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Kefir – a complex probiotic

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Abstract

Kefir is a fermented milk drink produced by the actions of bacteria and yeasts contained in kefir grains, and is reported to have a unique taste and unique properties. During fermentation, peptides and exopolysaccharides are formed that have been shown to have bioactive properties. Moreover, *in vitro* and animal trials have shown kefir and its constituents to have anticarcinogenic, antimutagenic, antiviral and antifungal properties. Although kefir has been produced and consumed in Eastern Europe for a long period of time, few clinical trials are found in the scientific literature to support the health claims attributed to kefir. The large number of microorganisms in kefir, the variety of possible bioactive compounds that could be formed during fermentation, and the long list of reputed benefits of eating kefir make this fermented dairy product a complex probiotic.

Keywords: kefir, probiotics, kefir grains, kefiran, human health, bioactive ingredients

1. Introduction

Archaeological evidence has indicated that the process of fermentation in foods was discovered accidentally thousands of years ago. However, over time, it soon became apparent that many fermented foods had longer storage lives and improved nutritional values compared to their unfermented equivalents, making this form of food processing a popular technique. It is not surprising, therefore, to find that many foods including vegetables, fruits, cereals, meat and fish have all been converted into desirable food products by fermentation and are still being consumed throughout the world today (Farnworth 2004).

Certain bacteria, either alone or through the changes they bring about during fermentation, have been shown to have positive effects on health as well as resistance to disease. Interest in such probiotic species has increased in recent years as more is learned about the microorganisms used in the fermentation process, and the possibility of adding beneficial bacteria to food products. Furthermore, consumers are increasingly looking to improve their health and increase their resistance to disease through dietary means.

Fermented dairy products from milk from a variety of animals are perhaps the most common fermented foods worldwide. Yoghurt, which is known by many different names in different countries, is a fermented product which is familiar to consumers. Kefir, meanwhile, is less well known than yoghurt; however, an analysis of its composition indicates that it may contain bioactive ingredients that give it unique health benefits, which means that kefir may be an important probiotic product (Farnworth 1999).

2. Origins of kefir

Kefir is a viscous, slightly carbonated dairy beverage that contains small quantities of alcohol and, like yoghurt, is believed to have its origins in the Caucasian mountains of the former USSR. It is also manufactured under a variety of names including kephir, kiaphur, kefer, knapon, kepi and kippi (Koroleva 1988a), with artisanal production of kefir occurring in countries as widespread as Argentina, Taiwan, Portugal, Turkey and France (Thompson *et al*. 1990; Angulo *et al*. 1993; Lin *et al*. 1999; Garrote *et al*. 2001; Santos *et al*. 2003; Gulmez and Guven 2003). It is not clear whether all kefirs originate from a single original starter culture, since microbial analyses of kefir samples taken from different locations indicate microflora population differences.

The FAO/WHO (2001) have proposed a definition of kefir based on the microbial composition of both kefir grains (the starter culture used to produce kefir) and the final kefir product (see Table 1).

3. Kefir manufacture

Although commercial kefir is traditionally manufactured from cows' milk, it has also been made from the milk of ewes, goats and buffalos. Moreover, kefir produced using soy milk has also been recently reported (Ismail *et al*.

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Table 1. Codex Alimentarius description of kefir*

Definition	
Starter culture prepared from kefir grains, *Lactobacillus kefiri*, and species of the genera *Leuconostoc*, *Lactococcus* and *Acetobacter* growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (*Kluyveromyces marxianus*) and non-lactose-fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*).	
Composition	
Milk protein (% w/w)	min. 2.8
Milk fat (% m/m)	<10
Titratable acidity, expressed as % of lactic acid (% m/m)	min. 0.6
Ethanol (% vol./w)	not stated
Sum of specific microorganisms constituting the starter culture (cfu/g, in total)	min. 10^7
Yeasts (cfu /g)	min. 10^4

*From Codex Standard for Fermented Milks CODEX STAN 243-2003

1983; Mann 1985; Zourari and Anifantakis 1988; Hallé *et al*. 1994; Kuo and Lin 1999). Traditionally, kefir is produced by adding kefir grains (a mass of proteins, polysaccharides, mesophilic, homofermentative and heterofermentative lactic acid streptococci, thermophilic and mesophilic lactobacilli, acetic acid bacteria, and yeast) to a quantity of milk (Koroleva 1982; Hallé *et al*. 1994; Tamime *et al*. 1999). The size of the initial kefir grain inoculum affects the pH, viscosity and microbiological profile of the final product (Koroleva and Bavina 1970; Garrote *et al*. 1998). Koroleva (1991) reported that grain to milk ratios of 1:30 to 1:50 were optimum. In some manufacturing procedures, a perculate of the grains from a coarse sieve is used as the mother culture to inoculate fresh milk. Fermentation of the milk by the inoculum proceeds for approximately 24 hours, during which time homofermentative lactic acid streptococci grow rapidly, initially causing a drop in pH. This low pH favours the growth of lactobacilli, but causes the streptococci numbers to decline. The presence of yeasts in the mixture, together with fermentation temperature (21–23°C), encourages the growth of aroma-producing heterofermentative streptococci. As fermentation proceeds, growth of lactic acid bacteria is favoured over growth of yeasts and acetic acid bacteria (Koroleva 1982).

Taiwanese researchers have shown that the lactic acid bacteria from kefir grains grow more slowly in soy milk compared to cows' milk (Liu and Lin 2000). This may be due, in part, to the slower production of growth factors at the beginning of fermentation when soy milk is the substrate rather than cows' milk. Addition of carbohydrate (e.g. 1% glucose) to soy milk increases yeast numbers, lactic acid production and ethanol production, compared to kefir produced from soy milk alone (Liu and Lin 2000). The grains used in this study were found to have α-galactosidase activity that helped explain how these kefir grains were able to use the galactose-based carbohydrates which occur in soy milk.

Kefir grains are key to kefir production, and it has been found that the finished product has a different microbiological profile from the grains and therefore cannot be used to inoculate a new batch of milk (Simova *et al*. 2002). Grains have been shown to possess a dynamic and complex flora which is not conducive to commercial production of a uniform, stable product; this has prompted groups to try to produce kefir from a mixture of pure cultures (Petersson *et al*. 1985). Duitschaever *et al*. (1987, 1988a) combined a yoghurt culture with three other lactic acid bacteria and *Saccharomyces cerevisiae* (a non-lactose fermenting yeast) to produce a fermented milk with kefir characteristics (which produced CO_2 and contained ethanol) under a variety of conditions. Rossi and Gobbetti (1991) produced a multistarter culture using four bacteria and two yeasts isolated from kefir grains in order to manufacture kefir under a continuous process. More recently, Beshkova *et al*. (2002) produced a starter consisting of two bacteria (*Lactobacillus helveticus* and *Lactococcus lactis* subsp. *lactis*,) and one yeast (*S. cerevisiae*) isolated from kefir grains and combined with two yoghurt strains (*Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*). Yeast was added to the starter with sucrose either at the beginning, or after lactic acid fermentation. The two resulting kefirs produced were found to have high numbers of viable cocci and lactobacilli and had chemical and organoleptic properties that were similar to traditional kefir. A commercial kefir is being produced in the United States using a mixture of defined microorganisms rather than kefir grains. This starter culture mixture has been reported to contain *Streptococcus lactis*, *L. plantarum*, *Streptococcus cremoris*, *L. casei*, *Streptococcus diacetylactis*, *Leuconostoc cremoris* and *Saccharomyces florentinus* (Hertzler and Clancy 2003).

