Milk kefir: composition, microbial cultures, biological activities, and related products

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In recent years, there has been a strong focus on beneficial foods with probiotic microorganisms and functional organic substances. In this context, there is an increasing interest in the commercial use of kefir, since it can be marketed as a natural beverage that has health promoting bacteria. There are numerous commercially available kefir based-products. Kefir may act as a matrix in the effective delivery of probiotic microorganisms in different types of products. Also, the presence of kefir’s exopolysaccharides, known as kefiran, which has biological activity, certainly adds value to products. Kefiran can also be used separately in other food products and as a coating film for various food and pharmaceutical products. This article aims to update the information about kefir and its microbiological composition, biological activity of the kefir’s microflora and the importance of kefiran as a beneficial health substance.

Keywords: kefir, biological activity, polysaccharides, kefiran, microbial composition

INTRODUCTION

Kefir is an acidic-alcoholic fermented milk product with little acidic taste and creamy consistency that was originated in the Balkans, in Eastern Europe, and in the Caucasus (Fontán et al., 2006; Serafini et al., 2014). Kefir can be produced by fermenting milk with commercial freeze-dried kefir starter cultures, traditional kefir grains, and the product that remains after the removal of kefir grains (Bensmira et al., 2010). Kefir grains are a kind of yogurt starter, which are white to yellow–white, gelatinous, and variable in size (varying from 0.3–3.5 cm in diameter) and are composed by a microbical symbiotic mixture of lactic acid bacteria (10⁸ CFU/g), yeast (10⁶–10⁷ CFU/g), and acetic acid bacteria (10⁵ CFU/g) that stick to a polysaccharide matrix (Garrote et al., 2010; Chen et al., 2015). After successive fermentations, kefir grains can break up to new generation grains, which have the same characteristics as the old ones (Gao et al., 2012).

Commercial kefir is produced by two methods: The “Russian method” and the pure cultures. In the “Russian method” kefir is produced on a larger scale, using a series fermentation process, beginning with the fermentation of the grains and using the percolate. The other method employs pure cultures isolated from kefir grains or commercial cultures (Leite et al., 2013). Also, the industrial or commercial process uses direct-to-vat inoculation (DVI) or direct-to-vat set (DVS) kefir starter cultures. In addition, Bifidobacterium sp., Lactobacillus sp. and probiotic yeast (Saccharomyces boulardii) may be used as adjunct cultures when blended with kefir grains or kefir
DVI cultures (Wszolek et al., 2006). On the other hand, whey may be a practical base for kefir culture production, and fermented whey has shown to be a suitable cryoprotective medium during freeze-drying. The freeze-dried culture retains a high survival rate and shows good metabolic activity and fermentation efficiency, indicating a good potential for its use as a value-added starter culture in dairy technology. All of these studies have shown promising perspectives for the application of kefir grains in whey valorization strategies (Bensmira et al., 2010; Cheirsilp and Radchabut, 2011).

Traditionally, kefir is manufactured using cow, ewe, goat, or buffalo milk. However, in some countries, animal milk is scarce, expensive, or minimally consumed due to dietary constraints, preferences, or religious customs. Therefore, there have been many attempts to produce kefir from a variety of food sources such as soy milk (Botelho et al., 2014). Historically, kefir has been linked with health, for example, in Soviet countries, kefir has been recommended for consumption by healthy people to restrain the risk of some diseases (Saloff-Coste, 1996; St-Onge et al., 2002; Farnworth and Mainville, 2003). The consumption of this fermented milk has been related to a variety of health benefits (Vujčić et al., 1992; McCue and Shetty, 2005; Rodrigues et al., 2005a) not only linked to its microflora, but also due to the presence of some metabolic products as organic acids (Garrote et al., 2001; Ismaiel et al., 2011). In addition, kefir cultures have the ability to assimilate cholesterol in milk (Yanping et al., 2009). On the other hand, there is a growing commercial interest in using kefir as a suitable food matrix for supplementation with health-promoting bacteria. Kefir may not only be a natural probiotic beverage, but also acts as an effective matrix for the delivery of probiotic microorganisms (Vinderola et al., 2006; Medrano et al., 2008; Oliveira et al., 2013).

