Kefir: A symbiotic yeasts-bacteria community with alleged healthy capabilities

Fernando Lopitz-Otsoa[1], Aitor Rementeria[2], Natalia Elguezabal[3] and Javier Garaizar[1]

[1]Facultad de Farmacia, [2]Facultad de Ciencia y Tecnología, Departamento de Immunología, Microbiología y Parasitología, Universidad del País Vasco-Euskal Herriko Unibertsitatea; [3]Cell Biology and Stem Cells Unit, CIC bioGUNE, Parque Tecnológico de Bizkaia, Spain

Summary

Kefir is a fermented milk beverage. The milk fermentation is achieved by the use of kefir grains, a cluster of microorganisms held together by a polysaccharide matrix named kefiran. Kefir grains are an example of symbiosis between yeast and bacteria. They have been used over years to produce kefir, a fermented milk beverage that is consumed all over the world, although its origin is Caucasian. A vast variety of different species of organisms forming the kefir grains, comprising yeast and bacteria, have been isolated and identified. Kefir is a probiotic food. Probiotics have shown to be beneficial to health, being presently of great interest to the food industry. Kefir has been accredited with antibacterial, antifungal and antitumoural activities among other beneficial attributes. This review includes a critical revision of the microbiological composition of kefir along with its beneficial properties to human health.

Key words

Kefir, Symbiosis, Antibacterial, Antifungal, Antitumoural, Probiotic

Kefir: una comunidad simbiótica de bacterias y levaduras con propiedades saludables

Resumen

El kéfir es una bebida láctea fermentada. Los gránulos de kéfir, comunidades de microorganismos que se agrupan en una matriz polisacárida denominada kefirano, son los responsables de esta fermentación. Estos gránulos son un ejemplo de simbiosis entre levaduras y bacterias y se han utilizado a través del tiempo para producir el kéfir, que es consumido por todo el mundo a pesar de su origen caucásico. En esa relación simbiótica, que son los gránulos de kéfir, se han aislado e identificado una amplia variedad de especies microbianas que comprenden levaduras y bacterias. El kéfir es un alimento probiótico.
Los probióticos han demostrado ser beneficiosos para la salud, siendo de gran interés para la industria alimentaria en la actualidad. Según se afirma, el kéfir ha mostrado actividades antibacterianas, antifúngicas y antitumorales, entre otros atributos beneficiosos. Este trabajo incluye una revisión crítica de la composición microbiológica del kéfir junto con sus propiedades beneficiosas para la salud humana.

Palabras clave

Kéfir, Simbiosis, Antibacteriano, Antifúngico, Antitumoral, Probiótico

Dirección para correspondencia:
Dr. Fernando Lopitz Otsoa
Farmazia Fakultatea
Inmunologia, Mikrobiologia eta Parasitologia saila
Unibertsitateen ibilbidea, 7
01006 Vitoria-Gasteiz (ARABA), Spain
Tel.: +34 945 013 904
E-mail: f.lopitz-otsoa@ehu.es

Apdo. 699, E-48080 Bilbao (Spain)
1130-1406/01/10.00 ∷

Kefir is a fermented milk originated in the Caucasus. This acidic fermented milk is slightly carbonated and presents small amounts of alcohol. What distinguishes kefir from the traditional fermented milks (yoghourt) is that it is made only from kefir grains, which are composed of yeast and bacteria [32,33]. Historically, kefir grains were considered a gift from Allah among the Muslim people of the northern Caucasian mountains. The word kefir is derived from the Turkish word "keif", which can be translated to good feeling for the sense experienced after drinkig it. Kefir grains were passed from generation to generation among the tribes of Caucasus being considered a source of family wealth. Today, traditional authentic kefir can be prepared by culturing fresh or pasteurized milk with kefir grains in homes all over the world [50].

Probiotics are foods that contain microorganisms which are beneficial to health [54]. A probiotic is a live microbial food supplement used in fermented dairy products and cheeses that beneficially affects the host animal by improving the microbial balance [15]. However, on the basis of recent advances of research in this field, the following revised definition has been proposed: "Probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well being of the host" [55]. A probiotic improves one of three main functions (colonization resistance, immunomodulation or nutritional contribution) of the normal gastrointestinal microbiota when ingested by human or animal hosts [35]. Kefir is actually considered an example of probiotic mixture of bacteria and yeasts [60].

Kefir has been believed to possess healing powers since the early eighteenth century. Because of its origin and the way it has been passed onto generations the beneficial attributes of kefir seem to have been underestimated by the scientific community. Although a great number of studies have shown its benefits, the lack of standarized protocols for clinical trials makes the interpretation of results difficult. In addition, the production of kefir in great amounts in industry using kefir grains is not standardized, making big batches with the same characteristics problematic. Nevertheless, different kefir products are commercially available [50].

This review intends to give a vision of the composition of kefir, regarding microbiota and their activity with a critical examination on the beneficial properties ascribed to it over years. The importance on the symbiosis of yeast with bacteria, supported by accessible documentated scientific work, is also addressed.

Figure 1. Kefir grains. Black rectangle is equivalent to 1 cm.

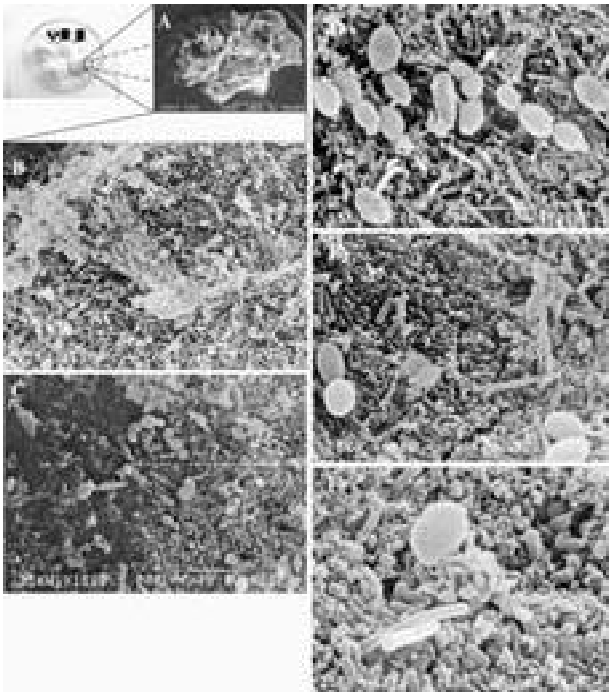

Figure 2. Microbiota of kefir grains by scanning electronic microscopy. A: 30x; B: 1100x; C: 1800x; D: 3600x; E: 4800x; F: 8600x.

