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Metagenomic comparison of bacterial diversity in kefir grains and liquid kefir

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Abstract. *Kurniawan, Milanda T, Kusuma SAF. 2025. Metagenomic comparison of bacterial diversity in kefir grains and liquid kefir. Biodiversitas 26: 6378-6388.* This study compared the bacterial community composition of kefir grains and their corresponding whey from cow's milk fermentation using 16S rRNA gene-based metagenomic sequencing. Next-Generation Sequencing (NGS) analysis revealed 405 bacterial species in kefir grains and 294 in whey, with 118 species shared between both matrices. *Lactobacillus kefiranofaciens* dominated both environments, followed by *Lactobacillus helveticus* and *Streptococcus thermophilus*. Three key ecological patterns emerged. First, kefir grains exhibited higher microbial richness and diversity, reaffirming their role as the primary microbial reservoir during fermentation. Second, a core microbiome common to both matrices indicated functional continuity between solid and liquid phases, ensuring the stable transfer of fermentative consortia. Third, distinct community structures in the liquid phase were shaped by ecological filtering, where oxygen-tolerant taxa such as acetic acid bacteria gained relative dominance. The study highlights the ecological complexity and regional uniqueness of Indonesian artisanal kefir, which showed greater microbial richness than most reported global datasets. The results provide insights for enhancing food safety, optimizing probiotic formulations, microbial succession, core community dynamics, and potential probiotic resources for food biotechnology. These findings underscore the ecological complexity and regional distinctiveness of Indonesian artisanal kefir, which exhibited higher microbial richness than most global reports.

Keywords: Kefir, lactic acid bacteria, metagenomics, microbial diversity, milk fermentation

INTRODUCTION

Fermented foods have long played a central role in human diets, offering enhanced preservation, safety, and nutritional value. In recent decades, scientific interest has expanded beyond these traditional functions to the health-promoting properties of fermented foods, many of which are attributed to their diverse microbial consortia (Rezac et al. 2018; Douglas et al. 2020). Among them, kefir has received particular attention as a traditional fermented milk originating from the Caucasus region and now consumed worldwide (Petrova et al. 2021). Kefir is produced by fermenting milk with kefir grains polysaccharide-protein matrices primarily composed of kefiran that harbor a symbiotic community of bacteria and yeasts (Apalowo et al. 2024; Tingirikari et al. 2024; Saady et al. 2025).

Kefir grains act as natural starter cultures, initiating milk transformation through the metabolic activity of lactic acid bacteria (LAB), acetic acid bacteria, and yeasts. These microbial consortia generate organic acids, exopolysaccharides, and bioactive metabolites that contribute not only to product safety and sensory characteristics but also to probiotic potential (Azizi et al. 2021; Li et al. 2023). Despite this shared framework, kefir is not a standardized product: its microbial composition is shaped by factors including milk type, grain origin, fermentation conditions, and geographic provenance (Avila-Reyes et al. 2022; Destro et al. 2024). This variability underscores kefir as a dynamic ecological system where microbial diversity is closely linked to functional properties.

A key aspect of kefir ecology lies in the distinction between the grains and the surrounding fermented liquid (often termed kefir whey). While grains provide the microbial reservoir, the liquid fraction represents the active product consumed. Evidence suggests that microbial transfer from grains to whey is selective, influenced by ecological pressures such as oxygen gradients, nutrient availability, and pH shifts during fermentation (Kayrei et al. 2022; Balivo et al. 2023; Wang et al. 2023). Consequently, the microbial composition of grains may not be faithfully reflected in the fermented liquid, raising questions about the persistence, adaptation, and functional roles of taxa across the two niches. Addressing this knowledge gap is critical for understanding how kefir's probiotic potential is shaped during fermentation.

Advances in high-throughput sequencing, particularly Next-Generation Sequencing (NGS), have transformed the ability to profile complex microbial ecosystems in fermented foods. Metagenomic analysis targeting the 16S rRNA gene enables the detection of both dominant and rare taxa, overcoming limitations of culture-based approaches (Douglas et al. 2020; Medrano et al. 2020; Kaur et al. 2022). Recent metagenomic studies have characterized kefir microbiota from diverse regions, revealing the consistent dominance of LAB such as *Lactobacillus kefiranofaciens* alongside substantial contributions from other bacterial and yeast taxa (Chen et al. 2022; Baars et al. 2023). These works highlight kefir as a model system for studying microbial interactions, resilience, and functional metabolite production. However, most prior studies have focused either on kefir grains alone or have not explicitly compared the microbial composition of grains and the corresponding fermented liquid (Nejati et al. 2022). Direct comparisons remain limited, and no study to date has systematically examined this relationship in artisanal kefir produced in Indonesia. This gap is significant given the increasing consumption of artisanal kefir in Southeast Asia, where production methods, milk sources, and environmental conditions may yield distinctive microbial community structures. Understanding how microbial diversity is distributed between grains and whey in these systems is vital both for validating kefir's probiotic potential and for guiding the development of microbiome-based fermentation technologies.

The present study addresses this gap by employing 16S rRNA gene-based metagenomic sequencing to compare bacterial biodiversity in kefir grains and kefir whey derived from cow's milk fermentation. Specifically, we aimed to (i) characterize and contrast microbial community structures in both samples; (ii) identify shared and unique taxa that constitute the core versus niche-specific microbiomes; and (iii) assess how ecological selection influences microbial distribution during fermentation. By integrating ecological and functional perspectives, this research provides new insights into the microbial ecology of artisanal kefir and establishes a scientific foundation for probiotic development and microbiome-driven innovations in food biotechnology.

MATERIALS AND METHODS

Study design and sample collection

This descriptive exploratory study investigated the bacterial biodiversity of kefir grains and kefir whey obtained from artisanal producers in Indonesia. Kefir was produced by fermenting fresh cow's milk with active kefir grains sourced from Aracaki (Bogor, Indonesia). Fermentation was carried out at ambient temperature (25-27°C) for 24 h. Following fermentation, the product was separated into two fractions: kefir grains and kefir whey (the liquid phase). All samples were stored at -20°C until DNA extraction.

DNA extraction and quantification

Total genomic DNA was extracted from both kefir grain and fermented whey samples using the DNeasy PowerFood Microbial Kit (Qiagen, Germany), which is optimized for complex food matrices. Extraction quality was confirmed based on concentration and purity measured using both NanoDrop 2000 spectrophotometry (Thermo Fisher Scientific, USA) and Qubit 4.0 fluorometry (Invitrogen, USA). DNA yields ranged between 20-35 ng/μL with A260/A280 ratios of 1.8-2.0, indicating high-quality DNA suitable for downstream metagenomic applications. Negative extraction controls were included to ensure the absence of contaminant DNA during sample processing.

16S rRNA gene amplification and sequencing

The bacterial 16S rRNA gene was amplified targeting the V3-V4 hypervariable regions using the primer pair 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GACTACHVGGGTATCTAATCC-3′). Amplicons were purified using AMPure XP beads (Beckman Coulter, USA), quantified, and pooled equimolarly for library construction. Library preparation followed the Illumina 16S Metagenomic Sequencing Library Preparation protocol, including adapter ligation and dual indexing. Sequencing was performed on an Illumina MiSeq platform (2 × 250 bp paired-end reads), generating an average of 65,000-70,000 reads per sample after quality control filtering (Wick et al. 2019; Estikomah et al. 2024). The choice of the V3-V4 region was based on its broad taxonomic resolution for lactic acid bacteria in fermented dairy matrices, as reported in previous kefir microbiome studies.

