Invited review: Milk kefir microbiota—Direct and indirect antimicrobial effects

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ABSTRACT

Kefir is a fermented dairy product with well recognized probiotic properties. Recently, consumer interest in fermented products with probiotic microorganisms has increased due to the accumulating evidence of the effects of kefir microorganisms on the modulation of gut microbiota and their antimicrobial activity. Although the health properties of kefir have been reviewed in other works, the present review addresses the antimicrobial effects of kefir microbiota and associated compounds. The antimicrobial activity of kefir microorganisms could derive from different mechanisms. The microorganisms' capacity to adhere to the intestinal epithelium, preventing the adhesion of pathogens, and their immunomodulation properties are among the mechanisms suggested. Bacteria and yeast isolated from kefir have been shown to have in vivo and in vitro antimicrobial activity against enteropathogenic bacteria and spoilage fungi. However, most reports have focused their approach on single-strain antimicrobial properties; evaluation of antimicrobial activity of cocultures of kefir microbiota and their potential mechanisms of action has been neglected. Kefir microbiota and associated compounds have shown promising antimicrobial effects; however, more research needs to be done to discern the mechanisms of action.

Key words: kefir microbiota, antimicrobial effect, consortia, potential probiotic, kefir

KEFIR GENERALITIES

Origin and Consumption Trends

Milk kefir is an acidic, slightly alcoholic fermented product widely consumed in eastern European and central Asian countries. It originated in the mountains of the Caucasus in the intersection between Europe and Asia thousands of years ago. Since then, kefir has been consumed mainly for its flavor profile and its potential to improve human health. The word derives from the

Slavic word *keif*, which means “well-being,” and kefir is traditionally made through the fermentation of milk with kefir grains as starter culture (Nielsen et al., 2014; Arslan, 2015; Rosa et al., 2017). Kefir grains have a cauliflower floret shape; they are elastic and irregular, with a creamy color, slimy texture, and variable size. Kefir grains are formed by a complex symbiotic microbial ecosystem comprised of lactic acid bacteria (LAB), acetic acid bacteria, and yeast embedded in an exopolysaccharide matrix known as kefir and composed in almost equal ratio of D-glucose and D-galactose (glucogalactan), which protects the grain microbiota from adverse environmental conditions and constitutes between 24 and 25% of the dry weight of the kefir grain (Rosa et al., 2017; Shen et al., 2018; Van Wyk, 2019). According to Wang et al. (2012), kefir grain formation starts with the self-aggregation of *Lactobacillus kefiranoformans* and *Saccharomyces* spp. to form small granules. Later, the biofilm producer *Lentilactobacillus kefir* begins to attach to the surface of granules and co-aggregates with other organisms and components in the milk to form the grains.

Kefir has been consumed for centuries and, due to its attributed health benefits, more consumers are incorporating kefir in their diets as a potential probiotic product; its rising popularity is also due to the increasing demand for products with low lactose content (lactose is broken down by microorganisms during the fermentation process; Egea et al., 2020). Kefir has recently been referred to as one of the “9 food trends to watch for in 2021” by the Institute of Food Technologist (IFT, 2021). Additionally, according to Hamida et al. (2021), the COVID-19 pandemic caused a consumer increase in health consciousness, and consumers are looking for products to enhance the immune system. In 2020 alone, global sales of functional foods and beverages associated with health benefits were estimated to reach US\$377.8 billion (Azizi et al., 2021). The market size of kefir products is expected to increase by US\$456 million from 2021 to 2025, with a compound annual growth rate of 4.37% in 2021 (Technavio.com, 2021). Despite the numerous reviews on kefir's general health benefits, there has been no integral review regarding the antimicrobial effects of kefir microbiota and the biological mechanisms behind this probiotic property.

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In this work, the potential antimicrobial effect of kefir and kefir grains' microbiota, as assessed by in vitro and in vivo techniques, are reviewed.

Preparation and Chemical Composition of Milk Kefir Beverage

Dairy kefir is prepared at the artisanal level by adding kefir grains (5–10%) as the starter culture to whole, semi-skimmed, or skimmed pasteurized goat, sheep, camel, buffalo, or—most commonly—cow milk. Fermentation takes place at 20 to 25°C for approximately 24 to 72 h. Lactose from milk is degraded during fermentation to lactic acid mainly by the β -galactosidase enzyme produced by LAB present in the grains, which causes a drop in the pH (4.0–4.6). CO₂ and ethanol (0.5–2.0%) produced by yeast and heterofermentative LAB, and aroma-flavor compounds such as aldehydes, diacetyl, acetic acid, and propionic acid are also generated during fermentation. After incubation, kefir grains are separated from the fermented product (Guzel-Seydim et al., 2011; Rosa et al., 2017; Azizkhani et al., 2021). However, some studies have suggested that artisanal kefir consumption may represent health risks for consumers due to kefir contamination associated with poor hygiene practices (Azizi et al., 2021). The commercial production of milk kefir starts with either milk fermentation with pure freeze-dried commercial kefir culture or, at a larger scale, with the “Russian back slopping method,” a serial fermentation process that begins with the kefir produced from grains as a natural starter culture, followed by subsequent fermentations with the fermented milk obtained from the grains as a starter (Garofalo et al., 2020). Producing kefir from commercial starter cultures allows for standardization of the process, which is not achieved by fermentation with kefir grains. However, the health-related properties are generally attributed to traditional kefir fermented by the complex microbiota present in the kefir grain matrix (Leite et al., 2013; Demir, 2020).

The chemical composition of the fermented product varies depending on the milk source, breed type, feeding conditions, fermentation conditions, and origin of the grains (Satir and Guzel-Seydim, 2016). For kefir produced from kefir grains and cow milk, the approximate composition is 90% moisture, 6% carbohydrates, 3.5% fat, 3% protein, and 0.7% minerals (Rosa et al., 2017). Kefir is also rich in vitamins A, C, and K, thiamine, riboflavin, cobalamin, and carotenes (Nogay, 2019).

The *Codex Alimentarius* (Codex Alimentarius Commission, 2003) establishes that fermented milk kefir obtained from kefir grains should contain at least 2.7% protein, 0.6% lactic acid, and less than 10% fat. The percentage of alcohol is not defined; the total number

of microorganisms in the product should be at least 10⁷ cfu/mL, and the yeast number not less than 10⁴ cfu/mL. However, kefir companies' claims of numbers of colony-forming units and taxa of microorganisms present in their products are not regulated by the United States Food and Drug Administration, since microorganisms added to products are considered dietary supplements by the FDA Code of Federal Regulations 21CFR131.111 (CFR, 2022). It has been reported that these claims are inaccurate in a significant percent of the cases and therefore are misleading to consumers (Metras et al., 2021).

Kefir Microbiota

Kefir has been associated with a myriad of health benefits, which include antihypertensive, hypocholesterolemic, antidiabetic, anticarcinogenic, anti-inflammatory, antioxidative, antiallergic, and immunomodulatory properties, and antiviral and antimicrobial activities (Ahmed et al., 2013; Hamida et al., 2021). These benefits are attributed to the variety of bioactive compounds generated during fermentation, including organic acids, bioactive peptides, bacteriocins, and exopolysaccharides, and to the very distinctive microbiota that could act either independently or in consortium (Rosa et al., 2017; Figure 1).

The microbial composition of kefir is considered a symbiotic community and varies dramatically depending on the milk source, fat content, origin of the kefir grains, fermentation parameters, and other factors (Farag et al., 2020). This variability has become problematic in the pursuit of a “generic” description of microorganisms present in kefir. Traditional culture-based methods to study kefir microbiota have been gradually shifted toward targeted and untargeted metagenomic approaches. Different works have investigated microbial species in kefir and kefir grains using high-throughput sequence-based analysis (Nalbantoglu et al., 2014; Korsak et al., 2015; Walsh et al., 2016). The microbial composition of kefir grain consists of 65 to 90% of bacteria of the *Lactobacillaceae* family, with members of *Lactococcus*, *Streptococcus*, *Leuconostoc*, and *Acetobacter* genera, and yeasts completing the remaining portion (Bourrie et al., 2016; Bengoa et al., 2019). Predominant bacteria species, updated with the new nomenclature, comprise *Lacticaseibacillus paracasei*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactiplantibacillus plantarum*, *Lactobacillus helveticus*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *Lentilactobacillus kefir*, *Lentilactobacillus parakefir*, and *Lactobacillus kefiranofaciens* as the predominant species in grains from different geographical regions (Italy, Korea, Germany, Turkey, the UK, Brazil, Argentina,

Belgium, and others; Nalbantoglu et al., 2014; Bengoa et al., 2019). These strains account for the majority of the bacterial population in grain but only 20% in the final fermented beverage; the remaining 80% consists of *L. kefir*, suggesting a major compositional change during milk fermentation (Shen et al., 2018; Blasche et al., 2021). During fermentation, kefir pH ranges from 3.0 to 5.0 due to high concentrations of organic acids; given this acidic pH, it is clear that kefir provides an environment that enhances the dominance of acid-resistant species (Kim et al., 2019). Dobson et al. (2011) also detected *Pseudomonadaceae*, *Enterobacteriaceae*, and *Clostridiaceae* bacteria family members.