Kefir grain composition and microbiota

Kefir is a fermented milk beverage, with a uniform creamy consistency and a slightly sour taste. The milk fermentation is acheived by kefir grains, a small cluster of microorganisms held together by a polysaccharide matrix named kefiran. The grains are a soft, gelatinous white biological mass, comprised of protein, lipids and a soluble-polysaccharide, the kefiran complex (Figure 1), that surrounds yeast and bacteria in the kefir grains. Kefiran is water-souble glucogalactan produced by *Lactobacillus kefiranofaciens.* This bacteria has demonstrated it's capability of producing kefiran under different conditions; when grown on rice hydrolysate medium [29] or PYG10 media [30]. However cell growth and kefiran production rates have been shown to be increased when *L. kefiranofaciens* is grown in a batch mixed culture together with *Saccharomyces cerevisiae* [6], and under the culture conditions established by mimicking the actions of yeast cells

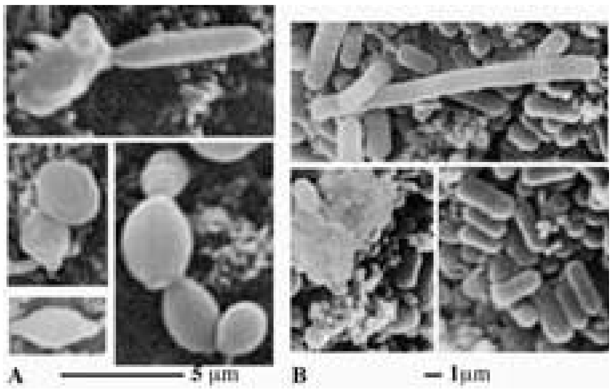

Figure 3. Scanning electronic micrographs, showing different yeast (A), cocci, bacilli and lactobacilli (B). Black bars indicate the sizes.

[66], demonstrating the importance of symbiosis between bacteria and yeast in kefir.

As described by some authors [44] a complex and tightly packed biofilm can be observed on the exterior of the grains, while the interior mainly comprises unstructured material. Microbiota is dominated by lemon-shaped or long filamentous yeast cells growing in close association with bacterial cocci and short or long rod shaped bacteria (lactobacilli). Cocci are observed preferentially on the surfaces of the yeast cells, while the rod-shaped bacteria are mainly found between the yeast cells. In figure 2, the association of the kefir microbiota can be observed by scanning electron microscopy.

A vast variety of different species of organisms comprising yeast and bacteria have been isolated and identified in kefir grains. In the figure 3, different microorganisms (yeast and bacteria) can be observed by scanning electron microscopy. Such species are among lactobacilli, streptococci, *Acetobacter* and yeasts. Yeast and bacteria share a symbiotic relationship, meaning that they survive or propagate by sharing their bioproducts as an energy source or growth-stimulating source. Some of the microorganisms isolated from kefir have been named after it, like *Lactobacillus kefiri, L. kefiranofaciens, Lactobacillus kefirgranum, Lactobacillus parakefir, Candida kefyr.* Also new species have been detected in kefir, such as *Saccharomyces turicensis* [71]. The taxonomic nomenclature of the different species of yeast and bacteria that compose kefir has varied along with the advances in taxonomic classification methods. Also complete knowledge of yeast life cycles (telemorphic and anamorphic phases) in some of these microorganisms has resulted in the use of different nomenclature to classify them. A complete description of the different yeast and bacteria that have been identified in different batches of kefir grains by different authors are shown on Table 1 and Table 2 respectively.

According to Hallé et al. [17] the predominant lactobacilli found in kefir grains are *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus plantarum* and *L. kefiranofaciens*, accounting for 20% of the lactobacilli in the final fermented beverage whereas the remaining lactobacilli (80%) is *Lactobacillus kefiri*. The predominant yeast in both the beverage and the kefir grains are *S. cerevisiae*, *Saccharomyces unisporus*, *Candida kefyr* and *Kluyveromyces marxianus* subsp. *marxianus*. Wyder [70] reviewed the literature on kefir and reported that 23 species of yeast could be found. However, the most frequently isolated species were *K. marxianus/C. kefyr* and *S. cerevisiae*. In a fairly recent study [23] once again, most of the microorganisms isolated from the kefir were identified as yeasts and lactic bacteria with *C. kefyr* and *Lactococcus lactis* making up 90% of each respective group.

From the data reviewed we can conclude that the microbiota composition in kefir seems to differ among different authors, although some of the reported species are always present. It is apparent that considerable modifications concerning microorganism species composition and its total representation occur between different cultures. Such modifications in different batches can be due to the variety of milk sources and also the period of time in which the kefir grains have been cultured. In addition, the microbiota from the same batch of kefir grains can be altered during different seasons or due to culture-conditions.

The characteristics of any spontaneously fermented milk product are a composite result of the metabolism of a variety of microorganisms [43]. The kefir, as fermented milk product, presents complex interactions between yeast and lactic bacteria that can influence product characteristics and quality. The activity of each microorganism and how they contribute to the symbiosis equilibrium has not been studied in depth. Narvhus et al. [43] have recently reviewed the possible interactions ocurring between yeast and bacteria in spontaneously fermented milk. According to these studies, since kefir is a fermented milk, its products are regarded as predominantly lactic fermentations by lactic

Table 1. Fungal microbiota isolates in different batches of kefir grains.