Starter cultures containing freeze-dried lactic acid bacteria and yeasts from kefir grains are now available commercially; some are supplemented with additional microorganisms to impart desirable characteristics in the finished kefir product (Piotr Kolakowski, private communication). It is evident that the final product, as produced from kefir grains, will have a larger number and variety of microorganisms than kefir produced from a mixture of a small number of pure cultures.

Kefir is still most familiar to consumers in Eastern Europe, although commercial production now occurs in North America. However, several patents can be found relating to commercial kefir production worldwide (Klupsch 1984; Dmitrovskaya 1986; Tokumaru *et al*. 1987; Kabore 1992).

Production/consumption figures for kefir are not readily available since statistics for fermented dairy products are not always broken down into separate items such as yoghurt and kefir (Mann 1989; Libudzisz and Piatkiewicz 1990; Serova 1997; Zimovetz and Boyko 2000). A survey of kefir products purchased on the retail market in Warsaw, Poland showed that 73% of products contained 10^7–10^9 cfu bacteria/g, and that 97% of samples were coliform-free (Molska *et al*. 2003). However, 48% of samples did not meet FAO/WHO requirements for yeast numbers (FAO/WHO 2001).

4. Characteristics of kefir

The flavour, viscosity and microbial/chemical composition of the final kefir product can be affected by the size of the inoculum added to the milk, the occurrence of any agitation during fermentation, and the rate, temperature and duration of the cooling and ripening stages following fermentation (Koroleva 1988b). Natural kefir has a refreshing, yeasty taste and a 'sparkling' mouth feel (Kemp 1984).

Modern manufacturing procedures for kefir result in ethanol levels in the finished product of 0.01–0.1% (Koroleva 1982), although kefir with ethanol concentrations as high as 0.25% have been produced from grains in the laboratory (Kuo and Lin 1999; Simova *et al*. 2002; Beshkova *et al*. 2002). The amounts of ethanol and CO_2 produced during fermentation of kefir depend on the production conditions used. CO_2 content of kefir has been said to be 'comparatively low' in relation to other fermented drinks (Koroleva 1982); values of 0.85–1.05 g/l have been reported for kefir produced from kefir grains (Beshkova *et al*. 2002; Simova *et al*. 2002) and 1.7 g/l for kefir produced from purified cultures (Gobbetti *et al*. 1990) However, the generation of CO_2 during kefir manufacture, especially after packaging, presents some practical problems, since the microorganisms (particularly yeasts) in the kefir continue to grow following packaging. The container used to package kefir must therefore be either strong enough to withstand any pressure build up (e.g. glass) or flexible enough to contain the volume of gas produced (e.g. plastic with an aluminium foil top (Kwak *et al*. 1996).

The distinctive taste of kefir results from the presence of several flavour compounds which are produced during fermentation (Beshkova *et al*. 2003). Kefir produced from pure cultures did not receive high sensory evaluation scores in Canada unless it was sweetened (Duitschaever *et al*. 1987, 1991); Duitschaever *et al*. (1987) also showed that only about 40% of people tasting natural kefir for the first time gave it a positive taste rating. Addition of peach flavour, or modification of the fermentation process (e.g. addition of lactococci, lactobacilli or yeasts) increased the acceptability of kefir, compared to traditionally made kefir (Duitschaever *et al*. 1991; Muir *et al*. 1999).

Acetaldehyde and acetoin have received particular attention with regard to their roles during kefir manufacture because of their contribution to taste; both have been found to increase in concentration during kefir fermentation. During storage, acetaldehyde increases in concentration and acetoin decreases (Güzel-Seydim *et al*. 2000a, 2000b). Yüksekgağ *et al*. (2004a), in their study of 21 isolates of lactic acid bacteria from various sources of Turkish kefir, were able to show that all 21 isolates produced acetaldehyde (0.88–4.40 μg/ml) when added to milk.

A whey beverage with an acceptable flavour has recently been developed using kefir yeasts (Athanasiadis *et al*. 2004), especially when fructose was added to fresh milk before fermentation, and final pH of the beverage was 4.1. Fructose was found to increase production of several flavour volatiles, but did not increase fermentation time.

5. Kefir grains

Kefir grains resemble small cauliflower florets: they measure 1–3 cm in length, are lobed, irregularly shaped, white to yellow-white in colour, and have a slimy but firm texture (La Rivière *et al*. 1967; Kosikowski and Mistry 1997; see Figure 1). Grains are kept viable by transferring them daily into fresh milk and allowing them to grow for approximately 20 hours; during this time, the grains will have increased their mass by 25% (Hallé *et al*. 1994). Grains must be replicated in this way to retain their viability, since old and dried kefir grains have little or no ability to replicate (La Rivière *et al*. 1967). Kefir grains repli-

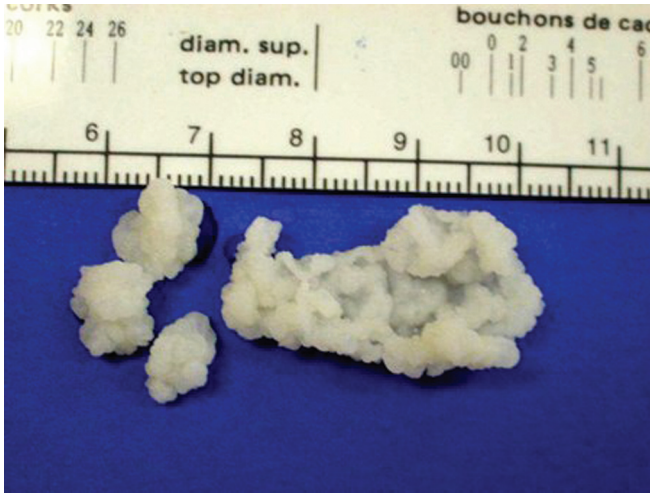

Figure 1. Kefir grains. Reproduced with permission from Handbook of Fermented Functional Foods, Farnworth, E.R. editor. Copyright CRC Press.

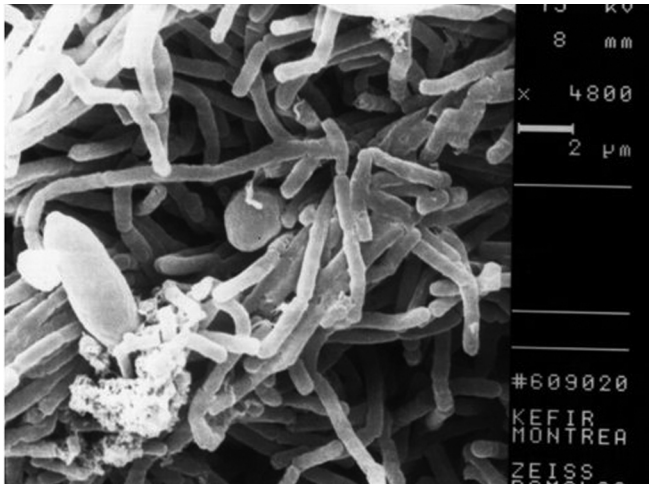

Figure 2. Electron micrograph of a kefir grain.

cated in milk 'at home with daily changes of milk' and stored for three months either at room temperature or at 4°C had microbiological profiles that were different to those of fresh grains (Pintado *et al*. 1996). In addition, washing grains in water also reduced viability. It has been recommended that in a commercial operation using grains to produce kefir, grains should be kept viable through daily transfers and should only be replaced if their ability to ferment milk becomes impaired. (Koroleva 1982). Low temperature storage appears to be the best way to maintain kefir grains for long periods. Garrote *et al*. (1997) showed that storage of kefir grains at −80 or −20°C for 120 days did not change their fermentation properties compared to grains that had not been stored; however, grains stored at −4°C did not produce acceptable kefir after thawing. Kefir grains replicated in soy milk have been reported to be smaller in size compared to grains replicated in cows' milk (Liu *et al*. 2002). There have been no reports of successful production of kefir grains from pure cultures.