In kefir grains the main polysaccharide is kefiran, which is a heteropolysaccharide composed by equal proportions of glucose and galactose and is mainly produced by Lactobacillus kefiranofaciens (Zajšek et al., 2011). It has been demonstrated that kefiran improves the viscosity and viscoelastic properties of acid milk gels (Rimada and Abraham, 2006), and is able to form gels that have interesting viscoelastic properties at low temperatures, because of that, kefiran can also be used as an additive in fermented products. Besides, kefiran can enhance the rheological properties of chemically acidified skim milk gels increasing their apparent viscosity (Zajšek et al., 2013).

Compared with other polysaccharides, kefiran has outstanding advantages such as antitumor, antifungal, antibacterial properties (Cevikbas et al., 1994; Wang et al., 2008) immunomodulation or epithelium protection (Serafini et al., 2014), anti-inflammatory (Rodrigues et al., 2005b), healing (Rodrigues et al., 2005a), and antioxidant activity (Chen et al., 2013).

This review presents the most recent advances about kefir and kefiran, their production and microbial cultures involved, biological activities and potential applications in health and food industries.

### MICROBIAL COMPOSITION OF KEFIR GRAINS AND KEFIR

Kefir grains have a complex composition of microbial species such as the predominance of lactic acid bacteria, acetic bacteria, yeasts, and fungi (Jianzhong et al., 2009; Pogacić et al., 2013). This microbial species are classified into four groups: homofermentative and heterofermentative lactic acid bacteria and lactose and non-lactose assimilating yeast (Cheirsilp and Radchabut, 2011). In that way, Lactobacillus paracasei ssp. paracasei, Lactobacillus acidophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus plantarum, and L. kefiranofaciens are predominant species. However, these species represent only 20% of the Lactobacillus in the final fermented beverage, with the remainder consisting of Lactobacillus kefir (80%; Yılmaz et al., 2004; Zanirati et al., 2015). Acetobacter aceti and A. rasens have also been isolated, such as the fungus Geotrichum candidum. More than 23 different yeast species have been isolated from kefir grains and from fermented beverages of different origins. However, the predominant species are Saccharomyces cerevisiae, S. unisporus, Candida kefyr, and Kluyveromyces marxianus ssp. marxianus (Withihuhn et al., 2004; Diosma et al., 2014; Zanirati et al., 2015; Table 1).

The microbial composition may vary according to kefir origin, the substrate used in the fermentation process and the culture maintenance methods. Tibetan kefir, which is used in China, is composed of Lactobacillus, Lactococcus, and yeast. Additionally, acetic acid bacteria have been identified in Tibetan kefir, depending on the region in China from where it was obtained (Gao et al., 2012), additionally, Tibetan kefir composition differs from that of Russian kefir, Irish kefir, Taiwan kefir, Turkey fermented beverage with kefir; however, it is known that this microbial diversity is responsible for the physicochemical features and biological activities of each kefir (Jianzhong et al., 2009; Kabak and Dobson, 2011; Gao et al., 2012; Altay et al., 2013).

