Microbiome and Metabiotic Properties of Kefir Grains and Kefirs Based on Them

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Abstract—The analysis of the literature on the microbiome composition and metabolic properties of kefir available at the RSCI and Web of Science was carried out. Kefir has been used by humans for centuries. It is a useful product of mixed lactic and alcoholic fermentation, produced using evolutionally established associative cultures, collected in an aggregated state termed kefir grains. General characterization of kefir grains from the territorial zones of different continents (Russia, Europe, Asia, and America) is provided. The methods for differentiation and identification of individual species are described, as well as their interactions within the community. The diversity of microbial composition of kefir grains depending on local cultivation conditions and storage processes is shown. The microorganisms present in kefir have a number of properties that determine their metabolism, interaction in the community, beneficial effects on human health and immune system, which is important for the prevention and control of bacterial and viral infections, especially during the COVID-19 pandemic.

Keywords: kefir, kefir grains, microbiota, differentiation, identification, community interaction, metabiotic properties

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Under pandemic conditions, a human organism is subject to a viral attack combined with a complex of unfavorable factors affecting the normal functioning of its major systems, which impairs the balance of the intestinal microbiome and suppresses immunity. Alterations in the composition of the microbial community of the organism caused by ecological, pharmacological, and other stress factors may be ameliorated by enrichment of the GIT microbiota with introduced beneficial microorganisms (Shenderov, 2014). This finding initiated development of a new field of microbiology, studies of probiotics, living microorganisms, certain amounts of which have beneficial effects on human and animal health when introduced into the organism with food (Meier and Steuerwald, 2005; Oleskin et al., 2020). The last decades of the previous century and the beginning of the present one are characterized by active development of nutrition science dealing with nutrition of healthy humans and development of functional foodstuffs containing probiotic microflora with desired beneficial properties. Nutraceutics include biologically active compounds positively affecting human health and may contribute to prevention of certain diseases (Shenderov, 2014; Oleskin et al., 2020).

The disciplines dealing with nutrition of patients (dietology) and healthy people (nutritiology) have been known since ancient times. Old manuscripts indicate that long ago (B.C.) Egyptians, Greeks, Jews, Romans, and Arabs have used various foodstuffs to cure and prevent diseases (Farnworth, 2005). Kefir is a useful, nutritious product with unique organoleptic properties, which exerts a positive effect on human health. Unlike other fermented milk products, it does not result from metabolic activity of a single microbial species (or closely related species), but is produced by a complex, naturally developed microbial community.
Kefir Grains: Structure and Functions

Kefir grains are discrete structures containing protein (4–5%), the polysaccharide termed kefiran (9–10%), and a complex lamellar microbiota (Leite et al., 2013; Gradova et al., 2014). They may be described as gelatinous, white or slightly yellowish irregular masses with elastic consistency, 0.3 to 3.5 cm in diameter (Figs. 1, 2). Microscopy of kefir grains revealed smooth, bumpy surfaces and a gelatinous matrix, which was overlaying the cell aggregates as a thin polysaccharide film.

The microbiota of kefir grains is represented by cocci and short and long rods, located close to elongated yeast cells. Short rods, probably *Lactobacillus kefiranofaciens* are distributed throughout the matrix volume, with their concentration increasing to the grain center. The cocci are mainly located at the surface of yeast cells, while the rods occupy the space between the yeast cells. The yeasts are most closely bound to the kefir grain stroma and are located both at the center and at the surface of the grains. The density of microbial cells is higher at the surface of the grain than in its center (Wang et al., 2012). The numbers of microorganisms at the surface and in the center of the grains depends on their relation to oxygen and on pH values. Inside the grains, pH is very low and inhibits the growth of lactococci. Due to weak adhesive properties of *Lactococcus lactis*, many electron microscopic studies failed to reveal them in kefir grains, although other isolation techniques showed that *L. lactis* was one of the dominant species in the same grains (Cheirsilp et al., 2003; Jianzhong et al., 2009).

The exact microbial composition of kefir grains remains debatable. In the grain base, up to 50 bacterial and yeast species were isolated from kefirs produced in different regions (Pogačić et al., 2013). The bacterial genera most common in kefir grains from milk belong to LAB, which are responsible for 37–90% of the microbial population (Yükselkay et al., 2004; Miguel et al., 2010; Zanirati et al., 2015); acetic acid bacteria, yeasts, and fungi are also present (Withuhn et al., 2005; Yang et al., 2007; Mayo et al., 2012; Gao et al., 2012, 2013). The microbial composition of kefir grains was reported to depend significantly on their origin and local cultivation conditions (Prado et al., 2001; Kotova et al., 2016) (Table 1).
Among LAB, lactobacilli, such as *Lactobacillus paracasei* ssp. *paracasei*, *L. acidophilus*, *L. delbrueckii* ssp. *bulgaricus*, *L. plantarum*, and *L. kefiranofaciens*, are predominant species constituting 20% of the total LAB number (Gao et al., 2007; Wang et al., 2012; Zanirati et al., 2015). Other microorganisms present include mesophilic homofermentative lactococci *Lactococcus* spp. (Magalhaes et al., 2011; Garofalo et al., 2015), thermophilic *Streptococcus thermophilus* (Simova et al., 2002; Kok-Tas et al., 2012; Guzel-Seydioglu, 2015), heterofermentative lactobacilli and *Leuconostoc* spp., streptococci producing lactic and acetic acids, CO₂, ethanol, dextran, and the substances responsible for specific aroma, such as acetoin and diacetyl (Diosma et al., 2014; Walsh et al., 2016). Acetic acid bacteria, e.g., *Acetobacter fabarum*, have been isolated in China (Yang et al., 2007; Gao et al., 2012; Jianzhong et al., 2009), while *Acetobacter pasteurianus* was found in kefirs produced in European countries (France, Belgium, Italy, and Switzerland) (Garofalo et al., 2015; Korsak et al., 2015; Kok-Tas et al., 2012). Acetic acid bacteria isolated from dairy products belong to the genus *Acetobacter*, comprising motile gram-negative rods, which occur singly, in pairs, or in chains. Some strains may exhibit involutionary forms: spherical, bent, filamentous, etc. They do not form spores or capsules. Ethanol is oxidized to acetic acid under oxic conditions (the so-called acetic acid fermentation); some species may oxidize acetate and lactate to CO₂ and H₂O. Lactose is not fermented (Montaghi et al., 1997).

