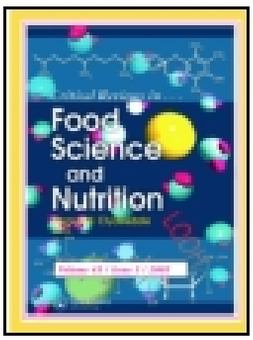

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**<Review>**

# Modern Perspectives on the Health Benefits of Kefir in Next Generation Sequencing Era: Improvement of the Host Gut Microbiota

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ACCEPTED MANUSCRIPT

**Abstract**

Kefir is a natural complex fermented milk product containing more than 50 species of probiotic bacteria and yeast, and has been demonstrated to have multiple properties conferring health benefits, including antiobesity, anti-hepatic steatosis, antioxidative, antiallergenic, antitumor, anti-inflammatory, cholesterol-lowering, constipation-alleviating, and antimicrobial properties. To better understand the underlying mechanisms of these benefits, we here review research on the effect of kefir (and kefir microorganisms) consumption to modulate the host gut microbiota. Owing to its excellent gastrointestinal resistance and colonization ability and wide ranges of microbial interaction, kefir has shown significant and wide-spectrum modulatory effects on the host gut microbiota. In particular, as a bacteria- and yeast-containing food, kefir can modulate both the gut microbiota and mycobiota. Since the association of this modulation with health benefit has only been addressed in a small number of recent studies thus far, further studies are needed to determine the precise mechanisms of the beneficial effects of kefir in relation to the modulation of the gut microbiota and mycobiota. Gaining this insight will surely help to take full advantage of this unique probiotic food.

**Keywords:** Kefir, probiotics, gut microbiota, gut mycobiota, lactic acid bacteria, yeast, next generation sequencing

 

## 1. Introduction

Kefir is a traditional fermented milk containing over 50 species of probiotic microorganisms, consisting of lactic acid bacteria, yeast, and acetic acid bacteria (Guzel-Seydim et al. 2011). According to the Codex Standard for Fermented Milks (www.codexalimentarius.net), kefir is defined as fermented milk prepared from a unique starter culture known as kefir grains, which contains lactic acid bacteria such as *Lactobacillus kefiri*; members of the genera *Leuconostoc* and *Lactococcus*; acetic acid bacteria, and the lactose-fermenting (*Kluyveromyces marxianus*) and non-fermenting yeasts (*Saccharomyces unisporus, Saccharomyces cerevisiae,* and *Saccharomyces exiguus*). Many beneficial effects have been reported of both kefir and these microorganisms individually, including cholesterol-lowering effects, regulation of blood pressure, antitumor effects, wound healing, immunomodulatory effects, anti-allergenic effects, improving liver function, anti-diabetic effects, and anti-obesity effects (Guzel-Seydim et al. 2011; Chon et al. 2013; Bourrie et al. 2016; Kim et al. 2017b).

Intestinal microbiota encompass a large variety of microorganisms located in the human intestinal tract with at least $10^{14}$ microbial cells originating from up to 1,000 different species (Sekirov et al. 2010). Recent studies have emphasized that the gut microbiota is not only composed of prokaryotic but also eukaryotic organisms, representing the gut mycobiota (Underhill et al. 2014). Both the microbiota and mycobiota play critical roles in immunity, energy homeostasis, and digestion, as well as in the pathogenesis of certain systemic diseases (De Filippo et al. 2010; Sekirov et al. 2010; Brown et al. 2015).

Despite the numerous reviews on the multitude of beneficial health effects of kefir, there has been no systemic review conducted on the direct effect of the consumption of kefir and its

 

microorganisms to modulate the host gut microbiota (Farnworth et al. 2003; Guzel-Seydim et al. 2011; Chon et al. 2013; Bourrie et al. 2016; AbdEl-Mogheith et al. 2017; Kim et al. 2017b). In addition, the key attribute of kefir that sets it apart from other fermented milk products is yeast fermentation (Irigoyen et al. 2005; Kim et al. 2017b). Although kefir is a rare case of a dairy-based lactic acid bacteria–yeast symbiotic fermented food, its modulatory effects on the gut microbiota and mycobiota have not yet been thoroughly reviewed (Soni et al. 2014; Lara-Hidalgo et al. 2017).

Therefore, we here provide an overview of the *in vitro* properties related to survival and colonization of the microbiota and mycobiota in the gut environment and the microbial interactions therein. In addition, the modulatory effects of kefir and kefir microorganisms on the gut micro- and mycobiota are reviewed to determine the target range, consensus modulatory trend, and its relationship to the host health. Note that we here use the term "kefir" to only refer to that derived from cow's milk kefir, and not soymilk or milk from another animal, water, or sugar kefir. In the light of preparation methods, traditional kefir prepared with kefir grain is reviewed in this study, not industrially manufactured kefir which contains only limited species of lactic acid bacteria but usually no kefir-isolated yeasts and acetic acid bacteria (Bourrie et al. 2016). In addition, colonization of a single kefir strain in the intestinal tract was not considered as a case of modulation of gut microbiota for the purpose of this analysis.

## 2. *In vitro* probiotic properties of kefir microorganisms

To understand the *in vivo* modulatory effects of kefir on the gut microbiota, it is first essential to evaluate the *in vitro* probiotic properties of kefir-derived microorganisms. Many *in vitro*

studies have been carried out to evaluate the possibility that kefir comprises probiotic strains, i.e., live microorganisms that when administered in adequate amounts confer a health benefit to the host (Hill et al. 2014; Vinderola et al. 2017), including analyses of survivability in the gastrointestinal system, colonization of the host gut, and interactions with other microorganisms (Yadav et al. 2016). To provide accurate information and best infer a significant relationship between kefir microorganisms and host health, we here focus only on strains that have been isolated from kefir.