Yeasts identified in kefir include members of *Candida*, *Debaryomyces*, *Kazachstania*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Torulaspora*, and other species. (Rosa et al., 2017; Farag et al., 2020). The most abundant yeast genera vary among reports. Korsak et al. (2015) reported *Kluyveromyces marxianus* and *Kazachstania* spp. as the most abundant yeasts, whereas Marsh et al. (2013) reported that the most abundant species were *Kazachstania barnettii* and *Kazachstania unispora*. Both works used a sequence-based analysis.

Extensive reviews on kefir beverage and grain microbiota composition can be found elsewhere (Nielsen et al., 2014; Arslan, 2015; Rosa et al., 2017; Bengoa et al., 2019). Figure 2 shows a phylogenetic tree of bacteria and yeast species reported to date in kefir and kefir grains. Kefir consortia potentially contain strains with widely accepted probiotic status, and genes encoding for probiotic functionalities have also been detected

in the kefir microbiota (Walsh et al., 2016; Slattery et al., 2019). Strong evidence suggests that the kefir microbiota depends on the geographical area of origin, fermentation conditions, and especially the milk source (Farag et al., 2020).

KEFIR AS A PROBIOTIC PRODUCT

Microorganisms advertised as probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (FAO and WHO, 2006). Probiotics can influence human health through different mechanisms of action. The inhibition of pathogenic bacteria, protozoa, and viruses has been one of the main traits of kefir microbiota; in some cases kefir has a potency similar to that conferred by conventional antibiotics in vitro (Azizi et al., 2021; Hamida et al., 2021). The antimicrobial mechanisms of kefir microbiota include modulation of the immune system, adhesion to the intestinal epithelium, stimulation of gut barrier function, toxin inactivation, competition for nutrients and adhesion sites, and secretion of antimicrobial substances (Collado et al., 2008; Monteagudo-Mera et al., 2019).

The consumption of kefir as a probiotic food presents some disadvantages. First, due to its variable nature, kefir defies standardization. As noted before, no legal standard currently exists for commercial kefir, which means that the amounts and characteristics of microorganisms provided by a product are unknown. Second, consumers may not be willing to consume kefir

Figure 1. Graphical depiction of the kefir grain and kefir product composition, health benefits, microbiota, and their antimicrobial properties.

Figure 2. Phylogenetic tree of bacteria and yeast species reported in kefir grains and kefir based on 16S rDNA (bacteria) and 18S rDNA (yeast) gene sequences. The phylogenetic tree was constructed using the maximum likelihood method in MEGA X software (Penn State University); maximum likelihood trees were constructed using the Kimura 2 parameter model. Bootstrapping was carried out with 1,000 replicates. The phylogenetic tree was edited using the online tool iTOL (<https://itol.embl.de/>).

as a probiotic food, since it has been reported that vinegary, yeasty, and sour tastes and flavors, along with the viscous texture of kefir, are unpleasant to consumers (Leite et al., 2013; Walsh et al., 2016). Additionally, some in vivo trials have reported that the daily volumes of kefir required for patients to ingest to observe a particular effective result are large and thus impractical for patients to ingest (Bakken, 2014). Therefore, other ways to deliver viable microorganisms with proven health-promoting properties are required, and the kefir-related microorganisms with potential as probiotics should be tested to provide evidence of their beneficial effects (Nielsen et al., 2014).

Adhesion Properties of Kefir Microbiota

Adhesion of probiotics to the intestinal epithelium can trigger antagonism to pathogens both directly and indirectly. Direct antagonism takes place by steric hindrance, by competitive exclusion for binding sites, and

by temporary colonization, where bioactive compounds produced by the microorganisms (organic acids, bacteriocins, and bioactive peptides) display an effect. Indirect antagonism occurs by modulation of the immune system, which induces pro- and anti-inflammatory signaling pathways (Wang et al., 2018; Monteagudo-Mera et al., 2019). Furthermore, the antimicrobial effect of probiotics could also be due to the co-aggregation of cells of different species, which bind pathogens into co-aggregates, thus preventing the growth and biofilm formation of pathogens. Auto-aggregation is also an antimicrobial mechanism of probiotics, since cell-barrier formation could prevent the colonization of pathogens (Collado et al., 2008).

Bacterial adhesion could be driven initially by non-specific hydrophobic interactions, followed by a specific adhesion through cell wall components such as cell wall-anchored proteinases, adhesins, fibronectin-binding proteins, collagen-binding proteins, surface-layer proteins (SLP), and mucus-binding proteins (Monteagudo-Mera

et al., 2019; Qu et al., 2021). The SLP are extracellular proteins that could act as structural components, virulence factors, or adhesion promoters. They can be found in members of the *Lactobacillaceae* family, but not in all species (Kim et al., 2019). The SLP have an important role in adhesion. It has been observed that with a chemical treatment that eliminates the proteinaceous cell envelope structure formed by SLP, adhesion to intestinal cells can be reduced (Monteagudo-Mera et al., 2019). Golowczyc et al. (2007) reported that the culture supernatants of *L. kefir* strains isolated from kefir grains contain significant amounts of SLP, and a *Salmonella* strain subjected to this supernatant showed a significant decrease in association and invasion to Caco-2/TC-7 cells. This latter finding suggests a protective role of these proteins.

The intestinal epithelium comprises a mucous layer that contains glycolipids and glycoproteins (mucins) that protect intestinal cells from mechanical damage. Together with antimicrobial peptides, antimicrobial molecules such as lysozyme and commensal microbiota represent the first line of defense against the colonization of pathogens (Liévin-Le Moal and Servin, 2006). Mucins are secreted by the goblet cells and are highly glycosylated proteins that secrete mucus through oligomerization; the glycan moieties act as ligands for bacterial receptors, and it has been reported that changes in the glycosylation pattern of these molecules could cause dysbiosis and inflammation, and promote bacterial infections (Monteagudo-Mera et al., 2019; Qu et al., 2021).

The membrane-bound mucins characterized by the presence of the Muc domain (Pro/Thr/Ser) belong to an ever-increasing group of type I membrane-anchored proteins. Based on their structure and localization at the cell surface, they are thought to act in cell-cell and cell-matrix interactions, and in cell signaling pathways (Brown and Hollingsworth, 2013). Muc domains can be found in many bacteria genera and fungi (Valle Arevalo and Nobile, 2020) but are almost exclusively found in *Firmicutes* and specifically LAB isolated from the gastrointestinal tract. Mucus-binding proteins confer bacteria of the *Lactobacillaceae* family with a strong affinity toward intestinal epithelium mucins, limiting the free access of pathogens (Chatterjee et al., 2018). Adhesion of the kefir microbiota to the intestinal epithelium has been described in vitro by cell lines and in vivo by animal models. Epithelial cell lines such as Caco-2 and HT29 have been helpful in identifying the adhesion mechanisms of probiotic microorganisms. Regarding microorganisms isolated from kefir, several reports have studied the in vitro adhesion of individual strains isolated from either kefir or kefir grains.

Lactiplantibacillus plantarum Lp27 isolated from Tibetan kefir grains exhibited an adhesion ratio of $9.5 \pm 2.5\%$ on Caco-2 cells and also was able to reduce cholesterol absorption in vitro and in vivo by inhibiting gene expression of Niemann-Pick C1-like 1, a key protein in cholesterol absorption in hypercholesterolemic rats (Huang et al., 2013). Bengoa et al. (2018) demonstrated that the gastrointestinal passage confers structural changes to the bacteria that allow them to significantly increase the adhesion to Caco-2/TC-7 cells of *L. paracasei* CIDCA 83123–83124 strains isolated from kefir. *Lacticaseibacillus paracasei* ssp. *paracasei* isolated from kefir grains exhibited desirable adhesion properties on the HT-29 cell line, similar to that of the reference strain *Lacticaseibacillus casei* ATCC 393 (Plessas et al., 2020). Golowczyc et al. (2008) showed that *L. plantarum* strains isolated from kefir had adhesion to Caco-2 cells, with the highest adhesion (10.5%) observed with the isolate CIDCA 8337. Finally, Toscano et al. (2017) administered *L. kefir* LKF01 isolated from water kefir grains as an oral probiotic to 20 Italian volunteers; their results showed that the microorganism was present in the feces of the subjects one month after treatment termination. The authors reported this result as evidence of its ability to adhere and colonize the gastrointestinal tract.

