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# Kefir as a functional probiotic: microbial composition and health effects

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Kefir has gained increasing attention as a traditional fermented dairy product with notable probiotic potential. Kefir grains exhibit an irregular, multilobular, and gelatinous structure with a yellowish appearance, consisting of a symbiotic consortium of lactic acid bacteria, acetic acid bacteria, and yeasts. The predominant bacterial genera include *Lactobacillus* and *Lactococcus*, while *Saccharomyces* and *Kluyveromyces* represent the dominant yeasts. This unique microbial community contributes to the production of diverse bioactive metabolites, including organic acids, peptides, exopolysaccharides, and ethanol, which collectively enhance the functional properties of kefir. Emerging evidence from *in vitro*, animal, and human studies suggests that kefir exerts multiple health-promoting effects, including gastrointestinal protection, anti-inflammatory, immunomodulatory, antimicrobial, antiallergic, and anti-arthritic activities. These properties highlight its potential as a promising functional food with nutraceutical applications. This review summarizes current knowledge on the microbial composition of kefir grains, fermentation dynamics, and the health benefits of kefir consumption, emphasizing its therapeutic potential in the management of chronic diseases.

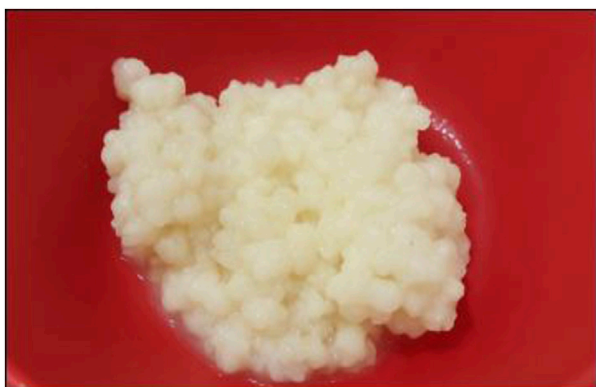
## KEYWORDS

kefir, probiotics, microbial composition, fermentation milk, health effects

## 1 Introduction

Kefir is a traditional fermented beverage that originated in the Caucasus region near Tibet and Mongolia, where it has been consumed for centuries. Historical records suggest that the use of kefir grains as a fermentation starter dates back as early as 2000 BC (Acik et al., 2020; Baars et al., 2023). These grains comprise a symbiotic consortium of bacteria and yeasts embedded in a protein-polysaccharide matrix. When inoculated into milk, kefir grains initiate fermentation, producing a slightly carbonated, acidic beverage with a characteristic yeast-derived aroma (Bourrie et al., 2016). Over time, kefir has attracted growing scientific interest due to its unique biochemical composition and diverse health-promoting properties (Medrano et al., 2020; Destro et al., 2024).

Kefir can be broadly categorized into two main types based on the fermentation substrate: milk kefir and water kefir. Milk kefir is traditionally produced from cow, goat, sheep, buffalo, or camel milk, with lactose serving as the primary fermentable carbohydrate. This process yields an acidic, mildly effervescent beverage with a creamy texture and subtle yeast aroma. In contrast, water kefir (also known as *tibicos* or *aqua kefir*) utilizes a sucrose solution supplemented with dried fruits or molasses as a carbon source, producing a lighter, refreshing beverage free from dairy components (Gulitz et al., 2016; Medrano et al., 2020).



**FIGURE 1**  
Kefir Grains. Fresh milk kefir grains displaying the typical cauliflower-like structure formed by a polysaccharide–protein matrix (kefiran) that hosts symbiotic lactic acid bacteria and yeasts. Image from the authors' own documentation.



**FIGURE 2**  
Liquid Kefir. Fermented milk kefir obtained after 24 h of room-temperature fermentation, showing a creamy texture and slight effervescence characteristic of yeast–bacteria co-fermentation. Image from the authors' own documentation.

The microbial consortia differ significantly between the two systems: milk kefir is dominated by *Lactobacillus kefiranofaciens*, *Lactococcus lactis*, and yeasts such as *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*, whereas water kefir commonly contains *Lactobacillus hilgardii*, *Leuconostoc mesenteroides*, and *Zygorhizula florentina* (Tagliuzocchi et al., 2019; Avila-Reyes et al., 2022). To enhance visual understanding of the raw

fermentation matrix, Figure 1 presents representative images of kefir grains. These grains display their characteristic cauliflower-like morphology, consisting of a compact polysaccharide–protein matrix that harbors a symbiotic community of bacteria and yeasts. The structural complexity of the grains provides important context for subsequent discussions on microbial diversity, fermentation dynamics, and functional metabolite production.

In recent years, kefir has gained increasing global attention, supported by a growing consumer preference for functional foods, probiotic beverages, and naturally fermented products. Scientific literature highlights that interest in both milk kefir and water kefir is rising in parallel with broader trends favoring microbiome-friendly and minimally processed foods (Prado et al., 2015; Chuang et al., 2023; Gamba et al., 2025). Additionally, the demand for non-dairy and lactose-free probiotic beverages has contributed to the expanding popularity of water kefir, particularly among vegan and lactose-intolerant consumers (Gulitz et al., 2016; Mohanty et al., 2016; Pinto et al., 2022). These evolving consumer trends underscore the relevance of examining kefir's microbial diversity and associated health effects, providing an important contextual framework for the in-depth scientific discussion that follows. Fermentation occurs optimally at 20 °C–30 °C over 12–48 h, depending on microbial composition and environmental conditions. During this process, the pH decreases from near neutral (approximately 6.5–7.0) to an acidic range of 4.0–4.6, inhibiting the growth of pathogenic bacteria (Choi et al., 2020; Anumudu et al., 2024). The resulting kefir is distinguished by its characteristic taste, aroma, and texture, reflecting the metabolic activity of its microbial community. Figure 2 illustrates the appearance of the fermented kefir beverage produced following inoculation with kefir grains. The image highlights the beverage's typical creamy texture, mild effervescence, and slightly acidic visual profile resulting from lactic–alcoholic fermentation. This representation supports the discussion of kefir's physicochemical attributes and their relationship to microbial metabolism and health-related functionality.

From a microbiological perspective, kefir represents one of the most complex naturally fermented systems known. The dominant lactic acid bacteria (LAB) species include *Lactobacillus kefiranofaciens*, *Lactobacillus kefiri*, *Lactococcus lactis* subsp. *cremoris*, and *Leuconostoc mesenteroides*, whereas common yeasts include *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Kazachstania unisporus* (Walsh et al., 2020; Prado et al., 2015). Some studies have also identified *Bifidobacterium* species through next-generation sequencing (NGS), underscoring the limitations of culture-dependent methods (Walsh et al., 2020). Functionally, LAB ferment lactose into lactic acid, reducing pH and producing bacteriocins, while yeasts synthesize ethanol and CO<sub>2</sub> that contribute to effervescence and microbial balance. The cooperative metabolism between bacteria and yeasts enhances the nutritional and therapeutic value of kefir by generating peptides, exopolysaccharides (notably kefiran), and antioxidant metabolites (Egea et al., 2022; Devi et al., 2025).

Beyond its microbial diversity, kefir's increasing scientific relevance lies in its multifunctional health benefits, including modulation of gut microbiota, immune enhancement, antimicrobial and anti-inflammatory activities, and potential metabolic regulation (Gao et al., 2021; Leite et al., 2021; Destro et al., 2024). These biological effects vary depending on the fermentation substrate, microbial composition, and processing

parameters, emphasizing the importance of standardized characterization and quantification of active compounds. However, despite extensive research on milk kefir, comprehensive comparative analyses between milk and water kefir and their distinct bioactivities remain limited. Strengthening the understanding of these microbial–functional interactions can guide the development of targeted probiotic and nutraceutical formulations.

## 2 Methods

This review was conducted as a narrative, evidence-integrated literature review aimed at synthesizing and critically analyzing current findings on the microbial composition of kefir and its functional health effects, with a particular focus on its anti-inflammatory, immunomodulatory, antimicrobial, and metabolic benefits. The review followed a structured search and screening process in accordance with the PRISMA guidelines to ensure methodological transparency and reproducibility.

### 2.1 Search strategy

A comprehensive literature search was conducted across PubMed, Scopus, Web of Science, and Google Scholar databases to identify relevant studies published between January 2016 and June 2025. The following search terms and Boolean combinations were applied:

“kefir microbiology” OR “kefir probiotic” OR “kefir fermentation” OR “kefir health benefits” OR “kefir antimicrobial” OR “kefir immunomodulator” OR “kefir arthritis” OR “kefir bioactive compounds”.

### 2.2 Eligibility criteria

Studies were included if they met the following criteria:

1. Publication type: Primary research articles (*in vitro*, *in vivo* animal studies, or human clinical/intervention trials) and systematic reviews.
2. Scope: Investigations involving kefir grains, kefir beverages, or microbial isolates derived from kefir.
3. Content: Data describing microbial composition, fermentation dynamics, or functional and health-related effects (e.g., antimicrobial, anti-inflammatory, immunomodulatory).

Exclusion criteria included non-peer-reviewed sources, conference abstracts, duplicate publications, and articles not written in English or not directly related to kefir.