Wang et al. (2012) examined a section of a whole kefir grain and found in the outer layer of the grain, lactococci, and yeasts, and, in the inner layer of the grain, the quantity of lactobacilli were much higher and more yeasts cells were found. There are little information about the mechanism of grain formation, so the same authors, proposed a hypothesis to explain that: “Initially, Lactobacillus kefiranofaciens and Saccharomyces turicensis start to auto-aggregate and co-aggregated to small granules.” The aggregation is enhanced when the pH drops. The biofilm producers, Lactobacillus kefiri, Kluyveromyces marxianus HY1, and Pichia fermentans HY3 then adhere to the surface of these small granules due to their cell surface properties and their strong aggregation ability, which gives rise to thin biofilms. After biofilm formation, the kefir yeasts and Lactobacillus continue to co-aggregated with the granule strains and associate with the granule biofilm to become a three dimensional microcolony. As the cell density due to the growth of kefir yeasts and Lactobacillus increases, cells and milk components that are present in the liquid phase accumulate on the granule surface.
| Microorganism | Source – Country | Reference |
|----------------|------------------|-----------|
| Lactobacillus kefir, Lactobacillus kefiranofaciens, Lactobacillus paracasei, Lactobacillus plantarum, Lactococcus lactis ssp. lactis, Kluyveromyces marxianus, Lactococcus lactis parakefiri, Saccharomyces cerevisiae, Saccharomyces unisporus, Leuconostoc mesenteroides, Acetobacter sp., Saccharomyces sp., Lactococcus lactis ssp. lactis biovar diacetylactis, Lactococcus lactis, Lactobacillus kefiri, Lactobacillus parakefiri | Kefir grains and beverage – Argentina | Garrote et al., 2001; Londero et al., 2012; Hamet et al., 2013; Diosma et al., 2014. |
| Lactobacillus kefiri, Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Lactococcus lactis ssp. cremoris, Gluconobacter frateurii, Acetobacter orientalis, Acetobacter lovaniensis, Kluyveromyces marxianus, Naumovozyma sp., Kazachstania kefiri, Lactobacillus brevis, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus helveticus, Streptococcus thermophilus, Lactobacillus casei ssp. pseudoplantrum, Kluyveromyces marxianus var. lactis, Saccharomyces cerevisiae, Candida inconspicua, Candida maris, Lactobacillus lactis ssp. lactis | Kefir grains and beverage – Belgium | Korsak et al., 2015 |
| Lactobacillus kefiri, Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Lactobacillus paracasei, Lactobacillus helveticus, Gluconobacter japonicus, Lactobacillus uvarum, Acetobacter syzygii, Lactobacillus satsumensis, Saccharomyces cerevisiae, Leuconostoc sp., Streptococcus sp., Acetobacter sp., Bilifodobacterium sp., Halococcus sp., Lactobacillus amylolaurus, Lactobacillus buchneri, Lactobacillus crispatus, Lactobacillus kefiranofaciens ssp. kefiranofaciens, Lactobacillus paracasei | Kefir grains – Brazil | Miguel et al., 2010; Leite et al., 2012; Zanirati et al., 2015 |
| Lactobacillus brevis, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus helveticus, Streptococcus thermophilus, Lactobacillus casei ssp. pseudoplantrum, Kluyveromyces marxianus var. lactis, Saccharomyces cerevisiae, Candida inconspicua, Candida maris, Lactobacillus lactis ssp. lactis, Lactobacillus paracasei, Lactobacillus parabuchneri, Saccharomyces cerevisiae, Pseudomonas sp. | Kefir grains and beverage – Bulgaria | Simova et al., 2002 |
| Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Lactobacillus helveticus, Kluyveromyces marxianus, Saccharomyces cerevisiae, Pseudomonas sp., Kazachstania unispora, Kazachstania exigua, Lactobacillus kefri, Lactobacillus casei, Bacillus subtilis, Pichia kudriavzevi, Leuconostoc lactis, Lactobacillus plantarum, Acetobacter fabarum, Pichia guilliermondii, Lactococcus sp., Lactobacillus sp., Acinetobacter sp., Shewanella sp., Leuconostoc sp., Streptococcus sp., Acinetobacter sp., Pelomonas sp., Dysgonomonas sp., Weissella sp., Shewanella sp., Acetobacter acetilis, Enterococcus faecalis, Enterococcus durans, Lactococcus lactis ssp. cremoris, Leuconostoc pseudomesenteroides, Leuconostoc paramestereoides, Lactobacillus brevis, Lactobacillus acidophilus, Saccharomyces sp., Brettanomyces sp., Candida sp., Saccharomyces cerevisiae, Lachancea meyersii | Kefir beverage – China | Jiahong et al., 2009; Gao et al., 2012, 2013a |
| Lactobacillus kefiranofaciens, Dekkara anomala, Streptococcus thermophilus, Lactococcus lactis, Acetobacter sp., Lactobacillus lactis, Enterococcus sp., Bacillus sp., Acetobacter fabarum, Acetobacter lovaniensis, Acetobacter orientalis | Kefir grains and beverage – Ireland | Dobson et al., 2011 |
| Leuconostoc sp., Lactococcus sp., Lactobacillus sp., Lactobacillus plantarum, Zygosaccharomyces sp., Candida sp., Candida lamberca, Candida kruusei, Saccharomyces sp., Cryptococcus sp. | Kefir grains and beverage – South Africa | Witthuhn et al., 2005 |
| Lactobacillus kefiri, Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Escherichia coli, Pseudomonas sp., Saccharomyces turicensis | Kefir grains – South Africa | Witthuhn et al., 2004 |
| Lactobacillus kefiri, Leuconostoc mesenteroides, Lactococcus lactis, Streptococcus thermophilus, Lactobacillus kefiranofaciens, Lactobacillus acidophilus | Kefir grains – Taiwan | Wyder et al., 1999; Chen et al., 2008; Wang et al., 2012; Simova et al., 2002 |
| Lactobacillus helveticus, Lactobacillus buchneri, Lactobacillus kefiranofaciens, Lactobacillus acidophilus, Lactobacillus helveticus, Streptococcus thermophilus, Bilifodobacterium bifidum, Kluyveromyces marxianus | Kefir grains – Turkey | Guzel-Seydim et al., 2005; Kesmen and Kacmaz, 2011 |
| Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Streptococcus thermophilus, Lactobacillus kefiranofaciens, Lactobacillus acidophilus | Kefir beverage – Turkey | Kok-Tas et al., 2012; Nalbantoglu et al., 2014 |
| Lactobacillus kefiranofaciens, Leuconostoc mesenteroides, Lactococcus lactis, Streptococcus thermophilus, Lactobacillus kefiranofaciens, Lactobacillus acidophilus | Kefir beverage – Turkey | Yuksel-Kdag et al., 2004 |
TABLE 2 | Kefir microorganisms and their biological activities.