Regarding yeast isolated from kefir, de Lima et al. (2017) reported that *Saccharomyces cerevisiae* strains isolated from kefir significantly adhere to mice ileal epithelial cells. *Kluyveromyces marxianus* CIDCA 8154 and *S. cerevisiae* CIDCA 8112 have been isolated from kefir grains and tested for adhesion on Caco-2/TC7 cells, yet, regardless of the concentration of cells added, the percent adhesion for both yeasts was not significant (Diosma et al., 2014). However, the adhesion capability of yeast as a probiotic criterion is still unclear, as other reports have suggested that yeast can modulate the immune system to reduce inflammation and also antagonize enteropathogenesis (Diosma et al., 2014). Tasteyre et al. (2002) reported that the non-adhesive strain *Saccharomyces boulardii* inhibited the adhesion of *Clostridium difficile* to Vero green monkey kidney cells by means of proteolytic activity and steric hindrance. Therefore, the authors concluded that nonadhesive yeast can still show probiotic properties in vitro. Of interest are the results reported by Xie et al. (2012), where *L. paracasei* H9 isolated from kefir grains increased the aggregation ability when co-cultivated with kefir grain's yeast *S. cerevisiae* at pH 7.2 in a Caco-2 cell model.

Although in vitro studies are a helpful tool to identify adhesion mechanisms and potential probiotic strains, the extrapolation to human gastrointestinal tract

conditions is not direct, since other mechanisms take place under in vivo conditions, including peristaltic movements, competition with natural microbiota, and the action of the host immune system (Youhanna and Lauschke, 2021). Therefore, in vivo experiments are required to verify adhesiveness and its associated mechanisms.

Immunomodulation Properties

The immunomodulatory properties of kefir can come either from bioactive compounds produced during fermentation or directly from the kefir microbiota. Inflammation is a state in which the immune system responds through a cascade of reactions and signals as a result of exposure of tissues and organs to various harmful stimuli, including microbial pathogens, injuries, or toxic cellular components (Turner et al., 2014; Zeng et al., 2017).

The inflammatory response is characterized by coordinate activation of various signaling pathways that regulate expression of pro- and anti-inflammatory cytokines such as interleukins and tumor necrosis factor- α (TNF α) in resident tissue cells and leukocytes. Together, IL-1 and TNF α represent the most characteristic pro-inflammatory cytokines that are rapidly released upon tissue injury or microbial infection. In turn these activate the nuclear factor (NF)- κ B pathway that mediates the expression of other proinflammatory genes encoding cytokines, chemokines, and adhesion molecules (Lawrence, 2009). Pathogenic colonization and attachment to intestinal epithelium trigger a strong defense response (i.e., release of cytokines and chemokines that trigger the recruitment of leukocytes and initiate the attraction of immune cells). However, the intestinal epithelium tolerates bacterial colonization by members of commensal microbiota (Liévin-Le Moal and Servin, 2006). The theory is that probiotic microorganisms restore the natural microbiota of the intestine, therefore causing a positive immunomodulation and a balance in the production of proinflammatory and anti-inflammatory cytokines (Carasi et al., 2015).

Chen et al. (2012) reported that *L. kefiranofaciens* M1 could strengthen the epithelial barrier function by increasing the transepithelial electrical resistance and significantly upregulated the level of a chemokine involved in epithelial repair in a Caco-2 cells model. Additionally, in vivo results from a mouse model showed that this strain could ameliorate induced colitis and decrease the production of proinflammatory cytokines as well as increasing the anti-inflammatory cytokine IL-10.

In vivo studies on mouse model showed that an *L. plantarum* strain isolated from kefir protected against

intestinal infection by *Yersinia enterocolitica* due to a defensive inflammatory status and an increase in the production of secreted IgA that is essential for optimal mucosal defense (De Montijo-Prieto et al., 2015). Carasi et al. (2015) showed an anti-inflammatory effect of *L. kefir* CIDCA 8348 isolated from kefir when administered daily to mice for 21 d by upregulation of anti-inflammatory cytokines [IFN- γ , granulocyte macrophage-colony stimulating factor (GM-CSF), and IL-1 β] and mucin genes in the ileum, whereas in the colon, proinflammatory mediators and cytokines (IL-6 and GM-CSF) were downregulated. Additionally, Romanin et al. (2010) reported that *K. marxianus* and *S. cerevisiae* yeast strains isolated from kefir downregulate NF- κ B signaling in Caco-2 cells, as well as downregulating the expression of other pro-inflammatory chemokines. In an in vivo *Caenorhabditis elegans* nematode model, kefir yeast *K. marxianus* CIDCA 8154 exhibited protection from oxidative stress and decreased intestinal inflammation (Romanin et al., 2016).

In addition to the direct immunomodulatory effects of the microbiota, microorganisms produce native proteases during fermentation that can hydrolyze milk proteins and produce peptides relevant for flavor development. Some of these peptides have shown antimicrobial, antihypertensive, and immunomodulatory properties (Biadala et al., 2020). In this sense, Dallas et al. (2016) found 25 peptides in kefir with potential biological activity, including antihypertensive, antimicrobial, immunomodulatory, opioid, and anti-oxidative functions, that are possibly the result of extracellular proteases in *L. acidophilus* and *L. helveticus* (casecidin-17; β -casein f.63–68 and f.192–209) with previously reported immunomodulatory properties. Also, recently an extracellular vesicle (lipid bilayer-enclosed particle) produced by kefir-derived *L. kefiranofaciens* ssp. *kefirgranum* PRCC-1301 showed downregulation in the expression of proinflammatory cytokines in Caco-2 cells alongside an enhancement of intestinal barrier function (Kang et al., 2020).

ANTIMICROBIAL EFFECTS

Antimicrobial Properties

Bacteriostatic as well as bactericidal effects have been shown against gram-positive and gram-negative bacterial pathogens and spoilage fungi (John and Deeseenthum, 2015; Rosa et al., 2017; Van Wyk, 2019). The antimicrobial activity of kefir could be attributable to a great number of factors, starting with the competition for nutrients and the production of antimicrobial compounds during carbohydrate metabolism of LAB, such as proteolytic enzymes, organic acids, CO₂, bacte-

riocins, bioactive peptides, hydrogen peroxide, and so on (Guzel-Seydim et al., 2011; Biadala et al., 2020). Furthermore, the reduction in the pH of kefir caused by the accumulation of organic acids results in a broad-spectrum inhibitory activity against gram-positive and gram-negative bacteria (Shen et al., 2018).

Nevertheless, it is difficult to attribute the inhibitory effects to only one of the compounds produced by the kefir microbiota. Additionally, different carbon source concentrations and different fermentation times of kefir grains influence the inhibition of pathogenic microorganisms, as reported by Silva et al. (2009). Furthermore, different inhibition compounds could be present in each state of the fermentation, as reported by Kim et al. (2016); in that work, the authors tested the antibacterial effects of kefir from different sources at different fermentation times against foodborne pathogens and spoilage bacteria. The authors reported antibacterial activity against *Staphylococcus aureus* after 48 h, and *Cronobacter sakazakii* and *Salmonella* Enteritidis at 36 and 72 h of fermentation, but not at 48 h. Therefore, considering that kefir supernatants contain different inhibitory compounds, the authors postulated that these compounds interact with each other to enhance or antagonize their antibacterial effects. An example of this mechanism would be the inactivation of bacteriocins by organic acids or enzymatic degradation during fermentation.

The antimicrobial activity of individual strains isolated from kefir has been extensively studied in vitro. Kakisu et al. (2013) showed the potential inhibitory properties of *L. plantarum* CIDCA 83114 against disease-producing species of *Shigella* (*Shigella flexneri* and *Shigella sonnei*) in the Hep-2 cell line. Three LAB present in spent culture supernatants of kefir showed inhibition of *Helicobacter pylori*, a bacterium that has been associated with chronic gastritis, ulcers, and gastric cancer (Lin et al., 2011). Santos et al. (2003) evaluated the antimicrobial activity of 58 strains of the *Lactobacillaceae* family isolated from kefir. Approximately 75% of the strains showed antimicrobial activity against enterohemorrhagic strain *Escherichia coli* and *Y. enterocolitica*; among them *L. acidophilus* CYC 10050 and CYC 10051 and *L. kefiranofaciens* CYC 10058 strains inhibited *E. coli* CECT 4076, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Shigella flexneri*, and *Y. enterocolitica*. Also, Zanirati et al. (2015) isolated different strains from Brazilian grains and found that *L. casei* 17U, *L. kefiranofaciens* 8U, and *Lactobacillus diolivorans* 1Z antagonized all the pathogens tested (*Pseudomonas aeruginosa* ATCC 25853, *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 29213, *Salmonella* Typhimurium ATCC 14028, *E. coli* ATCC 25723, and *Enterococcus faecalis*

ATCC 19433). However, those authors also remarked that even between strains of the same species, the antagonism differs considerably. Kefir bacteria strains (*L. kefiranofaciens* DD2, DD5, and DD6) inhibited the growth and biofilm formation of the *Streptococcus mutans* and *Streptococcus sobrinus* pathogens responsible for the formation of dental caries (Jeong et al., 2018).