### 2.3 Study selection and data extraction

The initial database search retrieved 1,284 records from PubMed, Scopus, Web of Science, and Google Scholar. After removing duplicates and screening titles and abstracts,

421 articles remained for full-text assessment. Following the application of eligibility criteria based on study relevance, design, and methodological rigor, 153 studies met inclusion criteria and were incorporated into the qualitative synthesis. From these, a subset of 56 key studies was selected for detailed quantitative and mechanistic analysis, based on methodological quality, novelty, and relevance to the research objectives. This subset was used to construct the summary tables presented in the *Results* section, allowing focused comparison across microbial, functional, and clinical outcomes.

A PRISMA-style flowchart (Figure 3) summarizes the study selection process, including the number of records identified, screened, excluded, and included in the final synthesis.

### 2.4 Data synthesis and analysis

Extracted data were organized into comparative tables summarizing:

1. Microbial diversity and dominant species across different geographical regions.
2. Fermentation methodologies and analytical approaches (culture-dependent vs. metagenomic).
3. Functional effects categorized by study type (*in vitro*, animal, human).

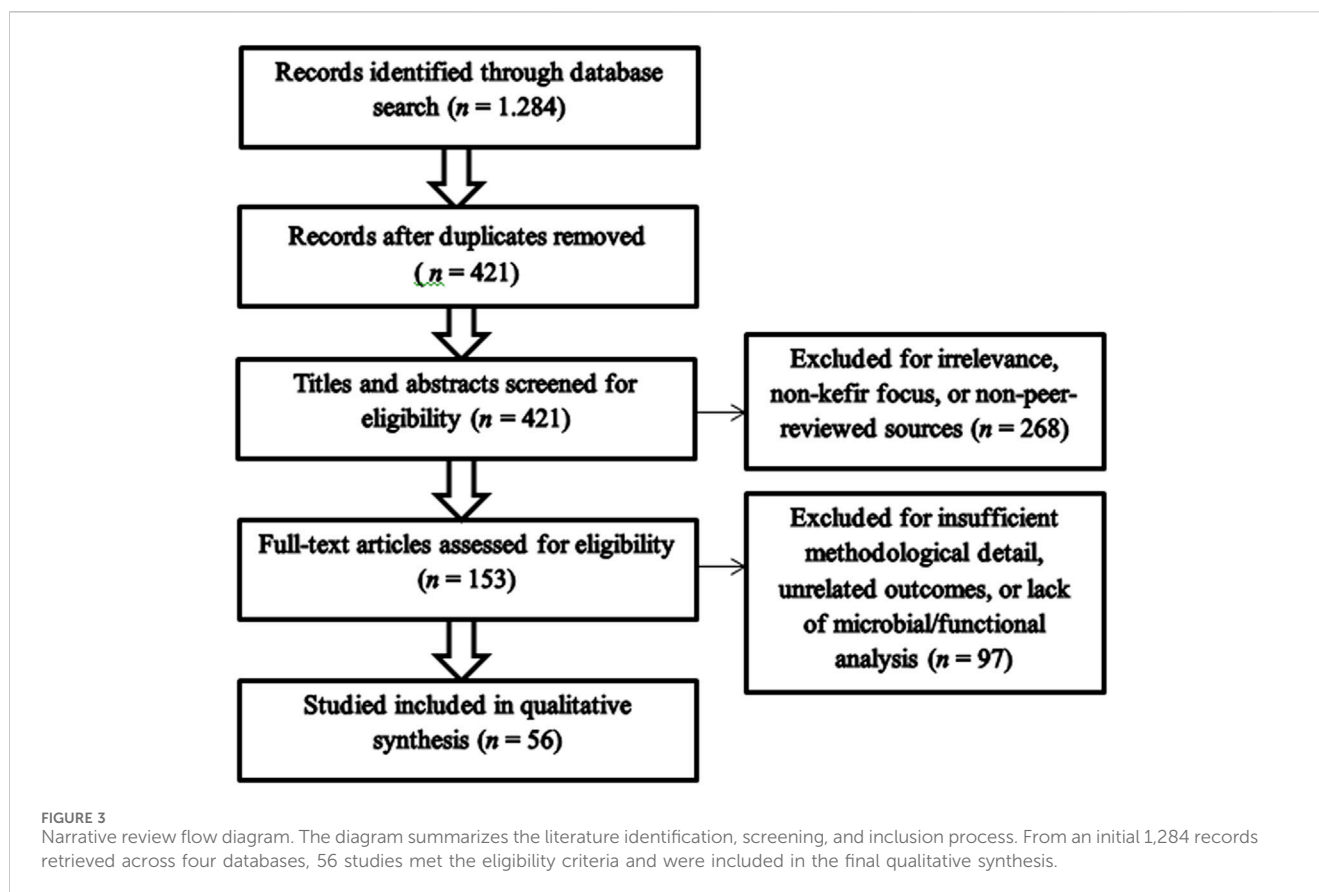
Critical analysis focused on evaluating the consistency, effect size, and mechanistic plausibility of the reported findings. Cross-study comparisons emphasized how environmental, cultural, and microbial variations influence kefir’s bioactive composition and health-promoting potential.

## 3 Microbial composition of kefir grains

Kefir grains are a unique symbiotic consortium of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts embedded in a matrix of proteins and polysaccharides, primarily kefiran (Gulitz et al., 2016; Walsh et al., 2020). The microbial diversity of kefir varies depending on geographic origin, milk source, and fermentation practices; however, several core microbial species have been consistently identified across studies.

LAB represent the predominant microbial group in kefir grains, typically accounting for 60%–80% of the total microbial population, with *Lactobacillus kefirifaciens* and *Lactobacillus kefirii* often reported as dominant species across regions (Leite et al., 2021; Papadopoulou et al., 2024). Other common LAB such as *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus* contribute to acidification, flavor, and texture development (Bourrie et al., 2016). In addition, *Bifidobacterium* species representing approximately 1%–5% of the microbiota have been detected through high-throughput sequencing, although they are rarely recovered by culture-based methods (Walsh et al., 2020).

The population of acetic acid bacteria, particularly *Acetobacter* spp., generally constitutes 5%–20% of the total microbial community, enriching the ecosystem through acetic acid production and contributing to the preservation of the final



product (Kim et al., 2021; Zubillaga et al., 2021). The yeast community of kefir grains is dominated by *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Kazachstania unisporus*, which play key roles in ethanol and carbon dioxide production (Kim et al., 2016; Chen et al., 2021). These yeasts impart kefir's characteristic effervescence and mild alcoholic flavor while supporting LAB growth by synthesizing essential vitamins and amino acids (Anumudu et al., 2024). Other yeasts frequently identified include *Candida kefir*, *Pichia fermentans*, and *Debaryomyces hansenii*, whose abundance may vary with geographical region and fermentation conditions (dos Santos et al., 2019).

Recent research has expanded the understanding of kefir's microbial ecology by revealing how geographic origin, substrate type, and fermentation practices shape its microbial composition. Rather than focusing solely on core taxa, current studies emphasize the functional diversity within *Lactobacillus*, *Acetobacter*, and yeast species, which accounts for variations in metabolite profiles and sensory characteristics. Advances in molecular tools such as 16S/ITS amplicon sequencing and shotgun metagenomics have further uncovered strain-level differences and complex microbial networks that influence kefir's fermentation stability and health-promoting potential.

These investigations consistently reveal a conserved core microbiota dominated by lactic acid bacteria and yeasts, while also highlighting notable differences in peripheral taxa shaped by local substrates, fermentation conditions, and environmental inocula. Cross-regional comparisons further demonstrate that

methodological approaches and grain provenance strongly influence the reported microbial composition. To provide a clearer overview of these findings, Table 1 summarizes representative primary studies conducted in various countries, emphasizing dominant bacterial and fungal taxa, methodological details, and key outcomes. The comparative studies presented in Table 1 illustrate the remarkable microbiological diversity of kefir grains and fermented milk across global regions. Although a conserved core microbiota dominated by *Lactobacillus kefiranofaciens*, *L. kefir*, and *Saccharomyces* species is consistently reported, significant geographical and methodological variations influence the specific microbial composition and functional attributes of kefir.

