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*Review Article*

# Exploring Holistic Wellness: Unveiling the Probiotic Wonders of Fermented Dairy – Meet Kefir and Its Health Benefits

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## Abstract

This article explores the rich historical background and emerging health advantages of kefir, a fermented dairy product renowned for its abundant probiotics and positive impact on gut health and overall well-being. Additionally, the research delves into the microbial composition of kefir, its potential probiotic benefits, and the intricate interplay of microorganisms within the kefir grains. Examining specific *Lactobacillus* strains, the study assesses their health-promoting benefits, ranging from improved digestion and immune system support to potential contributions to mental health. By scrutinizing the probiotic microorganisms found in kefir, this paper aims to offer valuable insights to researchers, food technologists, and entrepreneurs interested in the nexus of nutrition, health, and sustainable food production.

**Keywords:** Kefir, Kefiran, Microbes, Mutualism, Probiotics

## Introduction

Kefir, a fermented dairy product, is distinguished by its unique microbial composition resulting from the symbiotic co-cultivation of lactic acid bacteria and yeasts within a matrix of polysaccharides and proteins, primarily derived from milk. Traditionally fermented at ambient temperature, this process imparts distinct sensory and nutritional attributes to kefir, making it a rich source of probiotic microorganisms, bioactive peptides, vitamins, and minerals (Marco et al., 2017).

Originating from the Caucasus Mountains and consumed for centuries, kefir has gained global recognition for its potential health benefits. These benefits include improvements in gastrointestinal health, enhanced immune function, and the modulation of lipid metabolism (Liu et al., 2006). The positive effects are primarily attributed to the live probiotic microorganisms present in kefir, which have been demonstrated to exert beneficial influences on gut microbiota and host physiology (Leite et al., 2015).

The diverse microbial community of kefir includes lactic acid bacteria like *Lactobacillus* species (e.g., *Lactobacillus kefiranofaciens*) and *Streptococcus* species, as well as various yeasts such as *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* (Garofalo et al., 2015). Working collaboratively, these microorganisms break down lactose and other milk constituents, producing lactic acid, acetic acid, ethanol, carbon dioxide, and various volatile chemicals that contribute to kefir's distinctive flavor and aroma while maintaining its original color (Figure 1) (Zamberi et al., 2016).

**Figure 1:** Kefir produced from milk (left) and kefir grain (right).

## Brief History of Kefir

Kefir, the fermented milk product, is believed to have been discovered by shepherds in the Caucasus Mountains. These shepherds stored milk in leather pouches crafted from animal hides, noticing that over time, the milk would ferment, transforming into a tangy, effervescent beverage. They named this fermented milk "kefir," possibly derived from the Turkish word "keyif," meaning "feeling good" or "pleasure," reflecting both its delightful taste and potential health advantages (Ahmed et al., 2013).

The true origins of kefir remain elusive, but it is widely believed to have been consumed in the Caucasus region for over a millennium. Kefir grains, symbiotic colonies of bacteria and yeast, were passed down as invaluable artifacts through the centuries, and the kefir-making process was kept as a closely guarded secret. It wasn't until the late 19th century that kefir gained widespread attention. In 1880, Russian scientist Ilya Ilyich Mechnikov, a later Nobel Prize winner in Physiology or Medicine, conducted research on the health benefits of kefir consumption. He proposed that kefir contributed to the longevity of Caucasus region residents and hypothesized that the lactic acid bacteria in kefir played a role in promoting health (Smith et al., 2014).

Kefir's popularity surged in the early 20th century with its introduction to Europe and later North America. The production and consumption of kefir expanded globally, and today, it stands as a popular fermented dairy product enjoyed worldwide for its distinctive flavor and potential health benefits.

## Kefir Grains

Kefir grains, resembling cauliflower, are comprised of hollow globular structures forming a polyhedral network with diameters ranging from 2.0 to 9.0 mm. The diverse microbiota residing within these grains play a significant role in metabolism, community interactions, and provide crucial health benefits, particularly in supporting the immune system. This becomes especially pertinent in preventing bacterial and viral infections, such as during the COVID-19 pandemic (Lu et al., 2014). The matrix of kefir grains consists of kefiran, proteins, microbial cell debris, and other unspecified materials.