Actual nomenclature[a]	Obsolete nomenclature[b]	Cited in
Dekkera anomala (t) / Brettanomyces anomalus (a)		1,28,49,72
Torulaspora delbrueckii (t)	*Saccharomyces delbrueckii; Candida colliculosa*	72
Candida friedrichii		1
Candida humilis		22
Saccharomyces exiguus	*Torulopsis holmii; Candida holmii*	1,8,20,32,53
Candida inconspicua		60
Kluyveromyces marxianus (t) / Candida kefyr (a)	*Kluyveromyces marxianus* var. *marxianus*	23,32,72
Pichia fermentans (t) / Candida firmetaria (a)	*Candida lambica*	52,69
Issatchenkia orientalis (t) / Candida krusei (a)		69
Candida maris		60
Cryptococcus humicolus		69
Debaryomyces hansenii (t) / Candida famata (a)		22,28
Debaryomyces (Schwanniomyces) occidentalis		22
Galactomyces geotrichum (t) / Geotrichum candidum(a)		49,69
Kluyveromyces lactis var. *lactis*	*Kluyveromyces lactis; Kluyveromyces marxianus* var. *lactis*	1,32,60
Kluyveromyces lodderae		22
Saccharomyces cerevisiae		1,32,53
Saccharomyces pastorianus;	*Saccharomyces carlsbergensis*	21
Saccharomyces unisporus		28,32,72
Yarrowia lipolytica (t) / Candida lipolytica (a)		22
Zygosaccharomyces rouxii		28,69
Saccharomyces turicensis sp. *nov*[b]		71

[a] (t): teleomorph; (a): anamorph
[b] This species is proposed by the authors, indicating the place Zurich (*Turicum* in Latin).
Note: This nomenclature was based in:
The yeast: a taxonomic study. Amsterdam, Elsevier Science BV, 1998.
Guidelines - Classification of organisms. Fungi. Berne, The Swiss Agency for the Environment, Forests and Landscapes (SAEFL), 2004.
Dr. Fungus: http://www.doctorfungus.org/.

Table 2. Bacterial microbiota isolates in different batches of kefir grains.

Actual nomenclature[a]	Obsolete nomenclature	Cited in
Lactobacilli		
Lactobacillus acidophilus		1
Lactobacillus brevis		46,60
Lactobacillus casei subsp. *casei*	*Lactobacillus casei*	1
Lactobacillus paracasei subsp. *paracasei*	*Lactobacillus casei* subsp. *pseudoplantarum*	60
Lactobacillus fermentum		69
Lactobacillus delbrueckii subsp. *bulgaricus*		60
Lactobacillus helveticus		24,60
Lactobacillus kefiri		13,14
Lactobacillus kefiranofaciens subsp. *kefiranofaciens*	*Lactobacillus kefiranofaciens*	39,73
Lactobacillus kefiranofaciens subsp. *kefirgranum*	*Lactobacillus kefirgranum*	64
Lactobacillus delbrueckii subsp. *lactis*	*Lactobacillus lactis*	23
Lactobacillus parakefiri		14,64
Lactobacillus plantarum		13,69
Other bacteria		
Lactococcus lactis subsp. *cremoris*	*Lactococcus cremoris; Streptococcus cremoris*	75
Lactococcus lactis subsp. lactis	*Lactococcus lactis; Streptococcus lactis*	13,69,75
Streptococcus thermophilus		60,75
Enterococcus durans		75
Leuconostoc. mesenteroides subsp. cremoris		69
Leuconostoc. mesenteroides subsp. mesenteroides		69
Acetobacter aceti		1

[a] Garrity GM, Bell JA, Lilburn TG. Taxonomic outline of the prokaryotes release 5.0. Bergey's Manual of Systematic Bacteriology (2nd Ed). New York, Springer, 2004.

acid bacteria (LAB). LAB diminish pH rapidly with lactate acumulation until this production is inhibited and components that originate flavour are produced (acetaldehide, etc.), along with aromas of fermented milk. The presence of yeasts is crucial for the desirable properties of carbon dioxide and ethanol production in products such as kefir. The interaction between yeast and the lactic microbiota may be stimulation or inhibition of growth of one, or both, of the co-cultured strains. These organisms may compete for growth nutrients or they may produce metabolic products that inhibit or estimulate each other's growth. The growth of yeasts in milk and their employment in milk components is a source of speculation. Very few yeast isolates in kefir are lactose-positive, but most strains are able to utilize galactose, lactate or citrate. As seen in this review, when lactose is used by LAB it has been assumed that both monosaccharide moieties are metabolised, but an increase in galactose in fermented milk has been detected. This observation means that yeasts that can asimilate galactose, but not other milk components or lactate, and that they could grow together with galactose-exporting LAB. Some yeast species are also proteolytic or lipolytic and may obtain important cell growth components through this metabolism. Yeasts may change their metabolism according to carbohydrate concentration and also to available oxygen like *S. cerevisiae*. *K. marxianus* metabolised lactose and showed production of glucose, galactose and ethanol. The other species showed proteolytic and lipolytic activity that increase the free amino acids and fatty acids during growth and some species has shown much greater activities for example *Yarrowia lipolytica*. Yeasts may produce vitamins that enhance the growth of LAB. Several of the yeasts isolated on fermented milk products may assimilate lactate and lactate-positive yeast in co-culture with LAB in milk can originate slightly increases the pH, which then allows further growth and lactose metabolism by LAB leading to increased lactate production, which could initiate a metabolic cycle. Also, when yeast and lactobacilli were grown together the kefiran production rates have shown to be increased [6].