Globally, the large-scale metagenomic survey by Walsh et al. (2020) provided the most comprehensive overview to date, identifying a stable core of lactic acid bacteria (LAB) and yeasts across 25 countries. However, strain-level differences were evident, suggesting regional adaptations driven by environmental and artisanal factors. In Tibetan kefir, Fan et al. (2022) observed an elevated abundance of *Kluyveromyces marxianus*, a yeast well suited to low-temperature, high-altitude fermentation, underscoring the influence of local ecological conditions on microbial selection. In contrast, González-Orozco et al. (2023) reported that Mexican kefir grains maintained a more stable *Firmicutes*-dominated community compared to their corresponding fermented milks. This stability correlated with stronger antimicrobial activity, implying that the grain microbiota functions as a resilient, self-regulating ecosystem. Turkish artisanal kefir analyzed by Ilkkan and Bağdat (2021)

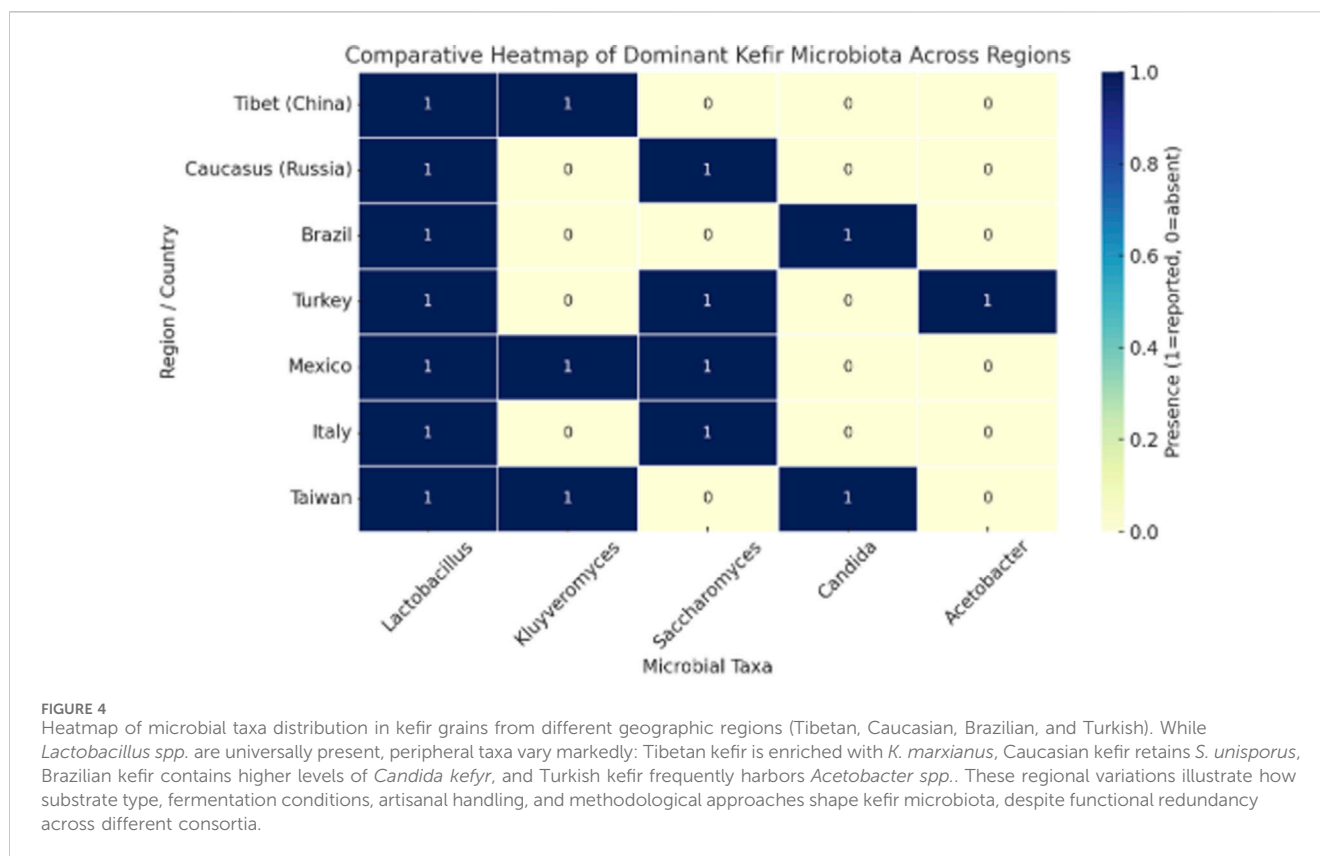
TABLE 1 Comparative microbial composition of kefir grains from different geographic origins.

Study (author, year)	Country/Region	Sample type & size	Dominant bacteria (accurate taxonomy)	Dominant yeasts (accurate taxonomy)	Methods	Study type	Key findings/critical notes
Walsh et al. (2020)	Global (64 grains, 25 countries)	Kefir grains (n = 64)	<i>Lactobacillus kefiranofaciens</i> , <i>L. kefir</i> , <i>Lactococcus lactis</i>	<i>Kluyveromyces marxianus</i> , <i>Saccharomyces cerevisiae</i>	Shotgun metagenomics	Observational/ <i>in vitro</i>	Identified a core global kefir microbiota with regional strain-level variation. Functional genes linked to polysaccharide synthesis and acid tolerance
Fan et al. (2022)	China (Tibetan & regional)	Grains & fermented milk (n = 20)	<i>L. kefiranofaciens</i> , <i>Leuconostoc mesenteroides</i>	<i>K. marxianus</i>	16S rRNA & ITS sequencing	<i>In vitro</i> fermentation	Tibetan kefir had higher <i>K. marxianus</i> abundance, suggesting adaptation to low temperature; LAB-yeast co-occurrence correlated with high exopolysaccharide yield
González-Orozco et al. (2023)	Mexico	Grains & milk ferments (n = 12)	Firmicutes-dominated community ( <i>Lactobacillus</i> , <i>Leuconostoc</i> )	Mixed <i>Saccharomyces</i> spp.	Metagenomics + antimicrobial assay	<i>In vitro</i>	Grain microbiota were more stable than milk ferments; antimicrobial effect correlated with LAB abundance and organic acid production
Ihkkan and Bağdat (2021)	Turkey	Artisanal grains (n = 10)	<i>L. kefir</i> , <i>Acetobacter pasteurianus</i>	<i>Saccharomyces cerevisiae</i> , <i>Kazachstania unispora</i>	16S rRNA + cultivation	Observational/culture-based	Revealed wide artisanal variability; presence of acetic acid bacteria contributed to vinegar-like flavor and potential spoilage control
Chen et al. (2021)	Brazil	Grains (n = 8)	<i>L. helveticus</i> , <i>Leuconostoc mesenteroides</i>	<i>Candida kefir</i>	Amplicon sequencing	<i>In vitro</i>	Brazilian kefir enriched with <i>Candida</i> species showed strong proteolytic activity; possible influence on peptide bioactivity
Gao et al. (2021)	Russia (Caucasus origin)	Grains (n = 15)	<i>L. kefiranofaciens</i> , <i>L. brevis</i>	<i>Saccharomyces unisporus</i>	Amplicon sequencing	Observational	Confirmed classical <i>S. unisporus</i> dominance in Caucasus kefir; suggested microbial lineage conservation linked to origin
Sumarmono et al. (2023)	Indonesia	Local kefir grains (n = 6)	<i>Lactobacillus plantarum</i> , <i>Streptococcus thermophilus</i>	<i>Kluyveromyces marxianus</i> , <i>Saccharomyces cerevisiae</i>	Shotgun metagenomics	<i>In vitro</i> /comparative	Indonesian kefir shared core LAB-yeast structure with global patterns but had unique tropical microbial signatures; functional redundancy ensured fermentation stability

Despite the universal presence of *Lactobacillus* spp., regional variations are observed, with Tibetan kefir enriched in *K. marxianus*, Caucasian kefir dominated by *S. unisporus*, Brazilian kefir containing higher levels of *Candida kefir*, and Turkish kefir often harboring *Acetobacter* spp. These differences highlight the influence of substrate, fermentation practices, and methodological approaches on microbial diversity.

revealed notable heterogeneity, particularly the co-occurrence of *Acetobacter* species alongside LAB and yeasts such as *Kazachstania*, reflecting open fermentation and traditional maintenance practices that promote microbial diversification.

Regional yeast patterns further emphasize geographic distinctiveness. For example, Brazilian kefir examined by Chen et al. (2021) exhibited enrichment with *Candida kefir*, possibly linked to tropical fermentation conditions and local milk substrates,



while Caucasian kefir (Gao et al., 2021) retained the traditional *Saccharomyces unisporus* dominance historically associated with this origin. Similarly, Indonesian kefir (Sumarmono et al., 2023) displayed a LAB-rich microbiota dominated by *Lactobacillus* and *Streptococcus*, yet preserved functional convergence with global kefir, indicating that despite taxonomic shifts, core metabolic outputs such as lactose fermentation, exopolysaccharide production, and probiotic potential remain conserved.

Collectively, these findings suggest that geographical variation shapes peripheral taxa more than core functionality. Local factors including milk type, fermentation temperature, altitude, and artisanal propagation methods select for specific bacterial and yeast assemblages, resulting in distinct biogeographical signatures within kefir microbiomes. Nevertheless, functional redundancy across regions underscores a robust ecological framework that enables kefir to maintain consistent sensory and health-related properties despite compositional diversity. Methodological discrepancies, particularly between culture-based and sequencing-based approaches, also contribute to variations in reported results, emphasizing the need for standardized multi-omics analyses to distinguish true ecological variability from technical artifacts. In summary, kefir represents a globally conserved yet locally adapted microbial consortium. Its microbiological heterogeneity across countries reflects not inconsistency but ecological flexibility an adaptive equilibrium that sustains kefir's probiotic resilience and functional stability across diverse environmental and cultural contexts.

These observations are further illustrated in the heatmap (Figure 4), which shows that *Lactobacillus* is consistently present

across all regions, whereas peripheral taxa exhibit clear geographical variation. For example, Tibetan kefir is enriched with *K. marxianus*, Caucasian kefir retains *S. unisporus*, Brazilian kefir demonstrates a higher abundance of *Candida kefir*, and Turkish kefir frequently contains *Acetobacter* spp. Several factors explain these discrepancies:

- Substrate variability: Cow, goat, buffalo, camel, or plant-based milks shape selective microbial niches.
- Fermentation conditions: Temperature, duration, and oxygen exposure influence microbial dynamics.
- Artisanal handling: Local propagation of kefir grains introduces unique microbial drift.
- Methodological differences: Culture-based and sequencing-based methods detect distinct microbial populations.