Enterococci *E. durans* were found in the microbiota of kefirs produced by Chinese and Turkish companies (Yang et al., 2007; Kesmen and Kacamaz, 2011). The numerous group of lactic acid bacteria of the genus *Enterococcus*, including *E. durans*, has previ-
Table 1. Bacterial composition of kefir grains from kefirs of various producers

| Bacterial component                                                                 | Source—country | Reference                        |
|-------------------------------------------------------------------------------------|----------------|----------------------------------|
| *Lactobacillus*: *L. kefiri*, *L. kefiranofaciens*, *L. paracasei*, *L. plantarum*, *L. parakefiri*; *Lactococcus*: *L. lactis* ssp. *lactis*, *L. lactis* ssp. *lactis* bv. *diacetylactis* | Argentina      | Garrote et al., 2001; Londero et al., 2012; Hamet et al., 2013; Diosma et al., 2014 |
| *Lactobacillus*: *L. brevis*, *L. delbrueckii* ssp. *bulgaricus*, *L. helveticus*, *L. casei* ssp. *pseudoplanatarum*; *Streptococcus thermophilus* *Lactococcus lactis* ssp. *lactis* | Bulgaria       | Simova et al., 2002             |
| *Lactobacillus* sp., *L. plantarum*; *Leuconostoc* sp., *Lactococcus* sp.           | South Africa   | Witthuhn et al., 2004; Witthuhn et al., 2005 |
| *Lactococcus*: *L. lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris* *Streptococcus thermophilus*, *Enterococcus durans* *Lactobacillus kefiri*, *Leuconostoc mesenteroides*, *Lactococcus*: *L. lactis*, *Streptococcus thermophilus* *Lactobacillus*: *L. kefiranofaciens*, *L. acidophilus*, *L. helveticus* *Streptococcus thermophilus* | Turkey         | Yüksekdag et al., 2004; Guzel-Seydim et al., 2005; Kesmen and Kacmaz, 2011; Kok-Tas et al., 2012; Nalbantoglu et al., 2014 |
| *Lactobacillus*: *L. kefiranofaciens*, *L. kefiri*, *L. parakefiri*, *Lactococcus lactis*, *Leuconostoc* sp. | Russia         | Mainville et al., 2006; Kotova et al., 2016 |
| *Enterococcus durans*, *Lactococcus lactis* ssp. *cremoris*, *Leuconostoc pseudomesenteroides*, *Leuconostoc paramesenteroides*, *Lactobacillus brevis*, *L. acidophilus*, *L. kefiranofaciens*, *Leuconostoc mesenteroides*, *Lactobacillus* sp., *L. kefiri*, *L. casei*, *L. plantarum*, *L. helveticus*, *Leuconostoc lactis*, *Lactococcus* sp., *L. lactis*, *Acetobacter fabarum*, *Bacillus subtilis* | China          | Yang et al., 2007; Jianzhong et al., 2009; Gao et al., 2012; Gao et al., 2013 |
| *Lactobacillus kefiri*, *L. kefiranofaciens*, *Leuconostoc mesenteroides*, *Lactococcus lactis*, *L. paracasei* and *L. helveticus*, *Gluconobacter japonicus*, *Lactobacillus*: *L. uvarum*, *L. satsumensis*, *L. amylovorus* *L. buchneri*, *L. crispatus*, *L. parakefiri*; *L. kefiranofaciens* ssp. *kefiranofaciens*, *L. kefiranofaciens* ssp. *kefirgranum*, *L. parakefiri*, *Lactobacillus parabuchneri*, *L. casei*; *Leuconostoc* sp. | Brazil         | Miguel et al., 2010; Leite et al., 2012; Zanirati et al., 2015; Magalhães et al., 2011 |
| *Lactobacillus lactis*, *L. kefiranofaciens*; *Lactococcus lactis* | Italy          | Garofalo et al., 2015           |
| *Lactobacillus*: *L. kefiri*, *L. kefiranofaciens*; *Leuconostoc mesenteroides*, *Lactococcus lactis* and *L. lactis* ssp. *cremoris* | Belgium        | Korsak et al., 2015             |
| *Lactobacillus kefiranofaciens* and *L. kefiri* | Malaysia       | Zambri et al., 2016             |
| *Lactobacillus kefiranofaciens*, *L. helveticus*, *Leuconostoc* spp., *Acetobacter pasteurianus* | France, Ireland, and England | Walsh et al., 2016              |
ously been assigned to streptococci of the serological groups D and E; during the first weeks of human life, they colonize the intestine and are a necessary culture involved in the processes of food transformation (Sycheva and Kartashova, 2015).

Unlike other fermented milk products, kefir is not the result of metabolic activity of one or several microbial species.

Kefir grains contain various species of yeasts, which ferment or do not ferment lactose, form or do not form spores (4 to 30 species, according to different sources). Those most often mentioned are Kluyveromyces marxianus, Candida kefyr, Saccharomyces cerevisiae, Saccharomyces unisporus, Torulaspora delbrueckii, Pichia fermentans, and the synonyms for these species (Table 2). Predominant species, however, are Saccharomyces cerevisiae, S. unisporus, Candida kefyr, and Kluyveromyces marxianus ssp. marxianus (Fleet, 1990; Assadi, 2000; Loretan et al., 2003; Witthuhn et al., 2004, 2005; Kok-Tas et al., 2012; Diosma et al., 2014). Unlike other fermented milk products, kefir grains contain considerable amounts of yeasts (Tamang et al., 2016). The key role of yeasts in the preparation of fermented milk product is accepted. In the course of this process, they produce the nutrients required for growth, including amino acids and vitamins, change the ambient pH, and release ethanol and CO2. The yeasts of kefir are less thoroughly studied than bacteria, although they are certainly responsible for establishment of the environment favoring growth of the kefir bacteria, as well as for production of the metabolites providing for the aroma and organoleptic properties of the product (Farnworth, 2005). Over 23 different yeast species have been isolated from kefir grains and from fermented beverages of various origin.

**Differentiation and Identification of the Kefir Grains Microbiome**

Initial differentiation of the microorganisms in the community includes a complex of phenotypic characteristics determined by investigation of their morphological, physiological, and biochemical properties. LAB are the most widespread bacteria in kefir and kefir grains (37 to 90% of the whole microbial population). These microbial species fall into four groups: homofermentative and heterofermentative lactic acid bacteria, and yeasts assimilating and not assimilating lactose (Gao et al., 2012). Anaerobic cultivation for the isolation of pure bacterial cultures was carried out under anoxic conditions at room temperature (21°C) on MRS agar in petri dishes. Under such conditions, bacterial colonies were formed after 3–5 days. Pure anaerobic colonies were obtained by streak inoculation. Inoculated plates were placed into anaerobic jars, in which gas packages were inserted. Complete isolation of the kefir grain components is difficult to achieve using the standard microbiological methods of plating on agar media. Morphological characteristics of the colonies of some microorganisms may be so similar that they may be mistaken for identical cultures, while the colonies with slight morphological differences may be formed by one microorganism (Sycheva and Kartashova, 2015). For example, under suboptimal unfavorable growth conditions, long-term action of physical, chemical, or biological stressors resulted in emergence of minor phenotypes (subpopulations) of lactic acid bacteria and of viable uncultured forms (Pachomov et al., 2018).

LAB are phylogenetically unrelated microorganisms of heterogeneous morphology: rods and orbs (cocci of spherical or ellipsoidal shape), which are characterized as gram-positive, not forming capsules or spores (except for the family Sporolactobacillaceae), not producing pigments (except for Leuconostoc citreum, which forms capsules and a yellow pigment), and not reducing nitrate to nitrite. They are catalase- and oxidase-negative, have no cytochromes, are aerob and acid-tolerant, nonmotile, and produce diverse amounts of lactic acid as the terminal metabolite (Lengeler et al., 2005).