The arrangement of microbiota on or within this structure is an ongoing subject of research. While some studies indicate that microorganisms occupy both interior and exterior surfaces of grains, variations in cultivation conditions and environmental factors contribute to diverse observations. Differences in cell sizes and chain lengths may result from various physiological stages or external stresses, such as cultivation conditions and nutrient limitations. For example, *Lactobacillus kefiranofaciens* exhibited two morphotypes, short rods (3.0 µm) and long rods (10.0 µm), colonizing outer or inner surfaces of kefir grains, underscoring the importance of careful interpretation of microscopy data in microbial community studies (Wang et al., 2018).

The reason behind the stable consortium of kefir microbiota, sustaining functionality indefinitely, remains unclear. All attempts to create kefir grains from pure starter cultures in fermentation mixtures have, so far, been unsuccessful (Kotova et al., 2016). Various hypotheses exist regarding the mechanisms behind grain formation in kefir. Initial auto- and co-aggregation of lactobacilli and yeasts, starting with self-aggregation of *L. kefiranofaciens* and *L. kazachstaniaturicensis*, are believed to be crucial. Biofilm-producing species like *L. kefiri* then attach to granule surfaces, co-aggregating with other microorganisms and milk components to form larger granules, potentially leading to kefir grains. Recent studies also emphasize the role of *Acetobacter orientalis*, indicating that LAB and AAB contribute to polysaccharide production and biofilm formation, while yeasts facilitate complex networks among the three microbes (Nejati et al., 2020).

## Chemical Composition of Kefir

Kefiran, the primary polysaccharide found in kefir grains, is a heteropolysaccharide consisting of equal proportions of glucose and galactose. The typical composition of kefir includes 89-90% moisture, 0.2% lipid, 3.0% protein, 6.0% sugar, 0.7% ash, and 1.0% each of lactic acid and alcohol. The chemical makeup of kefir is largely influenced by factors such as the type of milk used, the composition of grains or culture mixtures, additives, and the production technology employed (Arslan, 2015).

## Microbial Composition of Kefir and Kefir Grains

Kefir and kefir grains consist of a diverse symbiotic community of microbes. The composition of these microbes varies across different types of kefirs, influenced by factors such as growth conditions. Table 1 illustrates the microbial composition of some kefir varieties.

**Table 1:** Microbial Composition of kefir and kefir grains.

| Microorganisms | Source-Country | References |
|---|---|---|
| *Lactobacillus kefir, Lactobacillus kefiranofaciens, Lactobacillus paracasei, Lactobacillus plantarum, Lactococcus lactis* ssp. *lactis, Kluyveromyces marxianus, Lactobacillus parakefir, Saccharomyces cerevisiae, Saccharomyces unisporus, Leuconostoc mesenteroides, Acetobacter* sp., *Saccharomyces* sp., *Lactococcus lactis* ssp. *Lactis biovar diacetylactis, Lactococcus lactis, Lactobacillus kefiri, Lactobacillus parakefiri* | Kefir grains and beverage – Argentina | Garrote et al., 2001; Londero et al., 2012; Hamet et al., 2013; Diosma et al., 2014. |
| *Lactobacillus kefiri, Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Lactobacillus paracasei, Lactobacillus helveticus, Gluconobacter japonicus, Lactobacillus uvarum, Acetobacter syzygii, Lactobacillus satsumensis, Saccharomyces cerevisiae., Leuconostoc* sp., *Streptococcus* sp., *Acetobacter* sp., *Bifidobacterium* sp., *Halococcus* sp., *Lactobacillus amylovorus, Lactobacillus buchneri, Lactobacillus crispatus, Lactobacillus kefiranofaciens* ssp. *kefiranofaciens, Lactobacillus kefiranofaciens* ssp. *kefirgranum, Lactobacillus parakefiri* | Kefir grains – Brazil | Miguel et al., 2010; Leite et al., 2012; Zanirati et al., 2015 |
| *Lactobacillus kefiri, Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Lactococcus lactis* ssp. *cremoris, Gluconobacter frateurii, Acetobacter orientalis, Acetobacter lovaniensis, Kluyveromyces marxianus, Naumovozyma* sp., *Kazachastania khefir* | Kefir grains and beverage – Belgium | Korsak et al., 2015 |
| *Lactobacillus helveticus, Lactobacillus buchneri, Lactobacillus kefiranofaciens, Lactobacillus acidophilus, Lactobacillus helveticus, Streptococcus thermophilus, Bifidobacterium bifidum, Kluyveromyces marxianus* | Kefir grains- Turkey | Kok-Tas et al., 2012; Nalbantoglu et al., 2014 |
| *Acetobacter acetic, Enterococcus faecalis, Enterococcus durans, Lactococcus lactis* ssp. *cremoris, Leuconostoc pseudomesenteroides, Leuconostoc paramesenteroides, Lactobacillus brevis, Lactobacillus acidophilus, Saccharomyces* sp., *Brettanomyces* sp., *Candida* sp., *Saccharomycodes* sp., *Acetobacter rancens* | Kefir beverage – China | Yang et al., 2007 |
| *Lactobacillus paracasei, Lactobacillus parabuchneri, Lactobacillus casei, Lactobacillus kefiri, Lactococcus lactis, Acetobacter lovaniensis, Kluyveromyces lactis, Kazachstania aerobia, Saccharomyces cerevisiae, Lachanceame yersii* | Kefir beverage – Brazil | Magalhães et al., 2011 |

