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Review

Microbiological Exploration of Different Types of Kefir Grains

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Abstract: Many studies have been published lately verifying the probiotic character of kefir grains. Most of them focused on the benefits to human health through the consumption of fermented food with kefir grains. However, the challenge is to characterize and isolate specific probiotic microorganisms involved in the kefir microbiota. The main reason for this is that the food industry prefers to apply isolated probiotic microorganisms from kefir grains rather than kefir grains in order to produce respective fermented products with added value. Thus, modern molecular techniques such as polymerase chain reaction (PCR)-based amplification, new generation sequencing (NGS) or denaturing gradient gel electrophoresis (DGGE) analyses have been applied. Furthermore, this review emphasizes the latest outcomes regarding the health benefits of the consumption of foods fermented with kefir grains and particularly the isolation of microorganisms from kefir grains worldwide, some of them exhibiting probiotic properties.

Keywords: kefir grains; *Lactobacillus*; probiotics; novel foods; polymerase chain reaction

1. Introduction

Kefir is a traditional fermented dairy beverage, first consumed in Caucasus Mountains of Russia centuries ago. Nowadays, its popularity has been expanded worldwide and it is considered as a healthy product with high nutritional value in Europe, Asia, and South and North America [1,2]. It is a stimulating and self-carbonated beverage produced through fermentation of different types of milk such as sheep, cow, goat, etc. However, currently, alternative kefir drinks have been proposed, substituting milk and applying other carbohydrate sources, such as fruit juices, molasses, etc. [3,4].

The fermented kefir beverage contains white or yellow kefir grains which are insoluble in water; their size is irregular and the grains tend to stay on the bottom of the fermentation broth [5,6]. The size of kefir grains ranges between 0.3–3.5 cm in diameter and their typical chemical composition is: water (89–90% *w*/*w*), lipids (0.2% *w*/*w*), proteins (3.0% *w*/*w*), sugar (6.0% *w*/*w*), and ash (0.7% *w*/*w*) [7]. Kefir grains consist of a consortium of several exopolysaccharides, named kefiran, as well as various microorganisms [8]. Kefiran is a heteropolysaccharide (EPS) comprising glucose and galactose in high concentrations, and it is classified as a water-soluble glucogalactan exhibiting very good rheological properties and enhancing gel properties [9,10]

A wide variety of microorganisms, sharing symbiotic relationships, have been isolated from kefir microflora, including yeasts (*Kluyveromyces*, *Candida*, *Saccharomyces* and *Pichia*), lactic acid bacteria (LAB) (*Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Streptococcus*), and occasionally acetic acid bacteria [11,12].

Kefir grains are considered a multi-functional starter culture in the food industry since they have been applied in various fermented systems such as bread, cheese and milk with very promising results. It is noteworthy that kefir grains are feasible for upgrading cheese whey, which is a widely abundant waste from the dairy industry with a high organic load [13]. In addition, there are studies revealing that kefir grains can be successfully cultured in cheese whey in appropriate conditions, which results in the reduction of the cost of kefir grain culturing substituting synthetic mediums or even milk [4]. Likewise, the application of kefir grains as a starter culture is more attractive in the food industry since they can be easily cultured in cheese whey at a low cost [14].

The overall objective of this review, hence, is to find and assess data available in the literature regarding: (i) the main microorganisms that are isolated and characterized from different types of kefir grains worldwide with various methods; and (ii) the probiotic properties and health benefits of kefir grains.

2. Microbiology of Kefir Grains and Origin of Kefir Grains Worldwide

Many origins of kefir grains are reported in the literature, including Brazilian, Argentinean, Irish and Turkish kefir grains (Table 1). Even though, at first glance, the differences seem to be minor, in the last year noticeable differences have been revealed in the microbiological microflora through molecular analysis.

Table 1. Dominant LAB and yeasts isolated from kefir grains worldwide.

Origin Country	Dominant LAB	Dominant Yeasts	Reference
Taiwan	*Lactobacillus kefiranofaciens*	*Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Issatchenkia occidentalis*, *Kluyveromyces marxianus*,	[15]
Tibet, China	*Pseudomonas* spp., *Leuconostoc mesenteroides*, *Lactobacillus helveticus*, *Lactobacillus kefiranofaciens*, *Lactococcus lactis*, *Lactobacillus kefiri*, *Lactobacillus casei*	*Kazachstania unispora*, *Kluyveromyces marxianus*, *Saccharomyces serevisiae*, *Kazachstania exigua*	[16]
Ireland	*Lactobacillus kefiranofaciens*, *Lactobacillus kefiri*, *Lactobacillus parabuchneri*, *Lactobacillus kefiranofaciens* spp. *kefirgranum*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus parakefiri*		[17]
Turkey	*Lactobacillus kefiranofaciens*, *Lactococcus lactis*		[18]
Brazil	*Lactobacillus paracasei*, *Lactobacillus parabuchneri*, *Lactobacillus casei*, *kefiri*, *Lactococcus lactis*, *Acetobacter lovaniensis*	*Kazachstania aerobia*, *Saccharomyces cerevisiae*, *Lachancea meyersii*	[19]
Slovenia	*Lactobacillus kefiranofaciens* spp., *Kefirgranum*, *Lactobacillus parakefiri*, *Lactobacillus kefiri*	*Kluyveromyces marxianus*, *Kazachstania exiqua*, *Rhodosporidium kratochvilovae*	[20]
Argentin	*Lactobacillus plantarum*	*Saccharomyces cerevisiae*, *Saccharomyces unisporus*, *Kluyveromyces marxianus*	[21]
Russia	*Lactobacillus helveticus*, *Lactobacillus kefir*, *Lactobacillus parakefir*		[22]
Italy	*Lactobacillus kefiranofaciens*, *Streptococcus. thermophilus*, *Lactococcus lactis*	*Dekkera anomala*	[23]