While early studies of kefir grains employed light microscopy, later investigations used electron microscopy to describe the complex microbial community of which they were comprised (Ottogalli *et al*. 1973; Bottazzi and Bianchi 1980; Molska *et al*. 1980; Marshall *et al*. 1984; Duitschaever *et al*. 1988b; Toba *et al*. 1990; Neve 1992; Bottazzi *et al*. 1994; Rea *et al*. 1996). Figure 2 shows an electron micrograph of kefir grains obtained from the Moscow Dairy Institute. Ottogalli *et al*. (1973) showed that the chemical and microbiological compositions of kefir grains from four different sources were different, making comparisons between results published by different laboratories difficult.

The microbial population that makes up kefir grains appears to be relatively constant over time, although seasonal variations in the grain flora have been noted which can affect the final product consistency (La Rivière *et al*. 1967; Koroleva *et al*. 1978). Analysis has shown that the microbial profiles of the grains themselves, a percolate taken from the grains (mother culture), and the final product are not the same (see Table 2). This, in part, explains why production of kefir must start with kefir grains, since the final drink does not have the number or complexity of microorganisms as the grains, preventing the drink from being used as a starter culture for a new batch of kefir. Kandler and Kunath (1983) reported similar results when they compared the microflora of kefir, inoculated milk before incubation, and a mixture of kefir grains.

Table 2. Microorganisms* in kefir grains, mother culture and kefir drink

	Lactococci	Lactobacilli	Yeasts
Kefir grains	7.37	8.94	8.30
Mother culture (wash of grains)	8.43	7.65	5.58
Kefir drink	8.54	7.45	5.24

*log CFU/g

6. Microbiology of kefir grains

6.1 Bacteria

The microbial population found in kefir grains has been used as an example of a symbiotic community (Margulis 1995); this symbiotic nature has made identification and study of the constituent microorganisms within kefir grains difficult. Koroleva (1991) stated that kefir bacteria and yeasts, when separated as pure cultures, either do not grow in milk or have a decreased biochemical activity, which further complicates the study of the microbial population of kefir grains. Several media have been proposed for the isolation and identification of bacteria in kefir grains (Kojima *et al*. 1993). Linossier and Dousset (1994) showed that *Lactobacillus kefir* grew better when the yeast *Candida kefir* was added to the milk. Garrote *et al*. (2004) reported a similar observation when they attempted to grow *L. kefir* in milk. In general, lactic acid bacteria are more numerous (10^8–10^9) than yeasts (10^5–10^6) and acetic acid bacteria (10^5–10^6) in kefir grains, although fermentation conditions can affect this pattern (Koroleva 1991; Garrote *et al*. 2001) Table 3 shows a list of the various bacteria that have been reported in kefir and kefir grains from around the world.

Garrote *et al*. (2004) carried out several *in vitro* tests to try to explain how the bacteria in kefir grains function. They showed that two of the heterofermentative lactobacilli, *L. kefir and L. parakefir*, possessed S-layer proteins that can be used to explain in part their auto-aggregation

Table 3. Bacteria found in kefir grains and kefir

Lactobacilli	
Lactobacillus kefir[a,c,j,n,o,p,r]	*Lactobacillus delbrueckii*[a,h,p]
Lactobacillus kefiranofaciens[l,n,p]	*Lactobacillus rhamnosus*[a,r]
Lactobacillus kefirgranum[n]	*Lactobacillus casei*[h]
Lactobacillus parakefir[n,o]	*Lactobacilli paracasei*[p]
Lactobacillus brevis[g,h,p,r]	*Lactobacillus fructivorans*[k]
Lactobacillus plantarum[o,p]	*Lactobacillus hilgardii*[k]
Lactobacillus helveticus[a,b,h]	*Lactobacillus fermentum*[r]
Lactobacillus acidophilus[g,p,r]	*Lactobacillus viridescens*[r]
Lactococci	
Lactococcus lactis subsp. lactis[a,c,e,f,g,h,k,o,r]	
Lactococcus lactis subsp. cremoris[a,e,f]	
Streptococci	
Streptococcus thermophilus[e,h]	
Enterococci	
Enterococcus durans[d*,e*] (reported as *Streptobacterium durans* in ref. d; reported as *Streptococcus durans* in ref. e)	
Leuconostocs	
Leuconostoc sp.[r]	
Leuconostoc mesenteroides[a,b,g*,o] (reported as *Leuconostoc kefir* in ref. g)	
Acetic acid bacteria	
Acetobacter sp.[o]	
Acetobacter pasteurianus[g*] (reported as *Acetobacter rancens* in ref. g)	
Acetobacter aceti[a,d]	
Other bacteria	
Bacillus sp.[r]	*Micrococcus sp.*[r]
Bacillus subtilis[g]	*Escherichia coli*[r]

[a]Koreleva 1991; [b]Lin *et al*. 1999; [c]Pintado *et al*. 1996; [d]Rosi 1978; ref. [e]Yüksekdag *et al*. 2004; [f]Dousset and Caillet 1993; [g]Ottogalli *et al*. 1973; [h]Simova *et al*. 2002; [j]Kandler and Kunath 1983; [k]Yoshida and Toyoshima 1994; [l]Fujisawa *et al*. 1988; [n]Takizawa *et al*. 1994; [o]Garrote *et al*. 2001; [p]Santos *et al*. 2003; [r]Angulo *et al*. 1993.

and haemagglutination properties. In addition, these two bacteria were also shown to adhere to Caco-2 cells, raising the possibility that these bacteria would be good probiotics.

6.2 Yeasts

It has been recognized that yeasts play an important role in the preparation of fermented dairy products, where they can provide essential growth nutrients such as amino acids and vitamins, alter the pH, secrete ethanol and produce CO_2 (Viljoen 2001). The yeasts in kefir have been less well studied than kefir bacteria, although it is obvious that the yeasts in kefir grains provide an environment for the growth of kefir bacteria, producing metabolites that contribute to the flavour and mouthfeel of kefir (Clementi *et al*. 1989; Kwak *et al*. 1996; Simova *et al*. 2002). Table 4 lists the various yeasts that have been reported in kefir grains. To prevent excessive CO_2 production (particularly after fermentation), Kwak *et al*. (1996) suggested a two stage fermentation process starting with a non-lactose fermenting yeast such as *Saccharomyces cerevisiae*.