The long-known members of the genera Lactococcus, Enterococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Vagococcus, Tetragenococcus, Carnobacterium, Bifidobacterium, but Lactococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus, and Bifidobacterium form the nucleus of this group. The genera Oenococcus, Weissella, Fructobacillus were recently added to this group (Lengeler et al., 2005; Lahtinen et al., 2012; Stoyanova, 2017). The genus Lactobacillus is morphologically highly diverse, from short coccoid cells to long, filamentous rods, 0.7–1.1 to 3.0–8.0 μm, occurring singly or in chains. Cell length often depends on the cultivation medium. In the case of mixed microbial populations, application of biochemical identification techniques is limited by the fact that after plating a sample of a liquid culture on solid nutrient medium, the ratio of two bacterial species may be determined only after isolation of pure cultures and investigation of all formed colonies, which is cost- and labor-consuming. The search for a more efficient approach to this problem is therefore an urgent issue. Molecular genetic identification techniques proved reliable and independent on external factors.

For identification of lactobacilli, the classical microbiological techniques (using cultural characteristics, morphology, Gram reaction, motility, presence of catalase, and the spectrum of fermented carbohydrates) are supplemented by molecular genetic techniques based on analysis of the 16S rRNA gene sequences using the MegAlign 6.00 DNASTAR Inc. software package. However, high stability of the 16S rRNA gene does not provide for unequivocal identification of closely related species. Accurate identification of the numerous species and subspecies of the phylogenetically related groups L. casei, L. plantarum,
Table 2. Yeast components isolated from kefirs of different countries

| Yeast component                                                                 | Source—country | Reference                            |
|---------------------------------------------------------------------------------|----------------|--------------------------------------|
| Geotrichum candidum,                                                            | Argentina      | Garrote et al., 1997; Garrote et al., 1998; Garrote et al., 2001; Diosma et al., 2014 |
| Kluovermyces marxianus,                                                          |                |                                      |
| Saccharomyces cerevisiae,                                                        |                |                                      |
| Saccharomyces unisporus,                                                          |                |                                      |
| Issatchenkia occidentalis                                                        |                |                                      |
| Candida inconspicua,                                                             | Bulgaria       | Simova et al., 2002                  |
| Candida maris,                                                                   |                |                                      |
| Kluovermyces marxianus,                                                          |                |                                      |
| Yarrowia lipolytica                                                             |                |                                      |
| Candida kefyr,                                                                   | Iran           | Motaghi et al., 1997                 |
| Saccharomyces fragilis                                                           |                |                                      |
| Saccharomyces lactis                                                             |                |                                      |
| Kazachstania aerobia,                                                            | Brazil         | Magalhaes et al., 2011a              |
| Lachancea meyersii                                                               |                |                                      |
| Zygosaccharomyces rouxii,                                                        | South Africa   | Loretan et al., 2003; Witthuhn et al., 2004; Witthuhn et al., 2005 |
| Torulaspora delbrus                                                             |                |                                      |
| Torulaspora delbrueckii                                                         |                |                                      |
| Debaromyces hansenii                                                             |                |                                      |
| Zygosaccharomyces sp.,                                                           |                |                                      |
| Candida lipolytica,                                                               |                |                                      |
| Candida holmii,                                                                  |                |                                      |
| Candida kefyr,                                                                   |                |                                      |
| Candida lambica,                                                                 |                |                                      |
| Candida krusei,                                                                   |                |                                      |
| Cryptococcus humicolus                                                           |                |                                      |
| Saccharomyces cerevisiae                                                         |                |                                      |
| Geotrichum candidum                                                              |                |                                      |
| Kluovermyces marxianus                                                           | Turkey         | Kok-Tas et al., 2012                 |
| Brettanomyces anomalus                                                           | Switzerland    | Fröhlich-Wyder, 2003; Fleet, 1990    |
| Candida holmii,                                                                  |                |                                      |
| Candida kefyr,                                                                   |                |                                      |
| Candida lambica,                                                                 |                |                                      |
| Candida tenuis,                                                                  |                |                                      |
| Candida valida,                                                                  |                |                                      |
| Geotrichum candidum                                                              |                |                                      |
| Issatchenkia occidentalis                                                        |                |                                      |
| Kluovermyces bulgaricus                                                          |                |                                      |
| Kluovermyces fragilis                                                            |                |                                      |
| Kluovermyces marxianus                                                           |                |                                      |
| Pichia fermentans                                                                |                |                                      |
| Saccharomyces cerevisiae                                                         |                |                                      |
| Saccharomyces delbrueckii                                                        |                |                                      |
| Saccharomyces exiguus                                                             |                |                                      |
| Saccharomyces unisporus                                                           |                |                                      |
| Yarrowia lipolytica                                                              |                |                                      |
| Yeast component                              | Source—country | Reference                               |
|----------------------------------------------|----------------|-----------------------------------------|
| Brettanomyces anomalus                       | Canada         | Farnworth, 2005                          |
| Candida friedrichii, Candida holmii          |                |                                         |
| Candida inconspicua, Candida kefyr           |                |                                         |
| Candida lambica, Candida maris               |                |                                         |
| Candida tenuis, Candida valida               |                |                                         |
| Candida tannotelerans                        |                |                                         |
| Issatchenkia occidentalis                    |                |                                         |
| Kluyveromyces marxianus                     | Spain          | Lopitz-Otsoa, 2006;                     |
| Pichia fermentans                           |                | Latorre-Garcia et al., 2007             |
| Saccharomyces cerevisiae                    |                |                                         |
| Saccharomyces dairensis                     |                |                                         |
| Saccharomyces delbrueckii                   |                |                                         |
| Saccharomyces exiguus                       |                |                                         |
| Saccharomyces tunicensis                    |                |                                         |
| Brettanomyces anomalus                      | Spain          | Lopitz-Otsoa, 2006;                     |
| Candida famata, Candida firmetaria           |                | Latorre-Garcia et al., 2007             |
| Candida friedrichii, Candida humilis         |                |                                         |
| Candida inconspicua, Candida kefyr           |                |                                         |
| Candida krusei, Candida lipolytica          |                |                                         |
| Candida maris, Yarrowia lipolytica          |                |                                         |
| Cryptococcus humicolus                      |                |                                         |
| Debaryomyces hansenii                       |                |                                         |
| Dekkera anomala                             |                |                                         |
| Galactomyces geotrichum                     |                |                                         |
| Geotrichum candidum                         |                |                                         |
| Issatchenkia orientalis                     |                |                                         |
| Kluyveromyces lodderae                      |                |                                         |
| Kluyveromyces marxianus                     |                |                                         |
| Pichia fermentans                           |                |                                         |
| Saccharomyces cerevisiae                    |                |                                         |
| Saccharomyces exiguus                       |                |                                         |
| Saccharomyces humaticus                     |                |                                         |
| Saccharomyces pastorianus                   |                |                                         |
| Saccharomyces tunicensis                    |                |                                         |
| Saccharomyces unisporus                     |                |                                         |
| Zygosaccharomyces rouxii                     |                |                                         |
| Candida holmii                              | India          | Assadi, 2000                             |
| Candida kefyr                               |                |                                         |
| Saccharomyces cerevisiae                    |                |                                         |
| Saccharomyces fragilis                      |                |                                         |
| Saccharomyces lactis                        |                |                                         |
| Kazachstania aerobia                        | Italy          | Garofalo et al., 2015                   |
| Kazachstania salicola                       |                |                                         |
| Kazachstania serovazzii                     |                |                                         |
| Kazachstania tunicensis                     |                |                                         |
| Kazachstania unispora                       |                |                                         |
| Kazachstania exigua                         | China          | Jianzhong et al., 2009;                 |
| Pichia kudriavzevii,                        |                | Gao et al., 2012;                       |
| Pichia guilliermondii,                      |                | Gao et al., 2013                        |
| Kazachstania unispora                       |                |                                         |
| Kazachstania exigua                         |                |                                         |
L. buchneri, and L. acidophilus is difficult, which requires a search for new genetic markers. Identification of the marker genes, analysis of which enables assessment of whole genome relationships between microorganisms is recommended for analysis of nucleotide sequences for species identification (Blaiotta et al., 2008). Analysis of the genes groEL, rplB, and rpoB revealed high polymorphism of their nucleotide sequences in members of the L. casei phylogenetic group and resulted in reliable identification of phenotypically and genetically close species within this group of lactobacilli (Shvetsov et al., 2011). The discriminative abilities of application of these genes is several times higher than that of the 16S rRNA gene. The nucleotide sequences were analyzed and combined into a common sequence using the SeqScape 2.6.0 software package (Applied Biosystems).