Table 1. *Cont.*

Origin Country	Dominant LAB	Dominant Yeasts	Reference
Bulgary	*Lactobacillus delbrueckii. bulgaricus*, *Lactobacillus helveticus*	*Kluyveromyces marxianus*	[24]
Belgium	*Lactobacillus kefiri*, *Lactobacillus kefiranofaciens*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *Lactococcus lactis* spp. *cremoris*, *Gluconobacter frateurii*, *Acetobacter orientalis*, *Acetobacter lovaniensis*	*Kluyveromyces marxianus*, *Naumovozyma* sp., *Kazachastania khefir*	[25]
South Africa	*Leuconostoc* spp., *Lactococcus* spp., *Lactobacillus* spp., *Lactobacillus plantarum*	*Zygosaccharomyces* spp., *Candida* spp., *Candida lambica*, *Candida krusei*, *Saccharomyces* spp., *Cryptococcus* spp.	[12]
Malaysia	*Lactobacillus kefiranofaciens*, *Lactobacillus kefir*		[26]

Belgian and Irish kefir grains exhibit greater variety in dominant LAB, followed by Chinese and Brazilian grains. *Lactobacillus kefiri* and *Lactobacillus kefiranofaciens* spp. are two common dominant LAB in Ireland, Malaysia and Belgium.

China (Tibet) and Brazil are next on the list with dominant LAB to be *Lactobacillus* species (*helveticus*, *casei*, *kefiri*). Kefir grains from other countries (Slovenia, Italy, South Africa, Russia, Bulgaria, Turkey, Taiwan and Argentina) present their own microbial profiles with different percentages in dominant LABs and with reduced strain variety.

As far as dominant yeasts are concerned, Taiwan and Slovenia have a greater number of different yeasts, with *Kluyveromyces marxianus* and *S. cerevisiae* being the most common and dominant over others such as *S. unisporous*, *Rodosporidium kratochvilovae* and *Kazachstania exigua.*

South Africa, Belgium, Brazil, Italy and Bulgaria follow on the list with different dominant yeasts, such as *Candida* spp., *Cryptococcus* spp., *Dekkera anomala*, etc.

3. Microorganisms Isolated from Different Types of Kefir Grains Worldwide

In recent years, plenty of scientific surveys were published regarding the isolation and characterization of microorganisms from kefir grains all over the world, some of them exhibiting probiotic properties. All the updated data are represented in Tables 1 and 2.

Table 2. Fully characterized probiotic LAB and yeasts isolated from kefir grains.

Origin Country	Dominant LAB	Dominant Yeasts	Reference
Taiwan	*Lactobacillus kefiranofaciens*	*Kluyveromyces marxianus CIDCA 8154*, *Saccharomyces cerevisiae CIDCA 8112*	[15,27]
Tibet, China	*Lactobacillus casei*		[28]
Argentin	*Lactobacillus plantarum*	*Kluyveromyces marxianus CIDCA 8154*, *Saccharomyces cerevisiae CIDCA 8118*	[15]
Russia	*Lactobacillus helveticus*, *Lactobacillus kefir*, *Lactobacillus parakefir*		[22]

Gao et al. selected and evaluated four samples of Tibetan kefir, namely TK-ZJUJ01 from Tibet, TK-ZJUJ02 from Sichuan, TK-ZJUJ03 from Xinjiang and TK-ZJUJ04 from Qinghain. The aim was to identify bacteria and yeasts through conventional methods using HANGWEI trae biochemical reaction tube kits and through molecular methods using PCR amplification [16]. Regarding the TK-ZJU0Q1 sample, *Bacillus subtilis*, *Lactococcus lactis*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* were identified, whereas in the TK-ZJUJ024 sample *Lactobacillus kefiri*, *Pichia kudriavzevii*, *Kluyveromyces marxianus*, *Kazachstania unispora* were present. Analysis of the TK-ZJUJ03 sample led to the identification of *Leuconostoc lactis*, *Lactococcus lactis*, *Lactobacillus plantarum*, *Kazachstania unispora*, *Kluyveromyces marxianus*, and from the TK-ZJUJ04 sample, *Lactobacillus plantarum*, *Acetobacter fabarum*, *Kazachstania unispora*, *Pichia guilliermondii* were detected. The yeasts *Pichia kudriavzevii* and *Pichia guilliermondii* were isolated from kefir grains for the first time.