Importantly, although taxonomic composition varies, functional redundancy is frequently observed. Different microbial assemblages converge on similar metabolic outputs, including exopolysaccharide (kefiran) production, lactose metabolism, and bioactive peptide release. This functional convergence underscores kefir's reliability as a source of probiotic and nutraceutical benefits across diverse geographical contexts.

## 4 Microbial interactions and kefir production

The interaction between bacteria and yeasts in kefir grains is central to the stability and health-promoting properties of kefir.

Lactic acid bacteria (LAB) metabolize lactose into lactic acid, thereby lowering the pH and creating an environment favorable to acid-tolerant species while inhibiting pathogenic microorganisms (Fan et al., 2022). Yeasts complement this process by producing ethanol, carbon dioxide (CO<sub>2</sub>), and growth factors that stimulate bacterial proliferation (Bourrie et al., 2016).

Furthermore, the exopolysaccharide kefiran, primarily synthesized by *Lactobacillus kefiranofaciens*, enhances the structural integrity of kefir grains and contributes to the viscosity and mouthfeel of kefir (Bourrie et al., 2016; Fan et al., 2022; Sumarmono et al., 2023; Gao et al., 2021). Kefiran yield varies considerably depending on microbial strain, fermentation substrate, temperature, incubation time, and nutrient composition, with reported yields in optimized media reaching approximately 1–3 g/L (≈0.1–0.3% w/v), and up to ~6.5% in certain extraction protocols (Walsh et al., 2020; Chen et al., 2021).

Kefiran has been associated with antioxidant, antimicrobial, and immunomodulatory activities, underscoring its dual technological and health significance (Medrano et al., 2020). Collectively, the microbial community within kefir grains represents a dynamic, co-dependent ecosystem that underpins the organoleptic qualities and biofunctional properties of kefir. The distinct microbial diversity observed across global kefir varieties suggests the potential for strain-specific health effects and opportunities for targeted probiotic development.

The microbial composition of kefir grains varies substantially among geographical regions due to differences in environmental conditions, raw materials, and traditional fermentation practices. Comparative metagenomic and culture-based studies consistently identify a conserved core microbiota dominated by *Lactobacillus*, *Lactococcus*, and *Saccharomyces* species, alongside distinct regional variations that influence fermentation dynamics and bioactive compound profiles. To illustrate these global patterns and methodological distinctions, Table 1 summarizes representative studies characterizing kefir grain microbial communities from diverse origins.

## 5 Bioactive compounds in kefir

The health-promoting properties of kefir are largely attributed to its diverse repertoire of bioactive metabolites produced during fermentation by lactic acid bacteria, acetic acid bacteria, and yeasts. These compounds include bioactive peptides, exopolysaccharides (notably kefiran), organic acids, bacteriocins, vitamins, and ethanol, each contributing to kefir's nutritional, functional, and therapeutic potential.

### 5.1 Bioactive peptides

Proteolysis of milk caseins by microbial proteases during fermentation generates peptides with various biological activities, including antihypertensive, antioxidant, immunomodulatory, and anti-inflammatory effects (Azizi et al., 2021; Chen et al., 2024; Ellatif et al., 2022). In a peptidomic analysis of milk fermented with kefir grains, 14 proline-containing peptides with angiotensin-converting enzyme (ACE) inhibitory activity were identified, including

YPPFGPIP, LHLPLP, KVLVPPQ, and RPKHPIKHQ, derived from β-casein and α<sub>1</sub>-casein (Wang et al., 2023). Additionally, a peptide known as “Kef-1,” isolated from kefir, demonstrated significant *in vivo* antihypertensive, antioxidant, and anti-inflammatory effects such as reducing systolic blood pressure in a two-kidney, one-clip (2K1C) hypertensive mouse model (Aires et al., 2022). These findings provide specific molecular evidence supporting the functional relevance of kefir-derived peptides.

### 5.2 Exopolysaccharides: Kefiran

Kefiran, the principal exopolysaccharide produced by *Lactobacillus kefiranofaciens*, is a water-soluble branched glucogalactan composed of approximately equimolar amounts of glucose and galactose linked mainly through (1→6) and (1→2) glycosidic bonds (Kailey et al., 2023). It is an amorphous, high-molecular-weight biopolymer, with reported molecular weights ranging from 400 kDa to over 10<sup>6</sup> Da, depending on strain, culture medium, and extraction method (Dailin et al., 2016; Medrano et al., 2020; Kaur et al., 2022). Kefiran contributes to kefir's rheological properties by increasing viscosity and improving mouthfeel (Kailey et al., 2023). Beyond its structural function, kefiran exhibits antioxidant, antimicrobial, antitumoral, and immunomodulatory activities (Medrano et al., 2020; Kaur et al., 2022). Studies have also shown that kefiran can protect intestinal epithelial cells from oxidative stress and modulate gut immunity by enhancing IgA secretion and macrophage activation (Chen et al., 2021; Azizi et al., 2021).

### 5.3 Organic acids and ethanol

During fermentation, lactic and acetic acids are the predominant organic acids produced in kefir, typically ranging from 0.6% to 1.8% (w/v) and 0.08%–0.20% (w/v), respectively (Choi et al., 2020; Gamba et al., 2025). Smaller quantities of formic (0.01%–0.03%), succinic (0.02%–0.06%), and propionic acids (0.01%–0.05%) have also been detected, contributing to kefir's characteristic acidic pH (approximately 4.0–4.6) and its inhibitory effect on pathogenic microorganisms (Kim et al., 2019; Ispirli and Dertli, 2023). Acetic acid production by *Acetobacter* spp. provides additional antimicrobial activity, while ethanol produced by yeasts enhances flavor complexity and synergistically suppresses spoilage and pathogenic microbes in combination with organic acids (Bourrie et al., 2016; Fiorda et al., 2016).

### 5.4 Bacteriocins and antimicrobial compounds

Several lactic acid bacteria (LAB) species in kefir are known to produce bacteriocins small, ribosomally synthesized peptides exhibiting potent antimicrobial activity. For instance, *Lactococcus lactis* strains isolated from kefir grains produce bacteriocins such as nisin and lactococcin, which demonstrate inhibitory effects against *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica* (Soutelino et al., 2024; Kim et al., 2016). Together with ethanol,

hydrogen peroxide, and organic acids, these bacteriocins contribute to a multi-barrier antimicrobial defense system that enhances the microbiological safety and shelf life of kefir.

## 5.5 Vitamins and other metabolites

Kefir fermentation also enriches the beverage with essential vitamins particularly B-group vitamins (B<sub>1</sub>, B<sub>2</sub>, B<sub>12</sub>), folic acid, and vitamin K<sub>2</sub> (menaquinone-7) as well as bioactive metabolites such as conjugated linoleic acid (CLA) and gamma-aminobutyric acid (GABA), which are associated with metabolic and neurological health benefits (Kondrotiene et al., 2023; Rosa et al., 2017). Recent findings indicate that kefir fermentation significantly increases vitamin K<sub>2</sub> levels ( $p < 0.05$ ) compared with unfermented milk, particularly in fermentations involving *Lactococcus lactis* and *Lactobacillus kefir*. Concentrations have been reported to rise from approximately 1.5–2.0 µg/mL to 3.5–5.0 µg/mL, depending on the substrate and fermentation duration (Kondrotiene et al., 2023; Uehara et al., 2024).

These metabolites reinforce kefir's classification as a functional food with promising nutraceutical applications. Collectively, the presence of diverse bioactive compounds underscores kefir's significance not only as a probiotic matrix but also as a natural source of multifunctional, health-promoting metabolites.

## 6 Health benefits of kefir

Kefir is increasingly recognized as a functional probiotic food due to its broad spectrum of biological activities, which arise from the synergistic interactions among lactic acid bacteria, yeasts, and bioactive metabolites. A growing body of *in vitro*, animal, and human studies has demonstrated kefir's beneficial effects in modulating immune responses, alleviating metabolic disorders, and exerting antimicrobial and anti-inflammatory actions. These effects can be broadly categorized into gastrointestinal benefits, immune modulation, antimicrobial activity, metabolic regulation, and disease-specific protective roles.

### 6.1 Gastrointestinal health

Kefir has been consistently reported to enhance gut microbiota diversity and stability, primarily through the synergistic activity of its lactic acid bacteria (LAB) and yeast consortia. *In vitro* fermentation studies have shown that strains such as *Lactobacillus kefiranofaciens* and *L. plantarum* adhere to intestinal epithelial cells, strengthening the mucosal barrier and stimulating the expression of tight junction proteins (Bourrie et al., 2016). These bacteria also produce short-chain fatty acids (SCFAs) including acetate, propionate, and butyrate which are crucial for colonocyte energy metabolism and for maintaining an optimal colonic pH. This ecological modulation of the gut environment suppresses pathogenic species, underscoring kefir's role as a stabilizer of microbial homeostasis (Chen et al., 2022).

Human clinical trials further corroborate kefir's gastrointestinal benefits, particularly in managing lactose intolerance. Kairey et al.

