Microbial flora associated with South African household kefir

T. Loretan*, J.F Mostert† and B.C. Viljoen

The production of kefir relies on the symbiosis between yeasts, lactobacilli and streptococci. The presence of variable fermenting microflora, however, may be a constraint in producing consistent quality. Consequently, we attempted a systematic study of the microbial diversity (with special emphasis on the presence of yeasts) associated with kefir grains from different regions in South Africa. Yeasts were isolated, identified and enumerated from kefir, fermented by different indigenous kefir grains collected throughout the country. All the samples revealed relatively high yeast populations, with counts exceeding $1 \times 10^8 \text{ cfu ml}^{-1}$. Kluyveromyces marxianus, Saccharomyces cerevisiae and Kluyveromyces lactis were the dominant yeast species isolated. Other species encountered were Saccharomyces unisporus, Zygosaccharomyces rouxii (formerly Saccharomyces rouxii), Torulaspora delbrueckii and Debaryomyces hansenii. The yeast population of traditional South African kefir grains is relatively varied, consisting of non-lactose and lactose-fermenting species, with the latter group dominant. Furthermore, the different kefir grains each hosted a distinct group of microorganisms.

Introduction

Kefir is a fermented milk drink commonly consumed in the Commonwealth of Independent States (formerly the USSR), Poland, Czechoslovakia, Hungary and Scandinavia. This fermented milk product originated in the Caucasus Mountains of Russia, which lie between the Black Sea and the Caspian Sea and was prepared in leather bags or oak vats. It is not a cultured product and is produced by adding kefir grains to milk. The fermentation is initiated by these white to yellow grains, resembling cooked rice or small cauliflower heads. The grains are insoluble in water, gelatinous, and irregular in size, distributed on the inner surface of the vat or bag. Original kefir grains cannot be precisely reconstructed; they are recovered from sour milk and used repeatedly. When added to milk they swell and turn white, forming a slimy, jelly-like product. Kefir is a self-carbonated beverage that can be made with any kind of milk, such as cow, goat, sheep, camel, buffalo and, according to Abraham and De Antoni, even soya milk. The kefir beverage has a characteristic buttermilk taste and is the product of a mixed fermentation with yeasts and bacteria as a starter culture rather than yeasts. Kefir is an exception and is the product of a mixed fermentation with yeasts and bacteria. The grains contain lactobacilli, streptococci, micrococci and also yeasts in a specific symbiotic relationship to the bacteria. The kefir granules are held together by a polysaccharide, called kefiran.

The yeast flora of kefir vary according to source and production, but mixtures of lactose- and non-lactose-fermenting species, identified as Kluyveromyces marxianus, Candida kefir, Candida pseudotropicalis, Saccharomyces cerevisiae, Saccharomyces exiguus, Pichia fermentans and Torulaspora holmii have been reported. Two other yeast species and bacteria have been identified: Saccharomyces kefir and Torula kefir; certain lactobacilli: Lactobacillus caucasicus and Lactobacillus casei; and cocci: Leuconostoc spp. Spoiling microorganisms included: micrococci, spore-forming bacilli, and coliforms. Unfortunately, an authentic strain of Lactobacillus caucasicus no longer exists and the epithet ‘caucasicus’ is not recognized. A reinvestigation of this commonly isolated strain has led to a renaming and it is now known as Lactobacillus kefir. Traditional kefir, according to Obermann, contains 70% lactobacilli, 20% streptococci and 5% yeasts. In commercial kefirs, however, yeasts may be absent, or at a concentration of only $1 \times 10^3 \text{ ml}^{-1}$ (ref. 12).

South African naturally fermented kefir is often of inconsistent quality due to the presence of variable fermenting microflora. The stability of the kefir grain is reported to be dependent on the symbiosis between the yeasts, lactobacilli and streptococci. Hence there is need to develop starter cultures based on microorganisms isolated from the naturally sour milk. Under South African conditions, a yeast population may be found that is quite different from the kefir of its native countries. Such differences might be due to, for example, adaptions to ecological factors (yeast-product associations), as suggested by Fleet.

Kefir is scarcely known to the South African public. Apart from the few households where the drink has been manufactured for years as a ‘yogurt-plant’ in the kitchen, many people are not aware of the existence of such a product. It has, however, been established that kefir grains are used in a few households to ferment milk. Moreover, kefir is not commercially available in South Africa. The microbiological composition, and especially the diversity of yeasts in these products, are unknown. In the study reported here, we isolated, enumerated and characterized yeast strains present in kefir, manufactured with indigenous grains, to compare with results reported elsewhere.

