Some probiotic characteristics of a fermented milk product based on microbiota of “Tibetan kefir grains” cultivated in Ukrainian household

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Abstract The article describes some probiotic properties of fermented product made of natural association of Tibetan kefir grains cultivated in Ukrainian household (UTKG); also, the effect of UTKG microbiota on the growth of pathogenic microbiota and sensitivity to antibiotics was studied. It was found that the test-cultures of opportunistic pathogens (*Staphylococcus aureus*, *Bacillus mesentericus*, and *Mycobacterium luteum*) were sensitive; bacteriostatic zone of the test-culture ranged from 21 to 25 mm, and highly sensitive (*Proteus vulgaris* and *Aspergillus niger*) bacteriostatic zone exceeded 25 mm to probiotic bacteria of fermented product. UTKG microbiota is also moderately sensitive to multiple antibiotics that allows defining the obtained fermented milk product as functional with therapeutic properties. During the study of the influence of different NaCl and bile concentrations on acid-activity of UTKG it was found that active acid formation occurred at the concentrations up to 4% NaCl in cultivation medium (boiled milk) and at 20% bile and 0.45% phenol. It proves microbial association to be capable of withstanding adverse gastrointestinal conditions and continue developing.

Keywords Tibetan kefir grains · Fermented product · Probiotic bacteria

Introduction

The preparations made of beneficial bacteria, namely lactic acid bacteria and bifidobacteria, have been increasingly used both in general medicine and veterinary medicine in recent years. The action of such medicines aims at inhibiting pathogenic microbiota, reducing pathogenic adhesion, increasing useful effect, neutralizing toxins, and stimulating immunity. Despite the large number of currently produced probiotics, the problem of intestinal microflora correction and normalization still remains actual due the fact that not all of biotic medicines exhibit a stable activity after long-term use. Therefore, current requirements as to effectiveness and safety of the said medicines became stricter. According to legal requirements of WHO, there must be a clear identification of probiotic strains that are supposed to be genetically stable and able to survive in gastrointestinal medium, particularly at low pH, and under the actions of digestive enzymes and bile acids. Moreover, probiotic strains must show high adhesive and antagonistic activity against pathogenic organisms (Colado et al. 2007).

Probiotic characteristics of dairy products were described in numerous works (Rosa et al. 2017; Lourens-Hattingh and Viljoen 2001; Shah 1997; Angmo et al. 2016) but developing market needs new products with well-expressed sanative properties. In addition, fermented milk product has raised interest in the scientific community due to its suggested beneficial properties, including improved digestion and tolerance to lactose (Hertzler and Clancy 2003), antibacterial effect (Rodrigues et al. 2005),

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hypocholesterolaemic effect (Taylor and Williams 1998), control of plasma glucose (Hadisaputro et al. 2012), anti-hypertensive effect (Maeda et al. 2004), anti-inflammatory effect (Rodrigues et al. 2005; Lee et al. 2007), antioxidant activity (Guzel-Seydim et al. 2003), anti-carcinogenic activity (Gao et al. 2013) and anti-allergenic activity (Lee et al. 2007).

Therefore, further possible probiotic sources are being researched. Thus, a new polyculture of microorganisms *Oryzomyces indici*, as well as the technology of its production was offered in Belarus (Tsed and Koroleva 2007). Milk supplements based on Tibetan kefir grains (TKG) that mentioned in local Ukrainian sources as *Lactomyces tibeticus*—culture association of microorganisms are of significant interest due to wide range of biologically active compounds, relatively simple cultivation and possibility of maintaining the culture active for a long time (Yelinov and Larina 1999; Kukhtyn et al. 2016; Vichko et al. 2013).

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; Prado et al. 2015). Moreover, 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 physico-chemical features and biological activities of each kefir (Jianzhong et al. 2009; Kabak and Dobson 2011; Gao et al. 2012; Altay et al. 2013). It can be assumed that regional differences and cultivation conditions can strong change microbial diversity in microbial natural association of *Tibetan kefir grains*.

TKG that cultivate in Ukrainian household (shortly *UTKG*) looks like a white lump of 3–6 mm (young fungus) and up to 3.5 cm in diameter (before division), with no particular smell. In water they sediment to the bottom of the container. The lumps are formed of round or oval curded granules of 1–3 mm, with uneven surface. The moisture content in fungus is around 86–89% according to our research (Kukhtyn et al. 2016; Vichko et al. 2013). Generic and species composition of the *UTKG* includes yeast cells of *Saccharomyces* genus, *Candida* kefir species and bacteria: *Lactobacillus fermentum* (nearly 90% of the microbial association), *Leuconostoc lactis* and acetic bacteria *Acetobacter* (Kukhtyn et al. 2016; Vichko et al. 2013). That is important generic and species composition of the *UTKG* are strongly difference than traditional TKG association (Gao et al. 2012; Prado et al. 2015) that contributes some characteristics in taste and smell of the fermented milk product.