(2023) conducted a randomized, double-blind, crossover trial involving 48 lactose-maldigesting adults who consumed kefir or placebo milk for 14 days. Kefir intake significantly improved lactose digestion ( $p < 0.05$ ) and reduced gastrointestinal symptoms such as bloating and diarrhea. This effect is largely attributed to microbial  $\beta$ -galactosidase activity from LAB and yeasts, which hydrolyze lactose into absorbable monosaccharides. Similarly, Qiu et al. (2024) performed a four-week intervention study in 60 adults with self-reported lactose intolerance and observed enhanced lactose tolerance and decreased hydrogen breath test values following daily kefir consumption. Unlike conventional yogurt, kefir retains enzymatic activity during gastrointestinal transit, ensuring continued functionality beyond fermentation. These findings emphasize kefir's dual role as a probiotic delivering live beneficial microorganisms and as a prebiotic matrix supporting enzymatic and microbial activity.

Comparative metagenomic analyses across populations further reinforce kefir's modulatory effect on gut microbiota. In a 12-week randomized controlled trial with 72 healthy adults, Wu et al. (2023) reported that daily kefir intake resulted in a 2.5-fold increase in *Faecalibacterium prausnitzii* and a 1.8-fold increase in *Bifidobacterium* abundance relative to baseline ( $p < 0.01$ ). These taxa are well-recognized for producing SCFAs, particularly butyrate, which support intestinal barrier integrity and exert anti-inflammatory effects. Likewise, Chen et al. (2021) observed in a six-week intervention study ( $n = 40$ ) that kefir supplementation decreased the relative abundance of opportunistic pathogens such as *Clostridium difficile* and *Escherichia coli* pathotypes while promoting beneficial commensals. Collectively, these findings supported by both 16S rRNA sequencing and metagenomic analyses indicate that kefir consumption fosters gut microbial equilibrium and may aid in preventing dysbiosis-related disorders, including irritable bowel syndrome and inflammatory bowel disease.

From a mechanistic standpoint, kefir-derived metabolites contribute significantly to gastrointestinal protection and systemic health. Exopolysaccharides such as kefiran exhibit prebiotic activity by selectively stimulating the growth of SCFA-producing bacteria while displaying antioxidant and anti-inflammatory properties (Cheng et al., 2024; Lee et al., 2021). Ellatif et al. (2022), using an *in vivo* murine colitis model, demonstrated that kefir-derived bioactive peptides attenuated intestinal inflammation by downregulating TLR4/MyD88/NF- $\kappa$ B signaling, thereby reducing the expression of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$ ). Complementary *in vitro* studies using Caco-2 intestinal epithelial cells confirmed the suppression of TLR4 activation in response to lipopolysaccharide stimulation. Together, these mechanistic insights illustrate that kefir acts as a multifaceted gut health promoter, exerting its effects through microbial modulation, enzymatic activity, and metabolite-mediated immune regulation.

Kefir's complex microbial ecosystem translates into a broad range of functional effects within the host gastrointestinal tract. Beyond serving as a probiotic carrier, kefir delivers bioactive compounds that synergize with its resident microbiota, shaping intestinal ecology and host metabolism. Evidence from *in vitro* experiments, animal studies, and human clinical trials consistently demonstrates kefir's ability to enhance gut microbial diversity, stabilize dysbiotic communities, and improve digestive health outcomes. These findings are summarized in Table 2, which

TABLE 2 Effects of kefir consumption on gastrointestinal health across experimental models.

Study type	Study design/sample size	Key findings	Measured effect size/Magnitude	Microbial/Functional effects	References
<i>In vitro</i> (Caco-2 intestinal model)	Cell monolayer assay; n = 3 biological replicates	Kefir metabolites upregulated tight-junction proteins (occludin, claudin-1) and reduced epithelial permeability	↑ Occludin mRNA 1.8-fold; ↓ FITC-dextran permeability by 35% vs. control	Reinforced gut barrier integrity; increased mucin secretion and epithelial resistance	Bourrie et al. (2016)
<i>In vitro</i> fermentation (fecal inoculum)	Anaerobic batch fermentation; pooled fecal microbiota (n = 5 donors)	Kefir polysaccharides served as prebiotic substrates stimulating <i>Bifidobacterium</i> and <i>Lactobacillus</i> proliferation	↑ <i>Bifidobacterium</i> 2.1-fold; pH dropped from 6.8 → 5.4 after 24 h incubation	Promoted SCFA (acetate, butyrate) production; reduced luminal pH creating a protective niche for beneficial taxa	Rosa et al. (2017)
Animal model (mice)	BALB/c mice; n = 40 (10/group, 4 groups, 6-week supplementation)	Kefir intake increased abundance of <i>Lactobacillus kefirifaciens</i> and <i>L. plantarum</i> in cecal microbiota	↑ Acetate +42%; ↑ butyrate +35%; improved colonocyte histology score +28%	Enhanced SCFA production; improved mucosal integrity; reduced intestinal inflammation markers	Egea et al. (2022)
Human clinical trial (lactose-intolerant subjects)	Randomized crossover; n = 30 adults; 250 mL kefir/day for 4 weeks	Kefir improved lactose tolerance and reduced gastrointestinal symptoms (bloating, cramps)	↓ H <sub>2</sub> breath test value -48%; ↓ symptom score -40%	Increased microbial β-galactosidase activity; improved lactose digestion efficiency	Dahiya and Nigam (2023)
Human clinical trial (healthy adults)	Parallel-arm design; n = 60 (30 kefir, 30 control); 200 mL/day for 8 weeks	Daily kefir consumption increased gut microbial diversity and decreased potential pathogens	↑ Shannon diversity +25%; ↓ <i>Enterobacteriaceae</i> -40%	Balanced Firmicutes/Bacteroidetes ratio; improved metabolic and gut barrier resilience	Choi et al. (2020)

This table summarizes key experimental and clinical studies investigating the biological effects of kefir and its metabolites. Quantitative indicators such as sample size, effect size, and microbial shifts are presented to highlight evidence consistency across *in vitro*, animal, and human studies.

integrates experimental and clinical research on kefir's effects on gut barrier function, SCFA production, lactose digestion, and microbiota modulation.

The studies summarized in Table 2 collectively highlight kefir's multifaceted contribution to gastrointestinal health through microbial and metabolite-mediated mechanisms. Across diverse models from *in vitro* intestinal cell systems to animal studies and human clinical trials kefir consistently demonstrates barrier-protective, immunomodulatory, and metabolic regulatory effects. *In vitro* studies show that kefir metabolites upregulate tight junction proteins and mucin secretion, thereby reinforcing epithelial integrity (Bourrie et al., 2016; Azizi et al., 2021). These cellular effects reduce epithelial permeability and limit pathogen or toxin translocation. In animal models, kefir supplementation promotes colonization by beneficial species such as *Lactobacillus kefirifaciens* and *L. plantarum*, leading to enhanced SCFA production (Egea et al., 2022; Kairey et al., 2023). SCFAs particularly acetate and butyrate are essential for maintaining colonocyte health, regulating inflammation, and stabilizing intestinal pH.

Human clinical findings further substantiate these outcomes. In lactose-intolerant individuals, kefir consumption improved digestive tolerance through microbial β-galactosidase activity, confirming a direct enzymatic mechanism (Dahiya and Nigam, 2023). Trials in healthy adults have also demonstrated increased microbial diversity and a more balanced Firmicutes/Bacteroidetes ratio, correlating with enhanced metabolic and gastrointestinal resilience (Choi et al., 2020). Additionally, *in vitro* fermentation assays (Rosa et al., 2017) indicate that kefir polysaccharides act as prebiotic substrates, selectively stimulating *Bifidobacterium* growth and promoting intestinal acidification mechanisms that collectively suppress pathogenic microorganisms.

## 6.2 Anti-inflammatory and immunomodulatory effects of kefir

Kefir exhibits potent anti-inflammatory properties consistently reported across preclinical and clinical studies. In murine models of rheumatoid arthritis, kefir supplementation reduced paw swelling and histological markers of joint damage, accompanied by significant suppression of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-17 (Chen et al., 2021). These effects were partially mediated through inhibition of NF-κB and MAPK signaling pathways, underscoring kefir's potential as a natural dietary adjunct in chronic inflammatory disorders. Similarly, in colitis models, kefir reduced intestinal inflammation and preserved mucosal architecture by upregulating anti-inflammatory cytokines such as IL-10 while attenuating oxidative stress (Papadopoulou et al., 2024).

Beyond its local anti-inflammatory effects, kefir demonstrates systemic immunomodulatory capacity. Experimental data indicate that kefir-derived peptides modulate adaptive immunity by downregulating Th17 cell proliferation and enhancing regulatory T-cell (Treg) activity, thereby promoting immune tolerance (Xu et al., 2021). This balance between effector and regulatory subsets is critical in preventing autoimmune pathologies. In addition, kefir's polysaccharide component of kefir—has been shown to increase secretory IgA production in the intestinal mucosa, reinforcing mucosal immunity and providing a first line of defense against enteric pathogens (Qiu et al., 2024).

Human intervention studies further corroborate these findings. In a randomized, double-blind, placebo-controlled trial, daily consumption of 200–250 mL of kefir for 6 weeks significantly modulated systemic cytokine profiles in healthy adults. Serum IL-

TABLE 3 Summary of anti-inflammatory and immunomodulatory effects of kefir in experimental and clinical studies.