Bioinformatic processing

Raw paired-end sequences were processed using the QIIME2 v2024.2 pipeline. Adapter and primer sequences were trimmed, and low-quality bases (Phred score <25) were removed using the DADA2 plugin. Chimera filtering was performed to eliminate spurious reads, and only sequences with a minimum overlap of 20 bp were retained. High-quality reads were denoised to generate amplicon sequence variants (ASVs), which provide single-nucleotide resolution of bacterial taxa. Taxonomic classification was conducted using a Naïve Bayes classifier trained on the SILVA 138.1 16S rRNA reference database, restricted to the V3-V4 region. Samples with fewer than 20,000 reads after quality control were excluded from downstream analyses to ensure sequencing depth consistency. Rarefaction was applied to normalize sequence counts across samples, ensuring comparable diversity estimates. Alpha diversity indices (e.g., Observed ASVs, Chao1, Shannon) and beta diversity metrics (e.g., Bray-Curtis dissimilarity) were computed within QIIME2. Statistical significance between kefir grain and liquid fractions was assessed using non-parametric tests (Kruskal-Wallis for alpha diversity and PERMANOVA for beta diversity). Negative extraction and PCR controls showed negligible read counts (<0.01% of total reads), confirming minimal contamination during laboratory procedures (De Coster et al. 2018).

Sequence data were processed using the DADA2 pipeline (version X.X) within QIIME2 to infer high-resolution amplicon sequence variants (ASVs), ensuring single-nucleotide discrimination without additional OTU clustering. Chimeric sequences were removed using the consensus method, and low-quality reads were filtered based on a Phred score ≥ Q30. Taxonomic classification of ASVs was performed using the SILVA 138.1 reference database with a minimum confidence threshold of 80%. Alpha and beta diversity indices were calculated following rarefaction to an even sequencing depth, and community dissimilarities were visualized using Bray-Curtis distance-based principal coordinate analysis (PCoA).

Diversity and statistical analysis

Alpha diversity (Shannon, Simpson, Chao1) and beta diversity (Jaccard distance) indices were calculated using phyloseq and vegan packages in R. Relative abundances of taxa were visualized with heatmaps and stacked bar plots. A Venn diagram was generated to identify shared and unique taxa between kefir grains and whey. It is important to note that the present study was based on representative samples of artisanal Indonesian kefir grains and their corresponding fermented liquid, without biological or technical replication. Consequently, the diversity metrics and relative abundance data presented herein should be interpreted as descriptive rather than inferential. The primary objective of this research was to provide a baseline metagenomic characterization of Indonesian kefir microbiota, which has not been previously reported at this level of taxonomic resolution. While the trends observed, such as the higher bacterial richness in grains relative to the liquid fraction, are consistent with international findings, they are not intended to imply statistically significant differences. Future investigations will incorporate replicated sampling and statistical analyses (e.g., ANOVA, PERMANOVA, or non-parametric tests) to validate these observations across different production sources and fermentation conditions.

This study was designed as a descriptive exploratory analysis focusing on kefir grains and their corresponding fermented whey obtained from a single artisanal producer in Indonesia. As such, the results primarily reflect the microbial composition of this local production and do not aim to represent the full diversity of Indonesian artisanal kefir. While this sampling provides valuable baseline data for regional kefir microbiota, future studies incorporating multiple producers, geographic regions, and fermentation batches are needed to validate and expand these findings.

Metagenomic profiling

This study employed 16S rRNA gene amplicon sequencing targeting the V3-V4 regions to characterize bacterial community composition. The analysis provides taxonomic insights into the microbial ecology of kefir grains and liquid kefir but does not include functional gene prediction or whole-metagenome sequencing. Therefore, any references to microbial functionality are interpretative and based on the known metabolic roles of identified taxa reported in previous literature.

Ethical and biosafety statement

All experimental procedures were conducted in compliance with the institutional biosafety regulations under Biosafety Level 2 (BSL-2) standards at the Faculty of Health Science, University of Darussalam Gontor, Indonesia. Kefir samples were collected from artisanal producers with informed consent for research use. No human or animal subjects were involved in this study. All microbial handling and DNA extraction procedures were performed following aseptic techniques and laboratory biosafety protocols to prevent contamination or exposure.

RESULTS AND DISCUSSION

The metagenomic analysis revealed clear differences in bacterial richness between kefir grains and the fermented liquid fraction. A total of 405 bacterial species were detected in the grains, while only 294 species were identified in the whey. Notably, 118 species were shared between both matrices, representing the core microbiome that bridges the solid and liquid phases of kefir fermentation (Figure 1). The relatively high richness observed in this study likely reflects the use of ASV-based analysis rather than conventional OTU clustering, which provides finer taxonomic resolution. This methodological distinction, combined with adequate depth normalization and chimera filtering, supports that the reported diversity represents genuine biological variation within the kefir microbiome.

Figure 1 illustrates the overlap and distinctiveness of bacterial species between kefir grains and the fermented liquid fraction. The metagenomic comparison of Indonesian artisanal kefir revealed that kefir grains contained 405 bacterial species, while the fermented liquid harbored 194 species, with 118 species shared between both matrices. Accordingly, 287 species were unique to kefir grains (405-118) and 76 species were unique to the liquid fraction (194-118), resulting in a total of 481 distinct bacterial species across both samples. This distribution confirms that kefir grains function as a primary microbial reservoir, harboring a richer and more diverse community that continuously seeds the liquid fraction during fermentation. Similar trends have been reported internationally, though the richness observed in Indonesian kefir exceeds that of other regional products. For instance, Zielińska et al. (2019) identified approximately 200-250 operational taxonomic units (OTUs) in Polish kefir grains, whereas Sun et al. (2022) and Wang et al. (2021) observed reduced diversity in liquid fractions due to ecological filtering during fermentation. Likewise, Medvedeva et al. (2024) reported that kefir grains maintain a stable and functionally diverse microbiota dominated by *Lactobacillus* and *Acetobacter*, typically not exceeding 300 species-level taxa.

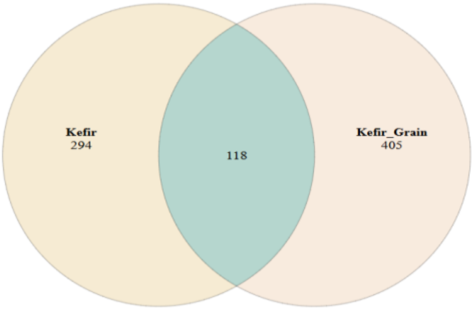

Figure 1. Venn diagram illustrating bacterial diversity in kefir grains and fermented kefir liquid

These findings highlight a research gap: while global studies emphasize the widespread dominance of lactic acid bacteria and the transfer of microbes from grain to liquid, few investigations have explored kefir from Southeast Asia, particularly Indonesia. Our data reveal that Indonesian artisanal kefir might contain greater microbial richness than previously documented in European or Chinese counterparts, suggesting a potential source of novel taxa with probiotic potential. This underlines the importance of regional studies in expanding the global kefir microbiome landscape and provides a scientific basis for harnessing locally adapted microbial consortia in functional food development (Bourrie et al. 2018; Tingirikari et al. 2024; Ströher et al. 2025).

The species-level visualization (Figure 2) provides a hierarchical overview of the bacterial community structure in Indonesian artisanal kefir grains and fermented whey. *L. kefiranofaciens* emerged as the overwhelmingly dominant species in both matrices, followed by *Streptococcus thermophilus* and minor taxa such as *Acetobacter* and *Weissella* (Cheng et al. 2024). This pattern confirms that lactic acid bacteria (LAB) form the functional and ecological core of kefir microbiomes, consistent with previous reports on traditional fermented dairy products. Notably, the relative distribution of secondary taxa differed between the solid and liquid fractions. Kefir grains retained higher proportions of *Lactobacillus kefiri* and *Lactobacillus helveticus*, consistent with their role as stable microbial reservoirs. In contrast, the whey fraction showed elevated levels of *S. thermophilus* and multiple *Acetobacter* species (*Acetobacter fabarum*, *Acetobacter lovaniensis*, *Acetobacter syzygii*), indicating ecological filtering that favors facultative and oxygen-tolerant taxa in the liquid phase. Minor species such as *Lactobacillus crispatus*, *Lactobacillus gallinarum*, and *Lactobacillus intestinalis* were also more pronounced in the whey, highlighting niche-specific adaptation during fermentation.