Another recent study suggested that a reduced *Firmicutes/Bacteroidetes* ratio could be related to the observed antiobesity effect of kefir and that reduced populations of *Proteobacteria* and *Enterobacteriaceae* could be related to the alleviation of systemic inflammatory responses; however, a direct correlation has not yet been proven (Kim et al. 2015d). In addition, kefir was suggested to increase the level of genus *Prevotella*, which may be linked to the development of mental diseases such as autism, although this association also has not been scientifically confirmed (Jeong et al. 2017b).

### *2.1. Gastrointestinal survivability*

Well-known kefir microorganisms such as *Lactobacillus kefiranofaciens (including* subspecies *kefiranofaciens* and *kefirgranum), Lactobacillus kefiri, Lactobacillus plantarum, Lactococcus lactis, Leuconostoc mesenteroides,* and *Kluyveromyces marxianus* have been employed in several studies aiming to evaluate the gastrointestinal survivability of kefir-derived microorganisms. To evaluate gastric resistance, many studies have employed acidic conditions in which the pH value ranged from 2.0 to 5.0, and the microorganisms were cultured with or

without pepsin for 30 min to 4 h (Table 1). With only mild reduction in the population observed (1–2 log reduction), most of the kefir lactic acid bacteria species were able to survive in simulated gastric conditions, except for *L. lactis* (Table 1). Considering an average of 2–9 log reduction of probiotic strains in the gastric conditions, these results indicate strong survivability of kefir lactic acid bacteria in the gut (Bezkorovainy 2001; McFarland et al. 2005). *Kluyveromyces marxianus* is the only kefir yeast that has been studied to date, and its survivability in the gastric environment was found to be superior when compared to that of *Saccharomyces cerevisiae*, the most well-known yeast probiotic worldwide (You et al. 2006; Lara-Hidalgo et al. 2017).

With regards to intestinal resistance, kefir microorganisms were cultured in oxgall or bile salts at a concentration of 0.1–1.0% for 12–24 h (Table 1), which showed more variable but excellent survivability in the intestinal environment (Table 1). Microorganisms belonging to the genus *Lactobacillus* showed better survivability than those of the genus *Lactococcus*, showing 1.3–2.6 log reduction (Table 1). Interestingly, kefir yeast showed very strong survivability in an artificial intestinal environment, in which 97.2% of the tested cells survived in a 1% bile salt-supplemented medium (Table 1).

Based on the results, it appears that kefir microorganisms have stronger resistance to the gastrointestinal environment than observed for traditional probiotic species (Rosa et al. 2017). Although the origin of kefir grain is still not clear, it is believed that kefir microorganisms are derived from the sheep or goat intestinal microbiota (Otles and Cagindi et al. 2003; Gaware et al. 2011). In addition, considering that the pH of kefir during fermentation varies from 3.0 to 5.0 (optimum pH 4.6) with high concentrations of organic acids, including lactic acid, acetic acid,

 

and carbonic acid, it could be inferred that kefir provides a natural selective environment for acid-resistant strains (Farnworth et al. 2003).

In addition, kefir microorganisms tend to produce protective molecules, including surface (S) layer proteins and exopolysaccharides, which could improve their survivability in harsh environments (Garrote et al. 2004; Jeong et al. 2017a). S layers are the outermost proteinaceous cell envelope structures and, in lactobacilli, S-layer proteins have been reported on many but not all species (Hynönen et al. 2013). It has been reported that the presence of the S-layer protein decreases the susceptibility of lactobacilli to gastric and pancreatic juice (Frece et al. 2005). In addition, exopolysaccharides is a polymer produced by some bacteria either on the cell surface or in the surrounding circumstance as a muco-substance (Ramchandran et al. 2009). For bacteria, the production of exopolysaccharide is a well-accepted strategy for survival in harmful environments such as low water activity, low acidity, and other stress conditions (Torino et al. 2001). The production of these protective compounds could be an important strategy for survival in both harsh physicochemical condition of kefir and the host gut.

*2.2. Colonization of host gut*

Compared to survivability, there have been relatively less studies focusing on colonization of kefir microorganisms in the host gastrointestinal system, and only a handful of studies have investigated the colonization of kefir microorganisms in the host gastrointestinal tract (Table 2). Colonization of kefir microorganisms in the host gut plays an essential role by exerting competitive exclusion of harmful microbiota (Bourrie et al. 2016). Most studies have focused on the colonization properties of kefir microorganisms, including epithelial adhesion,

autoaggregation, cell surface hydrophobicity, and *in vivo* excretion (Table 2). For example, lactic acid bacteria isolated from kefir showed adhesion to the intestinal epithelium, autoaggregation, and cell hydrophobicity (Santos et al. 2003; Garrote et al. 2004; Golowczyc et al. 2008; Zheng et al. 2013; Xing et al. 2017). Moreover, recent studies demonstrated that kefir microorganisms could be detected at 2 or 3 weeks after inoculation in the animal gastrointestinal tract, indicating their successful colonization (Zheng et al. 2013; Xing et al. 2017).

However, no studies have investigated the colonization of kefir yeast. With increasing global interest in the development and evaluation of probiotic yeasts, the colonization traits of kefir yeast should be evaluated toward development of a single probiotic agent (Czerucka et al. 2007). In addition, there has been no study demonstrating that kefir microorganisms have better adhesion ability to intestinal epithelium when compared to other probiotic strains although kefir microorganisms are believed to be originated from intestine as mentioned above. Future studies should address these limitations to understand the modulatory effects of kefir on the host gut microbiota and mycobiota.

### *2.3. Interaction with other microorganisms*

*In vitro* interactions of kefir or kefir-derived microorganisms with other microorganisms have been extensively investigated for decades (Table 3). Kefir showed a positive interaction with one of the most representative beneficial residents in the host gut, *Bifidobacterium* spp., which could also be described as a bifidogenic effect (Serafini et al. 2014). In addition, the kefir yeast *Kluyveromyces marxianus* showed a similar effect to *Bifidobacterium* spp., further highlighting

its function as a probiotic (Rada 1997; Maccaferri et al. 2012). These effects were also extended to *in vivo*, which will be discussed in section 3.