Among the important differentiating characteristics of yeasts is their ability to oxidize and ferment various carbohydrates, including maltose, sucrose, galactose, trehalose, etc. Yeasts grow within a relatively broad pH range (3 to 9), preferring, however, acidic tose, trehalose, etc. Yeasts grow within a comparatively small range of pH (pHopt 4.5‒5.5). Yeasts are osmophilic microorganisms, but they have a wide pH range (3 to 9), preferring, however, acidic conditions. Yeasts oxidize and ferment various carbohydrates, including maltose, sucrose, galactose, trehalose, etc.

The nucleotide sequences were analyzed and combined into a common sequence using the SeqScape 2.6.0 software package (Applied Biosystems).

**Metabolic and Structural Interactions of Yeasts and Bacteria**

Obtained data indicated that lactic acid bacteria of the physiological group which actively use lactose for lactic acid fermentation were probably the main producers of the system established in the kefir grains. The microorganisms belonging to another group use...
the products of lactose metabolism (glucose and galactose); the relations between members of this group may be either passive antagonism or cooperation. Investigation of the dynamics of lactic acid fermentation in the course of culture development revealed that the chemical transformations in the medium changed during this process. Two phases were clearly discernible in the course of carbohydrate fermentation. During the first one (the exponential growth phase), active synthesis of proteins and other cell components more reduced than hydrocarbons occurred. More oxidized products of metabolism were accumulated in the medium. The second phase was characterized by slower rates of biosynthesis and a
Fig. 4. Taxonomic tree constructed using MEGAN analysis for a kefir grain at the species level (by Zambelli et al., 2016). The spot indicates the most widespread species.
gradual decrease in the redox potential of the culture, which resulted in accelerated proton transfer to PGA with its subsequent reduction to lactic acid. These two phases reflect redistribution of redox reactions in the course of biosynthesis of the structural elements of bacterial cells (constructive processes) and fermentation (an energy process). Close symbiotic relationships between LAB and yeasts and a stimulatory effect of yeasts on LAB growth have been demonstrated by many researchers (Motaghi et al., 1997; Aziza et al., 2012; Stoyanova et al., 2017).

Complex interactions between yeasts and bacteria, as well as their dependence on the microbial composition of kefir grains, are presently not completely understood. However, when bacteria are removed from the grain, yeasts grow less efficiently (Cheirsilp et al., 2003; Farnworth and Mainville, 2008; Rattray and O’Connel, 2011). The interaction between yeasts and LAB is of pivotal importance for a broad spectrum of fermented products, including kefir (Han et al., 2018). Both groups of microorganisms naturally support each other by various means listed below.

**Lactic acid assimilation.** One interesting mechanism of interaction between yeasts and LAB is implemented in the presence of lactic acid-assimilating yeasts. Lactate accumulation damps and kills LAB, even when pH is maintained by addition of alkaline solutions (Katakura et al., 2010). However, yeasts not utilizing lactate, e.g. S. cerevisiae, may use lactate as a carbon source, which results in increased pH and long-term LAB growth. Acid resistance is a physiological feature of LAB resulting from their specific energy metabolism. Acid stress causes intracellular acidification, which decreases the activity of cytoplasmic enzymes (Miyoshi et al., 2013). Transcriptome and proteome studies showed that while a number of LAB were able to enhance activity of their glycolytic enzymes under acidic, thermal, and osmotic stresses, this did not result in elevated lactate synthesis. Although research on the mechanism of diacetyl formation has been carried out for a long time, there is still no unanimous understanding concerning the biosynthesis of this compound by lactic acid bacteria. One of the pathways of diacetyl production is its synthesis from L-acetolactate, one of the intermediate products of citrate metabolism. This is an unstable compound released from bacterial cells into the medium, where it undergoes oxidative decarboxylation to diacetyl and nonoxidative decarboxylation to acetoin. Another pathway involving condensation of acetaldehyde–thiamine pyrophosphate and acetyl-CoA is considered doubtful by many authors, since the enzymes catalyzing these reactions have not been isolated. Acetate is released into the medium, and oxaloacetate is decarboxylated to form pyruvate. Diacetyl is formed in the reaction of acetyl-CoA and “active acetaldehyde,” an enzyme–oxyethylaminopyrophosphate complex. Diacetyl reduction by acetaldehyde-generating results in acetoin formation (Fig. 5).

Such lactobacilli as Lactobacillus planatarum, L. reuteri, and L. rhamnosus and lactococci L. lactis modify pyruvate metabolism using lactate, and thus enhance the synthesis of the major energy-rich intermediates, such as ATP and NAD, EPS, and/or glycine. The level of lactate dehydrogenase (LDH), which is responsible for lactate synthesis from pyruvate, decreases significantly. Pyruvate oxidase and phosphoacetyl transferase, which are used to synthesize acetyl-CoA, are induced in Lactobacillus delbrueckii subsp. bulgaricus and L. rhamnosus under conditions of acid stress. Acetyl-CoA is redirected to biosynthesis of fatty acids, which may increase the strength and impermeability of the cell membrane (Leille et al., 2013).

**CO₂ production/O₂ removal.** CO₂ may be responsible for establishment of the suitable atmospheric (decreased oxygen and increased CO₂ concentrations) environment for growth of Lactobacillus spp. Although no works on the microorganisms isolated from kefir are available, research on other communities and microorganisms isolated from foodstuffs confirms this interaction. CO₂ produced by yeasts promotes development of the characteristic acidic and yeast taste of kefir (Karaçali et al., 2018).