Comparative analyses support these observations. *Lactobacillus* dominance has been reported globally, though the relative abundance of secondary genera varies regionally. *Leuconostoc* is more prevalent in Polish kefir (Kalamaki et al. 2022; Kondrotiene et al. 2023), while *Acetobacter* is more pronounced in Chinese and Russian kefir (Ding et al. 2022; Cheng et al. 2024). Interestingly, Indonesian kefir exhibited a higher proportion of *Acetobacter* than most international reports, suggesting local environmental and substrate-specific factors influence microbial balance (Sumarmono et al. 2023; Ströher et al. 2025). At the species level, *L. kefiranofaciens* was confirmed as the keystone organism due to its role in kefiran polysaccharide formation and probiotic functionality (Shukla et al. 2023; Wang et al. 2023; Chen et al. 2024). Other consistently abundant species, *L. helveticus, L. gallinarum, L. intestinalis,* and *S. thermophilus,* contribute to proteolysis, bioactive peptide production, gut microbiota modulation, and lactic acid synthesis (Burel et al. 2021; Tenorio-Salgado et al. 2021; Xu et al. 2021; Shi et al. 2024). Additionally, three minor taxa (<1%) were retained in the visualization to represent low-abundance but metabolically active members that may enhance flavor and stability. Collectively, these results highlight the LAB-dominated yet ecologically diverse microbiome of Indonesian kefir and its distinctive taxonomic balance shaped by environmental exposure, fermentation dynamics, and artisanal practices. The findings reinforce the view that Indonesian artisanal kefir represents a unique microbial ecosystem with promising functional and probiotic potential.

The ten most dominant bacterial community profiles of bacterial community profiling in kefir grains and fermented liquid are summarized in Table 1. The analysis demonstrates variation in species distribution, with some taxa showing dominance in both matrices, while others appear enriched only in the fermented liquid.

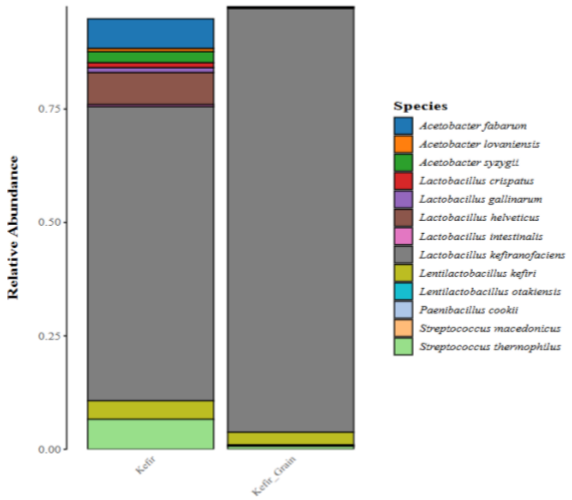

Figure 2. Top 10 species in kefir grains and fermented kefir liquid

Table 1. Ten dominant bacterial species in kefir grains and fermented kefir liquid based on metagenomic analysis

Bacterial species	Relative abundance (%) in kefir grains	Relative abundance (%) in fermented liquid	Dominance description
Lactobacillus kefiranofaciens	~65-70%	~35-40%	Dominant in both samples
Lactobacillus kefiri	~10-12%	~15-18%	More stable in grains
Lactobacillus helveticus	~5-7%	~20-22%	Dominant in fermented liquid
Lactobacillus gallinarum	~2-3%	~10-12%	Dominant in fermented liquid
Streptococcus thermophilus	~2%	~12-15%	Specifically dominant in fermented liquids
Acetobacter fabarum	~1%	~7-8%	Dominant in fermented liquid
Acetobacter lovaniensis	<1%	~4-5%	Minor in both samples
Acetobacter syzygii	<1%	~3-4%	Weakly detected in fermented liquid
Lactobacillus crispatus	<0.5%	~2-3%	Predominant in fermented liquid
Lactobacillus intestinalis	<0.5%	~4-5%	Dominant in fermented liquid

Metagenomic analysis revealed distinct differences in the relative abundance of bacterial species between kefir grains and the fermented liquid. While *L. kefiranofaciens* remained dominant in both matrices, several species, such as *L. helveticus, S. thermophilus,* and *A. fabarum*, were more enriched in the fermented liquid. Conversely, species with minor representation in the grains, including *L. intestinalis* and *L. crispatus*, showed higher prevalence in the liquid phase. These findings highlight the ecological transition of bacterial communities from the structured environment of kefir grains to the dynamic conditions of milk fermentation.

Figure 3 illustrates the bacterial community composition at the phylum level, highlighting clear differences between kefir grains and liquid kefir. In both sample types, Bacillota (formerly Firmicutes) overwhelmingly dominated, reflecting the central role of lactic acid bacteria (LAB) as the functional core of kefir microbiomes. These LAB taxa are primarily responsible for lactose fermentation, lactic acid production, exopolysaccharide synthesis, and other bioactive compounds (Sumarmono et al. 2023; Ströher et al. 2025). In contrast, Pseudomonadota (formerly Proteobacteria) was markedly more abundant in the liquid fraction but nearly absent in grains, consistent with observations that Proteobacteria taxa are enriched in liquid kefir due to their adaptation to more oxygenated and nutrient-rich environments (Gao et al. 2021; Qiu et al. 2024; Ströher et al. 2025). Minor phyla such as Actinomycetota, Bacteroidota, Acidobacteriota, Cyanobacteriota, and Synergistota were detected at trace levels (<0.5%) and are thus not visibly represented in the figure due to the proportional scaling of the visualization. The slight numerical difference between Table 2 and Figure 4 reflects normalization rounding from visual rendering software, which aggregates reads below the detection threshold. Overall, these findings confirm that while kefir grains and liquid kefir share a Bacillota-dominated core microbiome, the liquid fraction supports greater taxonomic diversity, particularly among Gram-negative taxa, shaped by environmental exposure, raw milk microbial variability, and distinct physicochemical conditions between the solid grain matrix and the surrounding liquid (dos Santos et al. 2019; Sumarmono et al. 2023; Ströher et al. 2025). For further clarity, the detailed relative abundances of the dominant bacterial taxa are presented in Table 2.

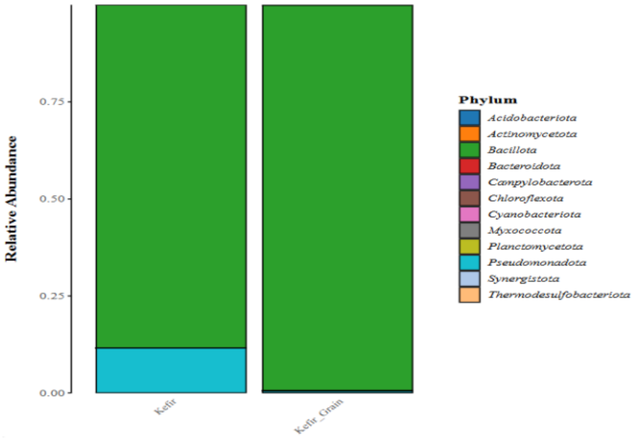

Figure 3. Top 10 of the phylum in kefir grains and fermented kefir liquid

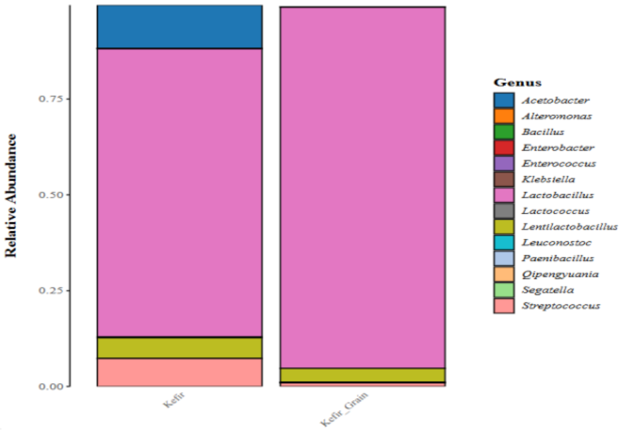

Figure 4. Top 10 genera in kefir grains and fermented kefir liquid

Table 2. Ten dominant phyla in kefir grains and fermented kefir liquid based on metagenomic analysis