In addition, the antimicrobial activity of kefir and its microorganisms is one of the most extensively investigated topics among studies focusing on the functionality of kefir (Bourrie et al. 2016). Kefir shows broad-spectrum and powerful antimicrobial activity against pathogenic bacteria, yeasts, fungi, protozoa, and viruses (Table 3). Indeed, this is one of the hallmark characteristics of kefir or its microbiota, which can inhibit many pathogenic microorganisms simultaneously with potency comparable to that of conventional antibiotics (Bourrie et al. 2016; Kim et al. 2016). In addition, a recent study reported that kefir also inhibited the growth of intestinal carcinogenic bacteria in a dose-dependent manner (Guzel-Seydim et al. 2016). These could be attributable to the complex and rich antimicrobial substances produced during kefir fermentation, including organic acids, alcohol, antimicrobial exopolysaccharides, carbon dioxides, and bacteriocin (Powell et al. 2007; Bourrie et al. 2016; Kim et al. 2016; Jeong et al. 2017a). Based on these interactions with other microorganisms, kefir administration exerts a powerful and broad-spectrum modulatory effect on the host gut microbiota.

## 3. Modulation of host gut microbiota and mycobiota by kefir and its microorganisms

Along with the increasing recognition of the gut microbiota in disease and health status of the host in the last few decades, kefir and its microorganisms have been investigated for their potential to positively modulate the host gut microbiota (Table 4). The early phase of this research focused on traditional culture methods to study the gut microbiota, which have been

gradually replaced with targeted metagenomics approaches such as quantitative real-time polymerase chain reaction (PCR) and semi-targeted diversity analysis tools, including PCR-denaturing gradient gel electrophoresis. More recently, untargeted metagenomics approaches have been introduced, including next-generation sequencing technology, which has helped to reveal many interesting and novel features of kefir (Table 4).

The essential beneficial health effects of kefir and its microbiota can be attributed to their ability to increase the populations of beneficial microorganisms such as *Lactobacillus, Lactococcus,* and *Bifidobacterium* in the host gut (Marquina et al. 2002; Liu et al. 2006; Yaman et al. 2006; Wang et al. 2009; Zheng et al. 2013; Carasi et al. 2015; Kim et al. 2015d; Hamet et al. 2016; Jeong et al. 2017b; Kim et al. 2017a; Kim et al. 2017b; Xing et al. 2017). This effect is largely attributed to the introduction of kefir lactic acid bacterial species or strains into the host gastrointestinal tract (Bourrie et al. 2016). Considering that kefir contains very high levels of lactic acid bacteria, ranging from 8 to 10 log CFU/ml, and their excellent survivability and colonization properties, it is not surprising that kefir can increase the lactic acid bacterial population in the host (Sarkar 2007; Guzel-Seydim et al. 2011; Kim et al. 2015d).

The increase of *Bifidobacterium* could be attributable to another mechanism by which the growth of resident *Bifidobacterium* in the host gut is promoted (Bourrie et al. 2016), because the presence of *Bifidobacterium* in kefir is not consistent among kefir grains – although *Bifidobacterium* has been detected in some kefir samples or added intentionally into kefir for improving the functionality (González-Sánchez et al. 2010; Guzel-Seydim et al. 2011; Kim et al, 2015c). Indeed, Hamet et al. (2016) found that kefiran, the major exopolysaccharide in kefir, showed a bifidogenic effect in an animal model, suggesting that kefir-originated bioactive

compounds could promote the indigenous *Bifidobacterium* population. It is known that kefiran administration increased the number of goblet cells which produce mucin on the surface of the intestinal lumen (Medrano et al. 2011). Increased mucin availability in the intestine could result in the bifidogenic effect by kefiran, as mucin is a well-known nutritional source for *Bifidobacterium* (Pokusaeva et al. 2011).

In addition to providing a favorable condition for the beneficial microbial population, kefir and its microorganisms inhibit the growth of opportunistic pathogens in the host gut (Marquina et al. 2002; Liu et al. 2006; Yaman et al. 2006; Kim et al. 2015d; Jeong et al. 2017b; Kim et al. 2017a; Xing et al. 2017; Zhang et al. 2017). For example, Kim et al. (2015d) evaluated the effects of kefir administration on chow diet-fed mice, revealing that 3 weeks of kefir administration significantly reduced the *Proteobacteria* and *Enterobacteriaceae*, which consists of a large family of gram-negative bacilli that includes many pathogens such as *Salmonella, Escherichia coli, Yersinia, Klebsiella, Shigella, Proteus, Enterobacter, Serratia,* and *Citrobacter* (Buffie et al. 2013). Consistent results were observed using different types of analysis tools. Marquina et al. (2002) and Yaman et al. (2006) demonstrated a decrease of the population of *Enterobacteriaceae* in mice and goslings using a culture method. Jeong et al. (2017b) also reported a decrease of Proteobacteria, Enterobacteriaceae, and *Clostridium* cluster I following the administration of *Lactobacillus kefiranofaciens* DN1, based on group-specific real-time PCR analysis. In addition, Xing et al. (2017) employed next-generation sequencing to analyze the modulatory effect of *L. kefiranofaciens* on host gut microbiota, revealing that *Proteobacteria* were reduced after 2 weeks.

This phenomenon of lactobacilli-*Enterobacteriaceae* antagonism is a well-established example of gut microbial interaction, in which as the proportion of lactobacilli in the host gut increases, the population of *Enterobacteriaceae* decreases, and vice versa (Reid et al. 1990; Juven et al. 1991; Yaman et al. 2006; Kim et al. 2015d). As enriched lactic acid bacteria acidify the host gut by producing a variety of organic acids, this provides an unfavorable environment for acid-sensitive *Enterobacteriaceae* (Edelson-Mammel et al. 2006; Dharmasena et al. 2016). In addition, kefir lactic acid bacteria compete with *Enterobacteriaceae* for nutrition and adhesion sites, and produce a bacteriocin or antimicrobial exopolysaccharides against them (Powell et al. 2007; Miao et al. 2014; Jeong et al. 2017b). Other opportunistic pathogens such as *Clostridium, Pasteurella, Flexispira,* and *Bacteroides* are also inhibited by the consumption of kefir and its constituent microorganisms (Marquina et al. 2002; Liu et al. 2006; Jeong et al. 2017b; Xing et al. 2017; Zhang et al. 2017).