**Supply of nutrients for bacteria.** Trophic interactions and metabolite exchange (crosswise nutrition) enable survival of several groups of microorganisms under resource limitation. Yeasts were shown to promote bacterial growth by supplying vitamins, growth factors, and essential amino acids (Pahva et al., 2010; Ponomarova et al., 2017). Zygo- torulaspora florentina was shown to produce essential amino acids, which support the growth of L. nagelii in mixed culture, but not in the case of cultivation as monocultures (Stadie et al., 2013).

To investigate the specifics of metabolite exchange between S. cerevisiae and two LAB groups (Lactobacillus plantarum or Lactococcus lactis), experiments were carried out with model systems using a combination of metabolic and genetic tools (Ponomarova et al., 2017). Nitrogen excess in the medium was found to favor the emergence of mutualism (a form of mutually beneficial coexistence, when the presence of a partner is a necessary condition for the existence of each of them) between yeasts and L. lactis. Interaction between L. lactis and S. cerevisiae is easily established when lactose is the major carbon source. This is another evidence of the important role of medium composition in formation of interspecific interactions.

Complex interactions between yeasts and bacteria, as well as their mutual dependencies in kefir grains, are still incompletely studied. However, when bacteria were separated from the grain, yeast growth became less efficient (Ratarura et al., 2010). Interaction of yeasts and LAB is of pivotal importance in a broad spectrum of fermented products, including kefir. Dif-
different groups of microorganisms naturally support each other by different means (Aziza et al., 2012).

Trophic interactions between microbial components were investigated using the strains isolated from kefir grains (~33 bacterial and 55 fungal isolates). Two physiological groups of lactic acid bacteria were revealed, differing in their ability to synthesize \( \beta \)-galactosidase, the enzyme required for lactose fermentation. The yeast isolates were shown to possess no \( \beta \)-galactosidase activity, did not utilize lactose, used glucose (actively) and galactose (at low activity), and do not form clods in milk. The approach based on assessment of the physiological activity of the isolates of lactic acid bacteria provided evidence that lactic acid bacteria of the first physiological group, possessing \( \beta \)-galactosidase activity, did not utilize lactose, used glucose (actively) and galactose (at low activity), and do not form clods in milk. The presence in the system of several LAB species possessing \( \beta \)-galactosidase activity indicates that certain regulatory factors should control development of bacteria of this group: either competition for the substrate, or shifts of the main producers depending on conditions, such as pH changes (Cheirsilp and Radchabut, 2011). Figure 6 presents a general scheme of the trophic chain of the kefir grain associated culture including three LAB group (synthesizing and not synthesizing \( \beta \)-galactosidase, and bacteria with repressive \( \beta \)-galactosidase synthesis), as well as acetic acid bacteria and yeasts.

Depending on the medium and cultivation conditions, the microbiota of kefir grains and kefir leaving exhibits unique abilities for autoregulation. Microbial symbiosis in kefir grains provides for the preservation of kefir quality and the microbial profile of kefir grains throughout the year with only insignificant changes in the ratios of the major microbial groups. Kefir microbial composition may differ from that of kefir grains due to the differences in pH and cultivation time; this difference may also be associated with location of

Fig. 5. Glucose fermentation by *Lactococcus lactis* under oxic conditions (by Miya et al., 2003): LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase; PFL, pyruvate-formate lyase; \( \alpha \)-ALS, \( \alpha \)-acetolactate synthase.
microorganisms within the grains. Thus, lactic acid bacteria of the genus *Lactococcus* are located on the surface of kefir grains, are easily desorbed into the culture liquid, and are therefore relatively numerous in kefir (Gradova et al., 2014).

**Fermentation and Preservation of Kefir Grains**

Increase in the biomass of a kefir grain during fermentation is the main marker for assessment of the symbiotic relationships between different microorganisms. The associative microbial culture of kefir grains is a stable, highly organized community with complex vertical and horizontal trophic relationships. The main products of fermentation of milk carbohydrates formed in the course of kefir production are lactic acid, ethanol (at a low concentration), and CO₂, which are responsible for viscosity, acidity, and pungency. The secondary components, including diacetyl, acetaldehyde, and amino acids, which may also be found in kefir, are responsible for its aroma. In the course of fermentation the size and number of the grains increase; they are usually removed from fermented milk for repeated use. Their activity may be retained for many years, provided they are stored correctly (Lopitz-Otsoa et al., 2006; Garrote et al., 2010; Leite et al., 2013). Dried grains remain active for 12–18 months, compared to 8–10 days for moist grains. Many preservation techniques were tested, and freezing is presently considered preferable. Lyophilization of the grains has also been tested; it, however, resulted in lower lactose metabolism and in changes in the bacterial profile, compared to the original one (Farnworth et al., 2008). Kefir may be either used immediately after grain separation or stored in a refrigerator for subsequent use (Otles et al., 2003). The properties of fermented milk should be retained during storage; however, continuous metabolic activity of the residual kefir microbiota may result in changes in the composition of cooled kefir during storage (Gronnevik et al., 2011). A drastic decrease in viscosity during storage in a refrigerator at 4°C has been reported (Magra et al., 2012), while total fat, lactose, dry matter, and pH remained constant during 14 days of storage (Vieira et al., 2015), and lactic acid concentration increased slightly after 7 days of storage. While the lipolytic activity of milk fat under laboratory conditions is limited, it may still contribute to production of free fatty acids (Kim et al., 2002).

Kefir production is affected by a number of factors: raw materials, production technology, and conditions of storage of kefir and kefir grains, which should be both optimized in parallel in order to achieve the best quality of the product. Increase in temperature from 20 to 30°C resulted in elevated amounts of yeasts (from $7.1 \times 10^6$ to $10^7$ CFU/g kefir grain and from $1.2 \times 10^5$ to $1.7 \times 10^6$ CFU/mL in the leaven) and acidic acid bacteria (from $10^5$ to $10^7$ CFU/g in the grains and from $4.2 \times 10^4$ to $7.0 \times 10^6$ CFU/mL in the leaven) and had
an insignificant effect on the numbers of mesophilic lactic acid bacteria in kefir grains (Schoevers and Britz, 2003; Khokhlacheva et al., 2006).