The properties of yeasts found in kefir grains vary. For example, some of the yeasts found in kefir grains are capable of fermenting lactose, while some are not. Also, it has been observed that some types of yeasts are located at the surface of the grain, while others inhabit the interior. It may be that yeasts located at different locations in the kefir grains play different roles in the fermentation process. (Iwasawa *et al*. 1982; Wyder *et al*. 1997). Iwasawa *et al*. (1982) showed that the electrophoretic pattern of the yeast *Torulopsis holmii* isolated from Danish kefir grains demonstrated patterns indicating the presence of ten different enzymes. Wyder *et al*. (1997) used restriction analysis of the two ITS regions to show that yeasts from five kefir grain samples of different origins had unique patterns, indicating the presence of different yeast species in kefir grains from different origins. Like kefir bacteria, the profile of yeasts is different in kefir grains when compared to the final kefir product (Wyder *et al*. 1997). Abraham and De Antoni (1999) showed that the yeast population in kefir produced from cows' milk using grains was two logs higher than when the same grains were added to soy milk.

7. Other uses of kefir grains

The ability of kefir grains to grow in milk whey prompted Rimada and Abraham (2001) to study whether kefir grains could be added to whey produced as a by-product of the dairy industry in Argentina, thereby producing a value-added product called kefiran. Kefiran was produced at a rate of 103 mg/l following fermentation at 43°C for 120 h, with an inoculation rate of 100 g grains per litre of milk.

Athanasiadis *et al*. (1999) showed that kefir yeast cells that had been immobilized on de-lignified cellulose were capable of producing commercially important quantities of ethanol from glucose over a wide variety of temperatures (5–30°C). Production of volatiles (e.g. ethanal, ethyl acetate, propanol-1, isobutyl alcohol and amyl alcohols) was found to depend on fermentation temperature. Ethyl acetate content did not change as fermentation temperature decreased, although contents of total volatiles during fermentations at 5°C were 38% of those carried out at 30°C. Using this system, it was shown that glucose produced the fastest fermentation compared to fructose or sucrose, although glucose-based fermentations also yielded lower concentrations of amyl alcohols, ethyl acetate and ethanol

Table 4. Yeasts found in kefir grains and kefir

Kluyveromyces marxianus [a,b,f*,g,h,i,j,k,m*,n] (reported as *Saccharomyces lactis* in ref. f; reported as *Kluyveromyces lactis* in ref. m)	*Candida friedrichii* [n]
Saccharomyces sp. [k]	*Candida pseudotropicalis* [f]
Saccharomyces cerevisiae [a,d,e,f*,g,j,m,n] (reported as *Saccharomyces carlbergensis* in ref. f)	*Candida tenuis* [f]
Saccharomyces unisporus [c,h,j,m]	*Candida inconspicua* [g]
Saccharomyces exiguus [l*] (reported as *Torolopsis holmii* in ref. l)	*Candida maris* [g]
Saccharomyces turicensis [h]	*Candida lambica* [j]
Saccharomyces delbrueckii [d]	*Candida tannotelerans* [e]
Saccharomyces dairensis [n]	*Candida valida* 6 [e]
Torulaspora delbrueckii [a,h,m]	*Candida kefyr* [a,j,n]
Brettanomyces anomalus [h]	*Candida holmii* [j,m]
Issatchenkia occidentalis [j]	*Pichia fermentans* [b,m,n]

[a]Koreleva 1991; [b]Lin *et al*. 1999; [c]Pintado *et al*. 1996; [d]Rosi 1978; [e]Dousset and Caillet 1993; ref. [f]Ottogalli *et al*. 1973; [g]Simova *et al*. 2002; [h]Wyder and Puhan 1997, 1999; [i]Yoshida and Toyoshima 1994; [j]Engel *et al*. 1986; [k]Garrote *et al*. 2001; ref. [l]Iwasawa *et al*. 1982; [m]Angulo *et al*. 1993; [n]Rohm *et al*. 1992

(Athanasiadis *et al*. 2001). The de-lignified cellulose material supporting kefir yeast cells were able to ferment a mixture of whey and raisins to produce a fermented product with an alcohol content of 4.4% v/v.

8. Composition of kefir

The composition of kefir depends greatly on the type of milk that was fermented (Kneifel and Mayer 1991). However, during the fermentation, changes in composition of nutrients and other ingredients have also been shown to occur. (Bottazzi *et al*. 1994). L(+) lactic acid is the organic acid in highest concentrations after fermentation and is derived from approximately 25% of the original lactose in the starter milk (Alm 1982d; Dousset and Caillet 1993). The amino acids valine, leucine, lysine and serine are formed during fermentation, while the quantities of alanine and aspartic acid increase when compared to raw milk (Alm 1982e). Bottazzi *et al*. (1994) reported the occurrence of acetic acid in their kefir, although others reported that no acetic acid was present (Güzel-Seydim *et al*. 2000a, 2000b).

Kneifel and Mayer (1991) found that appreciable amounts of pyridoxine, vitamin B12, folic acid and biotin were synthesized during kefir production, depending on the source of kefir grains used, while thiamine and riboflavin levels were reduced. These results contrast with Alm (1982b) who reported decreases in biotin, vitamin B_{12} and pyridoxine, and significant increases in folic acid, as compared to non-fermented milk.

Table 5. Definitions of functional foods and probiotics

Functional foods

A functional food is one that is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions. (Health Canada 2004)

Probiotics

Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. (FAO/WHO 2002). Report of a Joint FAO/WHO Working Group, 'Guidelines for the Evaluation of Probiotics in Food', London, Ontario, Canada, 2002.

9. Bioactive ingredients in kefir

The area of functional foods (see Table 5 for definition) has attracted a great deal of interest since it is now recognized that many foods contain bioactive ingredients which offer health benefits or disease resistance. A subset of functional foods is probiotic foods, from which there are several possible sources of bioactive ingredients. The microorganisms themselves (dead or alive), metabolites of the microorganisms formed during fermentation (including antibiotics or bactericides), or breakdown products of the food matrix, such as peptides, may be responsible for these beneficial effects (Ouwehand and Salminen 1998; Farnworth 2002; see Figure 3). Kefir has a long tradition of offering health benefits, especially in eastern Europe (Hallé *et al*. 1994). There are several compounds in kefir that may have bioactive properties.

9.1 Exopolysaccharides

Exopolysaccharides of differing structures and compositions are produced by a variety of lactic acid bacteria including *Lactobacillus, Streptococcus, Lactococcus* and *Leuconostoc* (De Vuyst and Degeest 1999; Ruas-Madiedo *et al*. 2002.). These cell-surface carbohydrates confer protective and adaptive properties on their bacterial producers; since they are often loosely bound to the cell membrane, they are, therefore, easily lost to their environment (Jolly *et al*. 2002). In food products, exopolysaccharides often contribute to organoleptic and stability characteristics. A unique polysaccharide called kefiran has been found in kefir grains; grains may also contain other exopolysaccharides.