Overall, we could conclude that kefir has broad and significant impacts on the gut bacterial population. It is noteworthy that although single probiotic agents generally colonize the host intestine, changes of the gut microbiota are not always guaranteed as a consequence (Fuller et al. 1997; Gareau et al. 2010; Hemarajata et al. 2013). Given the multiple advantages of kefir to host health, powerful improvement of the gut microbiota by kefir consumption could promote its reputation as a high-functional probiotic food.

Besides a focus on the gut bacterial population, a limited number of studies have also investigated the impact of kefir and its microorganisms on the host gut mycobiota (Marquina et al. 2002; Yaman et al. 2006; Kim et al. 2015d; Kim et al. 2017b). Although the gut mycobiota has attracted emerging interest due to its role in host health and balance with the bacterial

 

community (Underhill et al. 2014) and kefir is a representative lactic acid bacteria-yeast fermented milk, research on the gut mycobiota is still new (Kim et al. 2015c; Kim et al. 2017b; Kim et al. 2018). Marquina et al. (2002) attempted to analyze the number of total viable yeasts in mice after kefir consumption, but failed to enumerate any viable yeast by traditional culture methods. Subsequently, Yaman et al. (2006) evaluated the number of total yeasts of geese after kefir consumption and found an insignificant change when compared to the control group. About ten years later, Kim et al. (2015d) employed a group-specific real-time PCR method to quantify the yeast population in fecal samples, revealing that kefir-consuming mice had a significantly high level of yeast. In addition, the change of gut mycobiota was investigated by targeted and untargeted metagenomics approaches, indicating a significant increase of the yeast population, especially for the genus *Kluyveromyces* and class Saccharomycetes in high-fat diet-fed mice (Kim et al. 2017b). These findings are attributable to the fact that kefir contains a huge population of *Kluyveromyces* (one of the major kefir yeast (more than 90% of kefir mycobiota) is *Kluyveromyces marxianus*) and *Saccharomyces* (*Saccharomyces cerevisiae, Saccharomyces unisporus*, and *Saccharomyces exiguus*) (Kim et al. 2017b). However, it remains unclear whether this result is only due to the simple summation of kefir yeast to the host gut yeast population, or if they compete with members of the indigenous pathogenic yeast population, including *Candida albicans, Candida stellatoidea, Candida tropicalis,* and *Candida krusei,* which have a negative relationship with kefir *in vitro* (Cevikbas et al. 1994b; Rodrigues et al. 2005; Silva et al. 2009). Nevertheless, it is obvious that kefir is a rare type of fermented product with potential to modulate the mycobiota as well as the microbiota. Further studies are needed to obtain in-depth

knowledge on the change of gut mycobiota by kefir and its relationship with host health and disease status.

The main limitation of investigating the effect of kefir on host gut microbiota is that most studies estimate the microbiota population from fecal samples due to the ease of sampling. Although the fecal microbiota is considered as an acceptable surrogate of the gut microbiota, future studies should attempt to conduct these analyses with intestinal tissue samples or biopsy to best capture the intestinal ecology (distribution and colonization) of kefir microorganisms (Sekirov et al. 2010; Hamet et al. 2016). In addition, many studies have been carried out with animal models, including mice, rat, and geese, which might show variable modulatory effects on the gut microbiota compared to the situation in humans.

Next generation sequencing technology enables comprehensive microbial community analysis of food matrices with undefined microbiota, and subsequent changes of the host gut microbiota following the consumption of the foods (Zamberi et al. 2016; Cao et al. 2017). However, some limitations are derived from this platform. Although next-generation sequencing community analysis has been popularized in many countries, many of the reference libraries do not contain sequence information of kefir microorganisms, and there is difficulty in matching the operational taxonomic unit to the reference sequence using only restricted region of 16S rDNA sequence (usually one or two region of V1-V4 due to the short sequencing reads) (Shin et al. 2016). This does withdraw to track the change of the host gut microbiota at a species- and subspecies level (Sekirov et al. 2010; Lagier et al. 2012). More specifically, the colonization of major kefir species such as *Lactobacillus kefiranofaciens, Lactobacillus kefiri*, and *Lactobacillus parakefiri* could not be readily evaluated due to poor taxonomic assignments (Kim et al. 2017b). To

overcome this, species-specific analysis methods such as qPCR or immunoassays should be accompanied with the NGS platform (Garrote et al. 2005; Kim et al. 2015a; Kim et al. 2018), or the platforms which could generate long read length such as Pacific Biosciences, Ion Torrent, and Oxford Nanopore could be applied for the community analysis targeting full-length 16S rDNA sequences to allow more high-resolution taxonomic assignments (Weinstock 2012; Mardis 2017). By overcoming these drawbacks, we could obtain more complete knowledge on the modulatory effect of kefir and its microbiota on the host gut microbiota and mycobiota.

## 4. Link between the modulatory effect of kefir on the host gut microbiota and health benefits

It is well known that the gut microbiota is a virtual organ system that is important for the maintenance of health and well-being (Gareau et al. 2010). Dysbiosis in this consortium induced impaired host homeostasis, which ultimately led to the emergence of clinical diseases (Gibson et al. 1995; Fuller et al. 1997; Gareau et al. 2010). We have here provided a review on the emerging evidence of kefir to improve gut microbiota by increasing beneficial and decreasing harmful microbiota. In addition, many studies have reported the beneficial health effects of kefir consumption in the host, which have been experimentally and clinically demonstrated (Merenstein et al. 2009; Ostadrahimi et al. 2015; Fathi et al. 2017). However, only a few studies have suggested a relationship between these observed health effects and modulation of the intestinal microbiota (Kim et al. 2017b).