It is noteworthy that lactic acid bacteria contained in the TK-ZJUS01 sample constitute over 50% of the microflora in contrast to TK-ZJUJ04, in which the majority of microorganisms were yeasts [16]. In another study, three different Tibetan kefir grains were analyzed by culture-independent methods. The most frequently isolated species were *Pseudomonas* sp., *Leuconostoc mesenteroides*, *Lactobacillus helveticus*, *Lactobacillus kefiranofaciens*, *Lactococcus lactis*, *Lactobacillus kefiri*, *Lactobacillus casei*, *Kazachstania unispora*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae*, and *Kazachstania exigua*. Among the three different Tibetan kefir grains, the percentage of similarities was 78%–84% in bacteria and 80%–92% in yeasts [28]. Other studies in Tibetan kefir showed that *Lactococcus lactis* was the most commonly identified bacteria [16]. Furthermore, *Leuconostoc mesenteroides*, another heterofermentative lactic acid bacterium, was found to be prevalent.

In Turkey, three kefir grains and four kefir beverages were investigated for their microbial diversity using culture-independent and culture-dependent methods. According to the results, *Lactobacillus kefiranofaciens* was the most predominant species in kefir grains, while *Lactococcus lactis* predominated in kefir beverages. *Lactococcus lactis*, *Lactobacillus acidophilus* and *Streptococcus thermophilus* were also detected with both methods [18].

Culture-dependent and culture-independent methods were applied in order to analyze the microflora of tibico (sugary kefir) grains from eight different states in Brazil. In particular, through the application of amplified ribosomal DNA restriction analysis (ARDRA) and/or 16S rRNA gene sequencing, 108 isolates from both substrates were identified. The identified bacteria belonged to the genera *Lactobacillus*, *Acetobacter*, *Gluconobacter* and *Bacillus*, whereas the yeasts identified were *Pichia*, *Saccharomyces*, *Kazachstania*, *Candida*, *Zygosaccharomyces* and *Yarrowia*. It should be underlined that the bacterial species *Cluconobacter liquefaciens* and *Bacillus sereus*, as well as the yeasts *Pichia cecembensis*, *Pichia caribbica* and *Zygosaccharomyces fermentati*, were detected for the first time in tibico grains [2,29]. In another study, in eight Brazilian kefir grains, four of which were propagated in cow's milk and the rest in brown sugar, the dominant species were *Lactobacillus casei*, *Lactobacillus kefiri*, *Lactobacillus perolens*, *Lactobacillus kefiranofaciens*, *Lactobacillus satsumensis*, *Lactobacillus mali*, *Lactobacillus parafarraginis*, and *Lactobacillus diolivorans* [30]. Additionally, another study investigated the bacterial composition of a Brazilian kefir beverage using phenotyping and genotyping methods. The most frequently isolated species were *Lactobacillus paracasei*, *Lactobacillus parabuchneri*, *Lactobacillus casei*, *Lactobacillus kefiri*, *Lactococcus lactis*, *Acetobacter lovaniensis*, *Kluyveromyces lactis*, *Kazachstania aerobia*, *Saccharomyces cerevisiae* and *Lachancea meyersii*. It should be underlined that the presence of *Lachancea meyercii* and *Kazachstania aerobia* was confirmed for the first time in Brazilian kefir [19].

Argentinean kefir grains were also analyzed for their microbial composition. Yeast species were isolated from four milky and three sugary kefir grains and identified through classical microbiological and molecular-genetic methods. The microbial composition obtained consisted of *Zygosaccharomyces cerevisiae*, *Zygosaccharomyces unisporus*, *Issatchenkia occidentalis* and *Kluyveromyces marxianus*. Thirteen of these strains were tolerant to low pH and bile salts. Thus, *Kluyveromyces marxianus CIDCA 8154* and *Saccharomyces cerevisiae CIDCA 8118* were selected for further tests in order to investigate their ability to adhere to intestinal epithelial cells. As a result, both yeast strains were not detected in the gut after five days [15]. In another study, 11 isolated homofermentative lactobacilli were examined for potential probiotic activity. Restriction analysis of amplified ribosomal DNA (ARDNA) and analysis of the 16S rRNA internal spacer region were used to confirm that all 11 lactobacilli pertained to the species

Lactobacillus plantarum. RAPD-PCR analysis showed that they belonged to five groups and each was tested for potential probiotic activity. All strains showed high tolerance to bile salts. Regarding their antimicrobial activity, all strains were effective against *Salmonella typhimurium*, *Escherichia coli* and *Salmonella enterica* strains. In addition, most of the strains were effective against *Salmonella gallinarum*. It was also concluded that *L. plantarum* CIDCA 8327 had the highest potential probiotic ability [9].