Motivated by the strong progress in probiotic fermented milk product (Rosa et al. 2017; Lourens-Hattingh and Viljoen 2001; Shah 1997; Angmo et al. 2016; Rodrigues

et al. 2005; Lee et al. 2007), novel fermented food supplement obtained by fermentation of milk with microbial association *UTKG* is characterized here. The fermented milk produced using *UTKG* contains 10^8 – 10^9 CFU/cm³ of yeast and 10^4 CFU/cm³ of viable lactic acid bacteria. Components of fermented milk product showed high antagonistic activity to some species of fungi and bacteria. Moreover, *UTKG* microbiota is moderately sensitive to multiple antibiotics that allows ranging such fermented milk product as functional with sanative properties. In addition, bacteria of fermented product show resistance to unfavorable gastrointestinal factors. In fact, aim of this study is to determine some probiotic properties of the milk product fermented with microbial association *UTKG*.

Materials and methods

Product obtained by means of milk fermentation by *UTKG* was sour-milk traditional drink with fresh taste and aroma. The fermented product including *UTKG* only as starter culture was obtained by introducing 2.5–5% of *UTKG* association inoculum in sterile cow milk with milk fat content 2.5%. A titratable acidity of obtained product is nearly 85–120 °T (Therner Degrees).

Before each passage, the biomass was washed in sterile isotonic sodium chloride solution. For this, *UTKG* after milk fermentation was flushed out by aseptic physiological NaCl solution through two filtering septum: upper and down (the upper with bigger foramina). In this case there was an accumulation at the upper septum of a coarse-grained fungus layer (old fungus), which was utilized, and at the lower septum, a finely grained soft fungus layer (young fungus) accumulated that was then used for further milk souring. The acidity of the resulting food supplement was determined by the standard method (ISO 6092:1980).

Antagonistic activity of the fermented dairy products (*UTKG* drink) towards test-cultures of opportunistic pathogens was determined by “holes” in vitro conditions similar to (Mishra and Prasad 2005). The certified strains of *Escherichia coli* ATCC 25299, *Staphylococcus aureus* 209, *Bacillus cereus* ATCC 11778, *Mycobacterium luteum* VKM B-868, *Proteus vulgaris* VKM B-905 and *Aspergillus niger* ATSS 16404 were used as test cultures. Petri dishes with sterile nutrient medium [nutrient agar with 1% peptone (Chemtest, Ukraine) for bacteria and Sabouraud dextrose agar with maltose for *A. niger* (HiMedia Laboratories Pvt. Limited, India)] were filled with 1 cm³ of test-cultures suspension in 0.5% solution of NaCl (10^6 CFU/cm³). Then the suspension was distributed through the entire dish, and the excess was removed with pipette. After that, the dishes were dried in the incubator at 37 °C for 35–45 min. The drying was followed by making holes using No. 10 punch

(9 mm hole diameter). The formed hole was filled with milk product (fully filled). Petri dishes first were placed in a refrigerator with the temperature of 5 ± 0.5 °C for 10 h for diffusion of the drink in culture medium before the test organism starts to multiply rapidly; and then for 24 h in a thermostat at 37 °C for all bacteria and 25 °C for *A. niger*. In the end of incubation process, the result was defined based on bacteriostasis of the test cultures around the hole filled with fermented dairy product. The diameter of bacteriostatic zones of test-cultures less than 15 mm means insensitive microorganism; 16–20 mm—moderately sensitive, 21–25 mm—sensitive, 26 mm and more—highly sensitive.

Defining of antibiotic resistance of the studied fermented dairy product The suspension of the culture extracted from *UTKG* association (10^6 CFU/cm³) was prepared first. Then 0.1 cm³ of the suspensions was put in a Petri dish with solid medium [MRS-agar (Sigma-Aldrich, USA)] and spread with a sterile spatula throughout the dish. Then five discs with different antibiotics were places in the dishes. Petri dishes were placed in thermostat at 37 °C. After 24 h incubation the diameter of bacteriostatic zones around the disc was measured (Order of the Ministry of Healthcare of Ukraine No. 167 of April 05, 2007).