Kefir production and industrial utilities

The "traditional" way of producing kefir is using raw unpasteurized, pasteurized, or UHT treated milk (Figure 4). The milk is poured into a clean suitable container with the addition of kefir grains. The content is left to stand at room temperature for approximately 24 h. The cultured-milk is filtered in order to separate and retrieve the kefir grains from the liquid-kefir. This fermented milk is appropiate for consumption. The grains are added to more fresh milk, and the process is simply repeated. This simple process can be performed on an indefinite basis, since kefir grains are a living ecosystem complex that can be preserved forever as long as it is feeded. As

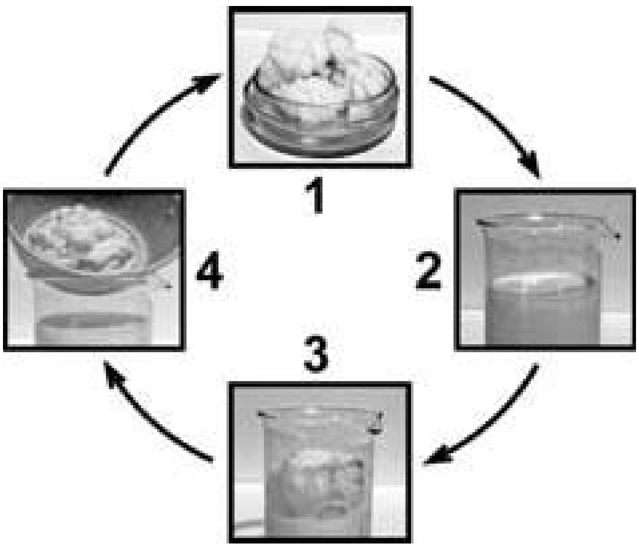

Figure 4. Kefir production. Kefir grains (1) are added to milk, (2) are left to stand at room temperature for fermentation 18-24 h, (3) after which they are filtered, (4) and ready to start another cycle. The fermented milk that results from step 4 is appropiate for consumption.

active kefir grains are continually cultured in fresh milk to prepare kefir, the grains increase in volume or in biological mass.

Although commercial kefir is available, the problem is that it doesn't share all the properties of "traditional" kefir. Different approaches have been taken to produce kefir in the industry. Some studies [29] have shown that kefir grains cannot be used as starter cultures in industry. The microbiota of a kefir grain is in symbiotic equilibrium but the species and quantitative structure of the various groups of microorganisms change significantly along the production procedure. Because of the complexity in the microbial relations in the communities in kefir, mutual influence of the microorganisms on each other's metabolism may lead to different profiles of organoleptically important compounds in the fermented milk. This is the reason why makes the employment of starter cultures difficult for its elaboration in industry.

Under certain conditions, by using a mixture of a limited number of representative microorganisms (lactobacilli, cocci and yeasts) isolated from kefir grains, the basic physicochemical and sensory characteristics of traditional kefir have been produced [4]. In another study [3], various ratios of starter cultures of kefir grains (lactic acid bacteria, yeasts and acetic acid bacteria) were used for production of kefir and the quality (colour, smell, flavour, acidity, effervescence and viscosity) of the product was assessed. Comparison of results with those of previous studies showed that kefir produced with kefir grains is more desirable than kefir produced with starter cultures.

Composition and sensory profiles of kefir using 'traditional' and 'modified' starter cultures were characterized finding differences between the sensory characteristics of the products [40]. In particular, the 'modified' kefir was less acidic, had little whey separation and was characterized by its creamy flavour.

Kefir beverage production is not the only industrial application being investigated. The conversion of agricultural and industrial waste materials into commercially valuable products is of great interest, especially when the final product can be a proteinaceous food source on regions with insufficient supply of traditional protein sources. For this reason kefir microorganisms were assessed for the production of single cell protein (SCP) on cheese whey [47]. The results of that study showed that the studied SCP was made of structures that were much stronger than those of gels made with soy flour.

Antifungal and antibacterial properties

In the early 1900s, an immunologist observed that life-long intake of yoghourt containing lactic acid producing microorganisms had a positive influence on the length of life. His idea was that the bacteria in the fermented products competed with microorganisms that are injurious to health [37]. Since then studies have been done demonstrating the antifungal and antibacterial activities of kefir.

Kefir is claimed to act against the pathogenic bacteria *Salmonella* [2,51,58], *Helicobacter* [58], *Shigella* [58], *Staphylococcus* [23,51,58], *Escherichia coli* [23,58,63], *Enterobacter aerogenes* [23], *Proteus vulgaris* [23], *Bacillus subtilis* [23], *Micrococcus luteus* [23], *Listeria monocytogenes* [51], *Streptococcus pyogenes* [51] and against the fungus *Candida albicans* [51].

In a recent study [2] assessing the survival of *Salmonella enteritidis* in the presence of kefir, 53 strains of this serovar were isolated from the Lugan river in Argentina, were cultured and inoculated into kefir at 22 °C and 4 °C. *S. enteritidis* was not detected after 24h incubation at 4 °C and after 18h incubation at 22 °C, demonstrating similar antibacterial activity of kefir at ambient and refrigerated temperature. In another study [63], the antibacterial activity of kefir against strains ATCC 25922 and O157:H7 of *E. coli* was analyzed under different pH conditions. Under neutral pH conditions bacterial growth was not influenced by the presence of kefir. However, in an acidic pH environment growth inhibition of both strains ocurred in short time, suggesting the existence of an antibacterial substance in kefir activated in an acidic environment.

This antimicrobial activity against pathogenic microorganisms has been recently applied. Kefir and kefiran have been tested for antimicrobial and cicatrizing activities against several bacterial species and *C. albicans* [51]. The most sensitive was *Streptococcus pyogenes* followed by *Staphylococcus aureus*, *Salmonella typhimurium*, *C. albicans* and *Listeria monocytogenes*. A kefir gel showed cicatrizing activity since a faster reduction of the wound diameter was observed compared to negative control in rats, indicating that kefir biofilms and their polysaccharide compounds may be good antimicrobial, anti-inflammatory and cicatrizing agents for use in a variety of infections.

The data reviewed could be, however, contradictory since apparently all microorganisms that comprise kefir seem inocuous and beneficial, but this could be deceptive. The absence of *C. albicans* in kefir can be explained because of the activity of some LAB found in kefir such as, *L. acidophilus* y *L. delbrueckii* [5,7,62] or kefir itself. However other *Candida* species such as *C. kefyr* have been detected. This species, apparently inocuous causes infections in burned patients [16] and it has been isolated form bloodstream [48]. The possibility that some strains of *C. kefyr* capable of causing infection could colonize kefir should therefore be considered. The symbiotic equilibrium of kefir may prevent the development of a possible infection by these strains and we must remember that *Candida* species are opportunistic and not pathogenic in all conditions.