Kefiran contains D-glucose and D-galactose only in a ratio of 1:1. Hydrolysis reactions followed by NMR analyses have been used to determine the chemical structure

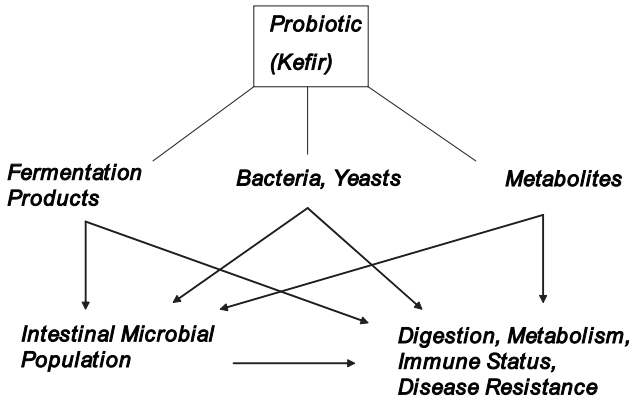

Figure 3. Probiotic effects on metabolism and health.

of kefiran (see Figure 4). The proposed structure is a branched hexa- or heptasaccharide repeating unit that is itself composed of a regular pentasaccharide unit to which one or two sugar residues are randomly linked (Kooiman 1968; Micheli *et al*. 1999). Subsequent methylation/hydrolysis studies have shown that the structure of kefiran may be more complex than first thought (Mukai *et al*. 1988; Mukai *et al*. 1990). Methylation and NMR analyses have also been used to verify the production of kefiran by new bacterial strains (Yokoi *et al*. 1991). A closer examination of chemical data published by Mukai *et al*. (1990) raises the question if, in fact, two exopolysaccharides are being produced that have very similar chemical structure and properties. La Riviére *et al*. (1967) reported that their kefiran had a 1:1 glucose to galactose ratio and an optical rotation of $+68.0^{\circ}$, while Mukai *et al*. (1990) isolated a kefiran with a glucose to galactose ratio of 0.9:1.1 and an optical rotation of $+54^{\circ}$. Examples can be found in the literature where the same bacterial strain produced different exopolysaccharides in different media (Grobben *et al*. 1995; Van Geel-Schutten *et al*. 1999). Furthermore, Santos *et al*. (2003) recently reported that they have also isolated an exopolysaccharide closely related to kefiran.

Kefiran dissolves slowly in cold water and quickly in hot water, and forms a viscous solution at 2% concentration (La Riviére *et al*. 1967). Carboxymethyl kefiran has a viscosity that is 14 times that of kefiran, although this is still much lower than those of other thickening agents used in the food industry, thus limiting any practical uses of kefiran or carboxymethyl kefiran (Mukai *et al*. 1990). Kefiran can form weak gels when added to κ-carrageenan (1% 1:4 kefiran/κ-carrageenan), which have gelation temperatures and melting temperatures similar to those of guar/κ-carrageenan gels (Pintado *et al*. 1996).

Since its initial isolation, it has been reported that kefiran may be produced by a variety of bacteria isolated from kefir grains which have been obtained from several sources (La Riviére and Kooiman 1967; Toba *et al*. 1987; Mukai *et al*. 1990; Hosono *et al*. 1990; Yokoi *et al*. 1991; Pintado *et al*. 1996; Mitsue *et al*. 1999; Micheli *et al*. 1999; Santos *et al*. 2003). Whether in fact the bacteria reported are the same has not been studied, nor has any definitive identification been published to fully characterize those bacteria reported as kefiran producers.

Bacteria which produce exopolysaccharides are often found in milk or milk products, although studies have shown that maximum production of exopolysaccharide may occur in chemically defined media (containing a carbohydrate source, mineral salts, amino acids/peptides, vitamins and nucleic acids) at a constant pH (Mozzi *et al*. 1996; Dupont *et al*. 2000). The potential health properties of kefiran have prompted several groups to develop media and growing conditions that optimize kefiran production (Toba *et al*. 1987; Yokoi *et al*. 1990;

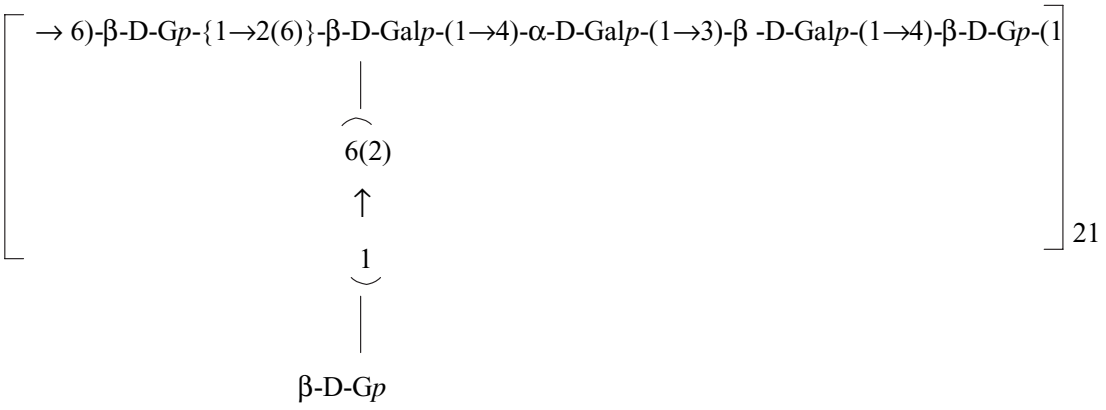

Figure 4. Proposed chemical structure of kefiran. Reprinted from Carbohydrate Research 7, Kooiman, P. The chemical structure of kefiran, the water-soluble polysaccharide of the kefir grain, pp 200–211. Copyright 1968 with permission from Elsevier.

