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Review Article

Kefir Grains and their Fermented Dairy Products

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Abstract

Kefir grains are multi-species natural starter culture and widely used in fermented dairy products. Kefir grains contain many bacterial species already known for their probiotic properties. Our review provides an overview of kefir grains' microbiological characteristics, microstructure, chemical composition, and their fermented dairy products.

INTRODUCTION

Kefir is a traditional drink obtained via fermentation of milk by kefir grains. The name kefir is derived from the Turkish language word keyif, meaning "good feeling" for the feelings experienced after drinking it [1]. Kefir grains are white to yellowish, cauliflower-like grains, with a slimy but firm texture (Figure 1). The grains are composed of an inert matrix made up of polysaccharides and proteins and some lactic acid bacteria species, acetic acid bacteria, and yeasts inhabit the matrix [2].

MICROBIOLOGICAL CHARACTERISTICS

The microflora of kefir grains is remarkably stable, retaining activity for years if preserved and incubated under appropriate cultural and physiological conditions [4,5]. Microbial distribution of kefir grains, grain-milk ratio, incubation time and temperature, sanitation during separation of kefir grains, washing of grains and cold storage all drastically affect the product quality and the microflora of the kefir grains [6]. However, their complex microbiological association makes kefir grains difficult to obtain defined and constant kefir starter culture appropriate for industrial kefir production [7]. The common microorganisms isolated from kefir grains at different regions have differences. The bacteria of the grains are usually various homo- and heterofermentative lactic acid bacteria species of Lactobacillus, Lactococcus, Leuconostoc and Streptococcus; acetic acid bacteria species of Acetobacter. The bacteria of the kefir grains and their fermented diary products from different countries or regions are listed in (Table 1).

Microstructure

The exterior surfaces of the kefir grains looked smooth and shiny with the naked eye (Figure 1). However, the grain surfaces, under scanning electron microscopy, were revealed to be very rugged (Figure 2) [35]. In the inner portion of the grain, a variety of lactobacilli (long and curved), yeasts and fibrillar material were observed. The short lactobacilli and yeast were observed on the outer portion [15]. The density of microbial cell on the inner portion was less than that on the outer portion. No lactococcus was found on scanning electron micrographs, which may be due to the bad attachment of lactococcus.

CHEMICAL COMPOSITION

Kefir grains are a soft, gelatinous white biological mass, comprised of protein, lipids and a soluble polysaccharide, the kefiran complex. Kefiran is water souble glucogalactian produced by *Lb. kefiranofaciens*, *Lc. Plantarum*, and others [36-38]. In general, kefir grains increase their weight with subcultures in milk due to the increase in microorganism biomass together with an increase in amount of the matrix that composed by protein and polysaccharide [31].

Figure 1 Macroscopic structure of kefir grains [3].

Table 1: Microflora species reported in kefir and kefir grains.