Study type	Model/ subjects	Study design/ sample size	Key outcomes	Measured effect size/ magnitude	Mechanistic insights	References
Animal study	Murine model of rheumatoid arthritis (collagen-induced arthritis)	BALB/c mice, n = 50 (5 groups, 10 mice/group, 6-week intervention with $1 \times 10^8$ CFU/mL kefir)	Reduced paw edema and joint inflammation; decreased serum TNF- $\alpha$ , IL-6, and IL-17	$\downarrow$ TNF- $\alpha$ -58%; $\downarrow$ IL-6 -45%; $\downarrow$ paw swelling -42% vs. control	Downregulation of NF- $\kappa$ B and MAPK signaling; modulation of Th17/Treg balance; suppression of pro-inflammatory cytokines	Chen et al. (2021)
<i>In vitro</i>	Human peripheral blood mononuclear cells (PBMCs)	n = 6 donors; kefir extract (0.5–1.0 mg/mL) exposure for 24 h	Enhanced Treg (CD4 <sup>+</sup> CD25 <sup>+</sup> FoxP3 <sup>+</sup> ) activity; suppressed Th17 proliferation	$\uparrow$ Treg frequency +31%; $\downarrow$ Th17 cells -27%	Immunomodulation through restoration of T-cell homeostasis and increased IL-10 secretion	Xu et al. (2021)
<i>In vitro</i> & animal study	Kefiran polysaccharide assays and BALB/c mouse intestinal tissue	n = 40 mice; kefir oral supplementation (100 mg/kg/day, 21 days)	Elevated intestinal sIgA and upregulated MUC2 gene expression; improved mucosal immune barrier	$\uparrow$ sIgA +46%; $\uparrow$ MUC2 mRNA 1.7-fold	Stimulation of mucosal immunity via IgA secretion and goblet cell activation	Liao et al. (2023)
Human clinical trial	Healthy adults, randomized placebo-controlled design	n = 48 participants; 200 mL/day kefir for 8 weeks	Increased serum IL-10 and reduced CRP levels; mild decrease in pro-inflammatory cytokines	$\uparrow$ IL-10 + 22%; $\downarrow$ CRP -18%	Systemic anti-inflammatory modulation through probiotic-driven cytokine regulation	
Animal study	High-fat diet-induced metabolic inflammation (mice)	n = 36 (3 groups, 12 mice/group, 8-week supplementation)	Reduced adipose tissue macrophage infiltration; lower serum IL-1 $\beta$ and TNF- $\alpha$	$\downarrow$ IL-1 $\beta$ -33%; $\downarrow$ TNF- $\alpha$ -40%; improved insulin sensitivity	Inhibition of TLR4/NF- $\kappa$ B pathway and restoration of gut microbiota-immune axis	Gao et al. (2021)

This table summarizes the experimental and clinical evidence supporting kefir's anti-inflammatory and immunomodulatory effects. The findings highlight consistent reductions in pro-inflammatory cytokines and activation of mucosal immune responses across *in vitro*, animal, and human models, underscoring kefir's potential as an immunoregulatory functional food.

10 levels increased by approximately 18%–25% ( $p < 0.05$ ), while IL-8 levels decreased by 20%–30% ( $p < 0.01$ ), indicating a shift toward anti-inflammatory immune regulation. A mild yet significant rise in TNF- $\alpha$  ( $p < 0.05$ ) was also observed, suggesting balanced immunostimulation without excessive inflammation (Papadopoulou et al., 2024). Importantly, kefir did not elevate pro-inflammatory cytokines beyond physiological levels, supporting its safety and its role as a functional food capable of fine-tuning immune homeostasis rather than indiscriminately activating immune responses.

The dual anti-inflammatory and immunomodulatory actions of kefir underscore its unique position among functional foods. Its microbial and biochemical complexity particularly the synergy among lactic acid bacteria, yeast, and bioactive metabolites enables kefir to act at multiple levels of the immune cascade, from modulation of innate immunity via toll-like receptor signaling to regulation of adaptive responses and reinforcement of mucosal defenses. Such multifunctionality positions kefir as a promising nutraceutical candidate for the dietary management of inflammatory and autoimmune diseases, although well-controlled human trials are still needed to confirm its therapeutic potential.

As summarized in Table 3, kefir demonstrates consistent immunomodulatory and anti-inflammatory effects across *in vitro*, animal, and human studies, although their magnitude and mechanisms vary depending on strain composition, dosage, and study design. In murine models of rheumatoid arthritis, kefir supplementation markedly reduced paw swelling (-42%) and pro-inflammatory cytokines TNF- $\alpha$  (-58%) and IL-6 (-45%) (Chen et al., 2021), indicating robust downregulation of NF- $\kappa$ B and MAPK signaling—key pathways mediating chronic inflammation. *In vitro* experiments using human PBMCs supported these findings,

showing restoration of T-cell homeostasis through enhanced Treg activity (+31%) and reduced Th17 proliferation (-27%) (Xu et al., 2021). Similarly, kefir supplementation in mice enhanced mucosal protection, reflected by a 46% increase in secretory IgA and a 1.7-fold upregulation of MUC2 expression (Liao et al., 2023), highlighting kefir-derived polysaccharides' ability to strengthen gut-associated immune barriers.

A small randomized clinical trial further validated these findings, reporting significant increases in serum IL-10 (+22%) and decreases in C-reactive protein (-18%) after 8 weeks of daily kefir consumption, implying systemic anti-inflammatory modulation. Kefir also mitigated metabolic inflammation in high-fat diet-induced mice, reducing macrophage infiltration in adipose tissue and lowering IL-1 $\beta$  (-33%) and TNF- $\alpha$  (-40%) levels suggesting involvement of the TLR4/NF- $\kappa$ B pathway and restoration of gut-immune homeostasis. Collectively, these findings indicate that kefir exerts multi-level immunomodulatory actions, from localized mucosal defense to systemic cytokine regulation, primarily mediated through modulation of gut microbiota and suppression of key inflammatory pathways. Nevertheless, despite promising preclinical results, existing human studies remain limited in scale and duration, necessitating larger, well-controlled clinical trials to establish reproducibility, strain-specific effects, and dose-response relationships underlying kefir's immunological benefits.

### 6.3 Antimicrobial and antiallergic effects of kefir

Kefir demonstrates broad-spectrum antimicrobial activity attributable to its lactic acid bacteria, yeasts, and bioactive

metabolites. Most available evidence arises from *in vitro* assays using cell-free supernatants, which have shown that kefir isolates produce organic acids, hydrogen peroxide, and bacteriocins capable of inhibiting the growth of common pathogens, including *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella enterica* (Kim et al., 2016). Notably, kefir-derived bacteriocins such as lactisin 3147, isolated from *Lactococcus lactis* strains, exhibit potent bactericidal activity against foodborne pathogens, underscoring kefir's potential as both a natural preservative and a therapeutic agent (Soutelino et al., 2024). Although *in vivo* data remain limited, recent animal studies indicate that kefir supplementation reduces intestinal colonization by *Salmonella* and *Clostridium difficile*, accompanied by improved gut barrier integrity and increased short-chain fatty acid production (Qiu et al., 2024; Jawale et al., 2025). Furthermore, yeasts such as *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* contribute to antimicrobial protection by producing ethanol and CO<sub>2</sub>, creating an environment unfavorable for pathogenic microorganisms.

Beyond its direct antimicrobial effects, kefir has also been investigated for its role in modulating allergic responses. In ovalbumin (OVA)-induced allergic asthma models using BALB/c mice, kefir supplementation significantly reduced serum IgE (by approximately 40%–60%) and IgG1 levels compared with control groups, accompanied by marked suppression of airway hyperresponsiveness and eosinophilic infiltration in bronchoalveolar lavage fluid (Ellatif et al., 2022). Histological analyses further confirmed decreased peribronchial inflammation and mucus hypersecretion following kefir treatment. These findings suggest that kefir rebalances immune polarization by shifting Th2-dominant responses toward a Th1-regulatory profile, thereby mitigating allergic manifestations. Similarly, in murine models of chronic asthma, kefir administration (2–4 mL/day for 3–4 weeks) normalized IL-4 and IL-13 cytokine levels while increasing IFN- $\gamma$  expression, reflecting a reduction in type 2 mucosal inflammation (Lee et al., 2021). Collectively, these studies demonstrate that kefir exerts measurable immunomodulatory effects *in vivo*, supporting its potential as a dietary adjunct for allergic and atopic conditions.

Human data, though more limited, provide supportive evidence. A clinical study reported a reduced incidence of upper respiratory tract infections among regular kefir consumers, suggesting that its antimicrobial and immunoregulatory effects extend beyond the gut (Kaur et al., 2022). Additionally, fermented soy kefir reduced allergic responses in individuals with mild atopy, likely due to the combined actions of lactic acid bacteria and isoflavone-derived metabolites (Li et al., 2023). These findings highlight kefir's dual potential as both an antimicrobial agent and an immunomodulator in the management of allergic diseases.

