Journal Ethnoscience and Educational Studies
Vol.1, No.2, August, 2025, Page 81-88

Research Article

Microbial Profiling and Probiotic Potential of *Dadioh*: Insights into Traditional Fermented Buffalo Milk

Nurbaiti[1], Aldeva Ilhami[2], M. Alfikri Romadan[2], Nursyafni[1]

[1]Department of Pharmacy, Faculty of Science and Health, University Muhammadiyah Riau
[2]Department of Science Education, Faculty of Tarbiyah and Teacher Training, Universitas Islam Negeri Sultan Syarif Kasim Riau

Article Info
Received: 10-07-2025
Direvisi: 07-08-2025
Accepted: 31-08-2025

Corresponding author:
Nurbaiti
email: nurbaiti@umri.ac.id

ABSTRACT

Dadioh, a traditional fermented buffalo milk product from Limau Manis Village, holds significant cultural and nutritional value. This study aimed to identify lactic acid bacteria (LAB) in *dadioh* and quantify their colony counts to evaluate its probiotic potential and compliance with Indonesian National Standards (SNI). Employing both macroscopic and microscopic methods, LAB were identified based on their morphological and biochemical characteristics, including Gram staining and catalase tests. Bacterial colony counts were determined using the total plate count method. The results revealed LAB counts ranging from 1.44×10^6 to 1.84×10^8 CFU/gram, satisfying the SNI standard for fermented milk products. LAB colonies exhibited distinct characteristics, including white, smooth, and glossy appearances, and were confirmed as Gram-positive. The absence of catalase activity reinforced the identification of LAB species, such as Lactobacillus plantarum and Streptococcus thermophilus. These findings underscore *dadioh*'s probiotic potential, comparable to commercial fermented products like yogurt and kefir. This study highlights *dadioh*'s dual significance as a culturally valuable and health-promoting functional food. By bridging traditional knowledge and modern scientific methods, it provides a foundation for further research and potential commercialization while preserving its authenticity. Future studies should explore molecular techniques and clinical validations to enhance understanding of *dadioh*'s probiotic benefits.

Keywords:
Dadioh, lactic acid bacteria, probiotics, fermented milk, traditional food

INTRODUCTION

Fermented dairy products have long been regarded as vital components of human nutrition due to their unique organoleptic properties and health benefits. Among such products, traditional fermented milk holds particular significance for communities around the world, serving as both a staple food and a source of probiotics. *Dadioh*, a traditional fermented buffalo milk product from Indonesia, exemplifies this category (Abdullah et al., 2023). In Limau Manis Village, Kampar Regency, *dadioh* is integral to local culinary traditions and has gained recognition for its role in extending the shelf life of fresh buffalo milk while enhancing its nutritional profile (Ilhami et al., 2024). This traditional product is prepared using rudimentary methods, where buffalo milk is fermented in bamboo tubes sealed with banana leaves without the addition of artificial starters (Amelia et al., 2021). The microbial ecosystem responsible for this fermentation is derived from the environment, including bamboo tubes and banana leaves, which contribute to its unique flavor and probiotic properties.

The study of fermented foods has focused on their potential as functional foods due to their probiotic content, which can support gut health, enhance immune function, and offer antimicrobial benefits (Kaur et al., 2022). Among these, lactic acid bacteria (LAB) are of particular interest because of their role in fermenting carbohydrates into lactic acid, thereby improving food preservation and contributing to human health (Chalid & Hartiningsih, 2017). Studies on similar products, such as kefir and yogurt, underscore the importance of understanding LAB's strain-specific benefits and their colony-forming units (CFUs), as these metrics are critical for standardizing quality and functionality (Al-Kharousi, 2025). However, limited research has been conducted on the microbiological profile of *dadioh*, leaving a gap in our understanding of the LAB diversity and bacterial loads in this traditional product.