## Symbiotic Interaction between Microorganism in the Kefir

### Yeast-bacteria interaction

The interaction between yeast and bacteria plays a pivotal role in kefir and various fermented foods. This collaboration encompasses:

**Assimilation of lactic acid:** Yeasts that assimilate lactic acid play a crucial role in facilitating the thriving of lactobacilli. This process helps prevent the accumulation of acid, ultimately leading to an increased production of kefiran (Katakura et al., 2010).

**$CO_2$ production / $O_2$ removal:** Yeasts, such as *S. cerevisiae*, contribute to creating a favorable environment for the growth of *Lactobacillus* spp. by generating carbon dioxide and reducing oxygen levels (Suharja et al., 2014).

**Nutrient provision:** The cooperation between yeast and bacteria involves trophic interactions and the exchange of metabolites. Yeast plays a vital role in supplying essential nutrients, such as vitamins and amino acids, to bacteria. This nutrient provision supports bacterial growth, especially when resources are limited (Ponomarova et al., 2017).

### Bacteria-bacteria interaction

Interactions among bacteria in food have received less exploration compared to yeast-bacteria interactions. Research focusing on yogurt bacteria, specifically *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, has unveiled protocooperative interactions. In the case of kefir bacterial species (e.g., *L. kefiranofaciens*, *L. kefiri*, *Lactococcus lactis*, *Acetobacter fabarum*, *Leuconostoc mesenteroides*), investigations have revealed dynamics of competition. *L. kefiranofaciens* was observed to suppress *L. kefiri*, promote *L. mesenteroides*, and have no impact on *L. lactis* and *A. fabarum*. These findings contribute to a broader understanding of microbiota interactions in the context of kefir (Ponomarova et al., 2017).

### Yeast-yeast interaction

Exploration of Quorum Sensing (QS) communication among yeasts is relatively limited. Studies conducted in various ecosystems, such as wine and sourdough, have illuminated the impact of environmental factors, including nitrogen content, cell density, and ethanol levels, on the production of QS-related molecules by *S. cerevisiae*. For example, the secretion of aromatic alcohols is most pronounced when ammonium sulfate levels are below 50 µM, diminishing above 500 µM. These aromatic molecules, known for their antioxidant and antimicrobial properties, play a role in quality control. Additionally, certain strains of *S. cerevisiae* release peptides that inhibit non-*Saccharomyces* strains, and this trait is dependent on the specific strain. Given the significance of these yeasts in kefir, comprehending their interactions is crucial for ensuring the quality and functionality of kefir (Avbelj et al., 2016).

## Importance of Kefir

Kefir is documented to have positive effects on various disorders, as illustrated in Figure 2, highlighting its diverse health benefits. Kefir exhibits anticancer, antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities. Additionally, it has the potential to offer benefits for gastrointestinal tract infections and other health-related conditions.

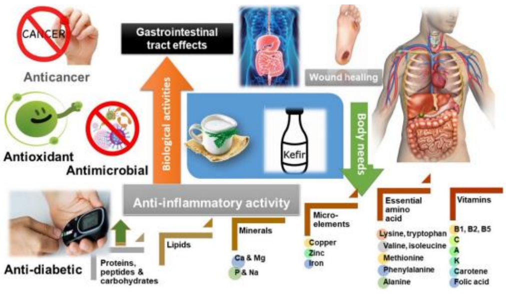

**Figure 2:** Kefir's biological characteristics, nutritional value, and macro- and micronutrient content (source: Farag et al., 2020).