However, higher fermentation temperature (25°C) resulted in a rapid pH decrease in the leaven, which inhibited growth of homo- and heterofermentative lactic acid streptococci. The leaven prepared at 25°C was shown to contain more lactic acid streptococci than that prepared at 18–20°C. Research revealed that all strains of this species grew actively within the temperature range from 20 to 30°C, while at 35°C growth was very poor (Londero et al., 2012). All strains produced maximal biomass at 25°C. The biomass produced at 25°C was 1.3–1.9 and 1.2–1.8 times higher than that produced at 20 and 30°C, respectively (Khiamagaeva and Vandanova, 2006). Shaking during the cultivation resulted in increased exopolysaccharide production by kefir fungal cultures and in significant differences if the qualitative and quantitative composition of the grains. Thus, shaking caused a decrease in abundance of yeasts and lactic acid bacteria in kefir grains, while the concentrations of carbohydrates and fats increased significantly (Schoevers and Britz, 2003). Screening of polysaccharide-synthesizing lactic acid bacteria revealed that out of 119 studied isolates, 60% were capable of polysaccharide synthesis. From these, 9 isolates were chosen, which synthesized polysaccharides most actively. Enhanced polysaccharide synthesis in the medium with sucrose was observed for the LAB capable of fermenting it. The cultures of Lactococcus lactis, and Leuconostoc mesenteroides were chosen as the most active exopolysaccharide producers in media with lactose and sucrose. Comparative study of kefir grain exopolysaccharides and those synthesized by monocultures, which involved IR spectroscopy and dynamic and statical light scattering revealed the similarity of EPS structure and the differences in the physicochemical properties of the polysaccharide samples with potential prebiotic activity.

Kefiran is the polysaccharide of kefir grains, which is produced by acetic acid bacteria and yeasts involved in milk fermentation. It possesses antimicrobial and wound healing activity and is able to decrease blood pressure and cholesterol level in blood serum. Kefiran in concentrations 5.9–14.3 g/L can form cryogels melting at 37°C, which may probably be applied for development of new foodstuff. Viscosity of the gels may be varied by addition of different concentrations of sucrose or fructose to kefiran solutions (Gradova et al., 2012; Zavala, 2015). When kefir grains were cultivated in milk, aeration promoted exopolysaccharide production and caused significant differences in the qualitative and quantitative composition of the grains.

Kefir grains are a complex symbiosis of several microbial species: lactic acid streptococci and lactobacilli, acetic acid bacteria, and yeasts. These grains may be used for daily kefir production at home. The popularity of kefir grains among the populace is presently steadily increasing.

The mass of kefir grains increases due to growth of microorganisms and biosynthesis of the grain components—proteins and polysaccharides. The kefir grain microbiota may be considered as a biofilm. The processes regulating biofilm formation include formation of the surface for cell attachment, intercellular interactions, and growth of a complex culture. Biofilm formation by some species has been reported, and a number of genes hypothetically responsible for adhesion or biofilm formation have been described. Biofilm formation helps the cells to survive environmental stresses, e.g., high concentrations of acid and ethanol.

Kefir contains easily digestible proteins. Minor essential acids, which are abundant in kefir, regulate the protein, carbohydrate, and lipid metabolism and have a positive effect on the regulation of human body mass, maintenance of immune response, and energy metabolism. The peptides exhibit antimicrobial and antioxidative activity in milk kefir produced by proteolysis of β-casein; 236 peptides with antimicrobial or antioxidant properties, inhibited the angiotensin converting enzyme (ACE), had immunomodulatory and antithrombotic effects (Hamet et al., 2013; Ebner et al., 2015). Peptide F3, which was isolated from Tibetan kefir and purified, exhibited antibacterial action against Escherichia coli and Staphylococcus aureus (Miao et al., 2016). In kefir prepared from cow milk, 35 peptides were identified, which had an anti-hypertensive effect mediated by inhibition of the ACE activity (Amorim et al., 2019). The product is rich in such amino acids as serine, threonine, alanine, lysine, valine, isoleucine, methionine, phenylalanine, and tryptophan, which play an important role in the functioning of the central nervous system; it also contains metabolites facilitating casein digestion and assimilation by the organism (Bensmira et al., 2015).

Kefir probiotic cultures are known to regulate the immune system, promoting suppression of viral infections. The antiviral mechanism of action of kefir includes enhance macrophage production, enhanced phagocytosis, elevated production with positive differentiation of CD4+/CD8+ as a biomarker of response to treatment, of immunoglobulins (IgG+ and IgA+), B-cells, T-cells, and neutrophiles, some of which may produce antibodies if required. Kefir LAB increase the cytotoxicity of natural killer cells against tumor cells (Yamane et al., 2018). Kefir may act as an antiinflammatory agent by decreasing the expression of interleukins IL-1 and IL-6 synthesized by macrophages and T-cells and stimulating the immune response, while interferons IFN-α and type II (IFN-γ) induce the antiviral defense mechanisms. In the presence of alien antigens, elevated amounts of cytokines are produced; they act as mediators of the inflammatory process and have regulatory functions, which, in turn, induce elevated IL-6 formation, cause activation and migration.
of T-lymphocytes and other immune cells, resulting in the symptoms of a cytokine storm during the coronavirus infection. Thus, kefir may be an important inhibitor of the cytokine storm, which favors COVID-19 development (Nakagaki et al., 2018; Boyoglu-Barnum et al., 2019; Bornstein et al., 2020).

To conclude, deterioration of the epidemiological situation worldwide resulted in greater demand for the products and safe preparations with beneficial health effects. Traditional fermented milk produce resulting from mixed alcohol and lactic acid fermentation, including kefir, have been known since antiquity as capable of countering infections and premature aging. Milk fermentation for kefir production is a process of combined metabolism of symbiotic microbial cultures promoting formation and stability of the kefir grain microecology. Analysis of the literature data indicates that, in spite of certain differences in quantitative ratios, four microbial groups are almost always present in kefir grains: lactic acid bacteria, lactococci, acetic acid bacteria, and yeasts. What are the synergistic or antagonistic effects of these microorganisms on each other in the course of mixed-culture metabolism? Is it possible to determine one or several indicator microorganisms or indicator metabolites for quantitative assessment and evaluation of the fermentation state of kefir bacteria? Answers to these questions will not only provide the theoretical basis for investigation of kefir communities, but may be used as a guide for investigation of other microbial consortia. The contradictory data used in the development of the conceptual model, including the results of investigation of microbial composition and trophic interactions between components of the established kefir grain consortium, which are required for construction of new communities and development of approaches to control of the kefir grain stability, which may be used to create new functional food products and pharmaceuticals with beneficial effects on human health.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants performed by any of the authors.

The authors declare that they have no conflicts of interest.

REFERENCES

Amorim, F.G., Coitinh, L.B., Dias, A.T., Friques, A.G.F., Monteiro, B.L., Rezende, L.C.D., Pereira, Th.M.C., Campagnaro, B.P., Pauw, E.D., Vasquez, E.C., and Quinton, L., Identification of new bioactive peptides from kefir milk through proteopeptidomics: bioprospection of antihypertensive molecules, Food Chem., 2019, vol. 282, pp. 109—119.

Assad, M.M., Pourahmad, R., and Moazami, N., Use of isolated kefir starter cultures in kefir production, World J. Microbiol. Biotechnol., 2000, vol. 16, pp. 541—543.

Aziza, M. and Amrane, A., Diauxic growth of Geotrichum candidum and Penicillium camemberti on amino acids and glucose, Braz. J. Chem. Engin., 2012, vol. 29, pp. 203—210.