Benefits for the digestive tract

As we have mentioned before, probiotics are defined as food with viable microorganisms that exhibit a beneficial effect on the host's health when they are ingested. The most popular probiotic microorganisms are bacteria but at the moment the usefulness of some yeast as probiotics is being studied [22,35]. Two important criteria are used for selection of probiotic microorganisms: they must be able to survive in the gastrointestinal environment and they must present at least one beneficial function (colonization resistance, immunomodulation or nutritional contribution). Studies have shown that probiotics can protect from gastrointestinal diseases [34,77]. Kefir seems to improve lactose digestion and tolerance in adults with lactose intolerance [18] and it also appears to have a stimulatory effect on the motor and emptying function of the gastric stump. In contrast, milk, milk whey, cheese and butter have inhibitory effects on this function [27]. Kefir comprises lactobacilli that have been associated to clear probiotic effects and some of the yeasts that are present in kefir such as *S. cerevisiae, Kluyveromyces lodderae, K. marxianus* and *Candida humilis* can show a colonizing effect on the intestine [22].

Immunological and antitumoural effects

In addition to antifungal and antibacterial activity, kefir [68] and kefiran [38] have been employed in studies to assess their effects on neoplams and their capability of being immunomodulators.

In a recent study the immunomodulating capacity of kefir on the intestinal mucosal immune response in mice was demonstrated [68]. Both kefir and pasteurized kefir showed to modulate the mucosal system in a dose-dependent manner. In addition, cell viability showed to be important since kefir was administred 10-times more diluted than pasteurized kefir and still induced an immunomodulation of similar magnitude. Kefir and sphingomielin isolated from the lipids in kefir have been reported to stimulate the immune system *in vitro* and *in vivo* [11,45]. Dietary supplementation with kefir has shown to act on the mucosal and systemic immune system of rats. In this case, animals with a dietary supplement of kefir and without it, were inmunized with the cholera toxin (CT). Kefir enhanced the serum anti-CT IgA antibody titer in rats and it was an age dependent effect [67]. Results suggest that kefir enhances the serum non-specific total IgG titer in both young and old rats whereas the non-specific IgA titer is similar in kefir fed and control groups.

A number of studies have assessed the anti-tumor activity of kefir [10-12,41,76]. The anti-tumor activity observed in kefir is thought to be originated in the polysaccharide content of the grains. For this reason the anti-metastatic activities of polysaccharides from kefir have been studied [12,41]. Water-soluble (WP) and water-insoluble (WIP) polysaccharide fractions of kefir grains against Lewis lung carcinoma and B16 melanoma cells were investigated in mice [12]. The WP fraction had a protective effect against pulmonary metastasis, but had little effect on metastasis of the melanoma cells, whereas the WIP fraction inhibited melanoma metastasis. The oral administration of kefiran, also named KGF-C, a water soluble polysaccharide from kefir grains, to mice showed a rise of antitumor activities and a delayed-type hypersensitivity induced by picryl chloride [41,59].

Although the previously commented studies show a protective effect on the formation of tumors, a recent study has demonstrated that nutritional facts are also important [19]. In this study three groups were studied of rats and were fed with kefir containing 3.5% fat, kefir containing 1.1% fat and milk containin 1.1% fat, respectively. The kefir 3.5% fat group had the highest number of adenocarcinomas whereas the lowest number of adenocarcinomas was found in the 1.1% milk fat group. From this study, the authors conclude that kefir may be protective but diet is also important since there was a high correlation between fat intake and adenocarcinoma formation.

The antimutagenic and antioxidant properties of kefir have also been assessed recently [25,26]. When milk kefir and soy-milk kefir were compared [25] both demonstrated significantly greater antimutagenic and anti-oxidative activity than milk or soy-milk alone. When cow-milk kefir and goat-milk kefir were compared [26], both showed substantial anti-oxidative activity. These findings suggest that kefir can be a promising food component for the prevention of mutagenic and oxidative damage and a potential candidate for the role of useful natural antioxidant supplements for the human diet.

It has also been suggested that kefir can suppress UV damage in cells [42]. In this study an aqueous extract of kefir suppressed morphological changes of human melanoma HMV-1 and SK-MEL cells and human normal fibroblast TIG-1 cells caused by UVC-irradiation. The kefir extract also stimulated unscheduled DNA synthesis and suppressed UVC-induced apoptosis of HMV-1 cells and exhibited strong thymine dimer repair-enhancing activity. Kefir has also shown to be beneficial on cells that have suffered damage by radiation [36]. When radiotherapy is employed for treatment of malignant pelvic disease, patients usually suffer rapid apoptotic death of the stem cells in the crypts of their intestine. In high doses, damage to the crypt is accompanied by functional changes. Kefir was able to decrease the apoptotic index in 2 h compared to controls indicating a protective effect on colonic crypt cells.

Effects on cholesterol

Cholesterol assimilation was studied in *Lactobacillus* strains from kefir and yoghourt and in non-starter strains; assimilation activity was also measured in several *Bifidobacterium* strains [74]. Cholesterol assimilation activity showed to be highly strain dependent, and was generally much higher in non-starter *Lactobacillus* strains than in starters. The highest activity detected (68.8%) was in *L. acidophilus* CU 673, isolated from kefir and yoghourt, meaning that kefir could enhance cholesterol assimilation.

The effects of a kefir produced by a mixture of 10 types of LAB and *S. cerevisiae* on serum and liver lipid concentration were analyzed in rats fed high-cholesterol diets [65]. Serum total cholesterol and phospholipid levels were significantly decreased in rats fed a high-cholesterol diet supplemented with fermented milk. However, there were no significant differences in the serum high density lipoproteincholesterol and triglyceride levels among the treatment groups. Total cholesterol and phospholipid levels in the liver showed no significant difference among the treated groups.