Materials and methods

Kefir production. Seven visually different kefir grains were obtained from rural and domestic households in various parts of South Africa, incorporating 56 different sites all over the country. The seven grains are shown in Fig. 1. All the grains were maintained in the laboratory by preparing kefir every third day. The grains were added to cold, heat-treated milk (autoclaved at 121°C for 10 min), incubated at 25°C for 18 h and then cooled to 7°C. The kefir grains were recovered from the milk by means of a household sieve, washed in quarter-strength, sterile Ringer’s solution (Merck, Darmstadt) and transferred to fresh, heat-treated milk.

Microbial enumeration. Kefir milk with grains was sampled in duplicate aseptically (100 ml) in sterile polyethylene sampling bags (Whirlpack, Nasco) and homogenized (Stomacher 400 Lab-blender, Seward Medical UAC House, London) for 2 min and serially diluted in sterile, quarter-strength Ringer’s solution. Viable plate counts were prepared in duplicate by the spread-plate method, using yeast-extract malt-extract agar (YM) for the enumeration of yeasts. All plates were incubated at 25°C for 72 h. Viable plate counts, according to the pour-plate method, for the lactic acid bacteria (LAB) and especially the thermophilic lactobacilli and streptococci, were determined on De Man, Rogosa and Sharp agar (MRS; Oxoid, CM 361, Basingstoke) and incubated aerobically at 25°C for 72 h. The lactococci were determined on M17 agar (Oxoid; CM 785) and incubated anaerobically by means of a CO$_2$/H$_2$ gas-generating kit (Oxoid; BR 038 B) placed in an anaerobic jar at 30°C for 48 h, whereas the lactobacilli plus leuconostocs, were determined on Rogosa agar. The plates were also incubated...
anaerobically at 35°C for 48 h. For the determination of the acetic acid bacteria, MYP medium was used\(^1\) and the plates incubated aerobically for five days at 28°C.

**Isolation and identification.** The predominant yeast isolates from the highest dilutions on the YM plates were isolated and pure cultures were obtained by three successive streakings onto YM agar plates. Stock cultures were maintained on YM slants and kept at 4°C, until they were identified.

The pH and physiological and biochemical characteristics of the yeasts were determined as described by Kreger-van Rij.\(^2\) The identifications were performed according to the keys proposed by Van der Walt and Hopsu-Havu,\(^3\) Barnett et al.,\(^4\) Van der Walt and Yarrow\(^5\) Each isolate was inoculated into six fermentation media, 33 carbon source assimilation media and a medium devoid of vitamins.\(^6\) Additional tests, included growth at 37°C, in 50% (m/v) D-glucose medium, urea hydrolysis, splitting of arbutin, and 0.01 and 0.1% cycloheximide.\(^7\) Assimilation of nitrogen compounds, as performed by means of the auxanographic method of Lodder and Kreger-van Rij,\(^8\) was also included.

Ascospore formation was examined on McClary’s acetate agar, potato glucose agar, Gorodkowa agar, corn meal agar (Oxoid, CM103), and malt extract agar (Biolab, C10).\(^9\) The inoculated media were incubated at 18°C for four weeks and examined at 4-day intervals. Cell morphology and mode of reproduction were examined on malt extract agar (Biolab, C10) and on Dalmau plates.\(^10\) The formation of pseudomycelium and true mycelium was examined on corn meal agar, according to the Dalmau plate technique.\(^4\)

**Results and discussion**

**Microbial enumeration.** The general microbiological composition of the kefir samples is depicted in Fig. 2. Yeasts were found in all seven visually different kefir samples. According to Mann,\(^11\) yeast counts of \(1 \times 10^7\) to \(1 \times 10^8\) cfu ml\(^{-1}\) were reported for kefir in Czechoslovakia, while lower counts (\(1 \times 10^3\) to \(1 \times 10^4\) cfu ml\(^{-1}\)) were found by Marshall et al.\(^12\) in subcultured fermented kefir milk without grains. The yeast count also declined after the third subculture and no yeasts could be recovered after the fifth subculture. This is a strong indication of the important role of the kefir grain matrix in supporting the microbial composition of kefir. Yeast counts ranging between \(6.4 \times 10^6\) and \(2.1 \times 10^7\) cfu ml\(^{-1}\) were found by Kuo and Lin,\(^13\) while in a study by Wyder,\(^14\) yeast counts varied between \(1 \times 10^6\) and \(1 \times 10^9\) cfu ml\(^{-1}\), respectively.

In our study, all the kefir samples had relatively high yeast counts that varied between \(2.2 \times 10^8\) and \(3 \times 10^8\) cfu ml\(^{-1}\). From Fig. 2, it is evident that the majority of the microbes are representatives of yeasts, lactic acid and acetic acid bacteria. The pH varied between 3.98 and 4.10, but no visual relationship was found between the yeast count and pH.