Phylum	Relative abundance in kefir grains (%)	Relative abundance in liquid kefir (%)	Notes/description
Bacillota (Firmicutes)	>95%	>85%	Dominant phylum; mainly LAB (*Lactobacillus*, *Streptococcus*), key role in lactose fermentation and exopolysaccharide production
Pseudomonadota (Proteobacteria)	<1%	~10-12%	Enriched in liquid; oxygen-tolerant and adapted to aqueous niches
Actinomycetota	<2%	<2%	Minor phylum, low abundance in both samples
Bacteroidota	<2%	<2%	Minor taxa, consistent with previous kefir studies
Acidobacteriota	<1%	~1-2%	Slightly enriched in the liquid phase; may reflect adaptation to pH/oxygen gradients
Cyanobacteriota	<1%	<1%	Detected sporadically; likely environmental origin
Synergistota	<1%	<1%	Very low abundance; non-core members
Chloroflexota	Trace	Trace	Transient/environmental contaminant
Campylobacterota	Trace	Trace	Sporadic detection; not central to kefir fermentation
Thermodesulfobacteriota	Trace	Trace	Very low abundance, consistent with transient microbiota

Based on metagenomic profiling through 16S rRNA sequencing, Figure 4 illustrates the dominant bacterial genera detected in both kefir grains and liquid kefir. Lactobacillus was overwhelmingly dominant in both matrices, confirming its central role as the functional core of the kefir microbiome. This genus, including species such as *L. kefiranofaciens* and *L. kefiri*, is responsible for key metabolic functions such as lactose fermentation, lactic acid production, exopolysaccharide synthesis, and the generation of bioactive metabolites with probiotic potential (Hong et al. 2019; Ganatsios et al. 2021; Li et al. 2023). In contrast, *Acetobacter* was consistently more enriched in the liquid fraction, reflecting its ecological adaptation to more oxygenated environments and its contribution to ethanol oxidation and acetic acid production, which influence flavor development and antimicrobial activity (Ellatif et al. 2022; Anumudu et al. 2024). Other genera, including *Lentilactobacillus*, *Lactococcus*, and *Streptococcus*, were detected in lower relative abundances but remain functionally relevant, contributing to early lactose metabolism, exopolysaccharide production, and synergistic interactions that support *Lactobacillus* growth (Kim et al. 2018; Gao et al. 2025). Several minor genera, such as *Leuconostoc*, *Bacillus*, and *Paenibacillus*, appeared sporadically at trace levels (<0.5%) and are thus not clearly visible in the stacked-bar representation, though included in the legend for completeness. The relative abundance of *Lactococcus* (~3-4% in liquid kefir) is also numerically presented in Table 3, but may not be distinctly visible in the figure due to scale overlap. Overall, these findings support the view that while kefir grains and liquid kefir share a LAB-driven core community, the liquid fraction harbors a broader diversity of secondary taxa shaped by environmental exposure, nutrient turnover, and fermentation dynamics (Vinderola et al. 2019; Wang et al. 2023). For further clarity, the detailed relative abundances of the dominant genera are presented in Table 3.

Figure 5 illustrates the bacterial community composition at the class level, showing clear differences between kefir grains and liquid kefir. Bacilli emerged as the most dominant class in both matrices, consistent with their central role as lactic acid bacteria (LAB), which include *Lactobacillus, Streptococcus,* and *Lactococcus*. These taxa are key drivers of fermentation, flavor development, and probiotic functionality (Devi et al. 2025; Wang et al. 2023). Alphaproteobacteria represented the second most prominent class, particularly enriched in the liquid fraction. Members of this class, such as *Acetobacter* and *Gluconobacter*, are typically associated with environmental microbiota and contribute to acetic acid production, co-fermentation with LAB, and modulation of flavor and pH (Hertzler et al. 2019; Kim et al. 2020). Gammaproteobacteria, once considered important, were detected only in minor proportions, suggesting a limited role in kefir ecosystems. Actinobacteria, represented mainly by *Bifidobacterium*, were consistently present across both sample types and are recognized for their health-promoting attributes, including modulation of the gut microbiota and immune support (Gao 2021; Wang et al. 2023). Other classes, including Bacteroidia, Negativicutes, and Coriobacteriia, occurred only in trace amounts, reflecting background or auxiliary taxa that may contribute to niche-specific metabolic processes or flavor complexity (Rosa et al. 2017). Although Figure 6 visualizes the dominant bacterial classes, minor taxa with relative abundances below 0.5% such as those associated with *Lentilactobacillus* and *Lactococcus,* are not distinctly represented due to graphical scaling in the Krona-based output. Consequently, the visual proportion of Bacilli appears slightly higher (~99%) than the quantitative values (~90-92%) reported in Table 3. For numerical accuracy, the corresponding relative abundances of dominant bacterial classes are presented in Table 3.

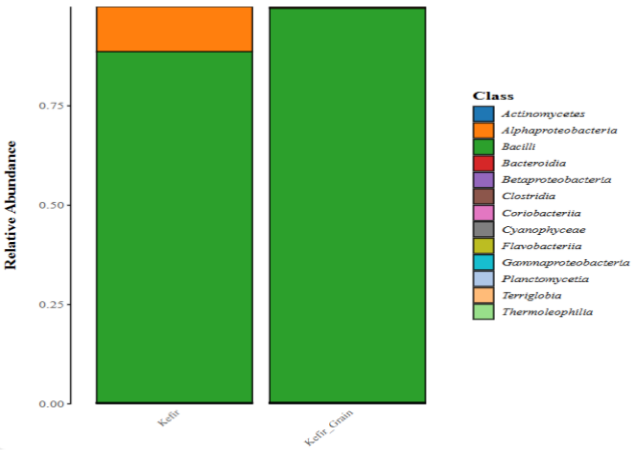

Figure 5. Top 10 of class in kefir grains and fermented kefir liquid

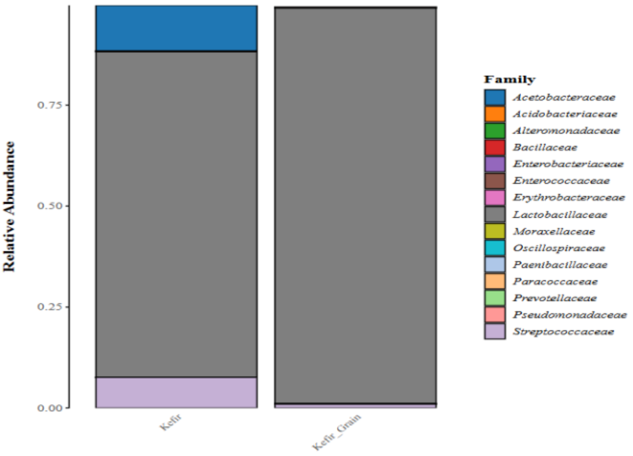

Figure 6. Top 10 of the family in kefir grains and fermented kefir liquid

Table 3. Ten dominant genus in kefir grains and fermented kefir liquid based on metagenomic analysis

Bacterial genus	Relative abundance in kefir grains	Relative abundance in liquid kefir	Notable roles/description
Lactobacillus	~90-92%	~80-82%	Core microbiota: lactose fermentation, lactic acid, and exopolysaccharide production; probiotic effects
Acetobacter	<1%	~10%	Ethanol oxidation to acetic acid contributes to flavor and antimicrobial activity
Lentilactobacillus	~2%	~3-4%	Secondary fermenter; flavor compound development
Lactococcus	~1-2%	~3-4%	Early fermentation initiator; rapid lactose fermentation and lactic acid production
Streptococcus	~1-2%	~2-3%	Synergistic role (e.g., *S. thermophilus* enhances Lactobacillus growth via formate and CO_2)
Bacillus	<1%	<1%	Likely from raw milk microbiota; minor presence
Leuconostoc	<1%	~1%	Produces diacetyl and acetoin; contributes to aroma
Paenibacillus	<1%	<1%	Environmental origin; non-core member
Weissella	<1%	<1%	Occasionally reported in kefir; role in exopolysaccharide production
Enterococcus	<1%	<1%	Transient; may contribute to proteolysis and flavor compounds