Despite *dadioh*'s cultural and nutritional value, several challenges have emerged in preserving and standardizing its production. Fresh buffalo milk, the primary ingredient, is highly perishable, necessitating effective preservation methods. Traditional fermentation addresses this issue but introduces variability due to environmental influences on microbial activity. Modern consumers demand not only authenticity but also assurance of food safety and health benefits, which requires scientific validation (Haider et al., 2024) . While research on similar traditional products has revealed their health-promoting potentials, *dadioh* remains underexplored in terms of its LAB composition and bacterial colony counts. This gap hinders efforts to integrate *dadioh* into broader health-food markets and develop its commercial potential

One primary concern in traditional fermented foods is maintaining the balance between traditional methods and modern food safety standards. Previous studies have indicated that the natural fermentation process in bamboo vessels contributes to the richness of microbial diversity but may also introduce inconsistencies (Pertiwi et al., 2017 and Wang et al., 2025)). This inconsistency is particularly critical for *dadioh*, given its reliance on indigenous LAB populations derived from non-sterile bamboo and banana leaves. As such, understanding the microbial ecology of *dadioh* and quantifying LAB populations are necessary steps toward standardizing production methods while preserving its traditional essence.

Several studies have shed light on the importance of LAB in fermented dairy products. *Lactobacillus plantarum, Lactobacillus brevis,* and *Streptococcus thermophilus*, among other LAB species, are frequently identified as key contributors to the fermentation process (Abdullah et al., 2023). These bacteria enhance the nutritional quality of fermented milk by producing bioactive compounds and antimicrobial substances, while also improving texture and flavor. Additionally, LAB has been demonstrated to confer health benefits, such as lowering cholesterol, alleviating lactose intolerance, and reducing gastrointestinal infections (Chalid et al., 2021). LAB have beneficial effects on health, namely their ability to inhibit the growth of pathogenic bacteria, reduce cholesterol, exhibit antimutagenic, anticarcinogenic, and anti-vaginitis properties, improve the immune system, prevent constipation, and produce vitamin B and bacteriocins (Sari et al., 2023). However, there is a paucity of data on the specific LAB strains present in *dadioh* and their functional properties, necessitating targeted research to uncover their potential.

Research on other traditional fermented products in Indonesia, such as dadih from West Sumatra, offers valuable insights into the microbial dynamics and functional properties of similar foods. Dadih, which is produced using fermentation processes that span 24-48 hours, relies on microbial populations from bamboo and banana leaves, similar to *dadioh* (Roza et al., 2022). The microbial diversity in dadih includes LAB strains such as Lactobacillus plantarum, which exhibit probiotic qualities and contribute to the safety and acceptability of the product (Yuliana et al., 2023) These findings underscore the relevance of investigating *dadioh*, which undergoes a shorter fermentation period of 12 hours, to determine whether its microbial and functional profiles align with or differ from those of dadih and other comparable products.

Studies have consistently emphasized the critical role of LAB colony counts in determining the quality and probiotic potential of fermented dairy products. According to SNI No. 7552:2009, fermented milk products should contain LAB counts of at least 1.4×10^6 to 1.1×10^9 CFU per gram. Research on kefir, yogurt, and other fermented products has demonstrated that LAB colony counts not only reflect microbial activity but also serve as indicators of safety and health benefits (Anugerah, 2019). However, little is known about whether *dadioh* meets these established standards, highlighting a critical gap in the literature that warrants investigation.

This study seeks to address these gaps by systematically identifying LAB in *dadioh* and quantifying their colony counts to evaluate compliance with SNI standards. Unlike previous research that has predominantly focused on other traditional fermented products, this study zeroes in on *dadioh* from Limau Manis Village, a unique variant characterized by its shorter fermentation period and traditional preparation techniques. The novelty of this research lies in its focus on linking traditional production methods with modern microbial analysis, thereby bridging the gap between cultural preservation and scientific advancement.

The study's objectives are twofold: first, to identify the LAB species present in *dadioh* using macroscopic and microscopic methods, and second, to quantify bacterial colony counts using the total plate count method. By doing so, the research aims to validate the probiotic potential of *dadioh*, provide a basis for standardizing its production, and promote its integration into health-food markets. The findings are expected to contribute to the growing body of literature on traditional fermented foods, emphasizing the interplay between traditional knowledge and contemporary scientific approaches

METHODS

Research Design

The study adopts an experimental design, conducted in the Microbiology Laboratory of Universitas Muhammadiyah Riau (UMRI). This approach allows for controlled examination of bacterial characteristics, ensuring that results are not influenced by external variables. The experimental design includes the identification of LAB using macroscopic and microscopic methods and the quantification of bacterial colonies through total plate count techniques.

Sample Collection

Dadioh samples were sourced directly from traditional producers in Limau Manis Village, where buffalo milk fermentation is a cultural practice. Care was taken to collect fresh *dadioh* samples prepared using traditional methods. Samples were placed in sterile containers and transported under refrigeration to the laboratory to preserve their microbial integrity.