Bornstein, S.R., Rubino, F., Khunti, K., Mingrone, G., Hopkins, D., Birkenfeld, A.L., Boehm, B., Amiel, S., Holt, R.I., Skylar, J.S., DeVries, J.H., Re-nard, E., Eckel, R.H., Zimmet, P., Alberti, K.G. et al., Practical recommendations for the management of diabetes in patients with COVID-19, Lancet. Diabetes Endocrinol. Published online April 23, 2020.

Bourrie, B.C.T., Willing, B.P., and Cotter, P.D., The microbiota and health promoting characteristics of the fermented beverage kefir, Front. Microbiol., 2016, vol. 7, pp. 647—664.

Boyoglu-Barnum, S., Chirkova, T., and Anderson, L.J., Biology of infection and disease pathogenesis to guide RSV vaccine development, Front. Immunol., 2019, vol. 10, art. 1675. https://doi.org/10.3389/fimmu.2019.01675

Cheirisilp, B., Shimizu, H., and Shioya, S., Enhanced kefir production by mixed culture of Lactobacillus kefiranfaciens and Saccharomyces cerevisiae, J. Biotechnol., 2003, vol. 100, pp. 43—53.

Diosma, G., Romain, D.E., Rey-Burusco, M.F., Londo-ro, A., and Garrote, G.L., Yeasts from kefir grains: isolation, identification, and probiotic characterization, World J. Microbiol. Biotechnol., 2014, vol. 30, pp. 43—53.

Ehner, J., Asgi Arslan, A., Fedorova, M., Hoffmann, R., Kugukgetin, A., and Pischetsrieder, M., Peptide profiling of bovine kefir reveals 236 unique peptides released from caseins during its production by starter culture or kefir grains, J. Proteomics, 2015, vol. 117, pp. 41—57.

Farag, M.A., Jomaa, S.A., and El-Wahed, A.A., The many faces of kefir fermented dairy products: quality characteristics, flavor chemistry, nutritional value, health benefits, and safety, Nutrients, 2020, vol. 12, pp. 346—359.

Farnworth, E.R. and Mainville, I., Kefir—a fermented milk product, in Handbook of Fermented Functional Foods, Farnworth, E.R., Ed., 2008, no. 2, pp. 89—127.

Farnworth, E.R., Kefir a complex probiotic, Food Science and Technology Bull.: Functional Foods, 2005, vol. 2, pp. 1—17.

Fleet, G.H., Growth of yeasts during wine fermentation, J. Wine Res., 1990, pp. 211—223.

Fonseca, G.G., Heli Latorre-Garcia, L., del Castillo-Agu-do, L., and Polaina, J., Taxonomical classification of yeasts isolated from kefir based on the sequence of their ribosomal RNA genes, World J. Microbiol. Biotechnol., 2007, vol. 23, pp. 785—791.
from different areas of China, *J. Food Sci.*, 2012, vol. 77, pp. 425–433.

Gao, J., Gu, F., He, J., Xiao, J., Chen, Q., and Ruan, H., Metagenome analysis of bacterial diversity in Tibetan kefir grains, *Eur. Food Res. Technol.*, 2013, vol. 236, pp. 549–556.

Garofalo, C., Osimani, A., Milanović, V., Aquilanti, L., De Filippis, F., and Stellato, G., Bacteria and yeast microbiota in milk kefir grains from different Italian regions, *Food Microbiol.*, 2015, vol. 49, pp. 123–133.

Garrote, G.L., Abraham, A.G., and De Antoni, G.L., Characteristics of kefir prepared with different grain: milk ratios, *J. Dairy Res.*, 1998, vol. 65, pp. 149–154.

Garrote, G.L., Abraham, A.G., and De Antoni, G.L., Chemical and microbiological characterization of kefir grains, *J. Dairy Res.*, 2001, vol. 68, pp. 639–652.

GOST (State Standard) 31454-2012: Kefir. Technical Conditions.

Gradova, N.B., Khokhlacheva, A.A., Murzina, E.D., and Myasoedova, V.V., Microbial components of kefir fungi, as a producer of kefiran exopolysaccharide, *Biotechnology*, 2014, no. 6, pp. 18–26.

Guzel-Seydim, Z., Wyffels, J.T., Seydim, A.C., and Greene, A.K., Turkish kefir and kefir grains: microbial enumeration and electron microscopic observation, *Int. J. Dairy Technol.*, 2005, vol. 58, pp. 25–29.

Hamet, M.F., Londero, A., Medrano, M., Vercammen, E., Van, H.K., and Garrote, G.L., Application of culture-dependent and culture-independent methods for the identification of *Lactobacillus kefiranofaciens* in microbial consortia present in kefir grains, *Food Microbiol.*, 2013, vol. 36, pp. 327–334. https://ngs.arb-silva.de/silvangs.

Jianzhong, Z., Xiaoli, L., Hanhu, J., and Mingsheng, D., Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis, *Food Microbiol.*, 2009, vol. 26, pp. 770–775.

Karaçali, R., Özdemir, N., and Çon, A.H., Aromatic and functional aspects of kefir produced using soya milk and *Bifidobacterium* species, *Int. J. Dairy Technol.*, 2018, vol. 71, pp. 921–933.

Katakura, Y., Sano, R., Hashimoto, T., Ninomiya, K., and Shioya, S., Lactic acid bacteria display on the cell surface cytosolic proteins that recognize yeast mannan, *Appl. Microbiol. Biotechnol.*, 2010, vol. 86, pp. 319–326.

Kesmen, Z. and Kacamaz, N., Determination of lactic microflora of kefir grains and kefir beverage by using culture-dependent and culture-independent methods, *J. Food Sci.*, 2011, vol. 76, pp. 276–283.

Khamagayaeva, I.S. and Vandanova, Ye.V., Selection of conditions for the cultivation of symbiotic starter culture for the production of kefir, *Food and Processing Industry. Abstract Journal*, 2006, no. 2, pp. 95–98.

Khokhlacheva, A.A., Egorova, M.A., Kalinina, A.N., and Gradova, N.B., Trophic patterns of functioning and microbial profile of an evolutionarily established associative culture of kefir grains, *Microbiology* (Moscow), 2015, vol. 84, pp. 561–569.

Kim, Y.J. and Liu, R.H., Increase of conjugated linoleic acid content in milk by fermentation with lactic acid bacteria, *J. Food Sci.*, 2002, vol. 67, pp. 1731–1737.

Kok-Tas, T., Ekinçi, F.Y., and Guzel-Seydim, Z.B., Identification of microbial flora in kefir grains produced in Turkey using PCR, *Int. J. Dairy Technol.*, 2012, vol. 65, pp. 126–131.

Korsak, N., Taminiaw, B., Leclerc, M., Nezer, C., Crevecoeur, S., Ferauche, C., Detry, E., Delcenserie, V., and Daube, G., Short communication: evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments, *J. Dairy Sci.*, 2015, vol. 98, pp. 3684–3689.

Kotova, I.B., Cherdynetzva, T.A., and Netrusov, A.I., Russian kefir grains microbial composition and its changes during production process, *Adv. Exp. Med. Biol.*, 2016, vol. 4, pp. 93–121.