Name	Origin of products	Countries or regions	References
Lactobacilli			
Lb. acidophilus	Kefir grains, Kefir beverage	Spain, Argentina, Denmark, Turkey	[8-16]
Lb. amylovorus	Kefir grains	Denmark, Brazil	[14,17]
Lb. brevis	Kefir grains	Spain	[13]
Lb. buchneri	Kefir beverage	Argentina, Italy	[18,19]
Lb. casei	Kefir grains	Spain, Tibet	[4,13,15,20]
Lb. crispatus	Kefir beverage, Kefir grains	Argentina, South Africa, Ireland, Turkey	[15,16,21]
Lb. delbrueckii	Kefir grains	South Africa	[4,12,22,23]
Lb. fermentum	Kefir grains	Spain, South Africa	[13,23]
Lb. gasseri	Kefir grains	Spain	[13]
Lb. gallinarum	Kefir grains	South Africa, Ireland	[21]
Lb. helveticus	Kefir beverage, Kefir grains	Argentina, Tibet, Turkey	[15,16]
Lb. hilgardii	water kefir, kefir grains	German, Taiwan	[20,24]
Lb. hordei	water kefir, kefir grains	German, Taiwan	[20,24]
Lb. jensenii	Kefir beverage	Argentina, Tibet	[15]
Lb. kefiranofaciens	kefir grains, Kefir beverage	Italian, Belgium, Argentina, Taiwan, Tibet, Denmark, Brazil, Italy, Slovenia, Turkey	[8,14-16,18,19,25-27]
Lb. kefirgranum	kefir grains,	Italian, Belgium, Taiwan	[8,18,19]
Lb. kefiri	kefir grains, Milk kefir, Kefir beverage	Greece, Tibet, Brazil, Argentina, Taiwan, Italy, Slovenia	[6,8,19,25,27,29,30]
Lb. nagelii	water kefir	German	[20]
Lb. otakiensis	kefir beverage, kefir grains	Argentina, Italy	[15,19]
Lb. parabuchneri	kefir	Brazil	[18]
Lb. paracasei	kefir grains	Argentina	[27]
Lb. parakefir	Kefir grains	Argentina, Slovenia	[27,28]
Lb. plantarum	Kefir grains	Tibet	[29]
Lb. reuteri	Kefir grains	Turkey	[16]
Lb. rhamnosus	Kefir grains	Spain	[13,22]
Lb. satsumensis	Milk kefir, kefir beverage	Brazil, Mexico	[16,20]
Lb. sunkii	kefir beverage	Argentina, Italy	[15,19]
Lb. viridescens	Kefir grains	Spain	[13]
Lactococci			
Lc. lactis	Kefir grains, Kefir beverage	Taiwan, Argentina, Tibet, Brazil	[8,15,17,25]
Streptococci			
Leuconostoc lactis	Kefir grains	South Africa	[23]
Leu. mesenteroides	Kefir grains, Kefir beverage	Taiwan, Tibet, South Africa	[8,15,23]
Streptococcus thermophilus	Kefir grains	Turkey	[16]
Acetic Acid Bacteria			
A. fabarum	water kefir	German	[20]
A. lovaniensis	Kefir grains	Belgium	[18]
A. orientalis	water kefir	German	[20]
A. pasteurianus	Kefir grains	Argentina	[31]
A. syzygii	Kefir grains	Brazil	[32]
Yeast			
Candida albicans	Kefir grains	Spain	[13]
C. friedricchi	Kefir grains	Spain	[13]
C. holmii	Kefir grains	Spain	[13]
C. kefir	Kefir grains	Spain	[13]
C. lambica	Kefir grains	South Africa, Ireland	[21]
Hanseniaospora valbyensis	water kefir	German	[20]
Kazachstania aerobia	Kefir grains	Italy	[19]
Ka. servazzii	Kefir grains	Italy	[19]
Ka. solicola	Kefir grains	Italy	[19]
Ka. unispora	Kefir grains	Brazil, Italy	[17,19]

Kluyveromyces dobzhanskii	Kefir grains	Turkey	[16]
Kl. lactis	Kefir	Spain	[33]
Kl. marxianus	Kefir grains, Kefir beverage	Tibet, Brazil, Slovenia	[15,26,28]
Lachancea fermentati	water kefir	German	[20]
Pichia caribbica	Kefir	Brazili	[34]
P. cecembensis	Kefir	Brazili	[34]
P. fermentas	Kefir grains	Taiwan	[13]
Saccaromyces unisporus	Kefir grains	Taiwan, Portuguese	[13,25]
S. cerevisiae	Kefir grains, Kefir beverage	Tibet, South Africa, Brazil, South Africa, Ireland, Italy	[15,19,21,23]
S. exiguus	Kefir	Spain	[33]
S. humaticus	Kefir	Spain	[33]
S. turicensis	Kefir grains	Taiwan	[13]
Torulospora delbrueckii	Kefir grains	Spain	[13]
Zygosaccharomyces fermentati	Kefir grains	Brazil	[34]