Effect of sodium chloride (Sfera-Sim, Ukraine), bile (Sigma-Aldrich, USA) and phenol (Sigma-Aldrich, USA) on acid-forming activity of *UTKG* association was determined by adding bile at concentrations 0.1, 0.2 and 0.3%, NaCl at concentrations 2, 4, and 6.5%, or phenol at concentrations 0.15, 0.3, 0.45 and 0.6% to boiled milk (Vinderola and Reinheimer 2003).

In order to study the stability of the *UTKG* against gastric juice, 1.5 cm³ of daily culture was centrifuged for 5 min at 3 thousand rotations per minute; then it was washed in saline and centrifuged again. The precipitate was re-suspended in 1.5 cm³ of gastric juice (pH 2.0) (CJSC “Biopharma”, Ukraine) and incubated for 2 h at 37 °C. The degree of bacterial survival was determined on MRS-agar (Sigma-Aldrich, USA) with counting the number of CFU after 48 h of incubation at 37 °C. Saline of the same volume was used instead of gastric juice in control.

Results and discussion

The results and discussion describe probiotic properties (selective inhibition of the growth of pathogens, sensitive to antibiotics, sodium chloride, bile and phenol concentration) of natural association of *UTKG*.

Active selective inhibition of the growth of pathogens and opportunistic pathogens is the mandatory characteristic of dietary and health products. Microorganisms used as leaven, often are part of normal intestinal microflora. They

must be resistant to metabolic gastrointestinal substances, such as sodium chloride, bile, phenol or gastric juice and show the ability for intestinal colonization and functioning provided there is no negative impact (Rosa et al. 2017; Ahmed et al. 2013).

The antagonistic action of *UTKG* association in the test cultures of opportunistic microorganisms (see “Materials and methods” section) was studied. The results are presented in Fig. 1.

From the above, it becomes evident that nearly all test cultures of opportunistic pathogens were sensitive and highly sensitive towards probiotic microorganisms of *UTKG* association. However, the most antagonistic activity of microbiota was shown towards fungus *A. niger* and bacteria *P. vulgaris*. Bacteriostatic zone made 28 ± 1 and 26 ± 1 mm, respectively, and they were highly sensitive. *Bacillus cereus*, *M. luteum* and *S. aureus* with 21–23 mm zone were sensitive, *E. coli* as normal gastrointestinal bacteria was moderately sensitive. Consequently, the above studies indicate that *UTKG* bacterial microbiota showed good antagonistic properties in relation to pathogenic and opportunistic microorganisms.

It is known that one of the criteria of a high quality probiotic drink is quantitative and qualitative composition of active microflora. According to published data, the content of probiotic bacteria should be an average of 10^7 – 10^9 CFU/cm³ (Vichko et al. 2013; Kukhtyn et al. 2016). Inoculation on selective medium showed that the total microbial count of a studied drink varies within 1.7 – 2.3×10^8 CFU/cm³. Most of the identified bacteria were the ones with active acid-forming capacity.

Fig. 1 Sensitivity of opportunistic test-cultures to the microorganisms of the *TKG* association: 1—*Escherichia coli* ATCC 25299, 2—*Staphylococcus aureus* 209, 3—*Bacillus cereus* ATCC 11778, 4—*Mycobacterium luteum* VKM B-868, 5—*Proteus vulgaris* VKM B-905, 6—*Aspergillus niger* ATSS 16404. Note The diameter of bacteriostatic zones of test-cultures less than 15 mm means insensitive microorganism; 16–20 mm—moderately sensitive, 21–25 mm—sensitive, 26 mm and more—highly sensitive

The resistance of “beneficial” microorganisms to antibiotics allows using (with maximum benefit for the body) the products with probiotic properties during antibiotic therapy (Fernandez et al. 2003). Antibiotics treatment without probiotics almost always leads to dysbiosis. The results of studying resistance against antibiotic of the microbial cells that are the part of the association are shown in Table 1.

It is clear herewith that *UTKG* association microorganisms are sensitive to vancomycin, doxycycline, nystatin, ceftriaxone, and cefotaxime. Bacteriostatic zones ranged from 21.5 to 24.0 mm. It is moderately sensitive towards chloramphenicol, gentamicin, amoxicillin, streptomycin, and penicillin and resistant to kanamycin. Virtually, low and medium sensitivity is observed towards all antibiotics studied therewith.

Therefore, the results listed in Table 1 and Fig. 1 indicate that culture association of *UTKG* fermented milk drink show high antagonistic activity to pathogenic gastrointestinal microflora and are moderately susceptible to multiple antibiotics. This allows ranging such fermented milk product as functional with sanative properties.