For example, kefir consumption was found to prevent the development of obesity and non-alcoholic fatty liver diseases in high-fat diet-fed mice, based on a significant decrease in body

weight gain, adiposity, and hepatic accumulation of lipid droplets (Kim et al. 2017b). Moreover, correlation analysis indicated that these effects were significantly related to the kefir-induced increase in the populations of *Lactobacillus* and *Candida*, which promoted fatty acid oxidation in the adipose tissues and liver (Kim et al. 2017b). In addition, *Lactobacillus kefiri* isolated from the same kefir grain increased the population of *Lactobacillus* in high-fat diet-fed mice, and prevented obesity by dual functions, including direct reduction of cholesterol in the lumen and upregulation of fatty acid oxidation in adipose tissues (Kim et al. 2017a). In humans, Fathi et al. (2017) found that kefir consumption improved the serum lipid profile in overweight or obese women, which led to several hypotheses such as that kefir microorganisms in the gut might produce short-chain fatty acids and bile salt hydrolases, assimilate the exogenous cholesterol, or regulate gene expression to alter systemic lipid metabolism; however, unveiling of the exact mechanisms requires further study.

In addition, Jeong et al. (2017b) demonstrated the constipation-alleviating effects of kefir, suggesting that the decreased proportion of members of the genus *Clostridium* by *Lactobacillus kefiranofaciens* may play a role in improving the defecation of mice. With respect to anti-oxidative effects, Zhang et al. (2017) proved that the administration of *Lactobacillus plantarum* YW11 from kefir increased the short-chain fatty acid-producing genera *Blautia, Butyricicoccus,* and *Allobaculum*, resulting in an increased level of short-chain fatty acids in the gut. This was significantly correlated with reduced oxidative stresses in a D-galactose-induced aging mouse model. In addition, Tang et al. (2016) found that *Lactobacillus plantarum* MA2 from kefir successfully colonized the murine gut, decreased the serum and hepatic malondialdehyde levels, and increased the antioxidative enzyme activities, thereby exerting antioxidative effects. Along

with these examples, some other connections between the health benefits and modulation of gut microbiota by kefir are shown in Table 5. Further studies are needed to more comprehensively investigate the link between the modulation of gut microbiota by kefir and its beneficial health effects to elucidate the in-depth mechanisms.

## 5. Conclusion

The human and animal intestine is the largest reservoir of microorganisms in the body, and these microbiota have a close relationship with host health and disease states. By evaluating and comparing the findings from recent studies, it is concluded that kefir exerts broad-spectrum and significant effects in the modulation of gut microbiota and mycobiota, based on the excellent survivability, colonization ability, and microbial interaction of the microorganisms in kefir as well as its rich bioactive compounds. Although emerging studies have demonstrated that the beneficial effects of kefir are largely related to the modulation of gut microbiota, future studies are still needed to elucidate the systematic mechanism underlying the health benefits of kefir.

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**Conflict of interest**

The authors declare no conflict of interest.

 

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Table 1. Gastrointestinal survivability of kefir microorganisms.

| Properties | Species | Survivability (condition) | References |
|---|---|---|---|
| Gastric resistance | *Lactobacillus kefiranocaciens* | 4/4 strains survived (pH2.5, 4 h) | Santos et al. 2003 |
| | | Variable - growth, partial survival, or no survival (pH2.5, pepsin 1000IU/ml, 37˚C, 2 h) | Jeong et al. 2017b |
| | | Growth, >50% survival (pH2.5, pepsin 1000IU/ml, 37˚C, 2 h) | Kim et al. 2017a |
| | | 2 log reduction (pH3.5, 30˚C, 3 h) | Xing et al. 2017 |
| | *Lactobacillus kefiri* | 19/19 strains survived (pH2.5, 4 h) | Santos et al. 2003 |
| | | Variable – growth or >50% survival (pH2.5, pepsin 1000IU/ml, 37˚C, 2 h) | Kim et al. 2017a |
| | | 1.2 log reduction (pH2.0, 37˚C, 3 h) | Zheng et al. 2013 |
| | *Lactobacillus plantarum* | 41.1 to 67.4% survival (pH2.5, 3 h) | Golowczyc et al. 2008 |
| | | 1.2 log reduction (pH2.0, 37 ˚C, 3 h) | Zheng et al. 2013 |
| | *Lactobacillus acidophilus* | 0.9 log reduction (pH2.0, 37 ˚C, 3 h) | Zheng et al. 2013 |
| | *Lactococcus lactics* | Variable - <50% survival or no survival | Kim et al. 2017a |
| | *Leuconostoc mesenteroides* | <50% survival (pH2.5, pepsin 1000IU/ml, 37 ˚C, 2 h) | Kim et al. 2017a |
| | *Kluyveromyces marxianus* | 1 log reduction (pH2.0, 37 ˚C, 30 min) | You et al. 2006 |
| Intestinal resistance | *Lactobacillus kefiranocaciens* | 4/4 strains survived (0.3% oxgall, 24 h) | Santos et al. 2003 |
| | | Variable - growth, partial survival, or no survival (pH7.0, 0.3% oxgall, 24 h) | Jeong et al. 2017b |
| | *Lactobacillus kefiri* | 15/19 strains survived (0.3% oxgall, 24 h) | Santos et al. 2003 |
| | | Variable – growth or <50% survival (0.3% oxgall, pH7.0, 37˚C, 24 h) | Kim et al. 2017a |
| | | 2.6 log reduction (0.3% oxgall, 37 ˚C, 12 h) | Zheng et al. 2013 |
| | *Lactobacillus plantarum* | Variable | Zheng et al. 2013 |
| | | 1.5 log reduction (0.3% oxgall, 37˚C, 12 h) | Zheng et al. |

| | | |
|---|---|---|
| | | 2013 |
| *Lactobacillus acidophilus* | 1.3 log reduction (0.3% oxgall, 37˚C, 12 h) | Zheng et al. 2013 |
| *Lactococcus lactics* | Variable - >50% survival or <50% survival (0.3% oxgall, pH7.0, 24 h, 37 ˚C) | Kim et al. 2017a |
| *Leuconostoc mesenteroides* | Variable - >50% survival or <50% survival (0.3% oxgall, pH7.0, 24 h, 37 ˚C) | Kim et al. 2017a |
| *Kluyveromyces marxianus* | 97.2% survival (1% bile salts, 37 ˚C, 6 h) | You et al. 2006 |