## Potential Health Benefits

In a comprehensive exploration of probiotic strains, Kakisu et al. (2013) delved into the impact of *Lactobacillus plantarum* CIDCA 83114 on *S. flexneri* invasion in Hep-2 cells. The study revealed a significant reduction in pathogen invasion by 5% during co-incubation, while pre-incubation demonstrated superior protective effects. Notably, all probiotic strains, especially *L. plantarum* CIDCA 83114, exhibited enhanced performance after pre-incubation. Dose testing of *L. plantarum* CIDCA 83114 indicated a dose-dependent effect on *S.*

*flexneri*, with internalization rates of 3.7% at 109 CFU/mL and 12.7% at 108 CFU/mL. However, this dose dependence was less pronounced in the presence of *S. sonnei*.

Examining the interaction with *E. coli*, the study found that pre-treating Hep-2 cells with 108 CFU of Lb. plantarum per well effectively inhibited E. coli adherence, particularly when the *E. coli* concentration was below 106 per well. At higher concentrations (107 and 108), the difference was less substantial. The researchers proposed that optimal protection required at least one Lactobacillus cell for every 100 pathogen cells (Hugo et al., 2008).

Shifting focus to *L. kefiranofaciens* M1, the bacterium demonstrated promise in reducing allergies by influencing Th1 and Th2 responses. A preliminary study indicated that mouse immune cells exposed to bacterial cells produced more IL-6, while the supernatant alone elevated cytokine levels. Another investigation with a different cell line revealed that bacterial cells induced larger amounts of cytokines. Despite challenges in cell-line analysis, *L. kefiranofaciens* M1 affected cytokine production. IL-6 generation from bacterial cells was specifically inhibited when the TLR-2 receptor was disabled, indicating a different mechanism. However, this did not diminish the amount of IL-6 generated by the supernatant, suggesting the involvement of another receptor (Hong et al., 2009).

Chen and Lee et al. (2013) explored the positive impact of treating mice with *L. kefiranofaciens* M1 at 2 × 108 CFU/mL for seven days on enterohemorrhagic *E. coli* (EHEC) infection. The pretreatment improved histology scores, indicating enhanced intestinal health, prevented a decrease in food intake, and significantly reduced intestinal hemorrhage. This treatment also decreased renal glomeruli and kidney interstitial tissue congestion, particularly severe in untreated mice. However, heat treatment diminished the efficacy of the *Lactobacillus* strain. Additionally, *E. coli* was less likely to reach the liver and spleen after pre-treatment with *L. kefiranofaciens*, as evidenced by the absence of *E. coli* in the blood of pre-treated mice compared to considerable concentrations in untreated controls.

In a follow-up trial assessing the length and amount of treatment, *L. kefiranofaciens* M1 exhibited effects on the allergic airway response in mice. Over 32 days (Course A), mice receiving a daily dose of 108 CFU/mL showed a significant decrease in inflammatory markers (IL-4, IL-13, IL-6, IL-1β, CCL20, TNF-α) and an increase in T-regulatory cells. The same dosage was given to a third group for the final three days (Course C) and a second group (Course B) for the first fourteen days. Although Courses B and C exhibited less IL-6 and TNF-α, the advantages observed in Course B did not persist, emphasizing the necessity of ongoing microbial treatment for lasting results. Course C highlighted that the observed benefits could not be achieved through short-term intake (Hong et al., 2011).

Chen & Chen et al. (2013) observed increased ileac villi length, crypt depth, and greater goblet cell levels in the stomach of mice continually administered *L. kefiranofaciens* M1. In comparison to untreated mice, the histological score of treated mice significantly decreased from 9 to 2, suggesting a beneficial therapeutic action against DSS-induced colitis. *L. kefiranofaciens* also improved the immunological response to Toll-like receptor (TLR) agonists—lipopolysaccharide (LPS) and R848. Treated animals exhibited higher levels of IFN-γ and IL-12 compared to germ-free (GF) or singly inoculated mice, suggesting a strengthened immunological response.