In Slovenia, culture-dependent and culture-independent methods were used to characterize and evaluate the stability of lactobacilli and yeast microbial flora in kefir grains. The results showed that the dominant bacterial species were *Lactobacillus kefiranofaciens* ssp. *Kefirgranum*, *Lactobacillus parakefiri* and *Lactobacillus kefiri*. Regarding the yeasts, the most frequent species were *Kluyveromyces marxianus*, *Kazachstania exiqua* and *Rhodosporidium kratochvilovae*. For the first time, it the presence of *Rhodosporidium kratochvilovae* was reported. Culture-independent analysis indicated that the majority of the microorganisms were *Lactobacillus kefiranofaciens* ssp. *kefirgranum*, *Kluyveromyces marxianus* and *Kazachstania exigua*, but there was no detection of *Lactobacillus parakefiri*, *Lactobacillus kefir* and *Rhodosporidium kratochvilovae*. The authors concluded that it is important to use a combination of methods for more reliable and detailed results [20].

The composition of *Lactobacillus* spp. in kefir grains from France, Italy, Portugal, Russia, Turkey and Spain was studied and the strains were tested for adherence to human anterocyte-like Caco2-cells, resistance to acidic pH and bile acid, antimicrobial activities against enteropathogenic bacteria and inhibition of *Salmonella typhimurium* attachment to Caco-2 cells. The conduction of in vitro tests showed that *L. acidophilus* CYC 10051 and *Lactobacillus kefiranofaciens* CYC 10058 exhibited the best results. *Lactobacillus kefiranofaciens* CYC 10058 produced kefiran-related exopolysaccharides that retard tumor growth in vivo when administered orally [19]. The results suggested that this strain is of major interest due to its kefiran-related polysaccharide production. It is possible that the good health properties attributed to kefir may also be related to but not restricted by the therapeutic properties of kefiran. In other words, this study has clearly shown that the *Lactobacillus* strains isolated from kefir clearly showed probiotic properties [3].

In Taiwan, *Lactobacillus kefiranofaciens* M1, isolated and identified from Taiwanese milk kefir grains, demonstrated immune-modulating activity. In this study, an anticolitis effect was clearly demonstrated. The underlying mechanisms seem to affect intestinal epithelial cells directly. *Lactobacillus kefiranofaciens* M1 could restore barrier function and reduce the permeability of the intestine against DSS-induced damage by (i) increasing chemokine CCL-20; (ii) up-regulating the production of anti-inflammatory cytokine IL-10; and (iii) suppressing the secretion of proinflammatory cytokines through TLR2. The anticolitis effect of *Lactobacillus kefiranofaciens* M1 was investigated in vitro and in vivo. Based on these results, it was found that *Lactobacillus kefiranofaciens* M1 has the potential to be used in fermented milk products as an alternative therapy for intestinal disorders [27]. In another study, various yeasts were isolated from kefir and those exhibiting tolerance to acidic conditions and bile salts were selected for further study of their adhesion to epithelial cells and their transit through the mouse gut. More specifically, from milky and sugary kefir grains, 34 yeast strains were isolated and identified through classical microbiological and molecular-genetic methods (internal transcribed spacer amplification, whole-cell protein pattern, and analysis of restriction fragment length polymorphisms). Fifteen strains of *Saccharomyces cerevisiae*, six strains of *Saccharomyces unisporus*, four strains of *Issatchenkia occidentalis*, and nine strains of *Kluyveromyces marxianus* were isolated. The strains that were further studied were *Kluyveromyces marxianus* CIDCA 8154 and *Saccharomyces cerevisiae* CIDCA 8112. Both strains were able to adhere to epithelial intestine–derived cells in vitro and to survive passage through the gastrointestinal tract of BALB/c mice. At the end of the administration, both yeast strains were absent from the gut within five days [15]. A combination of conventional microbiological cultivation was used with PCR-DGGE and resulted in the identification of four yeast species from both cultures in Taiwan. Specifically, *Kluveromyces marxianus*, *Saccharomyces turicensis*, and *Pichia fermentans* were found in Taiwanese kefir grains with 76%, 22%, and 2% distribution, respectively, while *Kluveromyces marxianus*, *Saccharomyces unisporus*, and *Pichia fermentans* were identified with 58%, 11%, and 31% distribution in viili starters, respectively. In addition, a culture-independent method was applied to identify the viili and kefir yeasts applying DGGE analysis. *Pichia fermentans* in kefir grains and *Saccharomyces unisporus* in viili starters were not identified. This result confirms that only the predominant yeast populations in these starters could be identified. This is the first report demonstrating that a combination of conventional microbiological cultivation, PCR-DGGE, and sequencing methods could successfully identify species of yeast and facilitate the study of their distribution in kefir grains [9].

In Italy, six different milk kefir grains were investigated and the dominant species were *Lactobacillus kefiranofaciens* and *Dekkera anomala*. The presence of the sub-dominant species ascribed to *Sreptococcus thermophilus*, *Lactococcus lactis* and *Acetobacter* genera was also highlighted. In addition, *Lactococcus lactis*, *Enterococcus* spp., *Bacillus* spp., *Acetobacter fabarum*, *Acetobacter lovaniensis* and *Acetobacter orientalis* were identified as part of the cultivable community [23].