Consumption of functional milk products leads to improving health generally, as well as helps during treatment and prevention of dysbiosis. It is known that only 20–30% of lactic acid bacteria as part of the dairy products survive through gastrointestinal passing. They are lost due to unsuitable gastrointestinal medium (acidic gastric juice and bile in small intestine). Such losses reduce the beneficial effect of functional probiotics on animals or humans. The results of the effect of NaCl, bile, phenol and trypsin on acid-formation activity of *UTKG* microbiota during 24 h at the temperature 28 ± 0.5 °C are shown in Fig. 2.

According to the data shown in Fig. 2, active acid formation occurred with 4.0% sodium chloride in culture medium; but with 6.5% salt concentration, acidity was 58 ± 2 °T after 24 h of cultivation. It shows that this

Fig. 2 The effects of NaCl on acid-formation activity of *TKG* microbiota: 1—medium free of sodium chloride, 2—medium with 2% sodium chloride, 3—medium with 4% sodium chloride, 4—medium with 6.5% sodium chloride

concentration of sodium chloride inhibits some microorganisms of *UTKG* association, and it did not lead to intense expansion of lactose.

Figure 3 shows bile effect on acid-formation activity of *UTKG* during 24 h of culturing at the temperature of 28 ± 0.5 °C. The above data shows that cultivation of *UTKG* microbiota in milk with 20% bile does not significantly affect acid-forming activity. Acidity for the content of bile after 24 h was 87 ± 5 °T that is appropriate for milk drinks produced with *UTKG* microbiota. With 30% bile concentration, no acidity increase in the culture medium was noted.

Thus, despite the fact that microbial resistance to bile is mainly observed with bacteria extracted from mammals' gastrointestinal tract (Chou and Weimer 1999), *UTKG* microbiota well developed in milk with 20% bile concentration. It proves possible the ability of the microbial association to survive in intestinal medium with presence of bile.

Figure 4 shows the effect of phenol on acid-forming activity of *UTKG* during 24 h of culturing at the temperature of 28 ± 0.5 °C. According to Fig. 4, *UTKG* microbiota was resistant to phenol at 0.5% of its concentrations

Table 1 Antibiotic resistance of microorganisms included to *TKG* microbial association

Order no.	Antibiotic	Disc diameter (mm)	Bacteriostatic zone diameter (mm)	Microorganisms' sensitivity to antibiotics
1	Vancomycin 30.0 µg	5	22.5 ± 0.5	Sensitive
2	Chloramphenicol 30.0 µg	5	20.5 ± 0.5	Moderately sensitive
3	Gentamicin 10.0 µg	5	15.5 ± 0.5	Moderately sensitive
4	Doxycycline 10.0 µg	5	21.5 ± 0.5	Sensitive
5	Amoxicillin 10.0 µg	5	16.5 ± 0.5	Moderately sensitive
6	Kanamycin 30.0 µg	5	10.5 ± 0.5	Resistant
7	Streptomycin 10.0 µg	5	18.5 ± 0.5	Moderately sensitive
8	Penicillin 6.0 µg	5	16.5 ± 0.5	Moderately sensitive
9	Nystatin 80.0 µg	5	23.0 ± 1.0	Sensitive
10	Ceftriaxone 30.0 µg	5	22.5 ± 1.0	Sensitive
11	Cefotaxime 30.0 µg	5	24.0 ± 1.0	Sensitive

Fig. 3 Bile effect on acid-forming activity of TKG: 1—cultivation in bile-free boiled milk, 2—cultivation in 10% bile boiled milk, 3—cultivation in 20% bile boiled milk, 4—cultivation in 30% bile boiled milk

Fig. 4 The effect of phenol on acid-forming activity of TKG microbiota: 1—cultivation in phenol-free milk, 2—cultivation in 0.15% phenol milk, 3—cultivation in 0.3% phenol milk, 4—culturing in 0.45% phenol milk, 5—cultivation in 0.6% phenol milk

in milk. During cultivation, the acidity increased to 85.1 ± 4.7 °T, which is 1.3 times less than that compared with the acidity of the milk fermented phenol free. It is an important factor for probiotic drink and it indicates to the

ability of microbial association to withstand the adverse gastrointestinal medium and to develop in it.

Table 2 shows the results on the effect of gastric juice on *UTKG* microbiota resistance.

The number of viable cells of *L. fermentum* and *Lactobacillus* spp. strains after a 2-h hold in gastric juice decreased on average by three orders of magnitude, namely from $2.7\text{--}4.2 \times 10^7$ to $1.2\text{--}1.3 \times 10^4$ CFU/cm³.