Figure 6 illustrates the presence and relative abundance of bacteria at the family level. The metagenomic analysis at the family level revealed that Acetobacteraceae and Enterobacteriaceae emerged as the second most dominant bacterial families in the liquid kefir samples, following Lactobacillaceae. In contrast, their relative abundance was markedly lower in the kefir grains, indicating a distinct microbial community structure between the two sample types. The prevalence of Acetobacteraceae in liquid kefir can be attributed to its members, primarily Acetobacter and Gluconobacter, which are aerobic acetic acid bacteria capable of oxidizing ethanol into acetic acid. These bacteria not only contribute to the distinctive sour flavor of kefir but also play a role in microbial inhibition through the production of organic acids (Cheng et al. 2024; Que et al. 2024). The elevated presence of Enterobacteriaceae in liquid kefir may be associated with environmental exposure during processing, including potential contamination from raw milk, water, air, or equipment. Members of this family, such as *Klebsiella* and *Enterobacter*, are known to survive in the early stages of fermentation before being outcompeted by lactic acid bacteria (LAB). While some strains within Enterobacteriaceae have been recognized for their fermentative capacity, others pose potential safety risks due to their opportunistic pathogenic nature (Chen et al. 2021). Previous studies have also reported the presence of Enterobacteriaceae in kefir products, though typically at lower levels due to competitive exclusion by LAB during fermentation (Fan et al. 2022), while Figure 7 is titled to represent the "top ten" bacterial families, the legend contains 15 color-coded taxa. This discrepancy arises because several minor families, each representing less than 0.5% of total abundance, were automatically visualized in the Krona output to preserve data completeness. These minor families, though not dominant, provide ecological context to the broader kefir microbiome and do not significantly alter the proportional representation of the dominant groups.

Figure 7 presents a comprehensive heatmap visualization integrating species-, genus-, and phylum-level bacterial profiles based on Next-Generation Sequencing (NGS) analysis of kefir grains and fermented liquid kefir. The color gradient represents log10-transformed relative abundance, ranging from red (high abundance) to blue (low abundance), while hierarchical clustering groups taxa according to similarity in abundance patterns. The results reveal that *L. kefiranofaciens*, *L. kefiri*, *L. helveticus*, and *S. thermophilus* are the dominant species across both matrices, particularly in kefir grains, indicating their role as core members of the lactic acid bacteria (LAB) consortium. At the genus level, *Lactobacillus* and *Streptococcus* were predominant, while *Bifidobacterium*, *Leuconostoc*, and *Weissella* appeared in moderate abundance, supporting their contribution to flavor development and probiotic activity. In contrast, acetic acid bacteria such as *Acetobacter* were more abundant in the liquid fraction, consistent with their aerobic metabolism and preference for ethanol-rich environments, which may influence acidity and aroma. At the phylum level, *Firmicutes* overwhelmingly dominated both samples, followed by Proteobacteria, which appeared more prominently in the liquid fraction, likely due to oxygen exposure during fermentation. Minor phyla, including Actinobacteriota and Bacteroidota, were detected in low proportions, suggesting niche-specific or transient ecological roles. Overall, the heatmap underscores the higher microbial richness and compositional stability of kefir grains compared to the liquid fraction, reflecting ecological filtering during fermentation and confirming that the grain matrix acts as a primary microbial reservoir for the development of functional and diverse kefir microbiota (Chen et al. 2021; Ganatsios et al. 2021; Wang et al. 2023).

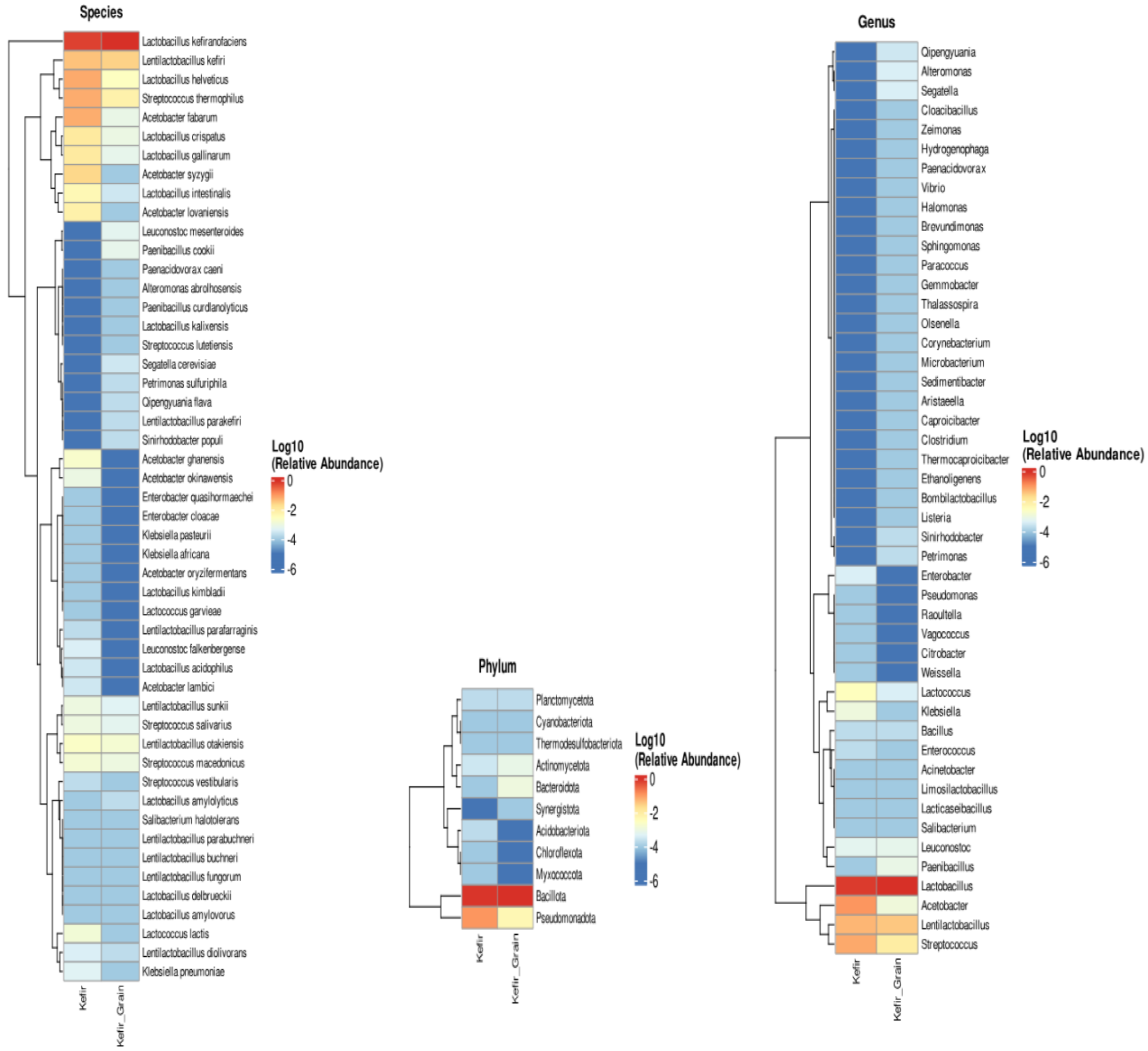

Figure 7. Heatmap of species, phylum, genus by NGS analysis

The boxplot in Figure 8 highlights significant differences in bacterial richness between kefir grains and liquid kefir. Grains consistently displayed higher richness, reflecting their function as a stable microbial reservoir supported by the protective grain matrix. In contrast, the liquid fraction exhibited reduced richness, consistent with ecological filtering during fermentation, where only a subset of microbes adapted to oxygen exposure, nutrient flux, and acidic conditions can persist. These findings align with global reports that describe grains as the primary microbial niche and liquids as filtered subsets, but the richness observed in Indonesian kefir exceeds values commonly reported elsewhere (Sumarmono et al. 2023). This suggests that artisanal kefir from Indonesia may harbor greater microbial diversity, underscoring its potential as a reservoir of novel probiotic taxa and offering opportunities for optimizing microbiome-based fermentation strategies.