The lactic acid bacteria, including the lactobacilli and leuconostocs, thermophilic lactobacilli and streptococci, and lactococci, were also present in relatively high numbers. The dominant LAB were lactobacilli and leuconostocs, however, in sample K3, the thermophilic lactobacilli and streptococci as well as the lactococci (\(1.2 \times 10^9\) and \(1.2 \times 10^9\) cfu ml\(^{-1}\), respectively) were found in higher numbers. The highest microbial counts of streptococci and lactobacilli, from a study by Kuo and Lin,\(^13\) were \(3.6 \times 10^9\) and \(8.3 \times 10^9\) cfu ml\(^{-1}\).

The acetic acid bacteria were present in lower numbers than the yeasts and LAB, and varied between \(1.2 \times 10^2\) (K2) and \(1.2 \times 10^5\) cfu ml\(^{-1}\) for sample K6.

**Isolation and identification of yeast isolates.** The different yeast species isolated from the kefir samples are listed in Table 1. Four different yeast genera were isolated from the kefir samples, with three different species belonging to *Saccharomyces*, two different species to *Kluyveromyces* and one each to *Debaryomyces* and *Torulaspora*. The lactose fermenting yeast *Kluyveromyces marxianus* was the most prevalent species followed by *Saccharomyces cerevisiae* and *Kluyveromyces lactis*. The other isolates were identified as repre-

![Fig. 1. The visually distinct kefir grain samples (×2.5).](image1)

![Fig. 2. Microbiological composition of seven kefir milk samples.](image2)
sentatives of Torulaspora delbrueckii, Debaryomyces Hansenii, Zygosaccharomyces rouxii and Saccharomyces unisporus. K2 and K5 were the only samples from which three different species were isolated. Samples K1, K3, K6 and K7 represented only single yeast species, namely Kluyveromyces marxianus, Saccharomyces cerevisiae and Kluyveromyces lactis. In contrast with other studies, our results indicated that despite the presence of single yeast strains, acceptable kefir can be produced. All the isolates were good candidates that one may expect in fermented single yeast strains, acceptable kefir can be produced. All the other studies, our results indicated that despite the presence of all the yeasts isolated from the kefir samples were unable to ferment lactose, which made them dependent on lactic acid bacteria capable of hydrolyzing this disaccharide. In a report on kefir production in Poland, the non-lactose fermenting yeasts were found in the deeper layer of kefir grains, whereas lactose fermenting yeasts were in the peripheral or outer layers.26,28 Koroleva29 in Russia confirmed this observation. According to Koroleva,29 C. kefir, the imperfect state of Kluyveromyces marxianus, is also frequently associated with kefir; however, no strains was detected in this study. In a similar study performed on milk products, 66% of the isolates from kefir were identified as Kluyveromyces marxianus, and the other species belonged to Pichia fermentans (19%), Saccharomyces cerevisiae (9%) and Saccharomyces dairensis (5%).30

### Conclusions

The kefir grains from South African households represented similar yeast strains to those reported for traditional kefir.28,29,30,33,34 The dominant yeast species were Kluyveromyces marxianus, Saccharomyces unisporus and Saccharomyces cerevisiae, but the dominance of specific strains varied among samples. Only samples K1 and K3 represented the same yeast strains, whereas the other kefir grains hosted different yeast strains. The high numbers of yeasts and LAB in kefir indicate that both are important and part of the overall microflora. The symbiotic relationship observed between the yeasts and LAB is a common phenomenon, in which the lactose is converted to lactic acid for use by the non-lactose-fermenting yeasts. The conserved diversity of yeasts — as only four genera were encountered — shows the ability of the kefir grain to self-regulate its microflora.35 However, Wyder26 found varying yeast compositions and counts in kefir and kefir grains, thus revealing completely different microbial systems.

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**Table 1. The diversity of yeast species associated with the different kefir samples.**

<table>
<thead>
<tr>
<th>Yeast isolates</th>
<th>%</th>
<th>K1</th>
<th>K2</th>
<th>K3</th>
<th>K4</th>
<th>K5</th>
<th>K6</th>
<th>K7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kluyveromyces marxianus</td>
<td>36</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Saccharomyces cerevisiae</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kluyveromyces lactis</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saccharomyces unisporus</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saccharomyces rouxii</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Torulaspora delbrueckii</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Debaryomyces hansenii</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Percentage of the total number of yeast isolates.
+ – + – indicate the presence or absence of a species, respectively.


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