Research in Ireland claims that the main species of Irish kefir grains belong to *Lactococcus* spp. and identified as *Lactobacillus kefiranofaciens*, *Lactobacillus kefiri*, *Lactobacillus parabuchneri*, *Lactobacillus kefiranofaciens* ssp. *kefirgranum*, *Lactobacillus helveticus*, *Lactobacillus acidophilus* and *Lactobacillus parakefiri* [17].

In addition, interesting findings were obtained through the application of rapid amplified ribosomal DNA restriction analysis (ARDRA) as a second culture-independent technique to evaluate the data obtained using the sequencing approach. These studies detected the presence of bifidobacteria as part of the sugary kefir consortium for the first time [31].

The microbiological consortium of communities in kefir grains of different origin have also been studied by the pyrosequencing of rDNA amplicons [32]. The outcome showed the prevalent strains were *Lactobacillus kefiranofaciens*, *Lactobacillus kefiri*, *Lactobacillus parakefiri*, *Lactobacillus buchneri*, while species of *Acetobacter* and *Lactococcus* were identified as minor bacterial constituents [17,33].

Next generation sequencing (NGS) was applied to investigate the predominant species in Malaysian kefir grains obtained locally. The taxonomic results analyses obtained when using BaseSpace (Illumina) and MEGAN were compared. The outcome showed that the predominant species was *Lactobacillus kefiranofaciens* (81.45%–91.93%), while *Lactobacillus kefiri* (2.01%–2.47%) was the second in abundance [26].

4. Health Benefits of Kefir and Kefir Grains

The complex microbiological consortium of kefir grains is responsible for the high numbers of produced metabolites that contribute to a wide range of health-promoting effects. The main established health benefits of kefir grains are represented in Table 3 and include antimutagenic, anticolitis, hypocholesterolemic and anti-atherogenic effects, as well as antimicrobial and antioxidant activities, protection against *C. difficile* infection, and inhibition of tumor growth and β-galactosidase activity.

Table 3. Presumed health benefits of kefir grains.

Biological Activity	Reference
antimutagenic effects against 2,3-dimethyl-4-aminobiphenyl (DMAB) and against acting *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG)	[27]
hypocholesterolemic and anti-atherogenic effects	[34,35]
inhibition of tumor growth	[36]
antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and *S. enteritidis*	[3]
antioxidant activity antifungal, antibacterial properties and epithelium protection due to kefiran	[9,37,38]
antimicrobial activity, all strains were effective against *Salmonella typhimurium* and *Escherichia coli*, and against *S. enterica* strains	[21]
anticolitis effect	[27]
protect against *C. difficile* infection	[39]
β-Galactosidase Activity	[40]

Kefir grains can inhibit the development of undesirable and pathogenic microorganisms, since the microbes contained in grains are capable of producing lactic and acetic acids, ethanol, peptides (bacteriocins), and other biologically active components [12]. Many studies have shown that species of lactobacilli contained in kefir grains can be successfully used in the treatment of vaginal infections, as they are able to produce a range of anti-microbial compounds [41]. More specifically, it has been reported that milk fermented with kefir grains exhibits antimutagenic effects against 2,3-dimethyl-4-aminobiphenyl (DMAB) and against *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG). Both of them are of the most potent mutagenic chemicals known [27]. In recent research data, the consumption of fermented yogurt and milk with kefir grains showed hypocholesterolemic and anti-atherogenic effects that protect against atherogenesis and the formation of plaques in arteries [34,35]. Liu et al. (2003) demonstrated that oral administration of kefir grains generated from milk or soy milk results in the significant inhibition of tumor growth [36]. It should also be mentioned that there are positive effects on the cholesterol metabolism [34].

Moreover, a bacteriocin (lacticin 3147), produced by *Lactococcus lactis* strain DPC3147 isolated from kefir grains, exhibited antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Salmonella enteritidis* [3]. On the other hand, new research evidence revealed that the health benefits of kefir grains are also attributed to kefiran. Specifically, kefiran presents antioxidant activity [37], antifungal and antibacterial properties [9] and epithelium protection [38].

According to Bolla et al. (2013), a mixture of kefir-isolated microorganisms (*Lactobacillus plantarum*, *Lactobacillus kefir*, *Lactococcus lactis*, *Kluyveromyces marxianus* and *Saccharomyces cerevisiae*) was administered to hamsters infected with *Clostridium difficile* and resulted in milder symptoms of *Clostridium difficile*–associated diarrhea (CDAD) and extinction of death among the hamsters examined [39].

The comparison was conducted between a group of mice that consumed the mixture of probiotic strains and another group administered clindamycin. The antibiotic showed more aggressive symptoms of diarrhea and almost all of the hamsters ended up dead. These results demonstrated that kefir-isolated bacteria and yeasts may contribute to protection against *C. difficile*–induced enterocolitis in hamsters.