Figures 9 and 10 show the hierarchical flow of microbial taxa from phylum to species level in both kefir grains and fermented liquid kefir. The left portion represents kefir grains, while the right portion depicts the corresponding liquid fraction, illustrating how microbial populations shift between matrices. *L. kefiranofaciens* remains the dominant species in both environments, supported by other lactic acid bacteria such as *S. thermophilus* and *L. helveticus*. In contrast, the liquid fraction shows increased representation of acetic acid bacteria (*A. fabarum*, *A. lovaniensis*) and reduced overall diversity, consistent with selective ecological pressures during fermentation. Minor taxa such as *Enterobacter* and *Klebsiella* appear only in trace abundance, suggesting possible environmental introduction. This visualization highlights the transition from the dense, microbially stable kefir grain structure to the more dynamic and oxygen-exposed liquid environment, reflecting ecological filtering and adaptation during fermentation.

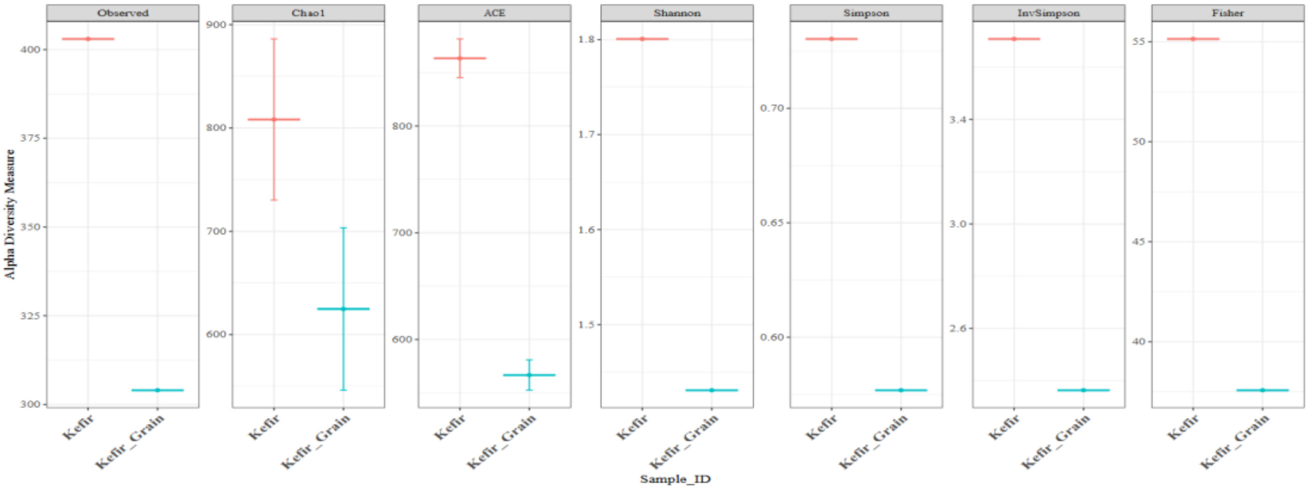

Figure 8. Boxplot of alpha diversity in kefir samples

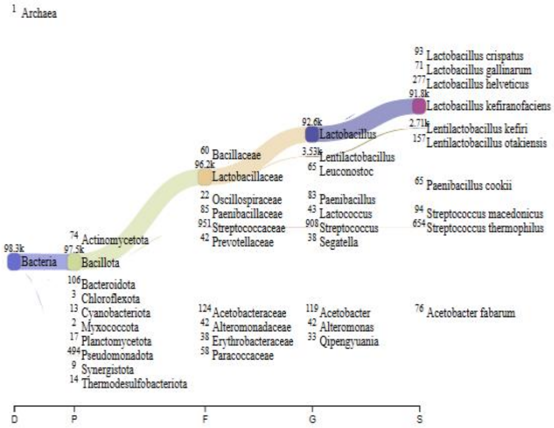

Figure 9. Sankey diagram of microbial in kefir grains

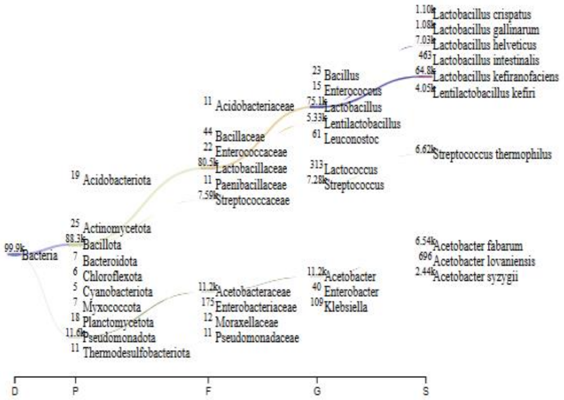

Figure 10. Sankey diagram of microbial in liquid kefir

This study provides the first metagenomic-based assessment of bacterial community structuring in Indonesian artisanal kefir by explicitly contrasting the ecological roles of kefir grains and the fermented liquid fraction. Rather than merely documenting taxonomic presence, the results reveal clear ecological stratification and selective pressures operating between the solid grain matrix and the surrounding liquid environment. Kefir grains harbored substantially higher bacterial richness (405 species) than the liquid fraction (294 species), with only 118 species shared between both matrices, indicating that the liquid represents a filtered subset of the grain microbiome rather than a mirror of its diversity. This pattern highlights the role of grains as a stable microbial reservoir that preserves both dominant and low-abundance taxa, while the liquid environment imposes physicochemical constraints that restrict community membership.

At the species and genus levels, *L. kefiranofaciens* consistently dominated both fractions, reinforcing its role as a keystone species responsible for kefiran synthesis, grain structural integrity, and fermentation stability. However, comparative abundance patterns demonstrate that community dominance alone does not equate to ecological uniformity. Kefir grains preferentially retained slow-growing, matrix-associated LAB such as *L. kefiri* and *L. helveticus*, whereas the liquid fraction showed enrichment of fast-growing and oxygen-tolerant taxa, including *S. thermophilus* and *Acetobacter* spp. This shift reflects ecological filtering driven by oxygen exposure, nutrient flux, and pH dynamics in the liquid phase, favoring taxa with rapid metabolic turnover and aerobic or facultative capabilities.

Importantly, this study extends beyond conventional taxonomic inventories by demonstrating that microbial divergence occurs across multiple taxonomic hierarchies (from class to species), indicating structured community assembly rather than stochastic variation. While Bacilli remained dominant overall, the relative expansion of Alphaproteobacteria in the liquid fraction and the persistence of minor taxa within grains suggest complementary ecological functions, including acid production, cross-feeding, and flavor modulation. These findings challenge the assumption common in previous kefir studies that grains and liquid share functionally equivalent microbiomes, instead supporting a model of functional partitioning between a conserved inoculum reservoir and a selectively enriched fermentation environment.

In contrast to many studies that report lower species richness and reduced taxonomic complexity, Indonesian artisanal kefir exhibited comparatively elevated diversity, particularly within the grain matrix. This discrepancy highlights an important research gap: regional production practices, milk substrates, and environmental exposure remain underexplored drivers of kefir microbial ecology, especially in Southeast Asia. Most prior studies have focused on European or East Asian kefir systems and have rarely quantified how artisanal handling and local conditions influence microbial assembly and persistence.