| Organism of interest | Origin | Biological activity | Reference |
|----------------------|--------|---------------------|-----------|
| Lactobacillus plantarum MA2 | Tibetan kefir | Hypcholesterolemic effect | Yanping et al., 2009 |
| Lactobacillus plantarum Lp27 | Tibetan kefir | Inhibited cholesterol absorption | Ying et al., 2013 |
| Lactobacillus plantarum CIDCA 83114 | Kefir grains – Argentina | Inhibit the growth of Shigella sonnei in vitro and also the cytotoxicity of C. difficile toxins on eukaryotic cells | Bolla et al., 2013 |
| Lactobacillus kefir CIDCA 8348 | Kefir grains – Argentina | Inhibit the growth of Shigella sonnei in vitro and also the cytotoxicity of C. difficile toxins on eukaryotic cells | Bolla et al., 2013 |
| Lactobacillus plantarum ST8KF | Kefir grains – South Africa | Bactericidal effect against: Lactobacillus casei, Lactobacillus salivarius, Lactobacillus curvatus, Listeria innocua | Powell et al., 2007 |
| Lactobacillus kefiranofaciens K1 | Kefir grains – Taiwanese milk | Antiallergenic effect | Chen et al., 2008; Wei-Sheng et al., 2010 |
| Lactobacillus kefiranofaciens M1 | Kefir grains – Taiwanese milk | Immunoregulatory effects – anticolitis effect | Hong et al., 2009; Chen et al., 2012 |
| Lactobacillus lactis CIDCA 8221 | Kefir grains – Argentine | Inhibit the growth of Shigella sonnei in vitro and also the cytotoxicity of Clostridium difficile toxins on eukaryotic cells | Bolla et al., 2013 |
| Kluyveromyces marxianus CIDCA 8154 | Kefir grains – Argentine | Inhibit the growth of Shigella sonnei in vitro and also the cytotoxicity of Clostridium difficile toxins on eukaryotic cells | Bolla et al., 2013 |
| Saccharomyces cerevisiae CIDCA 8112 | Kefir grains – Argentine | Inhibit the growth of Shigella sonnei in vitro and also the cytotoxicity of Clostridium difficile toxins on eukaryotic cells | Bolla et al., 2013 |
| Lactobacillus lactis ssp. cremoris | Kefir grains – India | Activity against food spoilage bacteria | Raja et al., 2009 |