Lahtinen, S., Ouwehand, A.C., Salminen, S., and von Wright, A., *Lactic Acid Bacteria: Microbiological and Functional Aspects*, CRC, 2011, 4th ed.

Latorre-García, L., del Castillo-Agudo, L., and Polaina, J., Taxonomical classification of yeasts isolated from kefir based on the sequence of their ribosomal RNA genes, *World J. Microbiol. Biotechnol.*, 2007, vol. 23, pp. 785–791.

Leite, A.M.O., Leite, D.C.A., del Aguilã, E.M., Alvarães, T.S., Peixoto, R.S., Miguel, M.A.I., Silva, J.T., and Paschoalin, V.M.F., Microbiological and chemical characteristics of Brazilian kefir during fermentation and storage processes, *J. Dairy Sci.*, 2013, vol. 96, pp. 4149–4159.

Leite, A.M.O., Miguel, M.A., Peixoto, R.S., Rosado, A.S., Silva, J.T., and Paschoalin, V.M.F., Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage, *Braz. J. Microbiol.*, 2013, vol. 44, pp. 341–349.

Lengeler, J., Drews, G., and Schlegel, G., Eds., *Biology of Prokaryotes*, Wiley-Blackwell, 1999.

Londero, A., Hamet, M.F., De Antoni, G.L., Garrote, G.L., and Abraham, A.G., Kefir grains as a starter for whey fermentation at different temperatures: chemical and microbiological characterization, *J. Dairy Res.*, 2012, vol. 79, pp. 262–271.

Lopitz-Otsoa, F., Rementeria, A., Elguezabal, N., and Garaiaraz, J., Kefir: a simbiotic yeasts-bacteria community with alleged healthy capabilities, *Rev. Iberoam. Micol.*, 2006, vol. 23, pp. 67–74.

Loretan, T., Mostert, J.F., and Viljoen, B.C., Microbial flora associated with South African household kefir, *South Afr. J. Sci.*, 2003, vol. 99, pp. 92–94.

Machado, A., Leite, D.O., Antonio, M., Miguel, L., Peixoto, R.S., Rosado, A.S., Silva, J.T., Margaret, V., and Paschoalin, F., Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage, *Braz. J. Microbiol.*, 2013, vol. 44, pp. 341–349.

Magalhães, K.T., Pereira, G.V.M., Campos, C.R., Dragone, G., and Schwan, R.F., Brazilian kefir: structure, microbial communities and chemical composition, *Braz. J. Microbiol.*, 2011, vol. 42, pp. 693–702.

Mainville, I., Robert, N., Lee, B., and Farnworth, E.R., Polyphasic characterization of the lactic acid bacteria in kefir, *Syst. Appl. Microbiol.*, 2006, vol. 29, pp. 59–68.

Mayoa, B., Rachid, C.T., Peixoto, R.S., Silva, J.T., and Paschoalin, V.M., Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis, *Food Microbiol.*, 2012, vol. 31, pp. 215–221.

Meier, R. and Steuerwald, M., Place of probiotics, *Curr. Opin. Crit. Care*, 2005, vol. 11, pp. 318–325.
Miao, J., Liu, G., Ke, C., Fan, W., Li, C., Chen, Y., Dixon, W., Song, M., Cao, Y., and Xiao, H., Inhibitory effects of a novel antimicrobial peptide from kefir against Escherichia coli, Food Control, 2016, vol. 65, pp. 63–72.

Miguel, M.G.C.P., Cardoso, P.G., Lago, L.A., and Schwan, R.F., Diversity of bacteria present in milk kefir grains using culture-dependent and culture-independent methods, Food Res. Int., 2010, vol. 43, pp. 1523–1528.

Mitra, S. and Ghosh, B.C., Quality characteristics of kefir as a carrier for probiotic Lactobacillus rhamnusus GG, Int. J. Dairy Technol., 2020, vol. 73, pp. 384–391.

Miyoshi, A., Rochat, T., Gratadoux, J.J., Le Loir, Y., Costa Oliveira, S., Langella, P., and Azevedo, V., Oxidative stress in Lactococcus, Gen. Mol. Res., 2003, vol. 3, pp. 348–359.

Motaghi, M., Mazaheri, M., Moazami, N., Farkhondeh, A., Fooladi, M., and Goltapeh, E., Kefir production in Iran, World J. Microbiol. Biotechnol., 1997, vol. 13, pp. 579–581.

Nakagaki, T., Nakano, Y., Yamane, T., Sakamoto, T., Nakagaki, T., and Nakano, Y., Lactic acid bacteria from kefir increase cytotoxicity of natural killer cells to tumor cells, Foods, 2018, vol. 7, p. 48.

Nalbantoglu, U., Cakar, A., Dogan, H., Abaci, N., Ustek, D., and Sayood, K., Metagenomic analysis of the microbial community in kefir grains, Food Microbiol., 2014, vol. 41, pp. 42–51.

Oleskin, A.V. and Shenderov B.A., Probiotics, prebiotics and metabolites: problems and prospects, Physical and Rehabilitation Medicine, Medical Rehabilitation, 2020, vol. 2, no. 3, pp. 233–243.

Pahwa, S., Kaur, S., Jain, R., and Roy, N., Design based on the structure of new histidinol dehydrogenase inhibitors from Geotrichum candidum, Bioorg. Med. Chem. Lett., 2010, vol. 20, pp. 3972–3976.

Pakhomov, Y.D., Blinkova, L.P., Dmitrieva, O.V., Berdyugina, O.S., and Stoyanova, L.G., Non-culturability and nisin production of Lactococcus lactis, J. Bacteriol. Parasitol., 2013, vol. 5, pp. 2–8.

Pogačić, T., Sinko, S., Zambertin, S., and Samarzija, D., Microbiota of kefir grains, Mijekarstvo, 2013, vol. 63, pp. 3–14.

Prado, M.R., Blandón, L.M., Vandenberge, L.P.S., Rodrigues, C., Castro, G.R., Thomaz-Soccol, V., and Soccol, C.R., Milk kefir: composition, microbial cultures, biological activities, and related products, Front. Microbiol., 2015, vol. 6, art. 01177.

Schoevers, A. and Britz, T.J., Influence of different culturing conditions on kefir grain increase, Int. J. Dairy Techn., 2003, vol. 56, pp. 183–187.

Shenderov, B.A., Microbial ecology of man and its role in maintaining health, Metamorphoses, 2014, no. 5, pp. 72–80.

Shevtsov, A.B., Kushugulova, A.R., Tynbyaeva, I.K., Kozhakhmetov, S.S., Azhahelov, A.B., Momynaliyev, K.T., and Stoyanova, L.G., Identification of phenotypically and genotypically related Lactobacillus strains based on nucleotide sequence analysis of the gro EL, rpo B, rpl B, and 16S rRNA genes, Microbiology (Moscow), 2011, vol. 80, pp. 672–681.

Simova, E., Beshkova, D., Angelov, A., Hristozova, T., Frengova, G., and Spasov, Z., Lactic acid bacteria and yeasts in kefir grains and kefir made from them, J. Ind. Microbiol. Biotechnol., 2002, vol. 28, pp. 1–6.