In addition, kefir grains can be successfully applied to a specific population suffering from lactose intolerance. The enzyme β-galactosidase is naturally present in kefir grains and reduces the lactose content, leading to a product suitable for lactose-intolerant persons. Likewise, the reduced lactose concentration and higher β-galactosidase activity in kefir make it suitable for lactose-intolerant persons by improving lactose digestion [40].

5. Future Prospective

Lately, kefir grains have been applied in the production of non-dairy probiotic beverages. Likewise, interesting results have been presented regarding the fermentation of apple (*Malus domestica* Borkh, cv Gala), quince (*Cydonia oblonga* Mill., cv Del Portogallo), grape (*Vitis vinifera* L., white-berry cv Italia), kiwifruit (*Actinidia chinensis* PL., cv Hayward), prickly pear (*Opuntia ficus-indica* L., cv Sanguigna) and pomegranate (*Punica granatum* L., cv Dente di cavallo) juices with kefir grains [42]. The products were of high added value and can be characterized as functional foods.

In the same manner, the fermentation of vegetable juices, such as carrot, onion, tomato and strawberry juices, was carried out by water kefir microorganisms. Likewise, the new products might represent important foods providing live microorganisms to vegetarians with a limited availability of fermented products [43]. Lately, mixtures of pomegranate and orange juice as well as *Cornus mas* L. juice were successfully fermented with kefir grains and isolated lactic acid bacteria from kefir grains, respectively, leading to low-alcohol beverages with high nutritional value and probiotic properties [44,45].

Taking into account that kefir grains obviously represent probiotic features, the aforementioned scientific studies might show the way for the future employment of kefir grains in the fermentation industry, either as a whole mixed culture or as single cultures isolated from kefir grains, leading to products with high added value that are ready to be consumed.

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References

1. Kakisu, E.; Irigoyen, A.; Torre, P.; de Antoni, G.L.; Abraham, A.G. Physicochemical, microbiological and sensory profiles of fermented milk containing probiotic strains isolated from kefir. *J. Dairy Res.* **2011**, *78*, 456–463. [CrossRef] [PubMed]
2. Miguel, M.G.C.P.; Cardoso, P.G.; Magalhães, K.T.; Schwan, R.F. Profile of microbial communities present in tibico (sugary kefir) grains from different Brazilian States. *World J. Microb. Biotechnol.* **2011**, *27*, 1875–1884. [CrossRef]
3. Santos, A.; San Mauro, M.; Sanchez, A.; Torres, J.M.; Marquina, D. The Antimicrobial Properties of Different Strains of *Lactobacillus* spp. isolated from Kefir. *Syst. Appl. Microbiol.* **2003**, *26*, 434–437. [CrossRef] [PubMed]
4. Harta, O.; Iconomopoulou, M.; Bekatorou, A.; Nigam, P.; Kontominas, M.; Koutinas, A.A. Effect of various carbohydrate substrates on the production of kefir grains for use as a novel baking starter. *Food Chem.* **2004**, *88*, 237–242. [CrossRef]
5. Plessas, S.; Trantallidi, M.; Bekatorou, A.; Kanellaki, M.; Nigam, P.; Koutinas, A.A. Immobilization of kefir and *Lactobacillus casei* on brewery spent grains for use in sourdough wheat bread making. *Food Chem.* **2007**, *105*, 187–194. [CrossRef]
6. Loretan, T.; Mosert, J.F.; Viljoen, B.C. Microbial flora associated with South African household kefir. *S. Afr. J. Sci.* **2003**, *99*, 92–94.
7. Garrote, G.L.; Abraham, A.G.; de Antoni, G.L. Preservation of kefir grains, a comparative study. *LWT—Food Sci. Technol.* **1997**, *30*, 77–84. [CrossRef]
8. Marquina, D.; Santos, A.; Corpas, I.; Muñoz, J.; Zazo, J.; Peinado, J.M. Dietary influence of kefir on microbial activities in the mouse bowel. *Lett. Appl. Microbiol.* **2002**, *35*, 136–140. [CrossRef] [PubMed]
9. Wang, S.Y.; Chen, H.C.; Liu, J.R.; Lin, Y.C.; Chen, M.J. Identification of Yeasts and Evaluation of their Distribution in Taiwanese Kefir and Viili Starters. *J. Dairy Sci.* **2008**, *91*, 3798–3805. [CrossRef] [PubMed]
10. Rimada, P.S.; Abraham, A.G. Effects of different fermentation parameters on quality characteristics of kefir. *Int. Dairy J.* **2006**, *16*, 33–39. [CrossRef]
11. Paraskevopoulou, A.; Athanasiadis, I.; Blekas, G.; Koutinas, A.A.; Kanellaki, M.; Kiosseoglou, M. Influence of polysaccharide addition on stability of a cheese whey kefir-milk mixture. *Food Hydrocoll.* **2003**, *17*, 615–620. [CrossRef]
12. Witthuhn, R.C.; Schoeman, T.; Britz, T.J. Characterisation of the microbial population at different stages of kefir production and kefir grain mass cultivation. *Int. Dairy J.* **2005**, *15*, 383–389. [CrossRef]
13. Magalhães, K.T.; Pereira, M.A.; Dragone, A.N.; Domingues, L.; Teixeira, J.A.; Almeida Silva, J.B.; Schwan, F. Production of fermented cheese whey-based beverage using kefir grains as starter culture: Evaluation of morphological and microbial variations. *Bioresour. Technol.* **2010**, *101*, 8843–8850. [CrossRef] [PubMed]
14. Londero, A.; Iraporda, C.; Garrote, G.L.; Abraham, A.G. Cheese whey fermented with kefir micro-organisms: Antagonism against Salmonella and immunomodulatory capacity. *Int. J. Dairy Technol.* **2016**, *68*, 118–126. [CrossRef]
15. Diosma, G.; Romanin, D.E.; Rey-Burusco, M.F.; Londero, A.; Garrote, G.L. Yeasts from kefir grains: Isolation, identification, and probiotic Characterization. *World J. Microbiol. Biotechnol.* **2013**, *30*, 43–53. [CrossRef] [PubMed]
16. Gao, J.; Gu, F.; Abdella, N.H.; Ruan, H.; He, G. Investigation on culturable microflora in Tibetan kefir grains from different areas of China. *J. Food Sci.* **2012**, *77*, 425–433. [CrossRef] [PubMed]
17. Dobson, A.; O'Sullivan, O.; Cotter, P.D.; Ross, P.; Hill, C. High-throughput sequence-based analysis of the bacterial composition of kefir and an associated kefir grain. *FEMS Microbiol. Lett.* **2011**, *320*, 56–62. [CrossRef] [PubMed]