In vivo studies have shown a reduction of *Clostridium* and *Enterobacteriaceae* in the gastrointestinal tract and a significant increase in *Lactobacillus* and *Bifidobacterium* species in the feces (Van Wyk, 2019). Kim et al. (2017) demonstrated the positive effects of kefir microbiota in an induced-obesity mouse model, as shown by decreasing members of the *Clostridiaceae* family. Xing et al. (2017) evaluated the modulatory effect of *L. kefiranofaciens* on mice gut microbiota, revealing that *Proteobacteria* were reduced after 2 wk of oral administration. This phenomenon of *Lactobacillaceae-Enterobacteriaceae* antagonism is a well-known gut microbiota interaction, as LAB acidify the environment, providing unfavorable conditions for proliferation of *Enterobacteriaceae* (Kim et al., 2015).

The antibacterial activity of kefir yeast has been less studied. Previous studies show that *Torulaspora* species from kefir exhibited antimicrobial activity against coliforms (Shen et al., 2018). de Lima et al. (2017) reported that *S. cerevisiae* strains isolated from kefir had activity against *Klebsiella pneumoniae*, *Ps. aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. More recently, *Kluyveromyces lactis* isolated from Mexican kefir grains showed antimicrobial activity against *E. coli* ATCC-25922, *Staphylococcus aureus* ATCC-BAA-42, and *Salmonella* Typhimurium BIOTEC019 by agar diffusion assay (Hurtado-Romero et al., 2021).

Toxigenic *C. difficile* is the main cause of antibiotic-associated diarrhea, and the resulting infection (*C. difficile* infection, **CDI**) is potentially fatal (Janoir, 2016). The use of kefir-derived microorganisms as an alternative treatment and prevention agent against CDI has been studied in vitro and in vivo (Liu et al., 2020). Banerjee et al. (2009) reported that *L. delbrueckii* ssp. *bulgaricus* B-30892 isolated from kefir reduced the cytotoxicity and the adhesion in a Caco-2 cell model. Trejo et al. (2010) reported that coculture of *C. difficile* with probiotic bacteria that included kefir isolated strains induced a reduction of the synthesis or secretion of clostridial toxins (TcdA and TcdB). Also, the surface proteins from *L. kefir* strains were shown to antagonize cytopathic effects of *C. difficile* toxins on Vero cells in vitro (Carasi et al., 2012). An in vivo study showed that the administration of commercial Lifeway kefir to patients with recurrent CDI, alongside staggered and tapered antibiotic withdrawal treatment, resulted in a complete resolution of symptoms in 84% of patients

(Bakken, 2014). Bolla et al. (2013) evaluated an oral treatment of a mixture of kefir isolated microorganisms (*L. plantarum*, *L. kefir*, *L. lactis*, *K. marxianus*, and *S. cerevisiae*) in a hamster model of CDI and were able to prevent diarrhea and enterocolitis triggered by *C. difficile*.

Organic Acids

The production of organic acids as antimicrobial compounds from LAB and acetic acid bacteria during fermentation has long been associated with the probiotic character of fermented products. In this sense, inhibition of gram-negative bacteria (*E. coli*, *Salmonella* spp., and *Shigella* spp.) mainly attributed to organic acids was reported by Garrote et al. (2000). More recently, Kim et al. (2018) showed that organic acids present on the culture supernatant of *L. kefir* significantly inhibited the growth of *Cronobacter sakazakii* ATCC 29544. Additionally, Gamba et al. (2016) showed the antifungal effect of kefir cell-free supernatant against *Aspergillus flavus* AFUNL5, the authors concluding that the inhibition was due to the combination of organic acids and low pH (3.3).

Bacteriocins

Regarding bacteriocins, Miao et al. (2014) reported that bacteriocin F1 produced by *L. paracasei* ssp. *tolerans* isolated from Tibetan kefir exhibited a wide range of antimicrobial activity against *E. coli*, *Salmonella enterica*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Bacillus thuringiensis*, and *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus nigricans*. Similarly, other bacteriocins isolated from kefir-related bacteria have previously shown antibacterial activity. Lacticin 3147 produced by *L. lactis* DPC3147 showed antibacterial activity against *Y. enterocolitica*, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Shigella flexneri* (Dobson et al., 2011), and another bacteriocin produced by *L. plantarum* ST8KF showed bacteriostatic activity against *Listeria innocua* (Powell et al., 2007). Leite et al. (2015) reported inhibition of *E. coli* ATCC 25922, *Salmonella* Enteritidis ATCC 13076, *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 15313 by a bacteriocin-like inhibitory substance produced by an *L. lactis* strain and *L. paracasei* isolated from Brazilian kefir grains.

Exopolysaccharides

The exopolysaccharide (EPS) kefiran also has demonstrated potential antimicrobial properties in wound infections against *Staphylococcus aureus* in a rat model.

Additionally, the authors reported antimicrobial activity against *Streptococcus pyogenes*, *Streptococcus salivarius*, *Salmonella* Typhimurium, *Candida albicans*, and *Listeria monocytogenes* in vitro (Rodrigues et al., 2005). The exopolysaccharide (EPS_DN1) produced by *L. kefiranofaciens* DN1, at a concentration of at least 1%, was able to exert bactericidal effects against *Listeria monocytogenes* and *Salmonella enteritidis* (Jeong et al., 2017). Kefiran also exerted biological activity to prevent damage in human enterocytes by *Bacillus cereus* B10502 in Caco-2 cells line (Medrano et al., 2008).

Wang et al. (2015) reported that EPS produced by *L. plantarum* YW32 isolated from Tibetan kefir grains exhibited a concentration-dependent inhibitory effect on the formation of biofilms by *E. coli* O157, *Shigella flexneri* CMCC, *Staphylococcus aureus* AC1, and *Salmonella typhimurium* S50333. Additionally, in an in vitro model the same EPS exhibited antitumor activity on HT-29 cells. The antimicrobial mechanism of the EPS may be associated with an induction of the immune response. Vinderola et al. (2006) showed that oral administration of kefir in mice increased the production of IgA⁺ B cells and the production of cytokines in the gut lamina propria and blood serum.

Other Antimicrobial Compounds

Recently, the antimicrobial fraction (FK-1000) composed of sugars and amino acids obtained from kefir serum showed bactericidal effects against *Ps. aeruginosa* ATCC-27853 and methicillin-resistant *Staphylococcus aureus* (Marques et al., 2020). Also, Purutoğlu et al. (2020) demonstrated that *L. kefiranofaciens* KF16 and *L. lactis* KF48 isolated from kefir grains showed the highest inhibitory effects against *Staphylococcus aureus* ATCC 25923, *Y. enterocolitica* ATCC 27729, *B. cereus* BC6830, *E. coli* BC1402, and *Salmonella typhimurium* RSSK95091. Additionally, these authors evaluated the antifungal effect of the strains against spoilage fungi species and reported antifungal activities of *L. diolivorans* KF2; *L. kefir* KF3, KF12, KF25, KF33; *Lactobacillus otakiensis* KF23; and *L. paracasei* KF83 strains. Figure 3 shows the antimicrobial properties of bacteria and yeast isolated from kefir grains and fermented product.