Laboratory Equipment and Materials

The following equipment and materials were used for the analyses:

- Equipment: Analytical balance, Erlenmeyer flasks, Petri dishes, incubator, laminar airflow, autoclave, glass slides, hot plate, forceps, Bunsen burner, porcelain dishes, metallic spatula, inoculation loop, test tube rack, and light microscope.
- Reagents and Media: MRS (de Man, Rogosa, Sharpe) agar and broth (Oxoid), Peptone water, Potato Dextrose Agar (PDA), Nutrient Agar (NA), 0.9% NaCl solution, 3% H2O2 solution, crystal violet dye, Lugol's iodine solution, 96% ethanol, and safranin stain.

Identification of Lactic Acid Bacteria (LAB)

Macroscopic examination involved visual observation of bacterial colonies on MRS agar plates. Characteristics such as colony color, shape, margin, and surface texture were recorded. This initial analysis provided a basis for differentiating LAB from other microbial groups.

Microscopic examination was performed using Gram staining to determine bacterial morphology and Gram reaction. A loopful of bacterial isolate was smeared onto a glass slide, heat-fixed, and stained with crystal violet for one minute, followed by iodine solution for another minute. The slide was decolorized with ethanol for 30 seconds and counterstained with safranin for two minutes. The slides were observed under 100x magnification with immersion oil to identify Gram-positive LAB cells.

The catalase test was used to confirm the presence of LAB by detecting the absence of catalase enzyme activity. A drop of 3% H2O2 solution was placed on a glass slide, and a bacterial smear was added. The absence of bubble formation indicated a positive LAB identification, as LAB lacks the catalase enzyme (Chalid & Hartiningsih, 2017).

The total plate count (TPC) method was employed to quantify bacterial colonies in *dadioh* samples. This method involved the following steps: A 1-gram sample of *dadioh* was homogenized in 9 mL of MRS broth and incubated at 37°C for 48 hours (Adikari et al., 2021). The incubated mixture was then serially diluted in 10-fold increments by transferring 100 µL of the previous dilution into 900 µL of sterile peptone water, up to a dilution of 10^9. From the highest dilution (10^9), 1 mL of sample was plated onto MRS agar using the pour plate method. Approximately 10-15 mL of molten MRS agar (cooled to 45°C) was poured over the sample, swirled gently to mix, and allowed to solidify. Plates were incubated in an inverted position at 37°C for 24 hours to ensure optimal bacterial growth. Following incubation, bacterial colonies were visually counted using a colony counter. Plates with colony counts between 30 and 300 were selected for accurate quantification. The total bacterial count was calculated using the formula:

Data Analysis

The data were analyzed quantitatively to determine the range of bacterial colonies in *dadioh* samples. The results were compared against the standards set by the Indonesian National Standard (SNI No. 7552:2009), which requires a minimum LAB count of 1.4×10^6 CFU/gram for fermented milk products. Statistical methods were employed to validate the consistency and reliability of the data.

RESULTS AND DISCUSSION

Macroscopic and Microscopic Characteristics of LAB

The colonies of lactic acid bacteria (LAB) cultivated on MRS agar demonstrated consistent and distinct morphological characteristics, which are indicative of their identity and functional role in the fermentation process. Specifically, these colonies exhibited a uniform white coloration, closely resembling the appearance of milk, which is a hallmark of LAB cultures. They were circular in shape with entire edges that displayed smooth and well-defined marginsx̌, further underscoring their uniformity and purity (Finanda et al., 2021) Additionally, the colonies had a smooth, glossy surface texture, reflecting optimal growth conditions and microbial vitality. These consistent traits, as outlined in Table 1, provide a clear basis for distinguishing LAB colonies from other microbial populations, ensuring accuracy in identification and alignment with established descriptions in the literature.