18. Kesmen, Z.; Kacmaz, N. Determination of Lactic Microflora of Kefir Grains and Kefir Beverage by Using Culture-Dependent and Culture-Independent Methods. *J. Food Sci.* **2011**, *76*, 276–283. [CrossRef] [PubMed]
19. Magalhaes, K.T.; de Melo Pereira, G.V.; Campos Cássia, R.; DragoneI, G.; Schwan, R.F. Brazilian kefir: Structure, microbial communities and chemical composition. *Braz. J. Microbiol.* **2011**, *42*, 693–702. [CrossRef] [PubMed]
20. Vardjan, T.; Mohar Lorbeg, P.; Rogelj, I.; Čanžek Majhenič, A. Characterization and stability of lactobacilli and yeast microbiota in kefir grains. *J. Dairy Sci.* **2013**, *96*, 2729–2736. [CrossRef] [PubMed]
21. Golowczyc, M.A.; Gugliada, M.J.; Hollmann, A.; Delfederico, L.; Garrote, G.L.; Abraham, A.G.; Semorile, L.; de Antoni, G. Characterization of homofermentative lactobacilli isolated from kefir grains: Potential use as probiotic. *J. Dairy Res.* **2008**, *75*, 211–217. [CrossRef] [PubMed]
22. Mainville, I.; Robert, N.; Lee, B.; Farnworth, E.R. Polyphasic characterization of the lactic acid bacteria in kefir. *Syst. Appl. Microbiol.* **2006**, *29*, 59–68. [CrossRef] [PubMed]
23. Garofalo, C.; Osimani, A.; Milanović, V.; Aquilanti, L.; de Filippis, F.; Stellato, G.; Mauro, S.D.; Turchetti, B.; Buzzini, P.; Ercolini, D.; et al. Bacteria and yeast microbiota in milk kefir grains from different Italian regions. *Food Microbiol.* **2015**, *49*, 123–133. [CrossRef] [PubMed]
24. Simova, E.; Beshkova, D.; Angelov, A.; Hristozova, T.; Frengova, G.; Spasov, Z. Lactic acid bacteria and yeasts in kefir grains and kefir made from them. *J. Ind. Microbiol. Biotechnol.* **2002**, *28*, 1–6. [CrossRef] [PubMed]
25. Korsak, N.; Taminiau, B.; Leclercq, M.; Nezer, C.; Crevecoeur, S.; Ferauche, C.; Detry, E.; Delcenserie, V.; Daube, G. Short communication: Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments. *J. Dairy Sci.* **2015**, *98*, 3684–3689. [CrossRef] [PubMed]
26. Zamberi, N.R.; Mohamad, N.E.; Yeap, S.K.; Ky, H.; Beh, B.K.; Liew, W.C.; Tan, S.W.; Ho, W.Y.; Boo, S.Y.; Chua, Y.H.; et al. 16S Metagenomic Microbial Composition Analysis of Kefir Grain using MEGAN and BaseSpace. *Food Biotechnol.* **2016**, *30*, 219–230. [CrossRef]
27. Chen, Y.P.; Hsiao, P.J.; Hong, W.S.; Dai, T.Y.; Chen, M.J. *Lactobacillus kefiranofaciens* M1 isolated from milk kefir grains ameliorates experimental colitis in vitro and in vivo. *J. Dairy Sci. Assoc.* **2012**, *95*, 63–74. [CrossRef] [PubMed]
28. Zhou, J.; Liu, X.; Jiang, H.; Dong, M. Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis. *Food Microbiol.* **2009**, *26*, 770–775. [CrossRef] [PubMed]
29. Leite, A.M.O.; Miguel, M.A.L.; Peixoto, R.S.; Ruas-Madiedo, P.; Paschoalin, V.M.F.; Mayo, B.; Delgado, S. Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian kefir grains. *J. Dairy Sci.* **2016**, *98*, 3622–3632. [CrossRef] [PubMed]
30. Ferreira, Z.D.; Abatemarco, M., Jr.; de Cicco Sandes, S.H.; Nunes, N.J.R.; Cantini, A.; Neumann, E. Selection of lactic acid bacteria from Brazilian kefir grains for potential use as starter or probiotic cultures. *Anaerobe* **2015**, *32*, 70–76.
31. Gulitz, A.; Stadie, J.; Ehrmann, M.A.; Ludwig, W.; Vogel, R.F. Comparative phylobiomic analysis of the bacterial community of water kefir by 16S rRNA gene amplicon sequencing and ARDRA analysis. *J. Appl. Microbiol.* **2013**, *114*, 1082–1091. [CrossRef] [PubMed]
32. Mayo, B.; Rachid, C.T.C.C.; Alegría, A.; Leite, A.M.O.; Peixoto, R.S.; Delgado, S. Impact of Next Generation Sequencing Techniques in Food Microbiology. *Curr. Genom.* **2014**, *15*, 293–309. [CrossRef] [PubMed]
33. Leite, A.M.; Mayo, B.; Rachid, C.T.; Peixoto, R.S.; Silva, J.T.; Paschoalin, V.M.; Delgado, S. Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. *Food Microbiol.* **2012**, *31*, 215–221. [CrossRef] [PubMed]
34. Xiao, L.L.; Dong, M.S. Screening and cholesterol-degrading activity of *Lactobacillus casei* KM-16. *China Dairy Ind.* **2003**, *31*, 7–10.
35. Yanping, W.; Nv, X.; Aodeng, X.; Zaheer, A.; Bin, Z.; Xiaojia, B. Effects of Lactobacillus plantarum MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on high-cholesterol diet. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 341–347.
36. Liu, S.Q. Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations. *Int. J. Food Microbiol.* **2003**, *83*, 115–131. [CrossRef]
37. Chen, Z.; Shi, J.; Yang, X.; Nan, B.; Liu, Y.; Wang, Z. Chemical and physical characteristics and antioxidant activities of the exopolysaccharide produced by Tibetan kefir grains during milk fermentation. *Int. Dairy J.* **2015**, *43*, 15–21. [CrossRef]