FUTURE PERSPECTIVES OF KEFIR MICROBIOTA STUDIES

In natural environments, including the human gut, microorganisms are not isolated entities. They occur within communities of hundreds or thousands of strains that provide crucial benefits, such as the division of work toward a metabolic purpose where more than one strain is involved. Additionally, consortia allow sym-

Figure 3. Outline of antimicrobial properties from bacteria and yeast isolated from kefir grains and kefir. (a) Species names in salmon pink represent the bacteria or yeast isolated from kefir grain or kefir; those in navy blue indicate pathogenic strains. The color and end of arrow indicates the pathogenic microorganism inhibited by the bacteria isolated from kefir grains or kefir (Garrote et al., 2000; Santos et al., 2003; Ulusoy et al., 2007; Golowczyc et al., 2008; Silva et al., 2009; Banerjee et al., 2009; Anselmo et al., 2010; Trejo et al., 2010; Ismaiel et al., 2011; Carasi et al., 2012; Kakisu et al., 2013; Bolla et al., 2013; Bakken, 2014; Jeong et al., 2018; Kim et al., 2018; Bengoa et al., 2018; Purutoğlu et al., 2020). (b) Antibacterial activity of exopolysaccharides produced from bacteria isolated from kefir grains or kefir, and the microorganisms inhibited (Rodrigues et al., 2005; Medrano et al., 2008; Ismaiel et al., 2011; Wang et al., 2015; Jeong et al., 2017). (c) Bacteriocins produced from bacteria isolated from kefir grains or kefir, and the different microorganisms inhibited (Powell et al., 2007; Dobson et al., 2011; Miao et al., 2014; Leite et al., 2015).

biotic relationships between strains for the support of growth by the utilization of different substrates, and the elimination of inhibitory compounds generated during metabolism. Regarding coculture of microorganisms isolated from kefir, the approach of Bolla et al. (2013) represents an example that a coculture of microorganisms (*L. plantarum*, *L. kefir*, *L. lactis*, *K. marxianus*, and *S. cerevisiae*) with previously tested probiotic properties can provide benefits to the host, in this case by reducing the levels of a *C. difficile*-induced enterocolitis in a hamster model. The same workgroup previously reported that this coculture was able to inhibit the growth of *Shigella sonnei* in vitro (Bolla et al., 2011). Additionally, another coculture of kefir microorganisms (*L. plantarum* CIDCA 8327, *L. kefir* CIDCA 8348, and *K. marxianus* CIDCA 8154) showed effects against *Salmonella* spp. in Caco-2/TC7 cells in an in vitro model (Londero et al., 2015). Therefore, the

use of natural or genetically engineered microbial consortia present advantages over individual strains, and the study of these complex microbial communities and the rules that determine their assembly and function is imperative for the designing of microbial consortia for biotechnological or medical purposes (Giri et al., 2021). Considering that the development of an artificial kefir grain is significantly important for the dairy industry, the potential knowledge provided by studying kefir microbial consortia becomes highly important regarding the choice of strains that could potentially be included in the artificial grain matrix (Slattery et al., 2019).

CONCLUSIONS

The physiological effects and antimicrobial properties of kefir microbiota were discussed in this review. There is still limited information and understanding

of the mechanisms of microbial inhibition of kefir microorganisms; however, advances in omics technologies may provide insight into the utilization of specific kefir microorganisms or consortia of bacteria and yeast to provide a targeted and measurable benefit against enteropathogenic bacteria and spoilage fungi. Future research in this area should be related to the antimicrobial properties of consortia from kefir microorganisms and the mechanisms of inhibition. Additionally, systematic clinical trials to better understand the antimicrobial properties of kefir microorganisms and associated compounds are required to establish adequate amounts for consumption. Using these approaches would enhance the possibility of producing kefir-based products containing consortia of specific kefir microbiota.

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REFERENCES

- Ahmed, Z., Y. Wang, A. Ahmad, S. T. Khan, M. Nisa, H. Ahmad, and A. Afreen. 2013. Kefir and health: A contemporary perspective. *Crit. Rev. Food Sci. Nutr.* 53:422–434. <https://doi.org/10.1080/10408398.2010.540360>.
- Anselmo, R. J., S. S. Viora, P. A. Ojeda, and L. I. Lausada. 2010. Antagonistic effect of the kefir on endospores and vegetative cells of *Bacillus cereus* and *Clostridium perfringens*. *Inf. Tecnol.* 21:131–138. <https://doi.org/10.4067/S0718-07642010000400017>.
- Arsilan, S. 2015. A review: Chemical, microbiological and nutritional characteristics of kefir. *CYTA J. Food* 13:340–345. <https://doi.org/10.1080/19476337.2014.981588>.
- Azizi, N. F., M. R. Kumar, S. K. Yeap, J. O. Abdullah, M. Khalid, A. R. Omar, M. A. Osman, S. A. S. Mortadza, and N. B. Alitheen. 2021. Kefir and its biological activities. *Foods* 10:1210. <https://doi.org/10.3390/foods10061210>.
- Azizkhani, M., P. E. J. Saris, and M. Baniyadi. 2021. An in vitro assessment of antifungal and antibacterial activity of cow, camel, ewe, and goat milk kefir and probiotic yogurt. *J. Food Meas. Charact.* 15:406–415. <https://doi.org/10.1007/s11694-020-00645-4>.
- Bakken, J. S. 2014. Staggered and tapered antibiotic withdrawal with administration of kefir for recurrent *Clostridium difficile* infection. *Clin. Infect. Dis.* 59:858–861. <https://doi.org/10.1093/cid/ciu429>.
- Banerjee, P., G. J. Merkel, and A. K. Bhunia. 2009. *Lactobacillus delbrueckii* ssp. *bulgaricus* B-30892 can inhibit cytotoxic effects and adhesion of pathogenic *Clostridium difficile* to Caco-2 cells. *Gut Pathog.* 1:8. <https://doi.org/10.1186/1757-4749-1-8>.
- Bengoa, A. A., C. Iraporda, G. L. Garrote, and A. G. Abraham. 2019. Kefir micro-organisms: Their role in grain assembly and health properties of fermented milk. *J. Appl. Microbiol.* 126:686–700. <https://doi.org/10.1111/jam.14107>.
- Bengoa, A. A., L. Zavala, P. Carasi, S. A. Trejo, S. Bronsoms, M. Á. Serradell, G. L. Garrote, and A. G. Abraham. 2018. Simulated gastrointestinal conditions increase adhesion ability of *Lactobacillus paracasei* strains isolated from kefir to Caco-2 cells and mucin. *Food Res. Int.* 103:462–467. <https://doi.org/10.1016/j.foodres.2017.09.093>.
- Biadala, A., T. Szablewski, M. Lasik-Kurdyś, and R. Cegielska-Radziejewska. 2020. Antimicrobial activity of goat's milk fermented by single strain of kefir grain microflora. *Eur. Food Res. Technol.* 246:1231–1239. <https://doi.org/10.1007/s00217-020-03483-2>.
- Blasche, S., Y. Kim, R. A. T. Mars, D. Machado, M. Maansson, E. Kafkia, A. Milanese, G. Zeller, B. Teusink, J. Nielsen, V. Benes, R. Neves, U. Sauer, and K. R. Patil. 2021. Metabolic cooperation and spatiotemporal niche partitioning in a kefir microbial community. *Nat. Microbiol.* 6:196–208. <https://doi.org/10.1038/s41564-020-00816-5>.
- Bolla, P. A., S. M. de los Angeles Serradell, P. J. de Urraza, and G. De Antoni. 2011. Effect of freeze-drying on viability and in vitro probiotic properties of a mixture of lactic acid bacteria and yeasts isolated from kefir. *J. Dairy Res.* 78:15–22. <https://doi.org/10.1017/S0022029910000610>.
- Bolla, P. A., P. Carasi, M. A. Bolla, G. L. De Antoni, and M. A. Serradell. 2013. Protective effect of a mixture of kefir-isolated lactic acid bacteria and yeasts in a hamster model of *Clostridium difficile* infection. *Anaerobe* 21:28–33. <https://doi.org/10.1016/j.anaerobe.2013.03.010>.
- Bourrie, B. C. T., B. P. Willing, and P. D. Cotter. 2016. The microbiota and health promoting characteristics of the fermented beverage kefir. *Front. Microbiol.* 7. <https://doi.org/10.3389/fmicb.2016.00647>.
- Brown, R. B., and M. A. Hollingsworth. 2013. Mucin Family of Glycoproteins. Elsevier.
- Carasi, P., S. M. Racedo, C. Jacquot, D. E. Romanin, M. A. Serradell, and M. C. Urdaci. 2015. Impact of kefir derived *Lactobacillus kefir* on the mucosal immune response and gut microbiota. *J. Immunol. Res.* 2015:1–12. <https://doi.org/10.1155/2015/361604>.
- Carasi, P., F. M. Trejo, P. F. Pérez, G. L. De Antoni, and M. A. Serradell. 2012. Surface proteins from *Lactobacillus kefir* antagonize invitro cytotoxic effect of *Clostridium difficile* toxins. *Anaerobe* 18:135–142. <https://doi.org/10.1016/j.anaerobe.2011.11.002>.
- CFR. 2022. CFR—Code of Federal Regulations 21CFR131.111. Accessed Jan. 28, 2022. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=131.111>.
- Chatterjee, M., A. C. Pushkaran, A. K. Vasudevan, K. K. N. Menon, R. Biswas, and C. G. Mohan. 2018. Understanding the adhesion mechanism of a mucin binding domain from *Lactobacillus fermentum* and its role in enteropathogen exclusion. *Int. J. Biol. Macromol.* 110:598–607. <https://doi.org/10.1016/j.ijbiomac.2017.10.107>.
- Chen, Y., P. Hsiao, W. Hong, T. Dai, and M. Chen. 2012. *Lactobacillus kefiranoferiens* M1 isolated from milk kefir grains ameliorates experimental colitis in vitro and in vivo. *J. Dairy Sci.* 95:63–74. <https://doi.org/10.3168/jds.2011-4696>.
- Codex Alimentarius Commission. 2003. Codex Standard for fermented milks, 243-2003. Accessed November 2021. http://www.alimentosargentinos.gob.ar/contenido/marco/Codex_Alimentarius/normativa/codex/stan/CODEX_STAN_243.asp.
- Collado, M. C., J. Meriluoto, and S. Salminen. 2008. Adhesion and aggregation properties of probiotic and pathogen strains. *Eur. Food Res. Technol.* 226:1065–1073. <https://doi.org/10.1007/s00217-007-0632-x>.
- Dallas, D. C., F. Citerne, T. Tian, V. L. M. Silva, K. M. Kalanetra, S. A. Frese, R. C. Robinson, D. A. Mills, and D. Barile. 2016. Peptidomic analysis reveals proteolytic activity of kefir microorganisms on bovine milk proteins. *Food Chem.* 197:273–284. <https://doi.org/10.1016/j.foodchem.2015.10.116>.
- de Lima, M. dos S. F., K. M. S. de Souza, W. W. C. Albuquerque, J. A. C. Teixeira, M. T. H. Cavalcanti, and A. L. F. Porto. 2017. *Saccharomyces cerevisiae* from Brazilian kefir-fermented milk: An in vitro evaluation of probiotic properties. *Microb. Pathog.* 110:670–677. <https://doi.org/10.1016/j.micpath.2017.05.010>.
- De Montijo-Prieto, S., E. Moreno, T. Bergillos-Meca, A. Lasserrot, M.-D. Ruiz-López, A. Ruiz-Bravo, and M. Jiménez-Valera. 2015. A *Lactobacillus plantarum* strain isolated from kefir protects against intestinal infection with *Yersinia enterocolitica* O9 and modulates immunity in mice. *Res. Microbiol.* 166:626–632. <https://doi.org/10.1016/j.resmic.2015.07.010>.
- Demir, H. 2020. Comparison of traditional and commercial kefir microorganism compositions and inhibitory effects on certain patho-