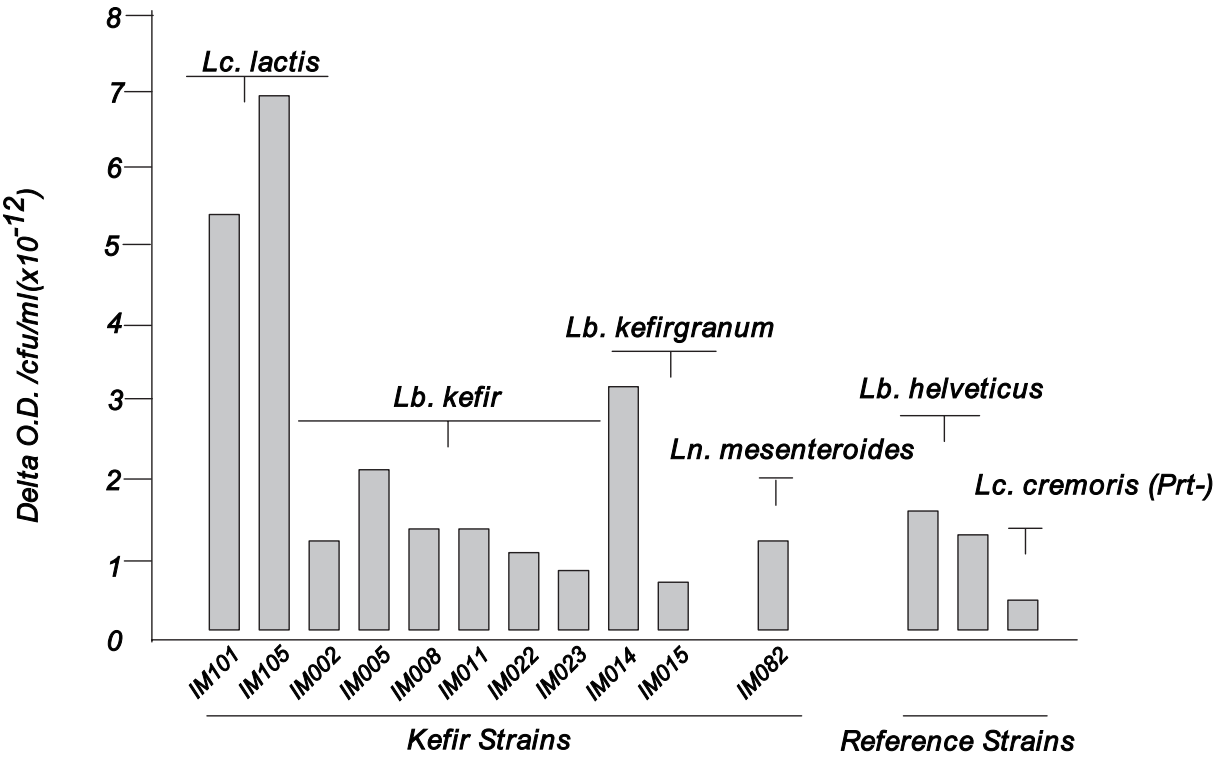

Figure 5. Proteinase activity of bacteria from kefir and kefir grains.

Yokoi and Watanabe 1992; Micheli *et al*. 1999; Mitsue *et al*. 1999). Media based on lactic acid whey have been found to be optimum for kefiran production. A batch procedure using a modified MRS media (MRSL) was reported by Micheli *et al*. 1999 to produce consistent yields of 2 g/l of kefiran. The best kefiran yields, however, have been reported by Mitsue *et al*. (1999) when they combined the kefiran producing bacterium, *Lactobacillus kefiranofaciens*, with the yeast *Torulaspora delbrueckii*. When these two organisms were grown in a 50 l fermentor in a fed-batch protocol, a yield of 3740 mg/l was obtained over a 7 day period.

No measurements have been reported with regard to kefiran concentration in the final kefir product. However, a comparison of the carbohydrate content of milk (USDA 2004) and that of kefir shows a more than doubling of the carbohydrate content; how much of this is kefiran is not known. Abraham and De Antoni (1999) did show that the polysaccharide content of kefir from cows' milk was almost twice that of kefir produced from soy milk.

Kefir grains grown in soy milk produce an exopolysaccharide that Liu *et al*. (2002) have shown to be primarily composed of D-glucose and D-galactose (ratio 1.00: 0.43), with a molecular weight of approximately 1.7×10^6 Da.

9.2 Bioactive peptides

Many organisms possess enzymes (e.g. proteinases and peptidases) that are able to hydrolyse the protein in a medium, thereby supporting growth of the organism by liberating peptides and amino acids (Thomas and Pritchard 1987; Matar *et al*. 1996). The action of proteinase and peptidase enzymes on milk proteins can theoretically result in a very large number of possible peptides. An analysis of the proteinase activity of kefir grain bacterial isolates has shown that several isolates have high proteinase activities (see Figure 5), which increases the possibility that bioactive peptides may be present in kefir. In their study of lactic acid bacteria in Turkish kefir, Yüksekdağ *et al*. (2004b) showed that 13 out of 21 lactococci strains had measurable proteolytic activity.

Initial studies on the peptide content of kefir drink have shown that kefir contains a large number of peptides and that the majority of kefir peptides have molecular weights of $\leq$5000 kDa (Farnworth 2005, unpublished results).

10. Health benefits of kefir

Kefir has had a long history of being beneficial to health in Eastern European countries, where it is associated with

general wellbeing. It is easily digested (Alm 1982c) and is often the first weaning food received by babies. Many of the studies regarding health benefits of kefir have been published in Russian and Eastern European journals and therefore are not easily accessible to Western science (Batinkov 1971; Ormisson and Soo 1976; Evenshtein 1978; Safonova *et al*. 1979; Ivanova *et al*. 1981; Sukhov *et al*. 1986; Besednova *et al*. 1997; Oleinichenko *et al*. 1999). However, the health benefits of kefir were demonstrated in Canada as early as 1932 (Rosell 1932).

10.1 Stimulation of the immune system

It has been proposed that stimulation of the immune system may be one mechanism whereby probiotic bacteria may exert many of their beneficial effects (De Simone *et al*. 1991; Gill 1998); this may be a direct effect of the bacteria themselves (Cross 2002). However, peptides formed during the fermentation process or during digestion have also been shown to be bioactive, and demonstrate a variety of physiological activities, including stimulation of the immune system in animal models (LeBlanc *et al*. 2002; Matar *et al*. 2003).

Thoreux and Schmucker (2001) fed kefir produced from grains to young (6 months) and old (26 months) rats and found an enhanced mucosal immune response in the young animals, as shown by a higher anti-cholera toxin (CT) IgA response compared to controls. Both young and old rats had significantly increased total non-specific IgG blood levels, and a decreased systemic IgG response to CT. Taken together, Thoreux and Schmucker concluded that kefir, like other probiotics, was exerting an adjuvant effect on the mucosal immune system, perhaps produced by bacterial cell wall components.

Stimulation of the immune system may also occur due to the action of exopolysaccharides found in kefir grains. Murofushi *et al*. (1983, 1986) used the method of La Riviére *et al*. (1967) for the extraction of kefiran from kefir grains to produce a water-soluble polysaccharide fraction that they fed to mice. The reduction in tumour growth that they observed was linked to a cell-mediated response, and it appeared that the total dose of the polysaccharide determined its effectiveness. Furukawa *et al*. (1992) have also shown that a water-soluble fraction of kefir grains may act as a modulator of the immune response.

The effect of kefir exopolysaccharides on the immune system may be dependent on whether the host is healthy or has developed any tumours. Furukawa *et al*. (1996) incubated kefir grain polysaccharides with Peyer's Patch (PP) cells from tumour-bearing mice and found that the supernatant of this mixture enhanced proliferation of splenocytes from normal mice and increased the mitogenic activities of lipopolysaccharides (LPS) and phytohaemagglutinin-P (PHA-P) in splenocytes. They concluded that the polysaccharide stimulated PP cells, causing them to secrete water-soluble factors that, in turn, enhanced the mitogenic response of thymocytes and splenocytes in normal mice.

10.2 Inhibition of tumour growth

Shiomi *et al*. (1982) were the first to report the antitumour effects of a water-soluble polysaccharide (approximate molecular weight 1 000 000 Da) isolated from kefir grains. Whether given orally or intraperitoneally, the polysaccharide was able to inhibit the growth of Ehrlich carcinoma or Sarcoma 180 compared to control mice receiving no kefir-derived polysaccharide (Shiomi *et al*. 1982; Murofushi *et al*. 1983). The mechanism of action was not clear, since *in vitro* incubation of the two cancer cell lines with the polysaccharide showed low cytotoxicity during 42 hours of incubation. This group then went on to show that this water-soluble polysaccharide was able to reach the spleen and thymus of mice and, based on the response to thymus-dependent and thymus-independent antigens, concluded that oral immune enhancement was mediated through T-cell, but not B-cell activity. (Murofushi *et al*. 1986). More recently, a water soluble polysaccharide fraction from kefir grains was shown to inhibit pulmonary metastasis of Lewis lung carcinoma, whether the kefir-derived polysaccharide was given orally before or after tumour transplantation. Murofushi *et al*. (1983) also reported the antitumour effectiveness of kefir grain polysaccharides regardless of the time of administration, although they cautioned that larger doses may only be more effective if administered after establishment of the tumours. A water-insoluble fraction containing kefir grain microorganisms, rather than the water-soluble polysaccharide fraction, significantly inhibited metastasis of highly colonized B16 melanoma. (Furukawa *et al*. 1993; Furukawa *et al*. 2000). It was suggested that the water-soluble polysaccharide suppressed tumour growth by means of the lymphokine activated macrophage (Mϕ) via the gut-associated lymphoid tissue, while the water-insoluble microorganism fraction acted through an increase of NK cell activity.