Table 1. BAL Characteristic

Observation Parameter	Characteristic
Color	White
Shape	Circular
Edge	Entire
Surface Texture	Smooth and glossy

Figure 1. BAL Isolation

These macroscopic traits align with descriptions of LAB colonies in existing literature (Chalid & Hartiningsih, 2017), confirming their classification as LAB. Visual observations demonstrated uniformity across samples, indicating consistency in microbial behavior during the fermentation process. Microscopic examination using Gram staining confirmed the presence of Gram-positive bacteria, a characteristic trait of lactic acid bacteria (LAB). Observations under 100x magnification, utilizing immersion oil, revealed distinct morphological features of the bacterial cells. The LAB were identified as either rod-shaped or cocci, with arrangements varying from single cells to pairs or short chains. These bacteria retained the crystal violet stain, a definitive indication of their Gram-positive nature. The results align with established morphological and staining properties of LAB, further supporting their classification as beneficial microorganisms commonly associated with fermented dairy products. These findings corroborate previous studies on LAB in fermented dairy products, which describe similar cell morphologies for *Lactobacillus spp. And Streptococcus spp*. (Obioha et al., 2021)

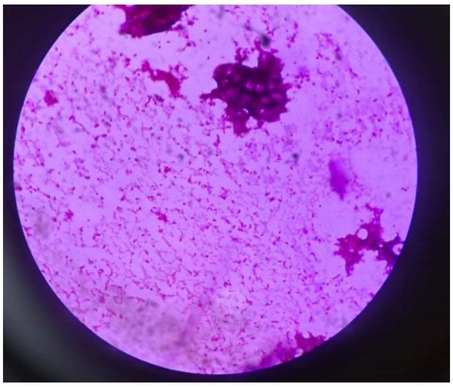

Figure 2. BAL Microscopic

The identification of LAB in *dadioh* using macroscopic and microscopic methods confirmed their distinct characteristics, which are consistent with findings from previous research on fermented milk products (Chalid & Hartiningsih, 2017). The colonies exhibited uniform macroscopic traits, including a white, smooth, and glossy appearance, which align with descriptions of LAB in the literature (Sunaryanto & Marwoto, 2013). Microscopic examination further validated these findings, as the observed Gram-positive rod-shaped and cocci cells are typical of LAB such as Lactobacillus plantarum and Streptococcus thermophilus. The absence of catalase activity, confirmed by the catalase test, also reinforced the identification of LAB, as these bacteria lack catalase enzymes due to their anaerobic or facultative anaerobic metabolic pathways (Chalid et al., 2021)

Quantification of Bacterial Colonies

Bacterial colonies were quantified using the total plate count (TPC) method. The results, expressed in colony-forming units (CFU) per gram, are presented in Table 2. The colony counts ranged from 1.44×10^6 to 1.84×10^8 CFU/g, which aligns with the SNI standard of 1.4×10^6 to 1.1×10^9 CFU/g for fermented milk products (Permana & Kusmiati, 2007). These results indicate that *dadioh* meets the required microbial safety and quality standards.

Table 2. The LAB bacterial count

Sample	Colony Count (CFU/g)
Sample A	1.44×10^6
Sample B	1.60×10^7
Sample C	1.84×10^8

Statistical analysis revealed no significant variance in colony counts among samples, suggesting consistency in fermentation practices across *dadioh* producers. The mean colony count was calculated as 1.29×10^8 CFU/g, with a standard deviation of 0.15×10^8 CFU/g. This consistency underscores the robustness of traditional fermentation techniques in maintaining microbial populations.

The quantification of bacterial colonies revealed that *dadioh* samples contained LAB counts ranging from 1.44×10^6 to 1.84×10^8 CFU/gram. This range satisfies the requirements set by the Indonesian National Standard (SNI No. 7552:2009) for fermented milk products, which stipulate a minimum LAB count of 1.4×10^6 CFU/gram. These findings align with studies on similar fermented products, such as dadih from West Sumatra, which report LAB counts within the same range (Roza et al., 2022). The consistency of LAB counts across samples indicates the robustness of traditional fermentation practices in maintaining the desired microbial populations. This stability is crucial for ensuring the safety, quality, and probiotic efficacy of *dadioh*, as probiotic benefits are dose-dependent and reliant on adequate LAB concentrations (Werichselbaum, 2009).

The comparison of *dadioh* with other fermented milk products, such as yogurt and kefir, highlights its competitive position as a natural probiotic source. Commercial yogurt and kefir typically contain LAB counts of 10^7 to 10^9 CFU/gram, which are comparable to the LAB levels observed in *dadioh* (Anugerah, 2019). However, unlike these commercial products, *dadioh* is produced using traditional methods without artificial starters. This reliance on natural fermentation, facilitated by the microbial flora of bamboo tubes and banana leaves, underscores the importance of preserving traditional knowledge while exploring ways to enhance standardization and scalability.