- gens. *Int. J. Food Prop.* 23:375–386. <https://doi.org/10.1080/10942912.2020.1733599>.
- Diosma, G., D. E. Romanin, M. F. Rey-Burusco, A. Londero, and G. L. Garrote. 2014. Yeasts from kefir grains: Isolation, identification, and probiotic characterization. *World J. Microbiol. Biotechnol.* 30:43–53. <https://doi.org/10.1007/s11274-013-1419-9>.
- Dobson, A., O. O'Sullivan, P. D. Cotter, P. Ross, and C. Hill. 2011. High-throughput sequence-based analysis of the bacterial composition of kefir and an associated kefir grain. *FEMS Microbiol. Lett.* 320:56–62. <https://doi.org/10.1111/j.1574-6968.2011.02290.x>.
- Egea, M. B., D. C. Santos, J. G. Oliveira Filho, J. C. Ores, K. P. Takeuchi, and A. C. Lemes. 2020. A review of nondairy kefir products: Their characteristics and potential human health benefits. *Crit. Rev. Food Sci. Nutr.* 0:1–17. <https://doi.org/10.1080/10408398.2020.1844140>.
- Farag, M. A., S. A. Jomaa, A. Abd El-Wahed, and H. R. El-Seedi. 2020. The many faces of kefir fermented dairy products: Quality characteristics, flavour chemistry, nutritional value, health benefits, and safety. *Nutrients* 12:346. <https://doi.org/10.3390/nu12020346>.
- FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization). 2006. Probiotics in Food: Health and Nutritional Properties and Guidelines for Evaluation. FAO food and nutrition paper. No. 85. Food and Agriculture Organization of the United Nations, World Health Organization.
- Gamba, R. R., C. A. Caro, O. L. Martínez, A. F. Moretti, L. Giannuzzi, G. L. De Antoni, and A. León Peláez. 2016. Antifungal effect of kefir fermented milk and shelf life improvement of corn arepas. *Int. J. Food Microbiol.* 235:85–92. <https://doi.org/10.1016/j.ijfoodmicro.2016.06.038>.
- Garofalo, C., I. Ferrocino, A. Reale, R. Sabbatini, V. Milanović, M. Alkić-Subašić, F. Boscaino, L. Aquilanti, M. Pasquini, M. F. Trombetta, S. Tavoletti, R. Coppola, L. Coccolin, M. Blesić, Z. Sarić, F. Clementi, and A. Osimani. 2020. Study of kefir drinks produced by backslipping method using kefir grains from Bosnia and Herzegovina: Microbial dynamics and volatiline profile. *Food Res. Int.* 137:109369. <https://doi.org/10.1016/j.foodres.2020.109369>.
- Garrote, G. L., A. G. Abraham, and G. L. De Antoni. 2000. Inhibitory power of kefir: The role of organic acids. *J. Food Prot.* 63:364–369. <https://doi.org/10.4315/0362-028X-63.3.364>.
- Giri, S., L. Oña, S. Waschina, S. Shitut, G. Yousif, C. Kaleta, and C. Kost. 2021. Metabolic dissimilarity determines the establishment of cross-feeding interactions in bacteria. *Curr. Biol.* 31:5547–5556. <https://doi.org/10.1016/j.cub.2021.10.019>.
- Golowczyc, M. A., M. J. Gugliada, A. Hollmann, L. Delfederico, G. L. Garrote, A. G. Abraham, L. Semorile, and G. De Antoni. 2008. Characterization of homofermentative lactobacilli isolated from kefir grains: Potential use as probiotic. *J. Dairy Res.* 75:211–217. <https://doi.org/10.1017/S0022029908003117>.
- Golowczyc, M. A., P. Mobili, G. L. Garrote, A. G. Abraham, and G. L. De Antoni. 2007. Protective action of *Lactobacillus kefir* carrying S-layer protein against *Salmonella enterica* serovar Enteritidis. *Int. J. Food Microbiol.* 118:264–273. <https://doi.org/10.1016/j.ijfoodmicro.2007.07.042>.
- Guzel-Seydim, Z. B., T. Kok-Tas, A. K. Greene, and A. C. Seydim. 2011. Review: Functional properties of kefir. *Crit. Rev. Food Sci. Nutr.* 51:261–268. <https://doi.org/10.1080/10408390903579029>.
- Hamida, R. S., A. Shami, M. A. Ali, Z. N. Almohawes, A. E. Mohammed, and M. M. Bin-Meferij. 2021. Kefir: A protective dietary supplementation against viral infection. *Biomed. Pharmacother.* 133:110974. <https://doi.org/10.1016/j.biopha.2020.110974>.
- Huang, Y., F. Wu, X. Wang, Y. Sui, L. Yang, and J. Wang. 2013. Characterization of *Lactobacillus plantarum* Lp27 isolated from Tibetan kefir grains: A potential probiotic bacterium with cholesterol-lowering effects. *J. Dairy Sci.* 96:2816–2825. <https://doi.org/10.3168/jds.2012-6371>.
- Hurtado-Romero, A., M. Del Toro-Barbosa, M. S. Gradilla-Hernández, L. E. García-Amezquita, and T. García-Cayuela. 2021. Probiotic properties, prebiotic fermentability, and GABA-producing capacity of microorganisms isolated from Mexican milk kefir grains: A clustering evaluation for functional dairy food applications. *Foods* 10:2275. <https://doi.org/10.3390/foods10102275>.
- IFT. 2021. 9 Food Trends to Watch For in 2021. Accessed Jun. 21, 2021. <https://www.ift.org/news-and-publications/blog/2021/2021-food-tech-predictions>.
- Ismaiel, A. A., M. F. Ghaly, and A. K. El-Naggar. 2011. Milk kefir: Ultrastructure, antimicrobial activity and efficacy on aflatoxin b1 production by *Aspergillus flavus*. *Curr. Microbiol.* 62:1602–1609. <https://doi.org/10.1007/s00284-011-9901-9>.
- Janoir, C. 2016. Virulence factors of *Clostridium difficile* and their role during infection. *Anaerobe* 37:13–24. <https://doi.org/10.1016/j.anaerobe.2015.10.009>.
- Jeong, D., D. H. Kim, I. B. Kang, H. Kim, K. Y. Song, H. S. Kim, and K. H. Seo. 2017. Characterization and antibacterial activity of a novel exopolysaccharide produced by *Lactobacillus kefirano-faciens* DN1 isolated from kefir. *Food Control* 78:436–442. <https://doi.org/10.1016/j.foodcont.2017.02.033>.
- Jeong, D., D.-H. Kim, K.-Y. Song, and K.-H. Seo. 2018. Antimicrobial and anti-biofilm activities of *Lactobacillus kefirano-faciens* DD2 against oral pathogens. *J. Oral Microbiol.* 10:1472985. <https://doi.org/10.1080/20002297.2018.1472985>.
- John, S. M., and S. Deeseenthum. 2015. Properties and benefits of kefir—A review. *Songklanakarin J. Sci. Technol.* 37:275–282.
- Kakisu, E., P. Bolla, A. G. Abraham, P. de Urraza, and G. L. De Antoni. 2013. *Lactobacillus plantarum* isolated from kefir: Protection of cultured Hep-2 cells against *Shigella* invasion. *Int. Dairy J.* 33:22–26. <https://doi.org/10.1016/j.idairyj.2013.06.004>.
- Kang, E. A., H. I. Choi, S. W. Hong, S. Kang, H. Y. Jegal, E. W. Choi, B. S. Park, and J. S. Kim. 2020. Extracellular vesicles derived from kefir grain *Lactobacillus* ameliorate intestinal inflammation via regulation of proinflammatory pathway and tight junction integrity. *Biomedicines* 8:522. <https://doi.org/10.3390/biomedicines8110522>.
- Kim, D., D. Jeong, H. Kim, and K. Seo. 2019. Modern perspectives on the health benefits of kefir in next generation sequencing era: Improvement of the host gut microbiota. *Crit. Rev. Food Sci. Nutr.* 59:1782–1793. <https://doi.org/10.1080/10408398.2018.1428168>.
- Kim, D. H., J. W. Chon, H. Kim, and K. H. Seo. 2015. Modulation of intestinal microbiota in mice by kefir administration. *Food Sci. Biotechnol.* 24:1397–1403. <https://doi.org/10.1007/s10068-015-0179-8>.
- Kim, D. H., D. Jeong, H. Kim, I. B. Kang, J. W. Chon, K. Y. Song, and K. H. Seo. 2016. Antimicrobial activity of kefir against various food pathogens and spoilage bacteria. *Korean J. Food Sci. Anim. Resour.* 36:787–790. <https://doi.org/10.5851/kosfa.2016.36.6.787>.
- Kim, D. H., D. Jeong, K. Y. Song, I. B. Kang, H. Kim, and K. H. Seo. 2018. Culture supernatant produced by *Lactobacillus kefir* from kefir inhibits the growth of *Cronobacter sakazakii*. *J. Dairy Res.* 85:98–103. <https://doi.org/10.1017/S0022029917000802>.
- Kim, D. H., H. Kim, D. Jeong, I. B. Kang, J. W. Chon, H. S. Kim, K. Y. Song, and K. H. Seo. 2017. Kefir alleviates obesity and hepatic steatosis in high-fat diet-fed mice by modulation of gut microbiota and mycobiota: Targeted and untargeted community analysis with correlation of biomarkers. *J. Nutr. Biochem.* 44:35–43. <https://doi.org/10.1016/j.jnutbio.2017.02.014>.
- Korsak, N., B. Taminiau, M. Leclercq, C. Nezer, S. Crevecoeur, C. Ferauche, E. Detry, V. Delcenserie, and G. Daube. 2015. Short communication: Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments. *J. Dairy Sci.* 98:3684–3689. <https://doi.org/10.3168/jds.2014-9065>.
- Lawrence, T. 2009. The nuclear factor NF- κ B pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* 1:a001651. <https://doi.org/10.1101/cshperspect.a001651>.
- Leite, A. M. O., M. A. L. Miguel, R. S. Peixoto, A. S. Rosado, J. T. Silva, and V. M. F. Paschoalin. 2013. Microbiological, technological and therapeutic properties of kefir: A natural probiotic beverage. *Braz. J. Microbiol.* 44:341–349. <https://doi.org/10.1590/S1517-83822013000200001>.
- Leite, A. M. O., M. A. L. Miguel, R. S. Peixoto, P. Ruas-Madiedo, V. M. F. Paschoalin, B. Mayo, and S. Delgado. 2015. Probiotic poten-