Jeong et al. (2017) explored the effects of *L. kefiranofaciens* DN1 given orally to mice for two weeks. The treatment improved the weight and water content of feces, indicating potential benefits for intestinal motility and constipation. Additionally, it adjusted the composition of gut bacteria by increasing beneficial bacteria like *Firmicutes*, *Bacteroidetes*, *Lactobacillus*, and *Prevotella*, while decreasing harmful bacteria like Proteobacteria, Enterobacteriaceae, and *Clostridium*. The production of a new exopolysaccharide by *L. kefiranofaciens* DN1 suggested potential benefits for gut health. In a second investigation, the same team found that the *L. kefiri* strain was a notable probiotic candidate, exhibiting encouraging probiotic traits by adhering to colon and small intestine mucus. Moreover, the *L. kefiri* strain demonstrated the ability to inhibit the growth of several pathogens, including *Salmonella enteritidis, P. aeruginosa, S. flexneri, B. cereus, L. monocytogenes, S. aureus,* and *E. faecalis*. This demonstrates that the *L. kefiri* strain may inhibit the growth of many harmful bacteria, supporting its probiotic properties (Carasie et al., 2014).

Kefir, renowned for its health benefits attributed to Lactobacilli, holds promise in various aspects such as immune system modulation, potential cancer and allergy risk reduction, oxidative stress mitigation, and cholesterol level control, along with potential aid in managing diabetes. Despite these potential advantages, the specific mechanisms and their applicability to humans remain unclear, necessitating further research, especially into the genetic factors responsible for health benefits. A comparative analysis of well-characterized kefir strains' genomes could offer valuable insights.

While studying individual strains has its limitations, the variation in effects between strains underscores the need for further investigation. Multi-species kefir models exhibit synergistic benefits, but understanding the primary contributors can be elucidated through single-strain models. The dairy industry's pursuit of artificial kefir grains underscores the potential application of such knowledge in selecting strains for specific functional properties. As personalized medicine gains prominence, such studies are poised to become increasingly significant (Slattery et al., 2019).

Examining the anti-inflammatory properties, studies involving rats' paws and cotton pellets revealed that kefir and kefiran treatments significantly reduced granuloma tissue and paw edema, akin to anti-inflammatory medications. The potential suppression of inflammatory mediators is suggested as a mechanism for these benefits. Recommendations for further research on their processes and active components highlight kefir and kefiran as potential natural treatments for inflammation (Rodrigues et al., 2005).

Huseini et al. (2012) delved into kefir's therapeutic properties, evaluating its wound healing and antimicrobial effects on *Pseudomonas aeruginosa*-infected burn injuries in rats. The 96-hour kefir gel and kefir grains 96-hour gel emerged as particularly effective in terms of antimicrobial and wound healing properties. Longer fermentation times, especially in kefir gel therapy, were deemed to improve clinical outcomes in thermal injuries.

Kurniati et al. (2020) conducted a study comparing lactic acid, protein, fat, and carbohydrate levels in curd kefir and colostrum kefir. Significant variations were observed, with good kefir exhibiting high lactic acid, proteins, and carbohydrates but low fat. Starter concentration and fermentation time notably influenced the content in both kefir types. Another investigation explored kefir's impact on gut microbiota and metabolic syndrome (MetS). While the study revealed no significant changes in body weight, lipid profile, glucose, or inflammatory markers, kefir ingestion led to a substantial reduction in fasting insulin, HOMA-IR, TNF-α, IFN-γ, and blood pressure, suggesting positive impacts on various MetS indicators, notably in gut microbiota (Belikci-Koyu et al., 2019). The resilience of lactic acid bacteria in kefir varieties to simulated digestion further emphasizes the potential benefits, with kefir grains exhibiting the highest probiotic potential (Ince et al., 2023).

Tu et al. (2020) investigated how kefir peptides derived from dairy milk proteins, produced by kefir probiotic bacteria, affected the gut microbiome in ovariectomized mice, preventing menopausal osteoporosis while having minimal impact on the gut microbiome.

## Conclusion

This analysis underscores kefir's rich microbial composition, historical roots, and diverse health benefits. Recognized for enhancing digestive health, boosting immunity, and influencing fat metabolism since its introduction from the Caucasus Mountains, kefir, particularly with strains like *Lactobacillus kefiranofaciens*, holds promise for the digestive and nervous systems. Emphasizing kefir's role in health management, the study envisions potential applications in personalized medicine and sustainable food production. Despite acknowledging the need for further research, kefir captivates academia, food technologists, and health enthusiasts as a complex and advantageous agent contributing to overall well-being.

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