Table 2. Colonization of kefir microorganisms in the host gastrointestinal tract.

| Properties | Species | Results | References |
|---|---|---|---|
| Epithelial adhesion | *Lactobacillus kefiranocaciens* | 4/4 strains adhere to Caco-2 cell | Santos et al. 2003 |
| | *Lactobacillus kefiri* | 19/19 strains adhere to Caco-2 cell | Santos et al. 2003 |
| | | Adhesion to Caco-2 cells | Garrote et al. 2004 |
| | *Lactobacillus plantarum* | 0.97 to 10.50 % adhesion to Caco-2/TC-7 cells | Golowczyc et al. 2008 |
| | *Lactobacillus parakefiri* | Adhesion to Caco-2 cells | Garrote et al. 2004 |
| Autoaggregation | *Lactobacilllus kefiranofaciens* | 27.8% | Xing et al. 2017 |
| | *Lactobacillus plantarum* | None | Golowczyc et al. 2008 |
| | *Lactobacillus kefiri* | Autoaggregation | Garrote et al. 2004 |
| | *Lactobacillus parakefiri* | Autoaggregation | Garrote et al. 2004 |
| Cell surface hydrophobicity | *Lactobacillus kefiranofaciens* | 79.9% | Xing et al. 2017 |
| | *Lactobacillus plantarum* | 0.3 to 5.8% | Golowczyc et al. 2008 |
| *in vivo* duration of excretion in feces | *Lactobacillus kefiranofaciens* | 3 weeks after single inoculation | Xing et al. 2017 |
| | *Lactobacillus kefiri* | 2 weeks after 4 weeks of successive inoculation | Zheng et al. 2013 |
| | *Lactobacillus plantarum* | 2 weeks after 4 weeks of successive inoculation | Zheng et al. 2013 |
| | *Lactobacillus acidophilus* | 2 weeks after 4 weeks of successive inoculation | Zheng et al. 2013 |

Table 3. *In vitro* microbial interactions of kefir and kefir-derived microorganisms with other microorganisms.

| Interaction | Materials | Target microorganisms | Classification (Phylum - family) | References |
|---|---|---|---|---|
| Positive | Kefir | *Bifidobacterium spp.* | Actinobacteria – Bifidobacteriaceae | Serafini et al. 2014 |
| | *Kluyveromyces marxianus* | *Bifidobacterium spp.* | Actinobacteria – Bifidobacteriaceae | Rada 1997; Maccaferri et al. 2012 |
| Negative | Kefir | *Bacillus cereus* | Firmicutes - Bacillaceae | Garrote et al. 2000; Ulusoy et al. 2007; Ismaiel et al. 2011; Kim et al. 2016 |
| | | *Staphylococcus aureus* | Firmicutes – Staphylococcaceae | Garrote et al. 2000; Carvalho 2004; Rodrigues et al. 2005; Ulusoy et al. 2007; Ismaiel et al. 2011; Kim et al. 2016; |
| | | *Listeria monocytogenes* | Firmicutes – Listeriaceae | Santos et al. 2003; Rodrigues et al. 2005; Ulusoy et al. 2007 |
| | | *Escherichia coli* | Proteobacteria – Enterobacteriaceae | Garrote et al. 2000; Santos et al. 2003; Carvalho 2004; Rodrigues et al. 2005; Ulusoy et al. 2007; Silva et al. 2009; Ismaiel et al. 2011; Londero et al. 2011; Kim et al. 2016 |
| | | *Salmonella spp.* | Proteobacteria – Enterobacteriaceae | Garrote et al. 2000; Santos et al. 2003; Carvalho 2004; Rodrigues et al. 2005; Ulusoy et al. 2007; Silva et al. 2009; Londero et al. 2011; Kim et al. |

| | | |
|---|---|---|
| | | 2016 |
| *Pseudomonas aeruginosa* | Proteobacteria – Pseudomonadaceae | Rodrigues et al. 2005; Ismaiel et al. 2011; Kim et al. 2016 |
| *Cronobacter sakazakii* | Proteobacteria – Pseudomonadaceae | Kim et al. 2015b |
| *Campylobacter jejuni* | Proteobacteria – Campylobacteraceae | Zacconi et al. 2003 |
| *Shigella flexineri/sonnei* | Proteobacteria – Enterobacteriaceae | Garrote et al. 2000; Santos et al. 2003; Carvalho 2004; Silva et al. 2009 |
| *Yersinia enterocolitica* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |
| *Helicobacter pylori* | Proteobacteria – Helicobacteriaceae | Carvalho 2004 |
| *Bacillus subtilis* | Firmicutes – Bacillaceae | Cevikbas et al. 1994a; Ryan 1996 |
| *Enterococcus faecalis* | Firmicutes – Enterococcaceae | Ryan 1996; Zanirati et al. 2015 |
| *Clostridium difficile* | Firmicutes – Clostridiaceae | Rea et al. 2007; Bolla et al. 2013 |
| *Klebsiella pneumonia* | Proteobacteria – Enterobacteriaceae | Cevikbas et al. 1994a |
| *Proteus vulgaris* | Proteobacteria – Enterobacteriaceae | Cevikbas et al. 1994a |
| *Streptococcus pyogenes* | Firmicutes – Streptococcaceae | Rodrigues et al. 2005 |
| *Streptococcus salivarius* | Firmicutes – Streptococcaceae | Rodrigues et al. 2005 |
| *Clostridium sporogenes* | Firmicutes – Clostridiaceae | Ryan 1996 |
| *Clostridium tyrobutyricum* | Firmicutes – Clostridiaceae | Ryan 1996 |
| *Enterococcus faecium* | Firmicutes – Enterococcaceae | Ryan 1996 |
| *Listeria innocua* | Firmicutes – Listeriaceae | Ryan 1996 |
| *Shigella sonnei* | Proteobacteria – Enterobacteriaceae | Golowczyc et al. 2008 |
| *Bacillus thuringiensis* | Firmicutes – Bacillaceae | Miao et al. 2014 |