However, in a recent study performed with 13 hypocholesterolemic men, kefir did not affect total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or trygliceride concentrations after a four-week supplementation [61]. The authors suggest that their results could be due to a low CFU (10^9) ingestion, when other authors [57] show that 10^{11} CFU is required to produce colonization in all subjects.

Controversy on results

A great controversy exists among the way the clinical trials are performed with kefir. The lack of a standardized protocol makes the interpretation of results difficult. In most studies sample sizes are not very representative. Also the treament periods with kefir vary although the general consensus suggests that probiotic organisms must be ingested continuously to exert their health benefits [9]. The viability of the microorganisms in the beverage seems to be another issue. While some authors claim that probiotic microorganisms may not be required to enhance the immune response since improved lactose digestion and the modulation of certain immune activities have been reported with non-viable probiotic organisms [56]. Other authors [2] affirm that the viability is important. Also controls seem to be a problem, since each study uses a different compound as placebo. Some authors [31] expose that a possible control for testing kefir in clinical trials would be a group consuming kefir in which microorganisms are inactivated. However, microorganism inactivation could also result in alterations of lipids and proteins that could be responsible for some of the beneficial properties. We enfatize that there should be a consensus concerning the way in vitro and in vivo studies are achieved in order to analyze the beneficial effects of kefir and be able to employ this product in medicine as a complementary dietary food.

Conclusions

Kefir is an example of coexistance of bacteria and yeast in the same environment, in equilibrium being beneficial for one another. The importance of the symbiotic relation in kefir between yeast and bacteria seems clear since they are both necessary in order to produce the components that are beneficial to health. Although the evidence might not result conclusive and much further research is required, the data reviewed constitute a promising evidence of the protective roles that kefir shows on health. The importance of probiotics in food industry is growing nowadays and further research should be performed on the symbiotic relations between different microorganisms and how these interactions can result in curing and preventing human diseases and other disorders.

The authors gratefully acknowledge the help of Dr. Ricardo Andrade (Servicio General de Microscopía Analítica y de Alta Resolución en Biomedicina, University of the Basque Country) for technical assistance and processing of the specimens to view under SEM.

References

1. Angulo L, Lopez E, Lema, C. Microflora present in kefir grains grown in milk and in soy milk. J Dairy Res 1993; 60:263-267.
2. Anselmo RJ, Viora SS, Lausada LI. Effect of kefir bactericide on *Salmonella* spp. Información Tecnológica 2001; 12: 91-95.
3. Assadi MM, Pourahmad R, Moazami N. Use of isolated kefir starter cultures in kefir production. World J Microbiol Biotech 2000; 16: 541-543.
4. Beshkova DM, Simova ED, Simov ZI, Frengova GI, Sapsov ZN. Pure cultures for making kefir. Food Microbiol 2002; 19: 537-544.
5. Brzozowski T, Zwolinska-Wcislo M, Konturek PC, Kwiecien S, Drozdowicz D, Kontureck SJ, Stachura J, Budak A, Pawlik WW, Hahn EG. Influence of gastric colonization with *Candida albicans* on ulcer healing in rats: effect of ranitidine, aspirin and probiotic therapy. Scand J Gastroenterol 2005, 40: 286-296.
6. Cheirsilp B, Shimizu H, Shioya S. Enhanced kefiran production by mixed culture of *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae*. J Biotechnol 2003; 100: 43-53.
7. Elahi S, Pang G, Ashman R, Clancy R. Enhanced clearance of *Candida albicans* from the oral cavities of mice following oral administration of *Lactobacillus acidophilus*. Clin Exp Immunol 2005; 141: 23-36.
8. Engel G, Krusch U, Teuber, M. Microbiological composition of kefir I. Yeast. Milchwissenschaft Milk Sci Intern 1986; 41: 418-421.