- tial of selected lactic acid bacteria strains isolated from Brazilian kefir grains. *J. Dairy Sci.* 98:3622–3632. <https://doi.org/10.3168/jds.2014-9265>.
- Liévin-Le Moal, V., and A. L. Servin. 2006. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: Mucins, antimicrobial peptides, and microbiota. *Clin. Microbiol. Rev.* 19:315–337. <https://doi.org/10.1128/CMR.19.2.315-337.2006>.
- Lin, W.-H., C.-R. Wu, T. J. Fang, J.-T. Guo, S.-Y. Huang, M.-S. Lee, and H.-L. Yang. 2011. Anti-*Helicobacter pylori* activity of fermented milk with lactic acid bacteria. *J. Sci. Food Agric.* 91:1424–1431. <https://doi.org/10.1002/jsfa.4327>.
- Liu, D., L. Zeng, Z. Yan, J. Jia, J. Gao, and Y. Wei. 2020. The mechanisms and safety of probiotics against toxigenic clostridium difficile. *Expert Rev. Anti Infect. Ther.* 18:967–975. <https://doi.org/10.1080/14787210.2020.1778464>.
- Londero, A., C. Iraporda, G. L. Garrote, and A. G. Abraham. 2015. Cheese whey fermented with kefir micro-organisms: Antagonism against *Salmonella* and immunomodulatory capacity. *Int. J. Dairy Technol.* 68:118–126. <https://doi.org/10.1111/1471-0307.12161>.
- Marques, V. D., M. R. Franzolin, S. S. Sanabani, H. Vigerelli, R. M. F. Piazza, D. C. Pimenta, T. Venâncio, I. V. Neves, H. G. de Sousa Silva, D. dos Santos Courrol, L. Mendonça-Previato, J. O. Previato, S. A. C. Jorge, and M. de Oliveira Domingos. 2020. A new class of antimicrobial molecules derived from kefir, effective against *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus* (MRSA) strains. *Sci. Rep.* 10:17434. <https://doi.org/10.1038/s41598-020-73651-7>.
- Marsh, A. J., O. O'Sullivan, C. Hill, R. P. Ross, and P. D. Cotter. 2013. Sequencing-based analysis of the bacterial and fungal composition of kefir grains and milks from multiple sources. *PLoS One* 8:e69371. <https://doi.org/10.1371/journal.pone.0069371>.
- Medrano, M., P. F. Pérez, and A. G. Abraham. 2008. Kefiran antagonizes cytopathic effects of *Bacillus cereus* extracellular factors. *Int. J. Food Microbiol.* 122:1–7. <https://doi.org/10.1016/j.ijfoodmicro.2007.11.046>.
- Metras, B. N., M. J. Holle, V. J. Parker, M. J. Miller, and K. S. Swanson. 2021. Commercial kefir products assessed for label accuracy of microbial composition and density. *JDS Commun.* 2:87–91. <https://doi.org/10.3168/jdsc.2020.0056>.
- Miao, J., H. Guo, Y. Ou, G. Liu, X. Fang, Z. Liao, C. Ke, Y. Chen, L. Zhao, and Y. Cao. 2014. Purification and characterization of bacteriocin F1, a novel bacteriocin produced by *Lactobacillus paracasei* subsp. *tolerans* FX-6 from Tibetan kefir, a traditional fermented milk from Tibet, China. *Food Control* 42:48–53. <https://doi.org/10.1016/j.foodcont.2014.01.041>.
- Monteagudo-Mera, A., R. A. Rastall, G. R. Gibson, D. Charalampopoulos, and A. Chatzifragkou. 2019. Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health. *Appl. Microbiol. Biotechnol.* 103:6463–6472. <https://doi.org/10.1007/s00253-019-09978-7>.
- Nalbantoglu, U., A. Cakar, H. Dogan, N. Abaci, D. Ustek, K. Sayood, and H. Can. 2014. Metagenomic analysis of the microbial community in kefir grains. *Food Microbiol.* 41:42–51. <https://doi.org/10.1016/j.fm.2014.01.014>.
- Nielsen, B., G. C. Gürakan, and G. Ünlü. 2014. Kefir: A multifaceted fermented dairy product. *Probiotics Antimicrob. Proteins* 6:123–135. <https://doi.org/10.1007/s12602-014-9168-0>.
- Nogay, N. H. 2019. Kefir beverage and its effects on health. Pages 273–296 in *Milk-Based Beverages*. A. M. Grumezescu and A. M. Holban, ed. Woodland Publishing.
- Plessas, S., D. E. Kiouisi, M. Rathosi, A. Alexopoulos, Y. Kourkoutas, I. Mantzourani, A. Galanis, and E. Bezirtzoglou. 2020. Isolation of a *Lactobacillus paracasei* strain with probiotic attributes from kefir grains. *Biomedicines* 8:594. <https://doi.org/10.3390/biomedicines8120594>.
- Powell, J. E., R. C. Witthuhn, S. D. Todorov, and L. M. T. Dicks. 2007. Characterization of bacteriocin ST8KF produced by a kefir isolate *Lactobacillus plantarum* ST8KF. *Int. Dairy J.* 17:190–198. <https://doi.org/10.1016/j.idairyj.2006.02.012>.
- Purutoğlu, K., H. İspirli, M. O. Yüzer, H. Serencam, and E. Dertli. 2020. Diversity and functional characteristics of lactic acid bacteria from traditional kefir grains. *Int. J. Dairy Technol.* 73:57–66. <https://doi.org/10.1111/1471-0307.12633>.
- Qu, D., G. Wang, L. Yu, F. Tian, W. Chen, and Q. Zhai. 2021. The effects of diet and gut microbiota on the regulation of intestinal mucin glycosylation. *Carbohydr. Polym.* 258:117651. <https://doi.org/10.1016/j.carbpol.2021.117651>.
- Rodrigues, K. L., L. R. Gaudino Caputo, J. C. Tavares Carvalho, J. Evangelista, and J. M. Schneedorf. 2005. Antimicrobial and healing activity of kefir and kefir extract. *Int. J. Antimicrob. Agents* 25:404–408. <https://doi.org/10.1016/j.ijantimicag.2004.09.020>.
- Romanin, D., M. Serradell, D. González Maciel, N. Lausada, G. L. Garrote, and M. Rumbo. 2010. Down-regulation of intestinal epithelial innate response by probiotic yeasts isolated from kefir. *Int. J. Food Microbiol.* 140:102–108. <https://doi.org/10.1016/j.ijfoodmicro.2010.04.014>.
- Romanin, D.E., S. Llopis, S. Genovés, P. Martorell, V.D. Ramón, G.L. Garrote, and M. Rumbo. 2016. Probiotic yeast *Kluyveromyces marxianus* CIDCA 8154 shows anti-inflammatory and anti-oxidative stress properties in in vivo models. *Benef. Microbes* 7:83–93. <https://doi.org/10.3920/BM2015.0066>.
- Rosa, D. D., M. M. S. Dias, E. M. Grzeskowiak, S. A. Reis, L. L. Conceição, and M. D. C. G. Peluzio. 2017. Milk kefir: Nutritional, microbiological and health benefits. *Nutr. Res. Rev.* 30:82–96. <https://doi.org/10.1017/S0954422416000275>.
- Santos, A., M. San Mauro, A. Sanchez, J. M. Torres, and D. Marquina. 2003. The antimicrobial properties of different strains of *Lactobacillus* spp. isolated from kefir. *Syst. Appl. Microbiol.* 26:434–437. <https://doi.org/10.1078/072320203322497464>.
- Satir, G., and Z. B. Guzel-Seydim. 2016. How kefir fermentation can affect product composition? *Small Rumin. Res.* 134:1–7. <https://doi.org/10.1016/j.smallrumres.2015.10.022>.
- Shen, Y., D.-H. Kim, J.-W. Chon, H. Kim, K.-Y. Song, and K.-H. Seo. 2018. Nutritional effects and antimicrobial activity of kefir (grains). *J. Milk Sci. Biotechnol.* 36:1–13. <https://doi.org/10.22424/jmsb.2018.36.1.1>.
- Silva, K. R., S. A. Rodrigues, L. X. Filho, and Á. S. Lima. 2009. Antimicrobial activity of broth fermented with kefir grains. *Appl. Biochem. Biotechnol.* 152:316–325. <https://doi.org/10.1007/s12010-008-8303-3>.
- Slattery, C., P. D. Cotter, and P. W. O'Toole. 2019. Analysis of health benefits conferred by *Lactobacillus* species from kefir. *Nutrients* 11:1–24. <https://doi.org/10.3390/nu11061252>.
- Tasteyre, A., M. C. Barc, T. Karjalainen, P. Bourlioux, and A. Collignon. 2002. Inhibition of in vitro cell adherence of *Clostridium difficile* by *Saccharomyces boulardii*. *Microb. Pathog.* 32:219–225. <https://doi.org/10.1006/mpat.2002.0495>.
- Technavio.com. 2021. Sample report: Kefir Products Market by Product and Geography - Forecast and Analysis 2021-2025. Accessed Nov. 24, 2021. <https://www.technavio.com/report/kefir-products-market-industry-analysis>.
- Toscano, M., R. De Grandi, V. L. Miniello, R. Mattina, and L. Drago. 2017. Ability of *Lactobacillus kefir* LKF01 (DSM32079) to colonize the intestinal environment and modify the gut microbiota composition of healthy individuals. *Dig. Liver Dis.* 49:261–267. <https://doi.org/10.1016/j.dld.2016.11.011>.
- Trejo, F. M., P. F. Pérez, and G. L. De Antoni. 2010. Co-culture with potentially probiotic microorganisms antagonizes virulence factors of *Clostridium difficile* in vitro. *Antonie van Leeuwenhoek* 98:19–29. <https://doi.org/10.1007/s10482-010-9424-6>.
- Turner, M. D., B. Nedjai, T. Hurst, and D. J. Pennington. 2014. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochim. Biophys. Acta* 1843:2563–2582. <https://doi.org/10.1016/j.bbamer.2014.05.014>.
- Ulusoy, B.H., H. Çolak, H. Hampikyan, and M.E. Erkan. 2007. An in vitro study on the antibacterial effect of kefir against some food-borne pathogens. *Turkish Microbiol. Soc.* 37:103–107.
- Valle Arevalo, A., and C. J. Nobile. 2020. Interactions of microorganisms with host mucins: A focus on *Candida albicans*. *FEMS Microbiol. Rev.* 44:645–654. <https://doi.org/10.1093/femsre/fuaa027>.