Although this study was based on 16S rRNA amplicon sequencing and thus does not directly measure functional genes or metabolites, the observed co-dominance of lactic acid bacteria and acetic acid bacteria strongly implies coordinated metabolic interactions, including lactose fermentation, exopolysaccharide synthesis, and organic acid production. These inferred interactions provide a mechanistic basis for the observed taxonomic structuring and underscore the need for future integrative multi-omics approaches. Expanding sampling across geographic regions and fermentation batches, incorporating yeast and archaeal communities, and applying metagenomic, metatranscriptomic, and metabolomic analyses will be essential to link microbial composition with functional outcomes such as flavor development, probiotic efficacy, and product safety. Overall, the results demonstrate that microbial differences between kefir grains and fermented liquid are not merely compositional but reflect underlying ecological mechanisms shaped by structural protection, environmental filtering, and metabolic specialization. By addressing an underrepresented geographic context and explicitly disentangling reservoir versus filter dynamics, this study advances current understanding of kefir microbiome assembly and positions Indonesian artisanal kefir as a valuable system for functional food and fermentation research.

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REFERENCES

Anumudu CK, Miri T, Onyeaka H. 2024. Multifunctional applications of lactic acid bacteria: Enhancing safety, quality, and nutritional value in foods and fermented beverages. Foods 13 (23): 3714. DOI: 10.3390/foods13233714.

Apalowo OE, Adegoye GA, Mbogori T, Kandiah J, Obuotor TM. 2024. Nutritional characteristics, health impact, and applications of kefir. Foods 13 (7): 1026. DOI: 10.3390/foods13071026.

Azizi NF, Kumar MR, Yeap SK, Abdullah JO, Khalid M, Omar AR, Osman M, Mortadza SA, Alitheen NB. 2021. Kefir and its biological activities. Foods 10 (6): 1210. DOI: 10.3390/foods10061210.

Avila-Reyes SV, Márquez-Morales CE, Moreno-León GR, Jiménez-Aparicio AR, Arenas-Ocampo ML, Solorza-Feria J, García-Armenta E, Villalobos-Espinosa JC. 2022. Comparative analysis of fermentation conditions on the increase of biomass and morphology of milk kefir grains. Appl Sci 12 (5): 2459. DOI: 10.3390/app12052459.

Baars T, van Esch B, van Ooijen L, Zhang Z, Dekker P, Boeren S, Diks M, Garssen J, Hettinga K, Kort R. 2023. Raw milk kefir: microbiota, bioactive peptides, and immune modulation. Food Funct 14 (3): 1648-1661. DOI: 10.1039/D2FO03248A.

Balivo A, Sacchi R, Genovese A. 2023. The Noble Method in the dairy sector as a sustainable production system to improve the nutritional composition of dairy products: A review. Intl J Dairy Technol 76 (2): 313-328. DOI: 10.1111/1471-0307.12941.

Burel C, Kala A, Purevdorj-Gage L. 2021. Impact of pH on citric acid antimicrobial activity against Gram-negative bacteria. Lett Appl Microbiol 72 (3): 332-340. DOI: 10.1111/lam.13420.

Bourrie BCT, Cotter PD, Willing BP. 2018. Traditional kefir reduces weight gain and improves plasma lipid profiles in a diet-induced obesity mouse model. J Funct Foods 43: 85-94. DOI: 10.1016/j.jff.2018.04.039.

Cheng T, Zhang T, Zhang P, He X, Sadiq FA, Li J, Sang Y, Gao J. 2024. The complex world of kefir: Structural insights and symbiotic relationships. Compr Rev Food Sci Food Saf 23 (4): e13364. DOI: 10.1111/1541-4337.13364.

Chen CF, Li HP, Chao YH, Tu MY, Yen CC, Lan YW, Yang SH, Chong KY, Lin CC, Chen CM. 2021. Suppression of dendritic cell maturation by kefir peptides alleviates collagen-induced arthritis in mice. Front Pharmacol 12: 721594. DOI: 10.3389/fphar.2021.721594.

Chen MY, Wu HT, Chen FF, Wang YT, Chou DL, Wang GH, Chen YP. 2022. Characterization of Tibetan kefir grain-fermented milk whey and its suppression of melanin synthesis. J Biosci Bioeng 133 (6): 547-554. DOI: 10.1016/j.jbiosc.2022.02.006.

Chen W, Wang J, Du L, Chen J, Zheng Q, Li P, Du B, Fang X, Liao Z. 2024. Kefir microbiota and metabolites stimulate intestinal mucosal immunity and its early development. Crit Rev Food Sci Nutr 64 (5): 1371-1384. DOI: 10.1080/10408398.2022.2115975.

De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics 34: 2666-2669. DOI: 10.1093/bioinformatics/bty149.

Destro TM, Junior HS, Scherer A, Spinosa WA. 2024. Characterization of milk and water-based kefir microbiota. Agron Sci Biotechnol 10: 1-19. DOI: 10.33158/ASB.r202.v10.2024.

Devi H, Singh TP, Siwach R, Chaudhary V. 2025. Development of nutri-functional paneer whey-based kefir drink. J Food Sci Technol 62 (2): 254-263. DOI: 10.1007/s13197-024-06023-y.

Ding F, Krasilnikova AA, Leontieva MR, Stoyanova LG, Netrusov AI. 2022. Analysis of kefir grains from different regions of the planet using high-throughput sequencing. Moscow Univ Biol Sci Bull 77 (4): 286-291. DOI: 10.3103/S0096392522040010.

dos Santos DC, de Oliveira Filho JG, Santana ACA, de Freitas BSM, Silva FG, Takeuchi KP, Egea MB. 2019. Optimization of soymilk fermentation with kefir and the addition of inulin: Physicochemical, sensory, and technological characteristics. LWT Food Sci Technol 104: 30-37. DOI: 10.1016/j.lwt.2019.01.030.

Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, Huttenhower C, Langille MG. 2020. PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 38 (6): 685-688. DOI: 10.1038/s41587-020-0548-6.

Ellatif SA, Abdel Razik ES, Abu-Serie MM, Mahfouz A, Shater AF, Saleh FM, Mahfouz AY. 2022. Immunomodulatory efficacy-mediated anti-HCV and anti-HBV potential of kefir grains; unveiling the in vitro antibacterial, antifungal, and wound healing activities. Molecules 27 (6): 2016. DOI: 10.3390/molecules27062016.

Estikomah SA, Suranto S, Susilowati A, Masykuri M. 2024. Bacterial diversity in cheese wastewater using Next-Generation Sequencing (NGS). Biodiversitas 25: 794-802. DOI: 10.13057/biodiv/d250206.

Fan D, Stoyanova LG, Netrusov AI. 2022. Microbiome and metabiotic properties of kefir grains and kefirs based on them. Microbiology 91 (4): 339-355. DOI: 10.1134/S0026261722100885.

Ganatsios V, Nigam P, Plessas S, Terpou A. 2021. Kefir, as a functional beverage gaining momentum towards its health-promoting attributes. Beverages 7 (3): 48. DOI: 10.3390/beverages7030048.

Gao J, Mao K, Wang X, Mi S, Fu M, Li X, Sang Y. 2021. Tibet kefir milk regulated metabolic changes induced by a high-fat diet via amino acids, bile acids, and equol metabolism in human-microbiota-associated rats. J Agric Food Chem 69 (23): 6720-6732. DOI: 10.1021/acs.jafc.1c02430.

Gao Y, Liu Y, Ma T, Liang Q, Sun J, Wu X, Mu G. 2025. Fermented dairy products as precision modulators of gut microbiota and host health: Mechanistic insights, clinical evidence, and future directions. Foods 14 (11): 1946. DOI: 10.3390/foods14111946.

Hertzler SR, Clancy SM. 2019. Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. J Am Diet Assoc 103: 582-587. DOI: 10.1053/jada.2003.50111.

Hong JY, Lee NK, Yi SH, Hong SP, Paik HD. 2019. Physicochemical features and microbial community of milk kefir using a potential probiotic *Saccharomyces cerevisiae* KU200284. J Dairy Sci 102 (12): 10845-10849. DOI: 10.3168/jds.2019-16384.

Kairey L, Leech B, El-Assaad F, Bugarcic A, Dawson D, Lauche R. 2023. The effects of kefir consumption on human health: A systematic review of randomized controlled trials. Nutr Rev 81 (3): 267-286. DOI: 10.1093/nutrit/nuac054.

