

Microbial Communities in Home-Made and Commercial Kefir

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Kefir is a popular traditional fermented dairy product in many countries. It has a complex and symbiotic culture made up of species of the genera *Leuconostoc*, *Lactococcus*, and *Acetobacter*, as well as *Lactobacilluskefirano**faciens* and *Lentilactobacillus kefir**i*. Though *kefir* has been commercialized in some countries, people are still traditionally preparing *kefir* at the household level. *Kefir* is known to have many nutritious values, where its consistent microbiota has been identified as the main valuable components of the product. Type 2 diabetes mellitus (T2DM) is a common diet-related disease and has been one of the main concerns in the world's growing population.

Kefir

T2DM

hypoglycemic

probiotics

Lactobacillus

1. Microbial Composition of Home-Made and Commercial Kefir

The microbial diversity of kefir has been thoroughly studied using culture-dependent techniques; however, in recent times, due to the application of sequence-based taxonomical tools, its microbial diversity has revealed more interesting findings among the homemade and the commercial varieties of kefir ^[1]. The widely reported microbiota present in *kefir* includes bacteria (lactic-acid bacteria, acetic-acid bacteria) and eukaryota (yeast and fungi) ^[2] make it a probiotic food product with a good source of probiotic microorganisms. Overall, the predominance of *Bacillota* (basonym: *Firmicutes*) ^[3] has been reported through various studies in both home-made and commercial *kefir* products ^[4], whereas the predominance of phylum *Actinomycetota* (basonym: *Actinobacteria*) ^[3] in the traditionally prepared *kefir* of Mexico has also been reported ^[5]. Metagenomics-based studies of *kefir* microbiota have been mostly studied using targeted amplicon sequencing and shotgun metagenomics ^{[6][7][8][9][10]}. So far, about 23 species (bacteria and eukaryota) have been reported from commercial *kefir* samples, among which *Streptococcus thermophilus*, *Lactococcus lactis*, and *Leuconostoc mesenteroides* are the main predominant species ^[9]. Among eukaryota, *Debaryomyces hansenii* and *Kluyveromyces marxianus* are the only two yeast species to have been reported ^[10]. Similarly, among the home-made *kefir* samples, the main predominant species belong to the lactic-acid bacterial group, where *Lactobacillus kefirano**faciens* and *Lentilactobacillus kefir**i* have been reported as the most abundant species through metagenomics (both amplicon and shotgun) studies ^[11]. Interestingly, in some varieties of *kefir* of Turkey, the predominance of *Bifidobacterium longum* is also reported ^[12]. To date, about 66 species (bacteria and eukaryota) have been reported from metagenomics studies from home-made *kefir* samples,

among which lactic-acid bacteria and acetic-acid bacteria are predominant [5][6][7][9][10][11][12][13][14][15][16][17][18][19]. Eukaryota (yeast and fungi) is also present to a significant degree in the home-made *kefir*, as compared to that of commercial *kefir*. However, homemade *kefir* shows the presence of many unwanted contaminants that would hamper the quality of the samples. From the above research studies, only the species viz., *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus*, and *Acinetobacter* sp. are found to be shared among the homemade and commercial *kefir*, and only one yeast *Kluyveromyces marxianus* has been reported from both the *kefir* varieties.

2. Hypoglycemic Properties of *Kefir*

Type 2 diabetes mellitus (T2DM) is a serious long-term disorder [20] that is primarily caused due to defective insulin secretion by pancreatic β -cells and the inability of insulin-sensitive tissues to respond to insulin [21], preventing the proper circulation of glucose for cell uptake [22]. It has been reported that some fermented milk products have hypoglycemic properties [23][24]. Biochemically, T2DM is mainly associated with glucose and lipid absorption in the human gut and is one of the main therapeutic measures to control the enzymes associated with these phenomena, such as lipid and carbohydrate hydrolyzing enzymes viz., lipases, and α -amylases [25]. Subsequently, it has also been shown that upon kefir administration, the reduction of α -amylase and lipases was significant [26]. *Kefir* exhibits a negative effect on α -amylase, one of the important enzymes that is regulated in the type two diabetes pathway [26], and reduces insulin resistance [27]. The intake of *kefir* has been demonstrated to have a great impact in the treatment of type 2 diabetes and increases the life span in treated rats [28][29] via the mechanisms that will be mentioned in following sections. Many probiotic bacteria have been shown to help ameliorate type 2 diabetes disorder through various means such as reducing insulin resistance and improving glucose homeostasis [30]. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate have been shown to be important targets in the treatment of type 2 diabetes [31], which has also been reported in *kefir* [19], showing positive colonic and immune modulation [32]. The MK-9 variant of menaquinone (Vitamin K₂) has been detected in *kefir* [33], which plays a role in improving insulin resistance and reducing type 2 diabetes by means of its anti-inflammatory and lipid-lowering effects [34]. Some studies on animal models and human clinical trials have been conducted to validate the hypoglycemic properties of both homemade and commercial *kefir*, which are reviewed below.

3. Animal Studies

Few animal models were investigated to validate the hypoglycemic properties of *kefir*. The blood glucose level of group 4 of rats was found to be lower than that of group 3 ($p < 0.001$) after feeding with *kefir*, indicating better control of oxidative stress and a decrease in the progression of diabetic nephropathy [35]. In another study, rats were divided into three different groups (the control group, fructose group, and fructose and *kefir* group), and fructose was given for 15 weeks, while *kefir* (1 mL/100 g/day) was given only for the last 6 weeks of the duration [36]. *Kefir* treatment improved insulin signaling in the liver via insulin receptor substrate 1 (IRS-1) and phospho-endothelial nitric oxide synthase (peNOS) [36]. The effect of *kefir* on metabolic syndrome was investigated [37]. Rats were divided into three different groups (negative control, without metabolic syndrome), positive control (with

metabolic syndrome), and *kefir* group (with metabolic syndrome) in a study. The fasting insulin, glucose, and HOMA- β values of the *kefir* group were found to be lower than in the positive control group. When compared before and after the study, the highest decrease in the AUC value was observed in the group fed with *kefir* [37]. The effects of *kefir* on oxidative stress in diabetic animals were studied, and it was observed that *kefir* may contribute to the better control of glycemia and oxidative stress, indicating its consumption to delay the progression of diabetic complications [38].

Based on an animal study, the effect of the combination of goat and soy milk on lipid profile, plasma glucose, glutathione peroxidase (GPx) activity, and the improvement of pancreatic β -cell in diabetic rats was noted [39]. It was found that *kefir* combination can maintain the serum triglyceride, decrease plasma glucose, increase GPx activity, and improve pancreatic β -cells [39]. The effects of the consumption of a prebiotic (catechin-rich wine grapeseed flour, GSF) and a probiotic (*Lactobacillus kefir* DH5) by obese mice on their hepatic steatosis were studied, and it was observed that the amelioration of high fat-induced hepatic steatosis after the consumption of GSF and *Lentilactobacillus kefir* was partially mediated via the alteration of cecum propionate and intestinal permeability [40]. Nurliyani et al. (2022) conducted another experiment to determine the effect of goat milk *kefir* supplemented with synbiotic *kefir* and goat milk *kefir* without probiotic *kefir* on blood glucose, haemoglobin A1c (HbA1c), and insulin-producing cells in rats fed a high-fat and high-fructose (HFHF) diet. There was a decrease in the HbA1c level of the group receiving symbiotic *kefir*, which improved the health of rats fed an HFHF diet [41].

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