Source: Soccol et al., 2014.

TABLE 3 | Biological activity of kefiran.

| Exopolysaccharide | Biological activity | Reference |
|-------------------|---------------------|-----------|
| Kefiran           | Reduction of blood pressure induced by hypertension | Maeda et al., 2004 |
|                   | Favors the activity of peritoneal macrophages | Duarte et al., 2006 |
|                   | Increase in peritoneal IgA | Liu et al., 2002 |
|                   | Antitumoral activity | Rodrigues et al., 2005a |
|                   | Antimicrobial activity | Medrano et al., 2008; Piernaria et al., 2010 |
|                   | Modulation of the intestinal immune system and protection of epithelial cells against Bacillus cereus exocellular factors | |

and the kefir grains are formed. There is a symbiotic relation between the microorganisms present in kefir grains, wherein the bacteria and yeast survive and share their bioproducts as power sources and microbial growth factors. This microorganism association is responsible for lactic and alcoholic fermentation (Witthuhn et al., 2005; Wang et al., 2012; Hamet et al., 2013).

After receiving its actual/present denomination, some of the microorganisms isolated and identified in kefir cultures were classified using the product name, as in Lactobacillus kefiri, L. kefiranofaciens, L. kefirgranum, Lactobacillus parakefir, and Candida kefyr (Wyder et al., 1999; Kwon et al., 2003; Yang et al., 2007; Kok-Tas et al., 2012). Table 1 demonstrates the microbial composition, which has been isolated from kefir and kefir grains of different origins.

**BIOCHEMICAL ACTIVITY OF KEFIR**

Due to its composition, kefir is mainly considered a probiotic resource (Nalbantoglu et al., 2014). "Probiotics are microbial cell preparations or components of microbial cells with a beneficial effect on the health of the host" (Lopitz et al., 2006). Some studies suggest that probiotic bacteria in kefir consumers' gut are abundant and are correlated with health improvement (Ahmed et al., 2013; Zheng et al., 2013); in that way, it had been demonstrated that the cell-free fraction of kefir enhances the ability to digest lactose relieving symptoms (Farnworth, 2005; Rizk et al., 2009).

Another reason for the increased interest in probiotic strains from kefir is its capacity to lower cholesterol levels. There are different ways in which bacteria can alter serum cholesterol: (i) through the binding to and absorption into the cell before it can
be absorbed into the body; (ii) producing free and deconjugating bile acids; (iii) inhibiting the enzyme HMG-CoA reductase (Yanping et al., 2009).

The microorganisms in the kefir grains produce lactic acid, antibiotics and bactericides, which inhibit the development of degrading and pathogenic microorganisms in kefir milk (Liu et al., 2002). Kefir acts against the pathogenic bacteria Salmonella, Helicobacter, Shigella, Staphylococcus, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Bacillus subtilis, Micrococcus luteus, Listeria monocytogenes, Streptococcus pyogenes, (Lopitz et al., 2006), Streptococcus faecalis KR6, Fusarium graminearum CZ1 (Ismail et al., 2011), and the fungus Candida albicans. On the other hand, it has been demonstrated that a mixture of kefir isolated bacteria and yeast is able to prevent diarrhea and enterocolitis triggered by Clostridium difficile (Bolla et al., 2013). Besides, kefir showed good efficacy in inhibiting spore formation and aflatoxin B1 produced by the fungus Aspergillus flavus, which is a toxic compound formed either in the field or during food storage. Therefore, kefir appears as a promising safe alternative natural food preservative offering protection against intoxication with aflatoxin B1 (Ismail et al., 2011).