Kalamaki MS, Angelidis AS. 2020. High-throughput, sequence-based analysis of the microbiota of Greek kefir grains from two geographic regions. Food Technol Biotechnol 58 (2): 138. DOI: 10.17113/ftb.58.02.20.6581.

Kaur H, Kaur G, Ali SA. 2022. Dairy-based probiotic-fermented functional foods: An update on their health-promoting properties. Fermentation 8 (9): 425. DOI: 10.3390/fermentation8090425.

Kim DH, Jeong D, Kim H, Seo KH. 2018. Modern perspectives on the health benefits of kefir in the next-generation sequencing era: Improvement of the host gut microbiota. Crit Rev Food Sci Nutr 59 (11): 1782-1793. DOI: 10.1080/10408398.2018.1428168.

Kim DH, Kim H, Seo KH. 2020. Microbial composition of Korean kefir and antimicrobial activity of *Acetobacter fabarum* DH1801. J Food Saf 40 (1): e12728. DOI: 10.1111/jfs.12728.

Kondrotiene K, Zavistanaviciute P, Aksomaitiene J, Novoslavskij A, Malakauskas M. 2023. *Lactococcus lactis* in dairy fermentation—health-promoting and probiotic properties. Fermentation 10 (1): 16. DOI: 10.3390/fermentation10010016.

Li X, He Y, Xie Y, Zhang L, Li J, Liu H. 2023. Effects of fermentation with kefir grains on nutrient composition, flavor volatiles, and product physical stability of a hemp seed (*Cannabis sativa* L.) beverage. LWT Food Sci Technol 183: 114934. DOI: 10.1016/j.lwt.2023.114934.

Medrano M, Gangoiti MV, Simonelli N, Abraham AG. 2020. Kefiran fermentation by human faecal microbiota: Organic acids production and in vitro biological activity. Bioact Carbohydr Diet Fibre 24: 100229. DOI: 10.1016/j.bcdf.2020.100229.

Medvedeva AS, Gudkova EI, Titova AS, Kharkova AS, Kuznetsova LS, Perchikov RN, Ivanov VR, Ryabkov YD, Tikhonova AA, Fomina ED, Naumova AO. 2024. Nanostructured copper electrodes–a new step in the development of microbial bioelectrochemical systems. Environ Sci: Nano 11 (11): 4562-4576. DOI: 10.1039/D4EN00440J.

Nejati F, Capitain CC, Krause JL, Kang GU, Riedel R, Chang HD, Kurreck J, Junne S, Weller P, Neubauer P. 2022. Traditional grain-based vs. commercial milk kefirs, how different are they? Appl Scie 12 (8): 3838. DOI: 10.3390/app12083838.

Petrova P, Ivanov I, Tsigoriyna L, Valcheva N, Vasileva E, Parvanova-Mancheva T, Arsov A, Petrov K. 2021. Traditional Bulgarian dairy products: Ethnic foods with health benefits. Microorganisms 9 (3): 480. DOI: 10.3390/microorganisms9030480.

Qiu Y, Yan F, Yu H, Li H, Xia S, Zhang J, Zhu J. 2024. The protective effects of kefir extract (KE) on intestinal damage in larval zebrafish induced by oxytetracycline: insights into intestinal function, morphology, and molecular mechanisms. Food Res Intl 190: 114642. DOI: 10.1016/j.foodres.2024.114642.

Que Z, Wang S, Wei M, Fang Y, Ma T, Wang X, Sun X. 2024. The powerful function of *Saccharomyces cerevisiae* in food science and other fields: A critical review. Food Innov Adv 3 (2): 167-180. DOI: 10.48130/fia-0024-0016.

Rosa DD, Dias MM, Grześkowiak ŁM, Reis SA, Conceição LL, Peluzio MD. 2017. Milk kefir: nutritional, microbiological and health benefits. Nutr Res Rev 30: 82-96. DOI: 10.1017/S0954422416000275.

Rezac S, Kok CR, Heermann M, Hutkins R. 2018. Fermented foods as a dietary source of live organisms. Front Microbiol 9: 1785. DOI: 10.3389/fmicb.2018.01785.

Saady EM, Elkot WF, Soliman SA, Helmy EA. 2025. Formulation of traditional Russian kefir and assessment of its microbiological, physicochemical, and sensory attributes. Aswan J Agric Biol Sci 1 (1): 64-76. DOI: 10.21608/ajabs.2025.403399.1013.

Shi J, Wang Y, Cheng L, Wang J, Raghavan V. 2024. Gut microbiome modulation by probiotics, prebiotics, synbiotics, and postbiotics: A novel strategy in food allergy prevention and treatment. Crit Rev Food Sci Nutr 64 (17): 5984-6000. DOI: 10.1080/10408398.2022.2160962.

Shukla P, Sakure A, Maurya R, Bishnoi M, Kondepudi KK, Das S, Hati S. 2023. Antidiabetic, angiotensin-converting enzyme inhibitory, and anti-inflammatory activities of fermented camel milk and characterisation of novel bioactive peptides from lactic-fermented camel milk with molecular interaction study. Intl J Dairy Technol 76 (1): 149-167. DOI: 10.1111/1471-0307.12910.

Ströher JA, Oliveira WD, de Freitas AS, Salazar MM, da Silva LD, Bresciani L, Flôres SH, Malheiros PD. 2025. A global review of the geographical diversity of the kefir microbiome. Fermentation 11 (3): 150. DOI: 10.3390/fermentation11030150.

Sumarmono J, Kusuma RJ, Rahayu N, Sukarno AS, Wulansari PD. 2023. Metagenomic analysis of the microbial community in kefir grains from different milk sources. Biodiversitas 24 (10): 5302-5308. DOI: 10.13057/biodiv/d241011.

Sun MC, Fan XJ, Wang JT, Yang FS, Yang L, Li Z, Fei P, Zhang T, Zhao C. 2022. Exploring the mechanism of milk kefir grain fermentation to improve the palatability of chokeberry juice. LWT 213: 117074. DOI: 10.1016/j.lwt.2024.117074.

Tenorio-Salgado S, Castelán-Sánchez HG, Dávila-Ramos S, Huerta-Saquero A, Rodríguez-Morales S, Merino-Pérez E, Roa de la Fuente LF, Solís-Pereira SE, Pérez-Rueda E, Lizama-Uc G. 2021. Metagenomic analysis and antimicrobial activity of two fermented milk kefir samples. Microbiologyopen 10 (2): e1183. DOI: 10.1002/mbo3.1183.

Tingirikari JMR, Sharma A, Lee HJ. 2024. Kefir: A fermented plethora of symbiotic microbiome and health. J Ethn Foods 11 (1): 35. DOI: 10.1186/s42779-024-00252-4.

Vinderola G, Perdigón G, Duarte J, Farnworth E, Matar C. 2019. Effects of kefir fractions on innate immunity. Immunobiology 211: 149-156. DOI: 10.1016/j.imbio.2005.08.005.

Wang Y, Zhang C, Liu F, Jin Z, Xia X. 2023. Ecological succession and functional characteristics of lactic acid bacteria in traditional fermented foods. Crit Rev Food Sci Nutr 63 (22): 5841-5855. DOI: 10.1080/10408398.2021.2025035.

Wick RR, Judd LM, Holt KE. 2019. Performance of neural network-based calling tools for Oxford Nanopore sequencing. Genome Biol 20: 129. DOI: 10.1186/s13059-019-1727-y.

Xu Z, Lu Z, Soteyome T, Ye Y, Huang T, Liu J, Peters BM. 2021. Polymicrobial interaction between *Lactobacillus* and *Saccharomyces cerevisiae*: Coexistence-relevant mechanisms. Crit Rev Microbiol 47 (3): 386-396. DOI: 10.1080/1040841X.2021.1893265.

Zielińska D, Marciniak-Lukasiak K, Karbowiak M, Lukasiak P. 2019. Effects of fructose and oligofructose addition on milk fermentation using novel *Lactobacillus* cultures to obtain high-quality yogurt-like products. Molecules 26: 5730. DOI: 10.3390/molecules26195730.