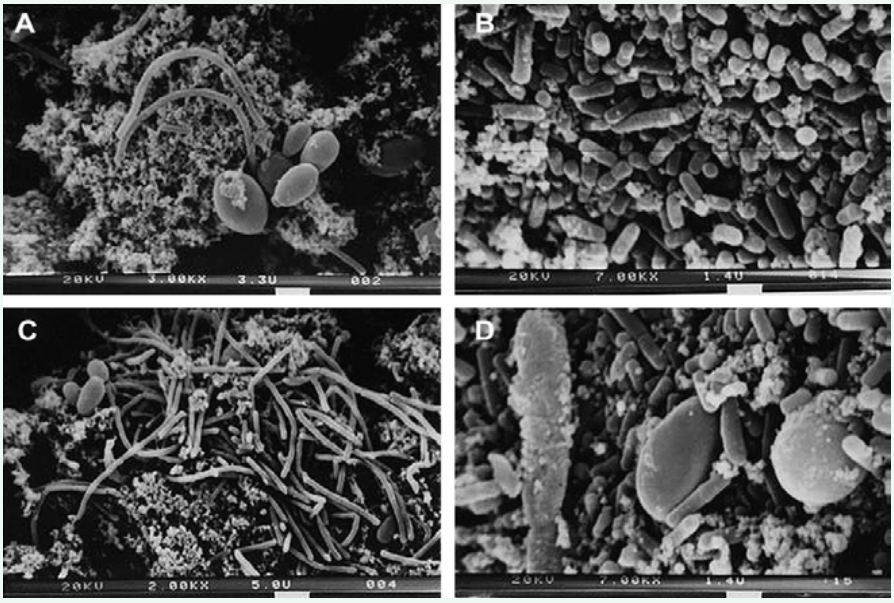

Figure 2 Scanning electron micrographs of Tibetan kefir grains. (A, C) The inside surface of Tibetan kefir grain. (B, D) The outside surface of Tibetan kefir grain [15].

FERMENTED DAIRY PRODUCTS

Cheese

The kefir culture has gained researchers' attention with regarding to cheese manufacturing due to its potential effect on quality, health, and safety properties of the product. Kefir grains or kefir has been used as a starter in many types of cheese. Goncu and Alpkent used kefir, yoghurt or a commercial cheese culture as a starter in the white pickled cheese production [39]. During 120 days ripenning, scores for appearance, structure, and odour were rated highest in white cheese samples produced by using kefir culture. However, for the commercial production of cheese, direct use of kefir grains is impractical regarding transportation, storage, and cell dosage. Freeze-dried or thermally-dried may be a solution for long-term preservation of microorganisms and convenience for shipping [40]. Dimitrellou et al. evaluated the use of a freeze-dried kefir culture in the production of whey-cheese similar to traditional Greek Myzithra-cheese [41]. The use of kefir culture as a starter led to increased lactic acid concentrations and decreased pH values in the final product compared with whey-cheese without starter culture. The cheeses produced were characterized as high-quality products during the preliminary sensory evaluation. The freeze-dried kefir culture added in the cheese seemed to suppress growth of pathogens and increased preservation time. Besides, Dimitrellou et al. also evaluated the use of thermally-dried immobilized kefir on casein as a starter culture for protein-enriched dried whey cheese [41,42]. Thermally-dried immobilized kefir starter culture resulted in an improved profile of aroma-related compounds. The preliminary sensory evaluation ascertained the soft, fine taste and the overall improved quality of cheese produced with the thermally-dried immobilized kefir. The free or immobilized freeze-dried kefir cells were used as a starter culture in hard-type cheese production. The freeze-dried kefir culture improved aroma, taste, and texture characteristics while increasing the degree of openness in comparison to traditional hard-type cheese products

[43]. Then the thermally-dried free and immobilized kefir cells were compared in the hard-type cheese production [44]. Both free and immobilised cells of kefir culture led to the production of improved cheese products as regards preservation time, sensory and textural characteristics. Thermal drying contributed to the volatile composition of the final product when compared to cheeses made with the alternate method of freeze-drying. The thermal drying process was simple, and of low cost, lower than that of freeze drying. A freeze-dried Tibetan kefir co-culture was used in the Camembert-type cheese production [35,45]. A total of 45 compounds were detected during ripenning. Volatile carboxylic acids were abundant in the headspace of the cheese. Lactobacillus paracasei represents the most commonly identified lactic acid bacteria isolates.

Kefir

Kefir is a self-carbonated, refreshing fermented yogurt which has a unique flavor due to a mixture of lactic acid, carbon dioxide, acetaldehyde, acetoin, slight alcohol, and other fermentation flavor products [46,47]. Kefir has been reported to contain 1.98 g/L of CO_2 and 0.48% alcohol, and the content of carbon dioxide (201.7-277.0 ml/L) positively correlated with the concentration (10-100 g/L) of kefir grains [48]. One feature of kefir that differs from other fermented yogurt products is that starter kefir grains are recovered after fermentation. The biomass of kefir grains slowly increases during the process of kefir fermentation [30]. Beyond its inherent high nutritional value as a source of protein and calcium, kefir has a long tradition of being regarded as good for health in countries where it is a staple in the diet [49]. Though cow's milk is most common, kefir can be made from any type of milk. For dairy kefir, cow, goat, or sheep milk are all commonly used [50]. Tratnik used the goat's milk to produce the kefir. When the goat's milk was fortified with 2g/100g skimmed milk powder, whey protein concentrate and inulin, the acidity level remained very stable in all the samples during the storage period. Goat's samples have significantly lower viscosity and slightly lower sensory profiles [51]. The pasteurised goat milk and goat milk kefir prepared using different amount of Indonesian kefir grains. The best chemical characteristics were obtained from goat milk kefir prepared with 7% (w/v) kefir grains and incubation time of 24 hours [52]. Varieties of kefir were made from bovine, caprine and ovine milk, using kefir grains and two direct-to-vat inoculation starter cultures [53]. Lactic acid bacteria and yeasts were the predominant flora in fresh and stored kefirs. The firmness and all the sensory attributes of the product were influenced by the type of milk used (ovine>bovine>caprine). Storage influenced mouth-feel characters. In general, the type of milk had greater influence on product characteristics than that of starter cultures. Kefir is best made with milk containing fat. As there is an established relationship between many health problems and the consumption of saturated fats and cholesterol, a non-fat choice in kefir is desirable; however, non-fat milk makes a kefir with significantly lower quality [47]. Ertekin and Guzel-Seydim experimented with non-fat milk supplemented with the fat substitutes inulin and Dairy-Lo® to improve the quality of kefir made with skim milk. They found that while kefir grains fermenting whole fat milk resulted in the best quality kefir, Dairy Lo® and inulin could be used without any adverse effect for the production of non-fat kefir [54].