 

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| | | *Shigella dysenteriae* | Proteobacteria – Enterobacteriaceae | Miao et al. 2014 |
| | | *Fusobacterium nucleatum* | Fusobacteria - Fusobacteriaceae | Guzel-Seydim et al. 2016 |
| | | *Candida albicans* | Ascomycota – Saccharomycetaceae | Rodrigues et al. 2005; Silva et al. 2009 |
| | | *Aspergillus flavus* | Ascomycota – Trichocomaceae | Miao et al. 2014 |
| | | *Aspergillus niger* | Ascomycota – Trichocomaceae | Miao et al. 2014 |
| | | *Rhizopus nigricans* | Zygomycota – Mucoraceae | Miao et al. 2014 |
| | | *Penicillium glaucum* | Ascomycota – Trichocomaceae | Miao et al. 2014 |
| | | *Candida stellatoidea* | Ascomycota – Saccharomycetaceae | Cevikbas et al. 1994a |
| | | *Candida tropicalis* | Ascomycota – Saccharomycetaceae | Cevikbas et al. 1994a |
| | | *Candida krusei* | Ascomycota – Saccharomycetaceae | Cevikbas et al. 1994a |
| | | *Saccharomyces cerevisiae* | Ascomycota – Saccharomycetaceae | Cevikbas et al. 1994a |
| | | *Rhodotorula glutinis* | Basidiomycota - Sporidiobolaceae | Cevikbas et al. 1994a |
| | | *Candida glabrata* | Ascomycota – Saccharomycetaceae | Cevikbas et al. 1994a |
| | | *Rotavirus* | Unassigned – Sedoreovirinae | Lee et al. 2011 |
| | | *Giardia intestinalis* | Metamonada – Hexamitidae | Franco et al. 2013 |
| | *Lactobacillus kefiranofaciens* | *Escherichia coli* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |
| | | *Listeria monocytogenes* | Firmicutes – Listeriaceae | Santos et al. 2003; Jeong et al. 2017a |
| | | *Salmonella spp.* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003; Jeong et al. 2017a |
| | | *Shigella flexineri* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |
| | | *Yersinia enterocolitica* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |
| | *Lactobacillus kefiri* | *Escherichia coli* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |

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|---|---|---|---|---|
| | | *Listeria monocytogenes* | Firmicutes – Listeriaceae | Santos et al. 2003 |
| | | *Salmonella spp.* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |
| | | *Shigella flexineri* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |
| | | *Yersinia enterocolitica* | Proteobacteria – Enterobacteriaceae | Santos et al. 2003 |
| | | *Cronobacter sakazakii* | Proteobacteria – Enterobacteriaceae | Kim et al. 2015b |
| | | *Clostridium difficile* | Firmicutes – Clostridiaceae | Carasi et al. 2012 |
| | *Lactobacillus plantarum* | *Salmonella spp. Shigella flexineri/sonnei Escherichia coli* | Proteobacteria – Enterobacteriaceae | Golowczyc et al. 2008 |
| | *Lactococcus lactis* | *Clostridium difficile* | Firmicutes – Clostridiaceae | Bolla et al. 2013 |

Table 4. Modulation of gut microbiota by kefir and kefir-derived microorganisms.

| No | Inoculum | Animal | Duration | Administration | Analysis | Samples | Modulation of gut microbiota | | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Increased | Decreased | |
| 1 | Kefir | Female BALB/c mice, 4-week old, normal diet | 3 weeks | Kefir 0.2 mL per head, orally, twice a day | Group specific real-time PCR | Feces | - Bacteroidetes<br>- Lactobacillus<br>- Lactococcus<br>- Total yeast | - Firmicutes<br>- Proteobacteria<br>- Enterobacteriaceae<br>- Firmicutes/ Bacteroidetes ratio | Kim et al. 2015d |
| 2 | Kefir | Male C57BL/6 mice, 4-week old 60% high fat diet | 12 weeks | Kefir 0.2 mL per head, orally, twice a day | Group specific real-time PCR Next generation sequencing (GS-FLX) | Feces | - Total yeast<br>- *Lactobacillus*<br>- *Lactococcus*<br>- *Candida* spp.<br>- Saccharomycetes | - *Bacteroides fragilis*<br>- Firmicutes/ Bacteroidetes ratio | Kim et al. 2017b |
| 3 | Kefir | Female BALB/c mice, 5-week old, normal diet | 4 weeks | 5mL/kg, orally, once a day | Culture methods | Feces | - *Lactobacillus spp.*<br>- *Bifidobacteroum spp.* | - *Clostridium spp.* | Liu et al. 2006 |
| 4 | Kefir | Female goslings, 2-week | 6 weeks | Drinking water added by kefir at 0.20% | Culture methods | Feces | - *Lactobacillus spp.* | - *Total aerobic bacteria*<br>- *Coliforms*<br>- | Yaman et al. 2006 |