Feeding kefir itself (2 g/kg body weight by intubation) was more effective in inhibiting tumour (Lewis lung carcinoma) growth than yoghurt, when given for 9 days after tumour inoculation (Furukawa *et al*. 1990). It was also shown that mice receiving kefir had an improved delayed-type hypersensitivity response compared to tumour-bearing mice receiving no kefir, although the mean survival time was not affected (Furukawa *et al*. 1991). Kubo *et al*. (1992) also reported that feeding kefir (100–500 mg/kg body weight) inhibited the proliferation of Ehrlich ascites carcinoma. In addition, kefir, from which the grains had been removed by filtration, were shown to kill or arrest the growth of fusiform cell sarcomas induced by 7,12-

dimethylbenzanthracene in mice when the kefir was injected intraperitoneally (Cevikbas *et al*. 1994). Examination of tissue in kefir-treated mice showed a small amount of mitosis, some stromal connections and, in some cases, disappearance of tumour necrosis.

Hosono *et al*. (1990) showed that isolates of *Streptococcus, Lactobacillus* and *Leuconostoc* in Mongolian kefir all showed strong *in vitro* binding to amino acid pyrolysates which are believed to be mutagens and are commonly found in food. Similarly, Miyamoto *et al*. (1991) reported that three slime-producing strains of *Streptococcus lactis* subsp. *cremoris* found in German kefir had strong desmutagenic properties, which they attributed to the ability of such strains to bind to a known mutagen. Using an Ames test, Yoon *et al*. (1999) showed that *Lactobacillus* spp. isolated from kefir and yoghurt had antimutagenic properties against the mutagen 2-nitrofluorene.

Liu *et al*. (2002) studied the effects of soy milk and cows' milk fermented with kefir grains on the growth of tumours in mice, using freeze-dried kefir (produced from either soy or cows' milk) from which the grains had been removed following fermentation. Mice were injected with 0.2×10^8 Sarcoma 180 cells one week prior to the start of the feeding portion of the experiment. Tumour growth (volume) was estimated for up to 30 days, after which tumours were removed and weighed. Both soy milk kefir (−70.9%) and cows' milk kefir (−64.8%) significantly inhibited tumour growth, compared to mice in the positive control group. Microscopic examination of the tumours indicated that apoptosis may have been responsible for reduced tumour growth. Similar effects of yoghurt on apoptosis have been reported (Rachid *et al*. 2002). Mice fed unfermented soy milk did not have reduced tumour volumes at day 30, and Liu *et al*. (2002) concluded that either the microorganisms themselves or any polysaccharides formed during fermentation by the kefir grains microflora were responsible for the antitumour response. Genistein itself has been shown to inhibit tumours (Murrill *et al*. 1996; Constantinou *et al*. 1996), although in this study genistein levels did not change during the fermentation process. Mice consuming kefir samples also had significantly increased levels of IgA in their small intestines compared to control animals, and it was proposed that the PP tissue was increasing IgA secretion into the intestine in response to food antigens.

Güven *et al*. (2003) proposed an alternative suggestion as to how kefir may protect tissues. They showed that mice exposed to carbon tetrachloride (a hepatotoxin to induce oxidative damage) and given kefir by gavage had decreased levels of liver and kidney malondialdehyde, indicating that kefir was acting as an antioxidant. Furthermore, their data showed that kefir was more effective than vitamin E (which is well known to have antioxidative properties) in protecting against oxidative damage.

10.3 Kefir and lactose intolerance

A proportion of the global population is unable to digest lactose (the major sugar found in milk), because of insufficient intestinal β-galactosidase (or lactase) activity (Alm 1982a). Research has shown, however, that lactose maldigestors are able to tolerate yoghurt, providing the number of live bacteria present in the yoghurt consumed is high enough (Pelletier *et al*. 2001). It is believed that the bacteria in the yoghurt matrix are protected by the buffering effect of the yoghurt. Bacterial cells remain viable, and the bacterial cell walls remain intact, and thus the β-galactosidase enzyme contained in the yoghurt-producing bacteria (*L. acidophilus*) is protected during transit through the stomach until it arrives at the upper gastrointestinal tract (Montes *et al*. 1995; De Vrese *et al*. 2001). It has also been shown that fermented milk products have a slower transit time than milk, which may further improve lactose digestion (Vesa *et al*. 1996; Labayen *et al*. 2001).

Some kefir grains have been shown to possess β-galactosidase activity which remains active when consumed (De Vrese *et al*. 1992). A recent study has shown that a commercial kefir produced using a starter culture containing six bacteria (but not *L. acidophilus*) and one yeast was equally as effective as yoghurt in reducing breath hydrogen in adult lactose maldigestors (Hertzler and Clancy 2003). Severity of flatulence in this group was also reduced when either yoghurt or kefir was consumed compared to milk.

De Vrese *et al*. (1992) showed that when pigs were fed kefir containing fresh grains, their plasma galactose concentrations rose significantly higher than pigs given kefir containing heated grains. The diet containing kefir and fresh grains had a β-galactosidase activity of 4.4 U/l, which was identified as being responsible for the hydrolysis of lactose in the intestine, thus yielding galactose that can be absorbed. Kefir itself contains no galactose (Alm 1982).

10.4 Antimicrobial properties of kefir

There are data to show that many lactobacilli are capable of producing a wide range of antimicrobial compounds, including organic acids (lactic and acetic acids), carbon dioxide, hydrogen peroxide, ethanol, diacetyl and peptides (bacteriocins) that may be beneficial not only in the reduction of foodborne pathogens and spoilage bacteria during food production and storage, but also in the treatment and prevention of gastrointestinal disorders and vaginal infections (Tahara and Kanatani 1997; Zamfir *et al*. 1999; Bonadé *et al*. 2001; Messens and De Vuyst 2002; Jamuna and Jeevaratnam 2004).

Garrote *et al*. (2000) tested the inhibitory activity of a supernatant of cows' milk fermented with kefir grains, against Gram-negative and Gram-positive bacteria. Gram-

positive microorganisms were inhibited to a greater extent than Gram-negative microorganisms; moreover, both lactic and acetic acids were found in the supernatants. Garrote *et al*. (2000) showed that milk supplemented with lactic acid or lactic acid plus acetic acid at the concentrations found in the kefir supernatant also had inhibitory activity against *E. coli* 3. They concluded that organic acids produced during kefir fermentation could have important bacteriostatic properties even in the early stages of milk fermentation. Cevikbas *et al*. (1994) found similar results against Gram-positive coccus, staphylococcus, and Gram-positive bacillus, and noted that kefir grains were more effective with regard to their antibacterial properties than the final kefir product.