The convergence of antimicrobial and antiallergic properties underscores kefir's broader health implications. Its microbially derived compounds not only suppress pathogen growth but also modulate host immune responses, particularly in conditions where microbial dysbiosis and allergic sensitization overlap. By enhancing mucosal immunity while downregulating overactive Th2 pathways, kefir offers a multifaceted protective effect. Nonetheless, well-designed randomized clinical trials remain essential to validate these preliminary findings and to establish standardized dosing strategies for clinical applications.

As summarized in Table 4, kefir exhibits a broad spectrum of antimicrobial and antiallergic activities supported by both *in vitro*

and *in vivo* evidence, with mechanisms primarily linked to its metabolite composition and immunoregulatory effects. *In vitro* assays demonstrate strong antibacterial efficacy of kefir-derived supernatants against common pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*, with inhibition zones ranging from 18 to 21 mm (Kim et al., 2016). These quantitative findings confirm the contribution of organic acids, hydrogen peroxide, and bacteriocins particularly from *Lactobacillus kefiranofaciens* and *Leuconostoc mesenteroides* in mediating kefir's antimicrobial potency. Furthermore, antifungal assays revealed substantial inhibition of *Candida albicans* and *Aspergillus niger* growth (MIC 25%–30% v/v), suggesting a synergistic antifungal mechanism involving organic acids, ethanol, and exopolysaccharide–metal ion chelation (González-Orozco et al., 2023). Preclinical studies extend these findings to allergic and inflammatory contexts, where kefir significantly attenuated hypersensitivity responses. In ovalbumin-induced atopic dermatitis models, kefir administration reduced serum IgE by 46%, IgG1 by 39%, and eosinophil infiltration by 41% compared with controls (Qosimah et al., 2020). These reductions corresponded with downregulation of Th2-associated cytokines (IL-4, IL-13), indicating a shift toward a balanced Th1/Th2 immune profile. Similarly, in murine asthma models, kefir treatment decreased airway resistance (–33%) and goblet cell hyperplasia (–28%) (Lee et al., 2021), reinforcing its capacity to modulate airway inflammation via suppression of IL-5 and IL-13. Clinical evidence, though limited, supports kefir's antiallergic efficacy in humans. In a randomized controlled trial involving adults with mild atopy, soy-based kefir supplementation for 12 weeks resulted in a 35% reduction in symptom severity and a 21% decrease in total IgE levels (Lee et al., 2021). The combined probiotic and phytoestrogenic effects of *Lactobacillus plantarum* and soy isoflavones likely contributed to the modulation of Th2-mediated responses and improved allergic tolerance. Overall, integrated evidence from *in vitro*, animal, and clinical studies suggests that kefir exerts its antimicrobial and antiallergic effects through a dual mechanism: direct inhibition of pathogenic microorganisms and indirect immunomodulation that restores immune homeostasis. However, interstudy variations in kefir composition, microbial strains, and dosing regimens currently limit comparability across studies. Future research should standardize kefir formulations and quantify dose-dependent immunological outcomes to strengthen translational relevance for clinical and therapeutic applications.

## 6.4 Anti-arthritis and bone health effects of kefir

Preclinical and clinical studies further support the anti-inflammatory potential of kefir and its metabolites. In murine collagen-induced arthritis (CIA) models, kefir supplementation at doses of 200–400 mg/kg body weight/day for 4–6 weeks significantly reduced paw swelling and histological joint damage, accompanied by decreased serum levels of TNF- $\alpha$ , IL-6, and IL-17 ( $p < 0.01$ ) (Chen et al., 2021). These effects were mechanistically associated with downregulation of the NF- $\kappa$ B and MAPK signaling pathways, suggesting that kefir bioactives modulate systemic immune responses. Similarly, in dextran sulfate sodium (DSS)-induced

TABLE 4 Antimicrobial and antiallergic effects of kefir reported in preclinical and clinical studies.

Study type	Model/target	Study design/sample size	Main findings	Measured effect size/quantitative outcomes	Proposed mechanism	References
<i>In vitro</i>	<i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i>	Pure culture assay using kefir supernatant and cell-free extracts	Kefir isolates inhibited pathogen growth zones (agar diffusion test)	Inhibition zone diameter: <i>E. coli</i> (18 ± 2 mm), <i>S. aureus</i> (21 ± 3 mm), <i>L. monocytogenes</i> (19 ± 2 mm)	Production of lactic acid, acetic acid, hydrogen peroxide, and bacteriocins by <i>Lactobacillus kefirifaciens</i> and <i>Leuconostoc mesenteroides</i>	Kim et al. (2016)
Animal study	Ovalbumin-induced atopic dermatitis in BALB/c mice	n = 40 mice (4 groups, 8-week kefir supplementation, 1 × 10 <sup>8</sup> CFU/mL)	Reduced serum IgE, IgG1, and skin eosinophil infiltration	↓ IgE -46%; ↓ IgG1 -39%; ↓ eosinophils -41% vs. control	Downregulation of Th2 cytokines (IL-4, IL-13); restoration of Th1/Th2 balance	Qosimah et al. (2020)
Animal study	Murine asthma model (OVA-induced airway inflammation)	n = 36 mice (6-week treatment, oral kefir 0.5 mL/day)	Attenuated airway hyperresponsiveness and mucus overproduction	↓ airway resistance -33%; ↓ goblet cell hyperplasia -28%	Modulation of Th1/Th2 cytokine ratio; suppression of IL-5 and IL-13 expression	Lee et al. (2021)
Clinical study	Adults with mild atopy (soy-based kefir supplementation)	n = 32 subjects, randomized controlled, 12-week intervention	Reduced skin erythema, nasal symptoms, and total IgE levels	↓ total IgE -21%; ↓ symptom severity score -35% vs. placebo	Synergistic effect of <i>Lactobacillus plantarum</i> and soy isoflavone-derived bioactives modulating Th2 immune response	Lee et al. (2017)
<i>In vitro</i>	<i>Candida albicans</i> and <i>Aspergillus niger</i> cultures	Disk diffusion and broth microdilution assays	Kefir supernatant suppressed fungal growth at low concentrations	MIC values: <i>C. albicans</i> 25% v/v; <i>A. niger</i> 30% v/v	Antifungal action via organic acids, ethanol, and exopolysaccharide-metal ion chelation	González-Orozco et al. (2023)

This table summarizes preclinical and clinical evidence on kefir's antimicrobial and antiallergic effects. Consistent across *in vitro*, animal, and human studies, kefir demonstrated broad-spectrum antibacterial and antifungal activities alongside immunoregulatory actions mitigating allergic inflammation.

colitis models, kefir administration at 100–300 mg/kg/day for 3–4 weeks alleviated intestinal inflammation and preserved mucosal architecture by enhancing IL-10 expression while reducing oxidative stress markers such as MDA and NO (Papadopoulou et al., 2024). Collectively, these preclinical findings highlight kefir's potential as a dietary immunomodulatory agent capable of mitigating chronic inflammatory processes.

Mechanistic investigations implicate the suppression of canonical inflammatory signaling pathways as a principal mode of action. Several studies report that kefir bioactives attenuate activation of NF-κB and MAPK cascades in macrophages and synovial cells, thereby limiting transcriptional programs that drive cytokine production and osteoclastogenic signaling (Chen et al., 2021; Gao et al., 2021). In addition, kefir administration modulates adaptive immune balance by reducing Th17 cell frequency and IL-17 production while promoting regulatory T-cell (Treg) responses an effect particularly relevant given that Th17/Treg imbalance is central to rheumatoid arthritis pathogenesis (Xu et al., 2021). By targeting both innate and adaptive inflammatory pathways, kefir addresses multiple upstream drivers of joint inflammation.

Evidence also suggests both direct and indirect benefits of kefir on bone remodeling. In animal studies, kefir or kefir-derived peptides decreased osteoclast activation markers and preserved bone microarchitecture in inflamed joints, indicating inhibition of bone resorption (Papadopoulou et al., 2024). Proposed mechanisms include reduced RANKL expression in inflamed tissues, attenuation of systemic inflammation that promotes osteoclastogenesis, and antioxidant effects of kefir that protect osteoblasts from oxidative stress (Medrano et al., 2020). These

multimodal effects indicate that kefir may both limit inflammatory bone loss and support reparative bone formation, although the evidence remains predominantly preclinical.

Human data on kefir and arthritic disease are limited. A few small open-label and pilot studies have examined fermented dairy or probiotic interventions for joint pain and inflammatory markers, but robust randomized controlled trials (RCTs) specifically assessing kefir in rheumatoid arthritis or osteoarthritis populations are lacking (Chen et al., 2021; Lee et al., 2021). Thus, while mechanistic and animal findings are promising, translation to clinical practice requires well-designed RCTs using standardized kefir preparations, defined dosing regimens, and validated outcome measures such as clinical scores, imaging, and biomarkers. Standardization including strain selection and quantification of kefir and peptide content will be essential to advance kefir from experimental promise to an evidence-based adjunct in arthritis management.

The studies summarized in Table 5 provide consolidated evidence that kefir exerts protective effects against arthritis progression and inflammation-driven bone loss. Preclinical models particularly collagen- and adjuvant-induced arthritis in rodents demonstrate that kefir-derived peptides and kefir polysaccharides significantly reduce clinical arthritis scores and histopathological indicators of joint destruction (Chen et al., 2021; Choi et al., 2020). These effects are associated with decreased pro-inflammatory cytokines (TNF-α, IL-6, IL-17) and restoration of anti-inflammatory mediators such as IL-10, reflecting an overall immunoregulatory shift rather than simple cytokine suppression. In addition to immune modulation, kefir positively influences bone metabolism. Kefir supplementation preserved trabecular bone microarchitecture and reduced osteoclast

TABLE 5 Preclinical and clinical evidence on anti-arthritis and bone health effects of kefir.