9. Fuller R. Probiotics in human medicine. Gut 1991; 32: 439-442.
10. Furukawa N, Matsuoka A, Takahashi T, Yamanaka Y. Effects of orally administered yogurt and kefir on tumor growth in mice. J Jpn Soc Nutr Food Sci 1990; 43: 450-453.
11. Furukawa N, Matsuoka A, Takahashi T, Yamanaka Y. Effects of fermented milk on the delayed-type hypersensitivity response and survival in mice bearing Meth-A. Anim Sci Technol 1991; 62: 579-585.
12. Furukawa N, Matsuoka A, Takahashi T, Yamanaka Y. Anti-metastatic effect of kefir grain components on Lewis lung carcinoma and highly metastatic B16 melanoma in mice. J Agric Sci Tokyo 2000; 45: 62-70.
13. Garrote GL, Abraham AG, De Antoni GL. Chemical and microbiological characterisation of kefir grains. J Dairy Res 2001; 68: 639-652.
14. Garrote GL, Delfederico L, Bibiloni R, Abraham AG, Perez PF, Semorile L, De Antoni GL. *Lactobacilli* isolated from kefir grains: evidence of the presence of S-layer proteins. J Dairy Res 2004; 71: 222-230.
15. Gorbach SL. The discovery of lactobacillus GG. Nutrition Today 1996; 31: 2S-4S.
16. Gupta N, Haque A, Lattif AA, Narayan RP, Mukhopadhyay G, Prasad R. Epidemiology and molecular typing of *Candida* isolates from burn patients. Mycopathologia 2004; 158: 397-405.
17. Hallé C, Leroi F, Dousset X, Pidoux M. Les kéfirs: des associations bactéries lactiques- levures. In: de Roissart H, Luquet FM (Eds.) Bactéries lactiques: Aspects fondamentaux et technologiques. Vol. 2. Uriage, Lorica, 1994: 169-182.
18. Hertzler SR, Clancy SM. Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. J Am Diet Assoc 2003; 103: 582-587.
19. Hlastan-Ribic C, Cerar A, Pokorn D, Perse M, Zebic A. Effects of kefir containing variuos levels of fat on chemically induced colorectal epithelial tumors in Wistar rats. Nutr Res 2005; 25: 55-63.
20. Iwasawa S, Ueda M, Miyata N, Hirota T, Ahiko K. Identification and fermentation character of kefir yeast. Agr Biol Chem 1982; 46: 2631-2636.
21. Koroleva NS. Technology of kefir and kumys. Bull Intern Dairy Fed 1988; 227: 96-100.
22. Kumura H, Tanoue Y, Tsukahara M, Tanaka T, Shimazaki K. Screening of dairy yeast strains for probiotic applications. J Dairy Sci 2004; 87: 4050-4056.
23. Kwon CS, Park MY, Cho JS, Choi ST, Chang DS. Identification of effective microorganisms from kefir fermented milk. Food Sci Biotech 2003; 12: 476-479.
24. Lin CW, Chen HL, Liu JR. Identification and characterisation of lactic acid bacteria and yeast isolated from kefir grains in Taiwan. Austral J Dairy Technol 1999; 54: 14-18.
25. Liu JR, Chen MJ, Lin CW. Antimutagenic and antioxidant properties of milk kefir and soy-milk kefir. J Agric Food Chem 2005; 53: 2467-2474.
26. Liu JR, Lin YY, Chen MJ, Chen LJ, Lin CW. Antioxidative activites of kefir. Asian-Australasian J Animal Sci 2005; 18: 567-573.
27. Loranskaia TI, Khoromskii LN, Benedikt VV. Effects of a series of food substances on motor and emptying function of the gastricstump and diverting intestinal loop after stomach resection and truncal vagotomy. Vopr Pitan 1986; 1: 19-22.
28. Loretan T, Mostert JF, Viljoen BC. Microbial flora associated with South African household kefir. South African J Sci 2003; 99: 92-94.
29. Maeda H, Zhu X, Omura K, Suzuki S, Kitamura S. Effects of an exoploysaccharide (Kefiran) on lipids, blood pressure, blood glucose, and constipation. Biofactors 2004; 22: 197-200.
30. Maeda H, Zhu X, Suzuki S, Suzuki K, Kitamura K. Structural characterization and biological activities of an exoploysaccharide kefiran produced by *Lactobacillus kefiranofaciens* WT-2B. J Agric Food Chem 2004; 52: 5533-5538.
31. Mainville I, Montpetit D, Durand N and Farnworth ER. Deactivating the bacteria and yeast in kefir using heat treatment, irradiation and high pressure. Int Dairy J 2001; 11: 45-49.
32. Marquina D, Santos A, Corpas I, Muñoz J, Zazo J, Peinado JM. Dietary influence of kefir on microbial activities in the mouse bowel. Lett Appl Microbiol 2002; 35: 136-140.
33. Marshall VM, Cole WM. Methods for making kefir and fermented milks based on kefir. J Dairy Res 1985; 52: 451-456.
34. Marteau PR, de Vrese M, Cellier CJ, Schrezemeir J. Protection from gastrointestinal diseases with the use of probiotics. Am J Clin Nutr 2001; 73: 430S-436S.