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|---|---|---|---|---|---|---|---|---|---|
| | | old, normal diet | | and 0.50% (v/v). | | | | *Enterobacteriaceae* | |
| 5 | Kefir | Female Swiss mice, 7-month old, normal diet | 7 months | 140 g lyophilized kefir powder was mixed with 200 g of the normal chow diet | Culture methods | Small intestine and large intestine | - *Streptococci*<br>- *Lactic acid bacteria* | - Sulfite-reducing clostridia<br>- *Enterobacteriaceae* | Marquina et al. 2002 |
| 6 | *Lactobacillus kefiranofaciens* | Female BALB/c mice, 4-week old, normal diet | 2 weeks | $2 \times 10^8$ cfu of *L. kefiranofaciens* DN1 per head, orally, once a day | Group specific real-time PCR | Feces | - Total bacteria<br>- *Firmicutes*<br>- *Bacteroidetes*<br>- Lactobacillus<br>- Prevotella | - *Proteobacteria*<br>- Enterobacteriaceae<br>- Clostridium cluster I | Jeong et al. 2017b |
| 7 | *Lactobacillus kefiranofaciens* | Male Balb/c mice, 16-week old, normal diet | 2 weeks | $4 \times 10^7$ cfu of *L. kefiranofaciens* XL10 per head, orally, once a day | Next generation sequencing (Illumina MiSeq system) | The contents of ileum and colon | - Lactobacillus<br>- Bifidobacteriaceae<br>- Ruminococcaceae | - Proteobacteria<br>- Pasteurella<br>- Prevotella<br>- Bacteroides<br>- Rikenellaceae | Xing et al. 2017 |
| 8 | *Lactobacillus kefiri* | Male C57BL/6 mice, 4-week old 60% high | 6 weeks | $2 \times 10^8$ cfu of *Lactobacillus kefiri* DH5 per head, orally, once a | Group specific real-time PCR | Feces | - *Lactobacillus*<br>- *Lactococcus* | - *Proteobacteria*<br>- Enterobacteriaceae | Kim et al. 2017a |

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|---|---|---|---|---|---|---|---|---|---|
| | | fat diet | | day | | | | | |
| 9 | *Lactobacillus kefiri* | Male Swiss albino mice, 4-week old, normal diet | 3 weeks | $10^8$ CFU of *L. kefiri* CIDCA 8348 per head, orally, once a day | PCR-DGGE Group specific real-time PCR | Feces | - *Lactobacillus spp.* | | Carasi et al. 2015 |
| 10 | *Lactobacillus plantarum* | Male Sprague–Dawley rat, 4-week old, 10% high cholesterol diet | 5 weeks | $10^{11}$ cfu of *L. plantarum* MA2 per head, orally, once a day | Culture methods | Feces | - *Lactobacillus spp.* - *Bifidobacteroum spp.* | | Wang et al. 2009 |
| 11 | *Lactobacillus plantarum* | Male Institute of Cancer Research mice, 8-week old, normal diet | 12 weeks | Fermented milk 20 mL/kg of BW, once a day (8.41 log cfu of *L. plantarum* YW11 per mL of fermented milk) | Next generation sequencing (Illumina MiSeq system) | Feces | - *Blautia* - *Prevotella* - *Butyricicoccus* - *Allobaculum* | - *Flexispira* | Zhang et al. 2017 |
| 12 | *Lactobacillus* | Male Sprague- | 4 week | $10^9$ cfu/ml of *L.* | Culture | Feces | -*Total anaerobes* | - *Coliforms* | Zheng et al. |

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|---|---|---|---|---|---|---|---|---|---|
| | *acidophilus* | Dawley rats, 4-week old, normal diet | s | *acidophilus* LA15, 2 ml per head, once a day, orally | methods | | -*Lactobacillus* | | 2013 |
| 13 | *Lactobacillus plantarum* | Male Sprague-Dawley (SD) rats, 4-week old, normal diet | 4 weeks | $10^9$ cfu/ml of *L. plantarum* B23, 2 ml per head, orally, once a day | Culture methods | Feces | -*Total anaerobes* -*Lactobacillus* | - *Coliforms* | Zheng et al. 2013 |
| 14 | *Lactobacillus kefiri* | Male Sprague-Dawley rats, 4-week old, normal diet | 4 weeks | $10^9$ CFU/ml of *L. kefiri* D17, 2 ml per head, orally, once a day | Culture methods | Feces | -*Total anaerobes* -*Lactobacillus* | - *Coliforms* | Zheng et al. 2013 |
| 15 | *Kefiran* | Female BALB/c mice, 6-8-week old, normal diet | 3 weeks | 300mg of kefiran per liter of drinking water | PCR-DGGE, FISH, Flow cytometry | Distal colon contents, Feces | -*Bifidobacterium* | | Hamet et al. 2016 |

Table 5. Suggested connection of the health-promoting effects of kefir to modulation of the gut microbiota.

| Health claim | Gut microbiota-related mechanisms |
|---|---|
| Anti-obesity and hepatic steatosis | - Decreased *Firmicutes/Bacterodetes* ratio decreased the energy harvest<br>- Increased population of *Lactobacillus* and *Candida* upregulated fatty acid oxidation in liver and adipose tissue<br>- Decreased population of *Enterobacteriaceae* reduced systemic low-grade inflammation |
| Anti-constipation | - Increased *lactic acid bacteria* and *Bifidobacterium* acidify the intestinal environment to stimulate bowel movement<br>- Increased population of lactic acid bacteria produces polysaccharides which confer a smoothing effect.<br>- Decreased population of *Clostridium* reduces the constipation by unknown mechanisms |
| Anti-oxidation | - Increased *Lactobacillus*, *Blautia*, *Butyricicoccus,* and *Allobaculum* enriches short chain fatty acid in the intestine<br>- Increased *Lactobacillus* increases the antioxidative enzyme activities |
| Anti-inflammation | - Increased *Bifidobacterium* and *Lactobacillus* and decreased gram-negative bacteria (*Gammaproteobacteria and Enterobacteriaceae*) prevent the translocation of bacteria and bacterial toxin from the intestine to the mesenteric lymph node and bloodstream |
| Anti-diabetes | - Increased *Bifidobacterium*, *Lactobacillus*, and decreased *Clostridium* reduce the mucosal oxidative stress and inflammatory responses preventing the islet destruction and improving insulin intolerance. |