38. Serafini, F.; Turroni, F.; Ruas-Madiedo, P.; Lugli, G.A.; Milani, C.; Duranti, S.; Zamboni, N.; Bottacini, F.; van Sinderen, D.; Margolles, A.; et al. Kefir fermented milk and kefiran promote growth of *Bifidobacterium bifidum* PRL2010 and modulate its gene expression. *Int. J. Food Microbiol.* **2014**, *178*, 50–59. [CrossRef] [PubMed]
39. Bolla, P.A.; Carasi, P.; Bolla Mde, L.; de Antoni, G.L.; Serradell Mde, L. Protective effect of a mixture of kefir-isolated lactic acid bacteria and yeasts in a hamster model of Clostridium difficile infection. *Anaerobe* **2013**, *21*, 28–33. [CrossRef] [PubMed]
40. Hertzler, S.R.; Shannon, M.C. Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *J. Am. Diet. Assoc.* **2003**, *103*, 582–587. [CrossRef] [PubMed]
41. Farnworth, E.R. Kefir—A complex probiotic. *Food Sci. Technol.* **2005**, *2*, 1–17. [CrossRef]
42. Randazzo, W.; Corona, O.; Guarcello, R.; Francesca, N.; Germanà, M.A.; Erten, H.; Moschetti, G.; Settanni, L. Development of new non-dairy beverages from Mediterranean fruit juices fermented with water kefir microorganisms. *Food Microbiol.* **2016**, *54*, 40–51. [CrossRef]
43. Corona, O.; Randazzo, W.; Miceli, A.; Guarcello, R.; Francesca, N.; Erten, H.; Moschetti, G.; Settann, L. Characterization of kefir-like beverages produced from vegetable juices. *Food Sci. Technol.* **2016**, *66*, 572–581.
44. Kazakos, S.; Mantzourani, I.; Nouska, C.; Alexopoulos, A.; Bezirtzoglou, E.; Bekatorou, A.; Plessas, S.; Varzakas, T. Production of low-alcohol fruit beverages through fermentation of pomegranate and orange juices with kefir grains. *Curr. Res. Nutr. Food Sci.* **2016**, *4*, 19–26. [CrossRef]
45. Nouska, C.; Kazakos, S.; Mantzourani, I.; Alexopoulos, A.; Bezirtzoglou, E.A.; Plessas, S. Fermentation of *Cornus mas* L. juice for functional low alcoholic beverage production. *Curr. Res. Nutr. Food Sci.* **2016**, *4*, 119–124. [CrossRef]

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