35. Martins FS, Nardi RM, Arantes RM, Rosa CA, Neves MJ and Nicoli JR. Screening of yeast as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. J Gen Appl Microbiol 2005; 51: 83-92.
36. Matsuu M, Shichijo K, Okaichi K, Wen CY, Fukuda E, Nakashima M, Nakayama T, Shiranata S, Tokumaru S, Sekine I. The protective effect of fermented milk kefir on radiation induced apoptosis in colonic crypt cells of rats. J Radiat Res 2003; 44: 111-115.
37. Metchnikoff E. The prolongation of life: Optimistic studies. London, W. Heinemann, 1907.
38. Micheli L, Uccelletti D, Palleschi C, Crescenzi V. Isolation and characterisation of a ropy *Lactobacillus* strain producing the exopolysaccharide kefiran. Appl Microbiol Biotech 1999; 53: 69-74.
39. Mitsue T, Tachibana K, Fujio Y. Efficient kefiran production by a mixed culture of *Lactobacillus kefiranofaciens* KF-75 and yeast strains. Seibutsu-Kogaku Kaishi J Soc Ferm Bioeng 1999; 77: 99-103.
40. Muir DD, Tamime AY, Wszolek M. Comparison of the sensory profiles of kefir, buttermilk and yogurt. Int J Dairy Technol 1999; 52: 129-134.
41. Murofushi M, Mizuguchi J, Aibara K, Matuhasi T. Immunopotentiative effect of polysaccharide from kefir grain, KGF-C, administered orally in mice. Immunopharmacology 1986; 121: 29-35.
42. Nagira T, Narisawa J, Teruya K, Katakura Y, Shim SY, Kusumoto K, Tokumaru S, Tokumaru K, Barnes DW, Shirahata S. Suppression of UVC-induced cell damage and enhancement of DNA repair by the fermented milk, Kefir. Cytotechnology 2002; 40: 125-137.
43. Narvhus JA, Gadaga TH. The role of interaction between yeast and lactic acid bacteria in African fermented milks: a review. Int J Food Microbiol 2003; 86: 51-60.
44. Neve H, Heller KJ. The microflora of water kefir: a glance by scanning electron microscopy. Kieler Milchwirtschaftliche Forschungsberichte 2002; 54: 337-349.
45. Osada K, Nagira K, Teruya K, Tachibana H, Shirahata S, Murakami H. Enhancement of interferon-ß production with sphingomyelin from fermented milk. Biotherapy 1994; 7: 115-123.
46. Ottogalli G, Galli A, Resmini P, Volonterio G. Composizione Microbiologique, chimica et ultraestructtura dei granuli di Kefir. Ann Microbiol Enzimol 1973; 23, 109-121.
47. Paraskevopoulou A, Athanasiadis I, Kanellaki M, Bekatorou A, Blekas G, Kiosseoglou V. Functional properties of single cell protein produced by kefir microflora. Food Res Int 2003; 36: 431-438.
48. Pfaller MA. Diekema DJ, International Fungal Surveillance Participant Group. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. Clin Microbiol Infect 2004; 10:11-23.
49. Pintado ME, Da Silva JAL, Fernandes PB, Malcata FX, Hogg TA. Microbiological and rheological studies on Portuguese kefir grains. Int J Food Sci Technol 1996; 31: 15-26.
50. Roberts M, Yarunin S. Danone moves into Russian kefir market. New Nutr Business 2000; 6: 22-24.
51. Rodrigues KL, Caputo LRG, Carvalho JC, Evangelista J, Schneedorf JM. Antimicrobial and healing activity of kefir and kefiran extract. Int J Antimicrob Agents 2005; 25: 404-408.
52. Rohm H, Eliskases-Lehner FY, Brauer M. Diversity of yeast in selected dairy products. J Appl Bacteriol 1992; 72: 370-376.
53. Rosi J. I microorganismi del kefir: I Lieviti. Scienza e Tecnica Lattiero-Casearia 1978; 29: 59-67.
54. Salminen S, Bouley C, Boutron-Ruault MC. Functional food science and gastrointestinal physiology and function. Br J Nutr 1998; 80: S147–S171.
55. Salminen SA, Ouwenhand A, Benno Y and Lee YK. Probiotics: How should they be defined?. Trends Food Sci Technol 1999; 10: 107-110.
56. Sanders ME. Considerations for use of probiotic bacteria to modulate human health. J Nutr 2000; 130: 384S-390S.
57. Saxelin S, Elo S, Salminen S, Vapaatalo J. Dose response colonisation of faeces after oral administration of *Lactobacillus casei* strain GG. Am J Clin Nutr 1991; 59: 929-934.
58. Schneedorf JM, Anfiteatro DN. Quefir, um probiotico produzido por microorganismos encapsulados e inflamaçao. In: Carvalho JCT (Ed.) Fitoterapicos Anti-inflamatorios. São Paulo, Tecmedd, 2004: 443-462.
59. Shiomi M, Sasasaki K, Murofishi M, Aibara K. Antitumor activity in mice of orally administered polysaccharide from Kefir grain. Jpn J Med Sci Biol 1982; 35: 75-80.
60. Simova E, Beshkova D, Angelov A, Hristozova Ts, 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.
61. St-Onge Mp, Franworth ER, Savard T, Chabot D, Maku A, Jones PJH. Kefir consumption dose not alter plasma lipid levels or cholesterol fractional synthesis rates relative to milk hyperlipidemic men: a randomized controlled trial. BMC Complementary and Alternative Medicine 2002; 2:1-7.
62. Strus M, Brzychczy-Wloch M, Kucharska A, Gosiewski T, Heczko PB. Inhibitory activity of vaginal *Lactobacillus* bacteria on yeast causing vulvovaginal candidiasis. Med Dosw Mikrobiol 2005; 57: 7-17.
63. Takahashi F, Kawakami H. Antibacterial action of kefir against *Escherichia coli* O157:H7. Jpn J Food Microbiol 1999; 16: 245-247.
64. Takizawa S, Kojima S, Tamura S, Fujinaga S, Benno Y, Nakase T. *Lactobacillus kefirgranum* sp-nov and *Lactobacillus parakefir* sp-nov, 2 new species from kefir grains. Int J Syst Bacteriol 1994; 44: 435-439.
65. Tamai Y, Yoshimitsu N, Watanabe Y, Kuwabara Y, Nagai S. Effects of milk fermented by culturing with various lactic acid bacteria and a yeast on serum cholesterol level in rats. J Ferment Bioeng 1996; 81: 181-182.
66. Taniguchi M, Tanaka T. Clarification of interaction among microorganisms and development of co-culture system for production of useful substances. Adv Biochem Eng Biotechnol 2004; 90: 35-62.
67. Thoreux K and Schmucker DL. Kefir milk enhances intestinal immunity in young but not old rats. J Nutri 2001; 131: 807-812.
68. Vinderola CG, Duarte J, Thangavel D, Perdigon G, Farnworth E, Matar C. Immunomodulating capacity of kefir. J Dairy Res 2005; 72: 195-202.
69. Witthuhn RC, Schoeman T, Britz TJ. Characterization of the microbial population at different stages of Kefir production and Kefir grain mass cultivation. Int Dairy J 2005; 15: 383-389.
70. Wyder MT. Identification and characterization of the yeast flora in kefir and smear ripened cheese: Contribution of selected yeast to cheese ripening. PhD thesis, ETH, Zurich, Switzerland, 1998.
71. Wyder MT, Meile L, Teuber M. Description of *Saccharomyces turicensis* sp. nov., a new species from kefyr. Syst Appl Microbiol 1999; 3: 420-425.
72. Wyder MT, Spillmann H, Puhan Z. Investigation of the yeast flora in dairy products: a case study of kefyr. Food Technol Biotechnol 1997; 35: 299-304.
73. Yokoi H, Watanabe T, Fuji Y. Isolation and characterization of polysaccharide-producing bacteria from kefir grains. J Dairy Sci 1990; 73: 1684-1689.
74. Yoon YH, Kang DG, Baek YJ, Huh CS. Cholesterol assimilation activity of *Lactobacillus* spp. from kefir and yoghurt and non starter strains. Korean J Dairy Sci 1998; 20: 143-152.
75. Yuksekdag ZN, Beyatli Y, Aslim B. Determination of some characteristics coccoid forms of lactic acid bacteria isolated from Turkish kefirs with natural probiotic. Lebensm Wiss. Technol Food Sci Technol 2004; 37: 663-667.
76. Zacconi C, Parisi MG, Sarra PG, Dallavalle P, Bottazzi V. Competitive exclusion of *Salmonella kedougou* in kefir fed chicks. Microbiol Alim Nutr 1995; 12: 387-390.
77. Zubillaga M, Weill R, Postaire E, Goldman C, Caro R, Bocci J. Effects of probiotics and functional foods and their use in different diseases. Nutr Res 2001; 21: 569-579.