Kefir beverage: Kefir grains successfully ferment the milk from most mammals and continue to grow in such milk. In addition, kefir grains ferment milk substitutes such as soy milk, rice milk and coconut milk, as well as other sugary liquids including fruit juice, coconut water, beer wort and ginger beer [55]. Carrot, fennel, melon, onion, tomato and strawberry juices underwent back slopping fermentations, could be carried out by water kefir microorganisms. Results indicated that lactic acid bacteria and yeasts were capable of growing in the juices tested. After fermentation, there was observance of a decrease of the soluble solid content and an increase of the number of volatile organic compounds. The overall quality assessment indicated that carrot kefir-like beverage was the product mostly appreciated by the judges [56]. Cocoa pulp was also used for for new cocoa beverages [57]. A microbial steady structure was detected in the analyzed kefir cocoa beverages and kefir grains. The beverages had the greater acceptance based on taste, odor, and appearance of the beverages. Based on the chemical characteristics and acceptance in the sensory analysis, these results open up perspectives for this innovative application of kefir grains for developing cocoa pulp-based beverages. Cui et al. experimented with walnut milk to produce kefir beverage [58]. The suggested optimum fermentation conditions are the following: fermentation temperature of 30°C, fermentation time of 12 h, inoculum size of 3 g of kefir grains (wet weight) and sucrose concentration of 8 g/100 mL.

Cheese whey is the liquid remaining after the precipitation and removal of milk casein during cheese-making. This by-product represents approximately 85-90% of the milk volume and retains 55% of milk nutrients [59]. Cheese whey represents an important environmental problem because of the high volumes produced and its high organic matter content [26]. The pressure of antipollution regulations together with whey nutritional value challenges the dairy industry to face whey surplus as a resource and not only as a waste problem [26]. The production of a functional beverage produced upon whey fermentation by kefir grains could be an interesting alternative for cheese whey utilisation. Cheese whey fermentation by kefir microrganisms could decrease the high lactose content in cheese whey, producing mainly lactic acid and other metabolites such as aroma compounds contributing to the flavour and texture and increasing carbohydrate solubility and sweetness of the end product [60]. Manufacture of beverages through lactic fermentations can provide desirable sensory profiles and have already been considered an option to add value to cheese whey [26,60-62]. Magalhães et al. made a tentative and more comprehensive study (including morphological and microbial variations, chemical composition and sensory analysis) of the kefir grains as a starter culture for cheese whey-based beverages production [26,60]. A steady structure and dominant microbiota, including probiotic bacteria, was detected in the analyzed kefir beverages. Besides, based on the chemical characteristics and acceptance in the sensory analysis, the kefir grains showed potential to be used for developing cheese whey-based beverages. Some researchers prepared fermented dairy fruits juice beverage making use of juice and whey. The dairy fruits juice beverage provided desirable sensory profiles and uses of whey can be applied to change it from a waste to a delicious beverage [63-65].

CONCLUSION

Kefir grains are unique symbiotic associations of different microrganisms, including lactic acid bacteria, yeasts and acetic acid bacteria, cohabiting in a natural polysaccharide and a protein matrix. Kefir is a distinctive fermented dairy product due to the unique, multi-species natural kefir grains used as the starter culture. The microbiological and chemical compositions of kefir provide a complex probiotic effect due to the inherent lactic acid bacteria and yeast. Kefir grains ferment the milk from most mammals and will continue to grow in such milk. Now, kefir grains have been widely used in fermented dairy products, including cheese, kefir, whey beverage, as well as other sugary liquids.

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