It had been proved that many species of lactobacilli present in kefir have S-layer proteins. Surface layers (S-layers) can be aligned in unit cells on the outermost surface of many prokaryotic microorganisms (Mobili et al., 2009). It has been demonstrated that these S-layer proteins can apply a protective action inhibiting the growth of Salmonella enterica serovar Enteritidis in Caco-2 cells, and also have the ability to antagonize the effects of toxins from Clostridium difficile on eukaryotic/eukaryotic cells in vitro (Carasi et al., 2012).

However, there are other important bioactivities that have been tested with kefir grains, the cell-free fraction of kefir or acid lactic bacteria isolated from kefir, such as antimutagenic (Guzel-Seydim et al., 2006), antimicrobial (Anselmo et al., 2010), immunoregulatory (Hong et al., 2009), antiallergenic (Wei-Sheng et al., 2010), wound healing (Huseini et al., 2012), antidiabetic (Young-In et al., 2006), and antigentoxic (Grishina et al., 2011). In that way, it had been demonstrated that kefir cell-free fraction has antiproliferative effects on human gastric cancer SGC7901 cells (Gao et al., 2013b), colon adenocarcinoma cells (Khoury et al., 2014), HUT-102 malignant T lymphocytes, sarcoma 180 in mice, Lewis lung carcinoma and human mammary cancer (Rizk et al., 2009), and reduce oxidative stress (Punaro et al., 2014). Another study has shown that suspensions after 24 h fermentation and mechanically disintegrated kefir grains cause a significant inhibition of granuloma tissue formation and a 43% inhibition of the inflammatory process (Diniz et al., 2003).

Nevertheless, there are other important studies performed with some microorganisms isolated from different types of kefir. Some microorganisms with their biological activities and origin are shown in Table 2.

**KEFIRAN, A POTENTIAL EXOPOLYSACCHARIDE**

The increased search for natural polysaccharides has been very significant due to their use in the food, pharmaceutical, and cosmetic industries as additives, bio-absorbents, metal removal agents, bioflocculants, and medicine delivery agents, among other functions (De Vuyst et al., 2001; Welman and Maddox, 2003; Badel et al., 2011). Many microorganisms, such as bacteria, fungi, and weeds, have the capacity/ability to synthesize and excrete extracellular polysaccharides, and these polysaccharides can be either soluble or insoluble (Wang et al., 2010; Badel et al., 2011).

The polysaccharides that are commonly used as food additives are xanthan, dextran, gellan, and alginates, while the exopolysaccharides (EPSs) produced by lactic acid bacteria
TABLE 4 | Marketed kefir-based products and their information.