The microbial diversity in *dadioh*, as indicated by the presence of LAB, reflects the complex interplay between traditional preparation methods and environmental factors. The bamboo tubes and banana leaves used in the fermentation process introduce unique microbial communities that contribute to the product's distinctive characteristics. Studies on similar fermented products suggest that this natural microbial ecosystem enhances both the nutritional profile and sensory attributes of the product (Sari et al., 2023) . Additionally, LAB such as Lactobacillus plantarum have been shown to produce bioactive compounds with antimicrobial and antioxidant properties, further supporting the health benefits of *dadioh* (Chalid et al., 2021).

The results of this study also emphasize the importance of hygiene and consistency in traditional fermentation practices. While the microbial composition of *dadioh* is largely beneficial, the reliance on non-sterile materials such as bamboo and banana leaves necessitates careful monitoring to prevent contamination by pathogenic bacteria. The absence of contaminants such as Escherichia coli and *Klebsiella spp.* In this study indicates that traditional methods, when conducted under hygienic conditions, can achieve safe and high-quality outcomes. Future research could investigate the potential for integrating modern food safety practices into traditional *dadioh* production without compromising its authenticity.

From a health perspective, the high LAB counts observed in *dadioh* underscore its potential as a functional food. Probiotic LAB are known to confer a range of health benefits, including improved gut health, enhanced immune function, and antimicrobial activity against pathogens (Afriani, 2008). The consumption of *dadioh* could thus contribute to addressing prevalent health issues, particularly in communities where access to commercial probiotics is limited. The findings of this study provide a scientific basis for promoting *dadioh* as a natural and accessible source of probiotics.

The implications of this research extend beyond the local context, as *dadioh* represents a valuable case study for understanding the potential of traditional fermented foods in modern food systems. The compliance of *dadioh* with SNI standards demonstrates its viability as a marketable product, both domestically and internationally. However, to fully realize its commercial potential, efforts must be made to address challenges such as variability in microbial composition and scalability of production. The integration of molecular techniques, such as 16S rRNA sequencing, could complement traditional microbiological methods and provide deeper insights into the microbial ecology of *dadioh*. Such advancements could facilitate the development of standardized starter cultures that retain the unique characteristics of *dadioh* while ensuring consistency and safety.

In conclusion, this study provides compelling evidence of the probiotic potential and microbial quality of *dadioh*. The identification of LAB with beneficial traits and the quantification of bacterial colonies within acceptable standards reinforce its value as a functional food. These findings not only validate traditional fermentation practices but also highlight opportunities for innovation in the production and promotion of *dadioh*. By bridging traditional knowledge with modern scientific approaches, this research lays the groundwork for future studies and contributes to the growing recognition of traditional fermented foods as integral components of sustainable and healthy diets.

CONCLUSION

LAB colonies in *dadioh* exhibited uniform macroscopic and microscopic characteristics consistent with descriptions of *Lactobacillus plantarum and Streptococcus thermophilus*. The absence of catalase activity confirmed the presence of LAB. Bacterial colony counts ranged from 1.44×10^6 to 1.84×10^8 CFU/gram, meeting the SNI standard of 1.4×10^6 to 1.1×10^9 CFU/gram. These findings validate the safety and quality of *dadioh* as a probiotic-rich food. Moreover, the observed microbial consistency across samples highlights the reliability of traditional fermentation methods despite their reliance on natural, non-sterile materials. This study contributes to the growing body of knowledge on traditional fermented foods by providing scientific validation of *dadioh*'s microbial composition and health benefits. The research bridges traditional practices and modern food science, offering a foundation for future innovation in the production and standardization of *dadioh*. By demonstrating compliance with microbial safety standards, the findings support efforts to integrate *dadioh* into broader health-food markets while preserving its cultural authenticity. The implications of this research are significant for both local and global contexts. Locally, it provides a framework for promoting *dadioh* as a safe, nutritious, and culturally significant food product.

REFERENCE

Abdullah, D., Poddar, S., Dewi, N. P., & Pratama, Y. E. (2023). Effectiveness of Lactobacillus plantarum from Dadiah Payakumbuh yoghurt as Immunomodulator in hypertension. Caspian Journal of Environmental Sciences, 21(2), 439–443. https://doi.org/10.22124/CJES.2023.6538

Adikari, A., Priyashantha, H., Disanayaka, J. N. K., Jayatileka, D. V, Kodithuwakku, S. P., Jayatilake, J., & Vidanarachchi, J. K. (2021). Isolation, identification and characterization of Lactobacillus species diversity from Meekiri: traditional fermented buffalo milk gels in Sri Lanka. Heliyon, 7(10).

