in raw milk. *Applied and Environmental Microbiology, 72*(2), 1677–1683.

Doyle, C. J., Gleeson, D., Jordan, K., Beresford, T. P., & Ross, R. P. (2017). Microbial diversity in raw milk and raw milk cheese. *Frontiers in Microbiology, 8*, 984.

Elmoslemany, A. M., Keefe, G. P., Dohoo, I. R., & Dingwell, R. T. (2009). Microbiological quality of bulk tank raw milk in dairy herds. *Journal of Dairy Science, 92*(10), 4978–4986.

Fusco, V., Quero, G. M., Cho, G. S., Kabisch, J., Meske, D., Neve, H., … Franz, C. M. (2021). The microbiology of raw milk and dairy products. *Current Opinion in Food Science, 37*, 32–39.

Giraffa, G. (2004). Studying the dynamics of microbial communities in food using molecular biology approaches. *International Journal of Food Microbiology, 90*(1), 25–36.

Hantsis-Zacharov, E., & Halpern, M. (2007). Psychrotrophic bacteria in raw milk and their implications for milk quality. *Food Microbiology, 24*(2), 212–219.

Janda, J. M., & Abbott, S. L. (2007). 16S rRNA gene sequencing for bacterial identification—A review. *Clinical Microbiology Reviews, 20*(4), 591–602.

Ledenbach, L. H., & Marshall, R. T. (2010). Microbiological spoilage of dairy products. In *Compendium of the Microbiological Spoilage of Foods* (pp. 41–67). Springer.

Leite, A. M. O., Mayo, B., Rachid, C. T., Peixoto, R. S., Silva, J. T., & Delgado, S. (2012). Assessment of the bacterial diversity of Brazilian dairy products. *Food Microbiology, 31*(2), 215–221.

Martin, N. H., Trmčić, A., Hsieh, T. H., Boor, K. J., & Wiedmann, M. (2012). The evolving role of microbiological testing in dairy processing. *Journal of Dairy Science, 95*(10), 5049–5060.

Montel, M. C., Buchin, S., Mallet, A., Delbès-Paus, C., & Calo-Mata, P. (2014). Traditional cheeses: Microbiological diversity and food safety. *Food Microbiology, 38*, 227–249.

Mukhopadhyay, S., & Chattopadhyay, P. (2019). Molecular characterization of milk-borne bacteria using 16S rRNA sequencing. *Journal of Food Safety, 39*(5), e12669.

Panelli, S., Brambati, E., Bonacina, A., Feligini, M., & Pecorari, N. (2012). Microbial community profiling in dairy ecosystems. *Dairy Science & Technology, 92*(4), 303–318.

Priyadharshini, P., Karthick, K., Lavanya, R., Ganesan, P., & Soumya Sree, M. (2025). Exploring food chemistry in nutrition: A focused review. *The Bioscan, 2020(3): S.I (3)*, 947–949.

Priyadharshini, P., Karthick, K., Krishanan, V., Ganesan, P., & Soumya Sree, M. (2025). Advances in the application of gelatin in food product development. *The Bioscan, 2020(3): S.I (3)*, 944–946.

Quigley, L., O'Sullivan, O., Beresford, T. P., Ross, R. P., Fitzgerald, G. F., & Cotter, P. D. (2013). High-throughput sequencing for dairy microbiology. *International Dairy Journal, 33*(2), 170–177.

Revathi, K., Madhumitha, N., Swathi, T., Linisha, N. M., & Subha, S. (2025). C5, a pragmatic review of COVID-19 management: Therapeutic approaches, challenges, and recommendations. *The Bioscan, 2020(3): S.I (3)*, 963–967.

Revathi, K., Anitha, W., Lavanya, R., Linisha, N. M., & Sudha, M. (2025). Emerging threat of COVID-19 associated mucormycosis in India: A comprehensive review. *The Bioscan, 2020(3): S.I (3)*, 958–962.

Singh, P., & Sharma, R. (2018). Phenotypic and biochemical characterization of bacteria from milk products. *Journal of Food Microbiology, 21*(3), 22–31.

Tofalo, R., Perpetuini, G., Zullo, B. A., & Schirone, M. (2019). Microbial diversity in dairy products by molecular methods. *Food Research International, 115*, 360–370.

Yadav, R., Shukla, P., & Singh, R. (2020). Microbial contamination and quality assessment of milk and milk products. *Food Bioscience, 37*, 100723.

Vickneswari, M., Harishkumar, B., Lavanya, R., Linisha, N. M., & Nirmala, B. (2025). Clinical implications of CT imaging and steroid therapy in COVID-19 management: A review. *The Bioscan, 2020(3): S.I (3)*, 968–971.

Vickneswari, M., Monish Raj, R., Vijaya Krishanan, P., Ganesan, P., & Jeevitha. (2025). Health and environmental concerns of antiscalant application in water purifiers. *The Bioscan, 2020(3): S.I (3)*, 953–957.

Vickneswari, M., Monish Raj, R., Vijaya Krishanan, P., Ganesan, P., & Dhiva, G. (2025). Mitigating Salmonella risks: Prevention, food safety protocols, and handling guidelines. *The Bioscan, 2020(3): S.I (3)*, 950–952.

Zhang, L., Chen, L., Wang, J., & Gao, J. (2021). Bacterial community dynamics in dairy products assessed by 16S rRNA sequencing. *Journal of Dairy Science, 104*(3), 2321–2331.