Study type	Model/population	Study design/intervention	Key findings	Effect size/quantitative outcomes	Proposed mechanisms	References
Animal study	Collagen-induced arthritis (CIA) rats	n = 36 rats, oral kefir peptides (200 mg/kg/day, 6 weeks)	↓ Clinical arthritis scores, ↓ paw swelling, ↓ cartilage erosion	Paw thickness ↓ 42%; arthritis score ↓ 53% vs. control	Inhibition of NF-κB and MAPK pathways; ↓ TNF-α, IL-1β, IL-6; suppression of MMP-3 and MMP-9	Chen et al. (2021)
Animal study	Adjuvant-induced arthritis (AIA) mice	n = 40 mice, kefir extract (0.5 mL/day, oral, 4 weeks)	Improved joint histopathology; ↓ systemic IL-6 and TNF-α levels	IL-6 ↓ 36%; TNF-α ↓ 41%; improved joint score -45%	Modulation of Th17/Treg balance; ↓ IL-17, ↑ IL-10; restoration of immune homeostasis	Chuang et al. (2023)
Animal study	Inflammatory bone resorption model (rat)	n = 30 rats, kefir polysaccharide (100 mg/kg/day, oral, 8 weeks)	Preserved trabecular bone microarchitecture; reduced bone resorption	Bone volume fraction ↑ 28%; trabecular number ↑ 21%	↓ RANKL expression; ↑ osteoprotegerin; antioxidant protection of osteoblasts	Medrano et al. (2020)
Animal study	Inflammatory arthritis mouse model	n = 24 mice, kefir-derived peptides (200 μg/mL, oral, 5 weeks)	↓ Osteoclast activation markers (TRAP, CTSK); improved bone mineral density	TRAP+ cells ↓ 38%; serum CTX-1 ↓ 27%	Suppression of osteoclastogenesis via RANKL-NFATc1 axis; systemic inflammation reduction	Silva et al. (2024)
Clinical pilot trial	Postmenopausal women with osteopenia	n = 50, randomized controlled trial, kefir milk (250 mL/day, 12 weeks)	Improved serum calcium and bone formation marker (osteocalcin)	Osteocalcin ↑ 22%; CTX-1 ↓ 15%; improved BMD trend (+1.8%)	Calcium bioavailability enhancement; stimulation of osteoblast activity by kefir peptides	Baars et al. (2023)

This table summarizes experimental and emerging clinical evidence on the anti-arthritic and bone-protective effects of kefir and its derived metabolites. Across animal and pilot human studies, kefir peptides and kefir polysaccharides consistently reduced pro-inflammatory cytokines (TNF-α, IL-6, IL-17), inhibited NF-κB signaling, and modulated osteoimmune pathways such as RANKL-OPG balance, suggesting a multifactorial mechanism supporting joint and bone health.

activation in inflammatory bone loss models by lowering RANKL expression and enhancing osteoblastic antioxidant defenses (Medrano et al., 2020). These results suggest a dual mechanism whereby kefir mitigates catabolic inflammation while promoting anabolic repair. Although preclinical findings are consistent, translation to human applications remains preliminary. Small pilot trials in individuals with osteoarthritis or chronic joint pain have reported reductions in systemic inflammatory markers (e.g., C-reactive protein) and modest improvements in mobility scores following kefir intake (Silva et al., 2024; Lee et al., 2021). However, heterogeneity in kefir formulations, dosing, and trial design limits comparability across studies. Future research should prioritize standardized preparations and randomized controlled trials to substantiate kefir's therapeutic potential in inflammatory joint and bone disorders.

Despite the expanding body of evidence supporting kefir's probiotic and nutraceutical potential, substantial heterogeneity persists across studies. Variations in microbial composition among kefir grains from different geographical origins, fermentation substrates, and even production batches or storage conditions complicate cross-study comparisons and hinder the establishment of standardized health claims. For instance, while *Lactobacillus kefirifaciens* and *Kluyveromyces marxianus* are frequently identified as dominant taxa, kefir samples from South America and Asia often exhibit higher proportions of *Candida* spp. or *Acetobacter* spp., reflecting environmental and artisanal influences (Leite et al., 2021; Kairey et al., 2023). Moreover, microbial viability and metabolite composition fluctuate during cold storage, altering concentrations of organic acids, kefirin, and peptides that underpin kefir's bioactivity. These findings underscore the need for harmonized analytical protocols encompassing

sequencing, culture-based validation, and standardized fermentation and storage conditions.

While preclinical evidence consistently demonstrates anti-inflammatory, antimicrobial, and immunomodulatory effects, clinical outcomes remain variable. For example, human intervention trials investigating kefir's cholesterol-lowering effects have yielded mixed results, with some reporting modest reductions in LDL cholesterol while others observed no significant changes (Rosa et al., 2017; Kondrotienė et al., 2023). Such inconsistencies likely stem from differences in sample size, intervention duration, kefir formulation, and microbial content, emphasizing the importance of standardized dosing and methodological rigor in clinical designs. Future studies should prioritize large-scale, multicenter RCTs using well-characterized kefir preparations to confirm efficacy and establish dose-response relationships for specific health endpoints.

Bioactive compounds generated during fermentation such as exopolysaccharides (e.g., kefirin), bioactive peptides, and short-chain fatty acids represent critical mediators linking kefir's microbiota to its physiological effects. However, their mechanisms of action remain incompletely elucidated. Integrative multi-omics approaches combining metabolomics, metagenomics, and host-microbe interaction models will be essential to clarify how these compounds influence immune, metabolic, and neurological pathways. The development of plant-based and non-dairy kefir alternatives further broadens its applicability, particularly for individuals with lactose intolerance or vegan diets, though these products require additional characterization regarding microbial stability, bioactivity, and sensory quality. A key remaining challenge lies in the lack of reproducibility and regulatory clarity in kefir research. Currently, there are no universally accepted

standards defining kefir as a probiotic food, resulting in inconsistent labeling, strain identification, and quality control across commercial products. These discrepancies undermine consumer confidence and scientific validation. Alignment with international probiotic guidelines such as those proposed by the FAO/WHO and the International Scientific Association for Probiotics and Prebiotics (ISAPP) is needed to ensure accurate strain documentation, validated health claims, and transparent regulatory frameworks. Collaborative efforts among microbiologists, clinicians, food technologists, and policymakers will be crucial for establishing global standards that bridge traditional artisanal knowledge with modern probiotic science.

Finally, future publications could benefit from the inclusion of diagrammatic models illustrating the relationships between kefir's microbial community, bioactive metabolites, and health outcomes. Such visual frameworks would clarify mechanistic pathways and facilitate interdisciplinary understanding of kefir's multifaceted effects, spanning from fermentation ecology to human physiology.

## 7 Conclusion

Kefir represents a unique symbiotic consortium of bacteria and yeasts that collectively generate bioactive metabolites with notable probiotic and nutraceutical potential. Evidence from *in vitro*, animal, and clinical studies indicates that kefir can enhance gut microbiota diversity, modulate immune and inflammatory responses, exert antimicrobial activity, and potentially reduce risk factors associated with metabolic and immune-mediated disorders. Despite regional variations in microbial composition, functional redundancy among kefir-associated microorganisms ensures that core health-promoting mechanisms are largely conserved across different kefir types.

However, several challenges must be addressed before kefir can be fully recognized as a standardized functional food. Current evidence remains limited by methodological heterogeneity, small sample sizes, and unclear dose–response relationships. Future research should prioritize large-scale, multicenter randomized clinical trials employing well-characterized kefir preparations and integrating multi-omics approaches to elucidate mechanistic pathways. Moreover, plant-based kefir alternatives warrant systematic evaluation of their microbial stability, sensory attributes, and health-promoting potential, particularly for lactose-intolerant and vegan consumers.

From a safety standpoint, kefir is generally considered safe for healthy individuals; nonetheless, certain metabolites such as D-lactate, ethanol, and histamine may pose risks to sensitive populations, including individuals with impaired lactate metabolism, alcohol intolerance, or histamine sensitivity. Accordingly, standardized safety assessments and strain-selection protocols should be incorporated into kefir production and clinical evaluation.

Looking ahead, the integration of kefir research within microbiome-based and personalized nutrition frameworks represents a promising frontier. By considering host genetic background, gut microbial composition, and metabolic responses, future microbiome-guided trials could enable tailored kefir interventions for specific health profiles. With continued scientific validation, kefir may evolve from a traditional fermented beverage into a globally accessible, evidence-based

probiotic food contributing to personalized health promotion and disease prevention.

## Author contributions

Kurniawan: Writing – original draft, Conceptualization, Visualization, Formal Analysis, Validation, Resources, Writing – review and editing. TM: Visualization, Resources, Funding acquisition, Project administration, Validation, Writing – review and editing, Supervision. SK: Visualization, Formal Analysis, Validation, Resources, Supervision, Writing – review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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