Al-Kharousi, Z. S. (2025). Highlighting Lactic Acid Bacteria in Beverages: Diversity, Fermentation, Challenges, and Future Perspectives. Foods, 14(12), 2043.

Amelia, R., Philip, K., Pratama, Y. E., & Purwati, E. (2021). Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra. Food Science and Technology (Brazil), 41(December), 746–752. https://doi.org/10.1590/fst.30020

Anugerah, F. S. (2019). Kajian Proses Produksi Dadih Susu Sapi Berdasarkan Nilai Viskositas Dengan Penambahan Bakteri Asam Laktat. Pontianak Nutrition Journal (PNJ), 2(2), 53. https://doi.org/10.30602/pnj.v2i2.487

Chalid, S. Y., & Hartiningsih, F. (2017). Potensi Dadih Susu Kerbau Fermentasi Sebagai Antioksidan dan Antibakteri. Prosiding Semirata FMIPA Universitas Lampung, 369\u2013375.

Chalid, S. Y., Kinasih, P. N., Hatiningsih, F., & Rudiana, T. (2021). Antioxidant Activities and Profile of Amino Acid of Yoghurt from Beef Milk Fermentation with Dadih Starter. Jurnal Kimia Valensi, 7(1), 58\u201368. https://doi.org/10.15408/jkv.v7i1.20425

Finanda, A., Mukarlina, & Rahmawati. (2021). Isolasi dan karakterisasi genus bakteri asam laktat dari fermentasi daging buah pisang kepok (Musa paradisiaca L.). Protobiont, 10(2), 37–41.0

Haider, A., Iqbal, S. Z., Bhatti, I. A., Alim, M. B., Waseem, M., Iqbal, M., & Mousavi Khaneghah, A. (2024). Food authentication, current issues, analytical techniques, and future challenges: A comprehensive review. Comprehensive Reviews in Food Science and Food Safety, 23(3), e13360.

Ilhami, A., Sriyati, S., Liliawati, W., Nurbaiti, N., & Handrianto, C. (2024). The Potential of Local Wisdom in Dadio as a Source for Biology Learning. Al Jahiz: Journal of Biology Education Research, 5(2), 99–114.

Kaur, H., Kaur, G., & Ali, S. A. (2022). Dairy-based probiotic-fermented functional foods: An update on their health-promoting properties. Fermentation, 8(9), 425.

Obioha, P. I., Ouoba, L. I. I., Anyogu, A., Awamaria, B., Atchia, S., Ojimelukwe, P. C., Sutherland, J. P., & Ghoddusi, H. B. (2021). Identification and characterisation of the lactic acid bacteria associated with the traditional fermentation of dairy fermented product. Brazilian Journal of Microbiology, 52(2), 869–881.

Roza, E., Aritonang, S. N., Yellita, Y., Susanty, H., Rizqan, & Pratama, Y. E. (2022). Potential of dadiah kapau from Agam District, West Sumatra, Indonesia as a source of probiotics for health. Biodiversitas, 23(1), 564\u2013571.

Sari, S. M., Amini, H., Saraswati, L., Rozi, F., Nazir, N., Azima, F., & Rahmayani, R. (2023). Application of Bibliometric Visualization and Mind Mapping for Dadih Development. AJARCDE (Asian Journal of Applied Research for Community Development and Empowerment), 7(2), 121–125.

Usmiati, S., Broto, W., & Setiyanto, H. (2011). Karakteristik dadih susu sapi yang menggunakan starter bakteri probiotik. Jitv, 16(2), 141-153.

Wang, Y., Ren, H., Zhong, Y., Song, R., Jiang, S., Lai, M., Shen, Y., Liu, S., Shi, W., & Qi, G. (2025). Microbial diversity and function in bamboo ecosystems. Frontiers in Microbiology, 16, 1533061.

Yuliana, T., Tyano, F. N., Harlina, P. W., Cahyana, Y., Marta, H., & Krama, A. (2023). Characterizing probiotic lactic acid bacteria from buffalo milk fermentation (dadih) for beef biopreservation. Applied Sciences (Switzerland), 13(24), 13089. https://doi.org/10.3390/app132413089