Kefir grains themselves have inhibitory power against bacteria that can be preserved during lyophilization, particularly when glycerol is added as a cryopreservative (Brialy *et al*. 1995). Fresh kefir grains were found to inhibit the growth of the bacteria *Streptococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*, but not the yeasts *Candida albicans* and *Saccharomyces cerevisiae*. *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, isolated from kefir grains, have both been shown to produce antimicrobial compounds that are present in kefir. Both inhibit Gram-positive and Gram-negative bacteria, have a molecular weight of approximately 1000 kDa and are heat stable, although their antimicrobial properties are reduced after exposure to proteolytic enzymes (Serot *et al*. 1990). Santos *et al*. (2003) showed that lactobacilli isolated from kefir grains had antimicrobial activities against *E. coli* (43/58 strains), *Listeria monocytogenes* (28/58 strains), *Salmonella typhimurium* (10/58 strains), *S. enteritidis* (22/58 strains), *S. flexneri* (36/58 strains) and *Yersinia enterocolitica* (47/58 strains). Bacteriocins were thought to be responsible, although they were not identified.

In a study in which foodborne bacterial pathogens (*E. coli O157:H7, L. monocytogenes 4b, Y. enterocolitica 03*) were added at the beginning of yoghurt or kefir fermentation, both kefir and yoghurt failed to inhibit pathogenic bacterial growth. For kefir, this was explained as being due to the slow acid development during fermentation. Interestingly, fermentations of kefir and yoghurt combinations proved to be more effective at pathogen suppression than single fermentation (Gulmez and Guven 2003)

Hydrogen peroxide is another metabolite produced by some bacteria as an antimicrobial compound. Yüksekdağ *et al*. (2004a) showed that all 21 isolates of lactic acid bacteria from Turkish kefir produced hydrogen peroxide (0.04–0.19 ug/ml). In a later paper, they reported that 11 out of 21 strains of kefir lactococci produced hydrogen peroxide (Yüksekdağ *et al*. 2004b). All lactococci strains were effective in inhibiting growth of *Streptococcus aureus*, but were less effective against *E. coli NRLL B-704* and *Pseudomonas aeruginosa*.

10.5 Behaviour of kefir bacteria in the gastrointestinal tract

One of the criteria for probiotic bacteria is that they should be able to withstand the harsh conditions of the gastrointestinal tract, including extreme pH conditions present in the stomach and the action of bile salts and digestive enzymes (Lee and Salminen 1995). It is also believed that one way in which probiotic bacteria could protect against pathogenic bacteria would be to compete with or displace pathogenic bacteria by adhering to intestinal epithelial cells. (Kirjavainen *et al*. 1998; Fujiwara *et al*. 2001; Gibson and Rastall 2003).

No results from human feeding trials have been published with regard to the ability of the microorganisms found in kefir to traverse the upper GI tract in large numbers and arrive at the large intestine. Kefir, because it is milk based, is able to buffer the pH of the stomach when ingested and thereby provide time for many of the bacteria to pass through to the upper small intestine (Farnworth *et al*. 2003). Santos *et al*. (2003) isolated 58 strains of *Lactobacillus spp*. and isolates of *L. paracasei, L. plantarum, L. delbrueckii, L. acidophilus* and *L. kefiranofaciens* from different sources of kefir grains and exposed them to an MRS medium at pH 2.5 and MRS containing 0.3% Oxgall (bile salts). They found that all strains survived 4 h incubation at pH 2.5, but did not grow. Eighty-five percent of isolates showed high resistance to Oxgall, but had delayed growth.

The caco-2 cell assay has been used to show that many of the lactobacilli isolated from kefir grains are able to bind to enterocyte-like cells (Santos *et al*. 2003), although the authors also cautioned that results using this model might not necessarily apply *in vivo*.

Human studies of the effects of diet on intestinal microflora are limited to the analysis of faecal samples, although no detailed human study has been published in which kefir has been used. Marquina *et al*. (2002) used mice to study the effect of consuming kefir (source not defined) in a feeding study that lasted 7 months. They were able to show that the numbers of lactic acid bacteria in the mouse small and large intestines increased significantly. Streptococci increased by 1 log, while sulfite-reducing clostridia decreased by 2 logs.

10.6 Kefir and cholesterol metabolism

Positive effects of yoghurt consumption on cholesterol metabolism have been reported (Kiessling *et al*. 2002; Xiao *et al*. 2003), although a review of the literature reveals that the results are at best moderate, and are often inconsistent (Taylor and Williams 1998; St-Onge *et al*. 2000; Pereira and Gibson 2002).

Several hypotheses have been proposed regarding the possible mechanism of action employed by bacteria to reduce cholesterol levels (St. Onge *et al*. 2002). Vujicic *et al*. (1992) showed that kefir grains from Yugoslavia, Hungary and the Caucase region were able to assimilate cholesterol in milk either incubated at 20°C for 24 h (reductions of up to 62%) or incubated and stored at 10°C for 48 h (reductions of up to 84%). These authors claimed that their results indicated that kefir grains had a cholesterol-degrading enzyme system. Similar results were reported for 27 lactic acid bacterial strains. However, it was pointed out that isolates from dairy products had lower cholesterol-assimilating capacity than strains isolated from infant faeces (Xanthopoulos *et al*. 1998).

In a clinical trial in which 13 subjects were fed 500 ml/day of kefir for 4 weeks in a placebo-controlled design, percentage changes in serum triglycerides compared to baseline levels were lower (although not significantly) than when subjects consumed unfermented milk; the percentage serum high-density lipoprotein (HDL) cholesterol change compared to baseline increased (although not significantly) when subjects consumed kefir compared to milk (St. Onge *et al*. 2002). Similarly, Kiessling *et al*. (2002) found that HDL levels increased after 6 months of feeding yoghurt supplemented with *Lactobacillus acidophilus* and *Bifidobacterium longum*, thereby producing an improved low-density lipoprotein (LDL)/HDL cholesterol ratio.

11. Conclusions

Many probiotic products have been formulated that contain small numbers of different bacteria. The microbiological and chemical composition of kefir indicates that it is a much more complex probiotic, as the large number of different bacteria and yeast found in it distinguishes it from other probiotic products. Since the yeasts and bacteria present in kefir grains have undergone a long association, the resultant microbial population exhibits many similar characteristics, making isolation and identification of individual species difficult. Many of these microorganisms are only now being identified by using advanced molecular biological techniques. The study of kefir is made more difficult, because it appears that many different sources of kefir grains exist that are being used to produce kefir.

The production of kefir depends on the synergistic interaction of the microflora in kefir grains. During the fermentation process, the yeasts and bacteria in kefir grains produce a variety of ingredients that give kefir its unique taste and texture. After fermentation, the finished kefir product contains many ingredients that are proving to be bioactive. At least one exopolysaccharide has been identified in kefir, although others may be present. Many bacteria found in kefir have been shown to have proteinase activity, and a large number of bioactive peptides has been found in kefir. Furthermore, there is evidence to show that kefir consumption not only affects digestion, but also influences metabolism and immune function in humans.

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