| Companies            | Product          | General information            |
|----------------------|------------------|-------------------------------|
| Lifeway              | Low Fat Kefir    | All-natural                   |
| • United States      |                  | 99% lactose-free              |
| • Canada             |                  | Gluten-free                   |
| • Great Britain      |                  | 12 probiotic cultures         |
| Veggie Kefir         |                  | High in protein and calcium   |
| Kefir Oats           |                  | All-natural                   |
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | Oat fiber enriched            |
|                      |                  | High in protein and calcium   |
| Perfect 12 Kefir     |                  | All-natural                   |
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
| Greek Style Kefir    |                  | No added sugar                |
|                      |                  | High in protein and calcium   |
| Low Fat Kefir (Organic) |      | USDA Certified Organic        |
|                      |                  | Oregon Tilth Certified Organic|
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | High in protein and calcium   |
| Whole Milk Kefir     |                  | USDA Certified Organic        |
| (Organic)            |                  | Oregon Tilth Certified Organic|
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | No added sugar                |
| Helios Kefir (Organic)|                  | USDA Certified Organic        |
|                      |                  | Oregon Tilth Certified Organic|
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | Seven probiotic cultures      |
|                      |                  | Contains Inulin               |
| Green Kefir (Organic) |                  | USDA Certified Organic        |
|                      |                  | Oregon Tilth Certified Organic|
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | No-spill pouch                |
| ProBugs (organic)    |                  | USDA Certified Organic        |
|                      |                  | Oregon Tilth Certified Organic|
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | No-spill pouch                |
| ProBugs Blast (Organic)|              | USDA Certified Organic        |
|                      |                  | Oregon Tilth Certified Organic|
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | High in protein and calcium   |
| Frozen ProBugs       |                  | All-natural                   |
| (Organic)            |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 10 probiotic cultures         |
|                      |                  | High in protein and calcium   |
| Frozen Kefir         |                  | All-natural                   |
|                      |                  | 99% lactose-free              |

(Continued)

TABLE 4 | Continued

| Companies            | Product          | General information            |
|----------------------|------------------|-------------------------------|
|                      |                  | Gluten-free                   |
|                      |                  | 10 probiotic cultures         |
|                      |                  | 90 calories per serving       |
|                      |                  | 1 g of fat                    |
|                      |                  | Frozen Kefir Bars             |
|                      |                  | All-natural                   |
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | 60 calories per serving       |
|                      |                  | 0.5 g of fat                  |
|                      |                  | BioKefir                      |
|                      |                  | All-natural                   |
|                      |                  | 20 Billion units of probiotics|
|                      |                  | 12 probiotic cultures         |
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | Farmer Cheese                 |
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | High in protein and calcium   |
|                      |                  | Evolve Kefir                  |
|                      |                  | 11 probiotic cultures         |
|                      |                  | Natural fruit flavors.        |
|                      |                  | Fiber.                        |
|                      |                  | Protein and calcium           |
|                      |                  | Wallaby                       |
|                      |                  | Lowfat Kefir                  |
|                      |                  | 12 different strains of Live andActive Kefir cultures.|
|                      |                  | CocoKefir                     |
|                      |                  | USDA Certified Organic        |
|                      |                  | Oregon Tilth Certified Organic|
|                      |                  | 99% lactose-free              |
|                      |                  | Gluten-free                   |
|                      |                  | 12 probiotic cultures         |
|                      |                  | No added sugar                |
|                      |                  | Contains valuable nutrients such as potassium, manganese, and magnesium. |
|                      |                  | Beneficial probiotic strains  |

show good physicochemical characteristics for their use as food additives. In addition to these characteristics, EPSs are obtained from microorganisms classified as GRAS (generally recognized as safe), such as lactic acid bacteria (Wang et al., 2008; Saija et al., 2010; Badel et al., 2011).

Many reports have demonstrated that the quantity and properties of EPSs depend on the microorganisms used in the fermentation process and on the fermentation conditions and the composition of the culture media (Kim et al., 2008). EPSs have physicochemical and rheological properties that make them suitable as additives, which can be used as stabilizers, emulsifiers, gelling agents, and viscosity improvers. Additionally, EPSs possess biological properties suggesting their use as antioxidants, antitumor agents, antimicrobial agents, and immunomodulators, among other roles (Suresh Kumar et al., 2008; Bensmira et al., 2010; Piermaria et al., 2010).

The EPS kefiran is produced by Lactobacillus kefiranofaciens (Kooiman, 1968; Wang et al., 2010) from kefir grains, which are composed of proteins, polysaccharides, and a complex symbiotic microbial mixture (Witthuhn et al., 2005; Jianzhong et al., 2009). These microorganisms grow in kefiran, which is a polysaccharide matrix consisting of glucose and galactose. Despite good kefiran production by L. kefiranofaciens alone, it has been observed that
the addition of *Saccharomyces* sp. to the culture improves the net quantity of kefiran, illustrating the importance of the symbiosis between the bacteria and yeast that are present in kefir (Cheirsilp et al., 2003).