- Van Wyk, J. 2019. Kefir: The Champagne of Fermented Beverages. Elsevier.
- Vinderola, G., G. Perdigón, J. Duarte, E. Farnworth, and C. Matar. 2006. Effects of the oral administration of the exopolysaccharide produced by *Lactobacillus kefiranoferiens* on the gut mucosal immunity. *Cytokine* 36:254–260. <https://doi.org/10.1016/j.cyto.2007.01.003>.
- Walsh, A. M., F. Crispie, K. Kilcawley, and O. O. Sullivan. 2016. Microbial succession and flavor production in the fermented dairy beverage kefir. *mSystems* 1:e00052-16. <https://doi.org/10.1128/mSystems.00052-16>.
- Wang, J., Y. Zeng, S. Wang, H. Liu, D. Zhang, W. Zhang, Y. Wang, and H. Ji. 2018. Swine-derived probiotic *Lactobacillus plantarum* inhibits growth and adhesion of enterotoxigenic *Escherichia coli* and mediates host defense. *Front. Microbiol.* 9:1364. <https://doi.org/10.3389/fmicb.2018.01364>.
- Wang, J., X. Zhao, Y. Yang, A. Zhao, and Z. Yang. 2015. Characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. *Int. J. Biol. Macromol.* 74:119–126. <https://doi.org/10.1016/j.ijbiomac.2014.12.006>.
- Wang, S. Y., K. N. Chen, Y. M. Lo, M. L. Chiang, H. C. Chen, J. R. Liu, and M. J. Chen. 2012. Investigation of microorganisms involved in biosynthesis of the kefir grain. *Food Microbiol.* 32:274–285. <https://doi.org/10.1016/j.fm.2012.07.001>.
- Xie, N., T. Zhou, and B. Li. 2012. Kefir yeasts enhance probiotic potentials of *Lactobacillus paracasei* H9: The positive effects of coaggregation between the two strains. *Food Res. Int.* 45:394–401. <https://doi.org/10.1016/j.foodres.2011.10.045>.
- Xing, Z., W. Tang, W. Geng, Y. Zheng, and Y. Wang. 2017. In vitro and in vivo evaluation of the probiotic attributes of *Lactobacillus kefiranoferiens* XL10 isolated from Tibetan kefir grain. *Appl. Microbiol. Biotechnol.* 101:2467–2477. <https://doi.org/10.1007/s00253-016-7956-z>.
- Youhanna, S., and V. M. Lauschke. 2021. The past, present and future of intestinal in vitro cell systems for drug absorption studies. *J. Pharm. Sci.* 110:50–65. <https://doi.org/10.1016/j.xphs.2020.07.001>.
- Zanirati, D. F., M. Abatemarco Jr., S. H. C. Sandes, J. R. Nicoli, Á. C. Nunes, and E. Neumann. 2015. Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starter or probiotic cultures. *Anaerobe* 32:70–76. <https://doi.org/10.1016/j.anaerobe.2014.12.007>.
- Zeng, M. Y., N. Inohara, and G. Nuñez. 2017. Mechanisms of inflammation-driven bacterial dysbiosis in the gut. *Mucosal Immunol.* 10:18–26. <https://doi.org/10.1038/mi.2016.75>.

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