Lactic acid bacteria can synthesize homopolysaccharides or heteropolysaccharides. The synthesized homopolysaccharides are glucans or fructans, which are composed of only one type of monosaccharide (glucose or fructose, respectively; Van Hijum et al., 2006; Badel et al., 2011), whereas the heteropolysaccharides contain different types of monosaccharides in different proportions (mainly glucose, galactose, and rhamnose), (De Vuyst and Degeest, 1999; Ruas-Madiedo et al., 2002).

Similarly to lactic acid bacteria, *Lactobacillus* sp. also produces glucan and fructan. The homopolysaccharides show a much higher performance compared with heteropolysaccharide production (Welman and Maddox, 2003; Badel et al., 2011).

The heteropolysaccharides excreted by *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus*, and *Lactobacillus helveticus* contain galactose, glucose, and rhamnose as the main monosaccharides, with other monosaccharides being present in smaller concentrations. They are also highly branched with different types of linkages, and their denominations are complex and generally dependent on the main monosaccharide (De Vuyst and Degeest, 1999; Badel et al., 2011).

*Lactobacillus plantarum* isolated from Tibetan kefir excretes EPS classified as heteropolysaccharides composed of galactose, glucose, and mannose. This EPS has the capacity/ability to reduce blood cholesterol and form a biofilm shape (Zhang et al., 2009; Wang et al., 2010).

Kefiran is an EPS classified as a heteropolysaccharide comprising glucose and galactose in high concentrations, and it is classified as a water-soluble glucogalactan, which makes it suitable to be used as an additive (Wang et al., 2008, 2010). Kefiran has excellent rheological properties and can significantly improve the viscosity of lacteous products by favoring and maintaining gel properties and avoiding the loss of water during storage (Rimada and Abraham, 2006). With respect to the biological activity of kefiran, several studies have demonstrated that this EPS can be used as a nutraceutical, as described in Table 3.

The first study about kefiran structure was published by Kooiman (1968), who proposed a structure composed of two units: kefiran (polysaccharide) and kefirose (pentasaccharide). Then, some authors analyzed the polysaccharide structure with current techniques such as chromatography and infrared spectroscopy (Wang et al., 2008; Chen et al., 2015) and nuclear magnetic resonance (NMR; Ghasemlou et al., 2012). The kefiran structure, according to them, is shown in Figure 1.

**KEFIR-BASED PRODUCTS**

Nowadays, the interest in developing functional foods is increasing because people want to improve their health and prevent diseases. Keeping in mind that kefir is a beverage with high probiotic activity, among other bioactivities, new companies are emerging around the world. One of the biggest kefir companies known is Lifeway, which started in 1986; their products can be obtained in the United States, Canada, and Great Britain, all of them based in kefir beverages, frozen, and cheese.

Other companies are Evolve Kefir with its principal product, a smoothie; Wallaby Yogurt Company with Low Fat Kefir; and CocoKefir LLC, which provides drinks/beverages based mainly on coconut water cultured with a comprehensive blend of probiotics. Table 4 summarizes the products provided these companies with some general information about each one.

**CONCLUSION**

Kefir, the traditional beverage, is now recognized as a potential source of probiotics and molecules with highly interesting healthy properties. The careful and detailed characterization of kefir composition has helped the scientific community to find new possibilities for its application. Kefiran, the EPS of kefir, has very important physicochemical and rheological properties. Besides, its biological properties suggest its use as an antioxidant, antitumor agent, antimicrobial agent, and immunomodulator, among other roles. Research is constantly being conducted to consolidate kefir and kefiran properties for the development of new important products to preserve consumer’s health.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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