Therefore, the results indicated that only lactobacilli strains were able to remain viable after holding in gastric juice. Despite the fact that lactococci strains were also acid-resistant and then appeared to be non-resistant to gastric juices.

Conclusion

It was established that bacteria of fermented milk product made of *UTKG* microbiota showed high antagonistic activity to *A. niger* and *P. vulgaris* bacteria (bacteriostatic zone made 28 ± 1 mm and 26 ± 1 mm, respectively); *B. cereus*, *M. luteum* and *S. aureus* (with bacteriostatic zone from 21 to 23 mm) were sensitive to the microbiota, and *E. coli* as normal gastrointestinal flora bacteria was moderately sensitive. *UTKG* microbiota was moderately sensitive to multiple antibiotics (chloramphenicol: 20.5 ± 0.5 mm bacteriostatic zone; gentamicin— 15.5 ± 0.5 mm, amoxicillin— 16.5 ± 0.5 mm, streptomycin— 18.5 ± 0.5 mm, penicillin— 16.55 ± 0.5 mm, kanamycin— 10.55 ± 0.5 mm). This allows ranging such fermented milk product as functional with sanative properties. The study revealed that bacteria of fermented product show resistance to unfavourable gastrointestinal factors: 4.0% sodium chloride concentration in culture medium; 20% bile concentration; 0.5% phenol concentration; the number of viable cells *L. fermentum* and *Lactobacillus* spp. strains after a 2-h hold in gastric juice decreased on average by three orders of magnitude, namely from $2.7\text{--}4.2 \times 10^7$ to $1.2\text{--}1.3 \times 10^4$ CFU/cm³.

Table 2 The effect of gastric juice on TKG survival

Microorganisms	Initial number of cells in suspension (CFU/cm ³)	Number of cells in suspension of control samples (CFU/cm ³)	Number of cells in suspension after treatment with gastric juice (CFU/cm ³)
<i>Saccharomyces</i>	$1.2 \pm 0.34 \times 10^7$	$1.0 \pm 0.56 \times 10^7$	0
<i>Candida kefir</i>	$1.9 \pm 0.22 \times 10^7$	$1.5 \pm 0.72 \times 10^7$	0
<i>Lactobacillus fermentum</i>	$2.7 \pm 0.51 \times 10^7$	$2.1 \pm 0.43 \times 10^7$	$1.3 \pm 0.17 \times 10^4$
<i>Lactobacillus</i> spp.	$4.2 \pm 0.65 \times 10^7$	$3.8 \pm 0.27 \times 10^7$	$1.2 \pm 0.1 \times 10^4$
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	$5.1 \pm 0.39 \times 10^7$	$4.7 \pm 0.17 \times 10^7$	0
<i>Leuconostoc lactis</i>	$7.7 \pm 0.49 \times 10^7$	$7.3 \pm 0.61 \times 10^7$	0

References

- Ahmed Z, Wang Y, Ahmad A et al (2013) Kefir and health: a contemporary perspective. *Crit Rev Food Sci Nutr* 53:422–434. doi:[10.1080/10408398.2010.540360](https://doi.org/10.1080/10408398.2010.540360)
- Altay F, Karbancioglu-Güler F, Daskaya-Dikmen C, Heperkan D (2013) Are view on traditional Turkish fermented non-alcoholic beverages: microbiota, fermentation process and quality characteristics. *Int J Food Microbiol* 167:44–56. doi:[10.1016/j.ijfoodmicro.2013.06.016](https://doi.org/10.1016/j.ijfoodmicro.2013.06.016)
- Angmo K, Kumari A, Bhalla TC (2016) Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. *LWT Food Sci Technol* 66:428–435. doi:[10.1016/j.lwt.2015.10.057](https://doi.org/10.1016/j.lwt.2015.10.057)
- Chou LS, Weimer B (1999) Isolation and characterization of acid and bile-tolerant isolates from strains of *Lactobacillus acidophilus*. *J Dairy Sci* 1:23–31. doi:[10.3168/jds.S0022-0302\(99\)75204-5](https://doi.org/10.3168/jds.S0022-0302(99)75204-5)
- Collado MC, Meriluoto J, Salminen S (2007) Role of commercial probiotic strains against human pathogenic adhesion to intestinal mucus. *Lett Appl Microbiol* 45:454–460. doi:[10.1111/j.1472-765X.2007.02212.x](https://doi.org/10.1111/j.1472-765X.2007.02212.x)
- Dried milk—determination of titratable acidity (Routine method). (ISO 6092:1980): /ICTY (Active from 01.01.2009), Kyiv, State Committee of Ukraine, (2009), p 8 (National Standard of Ukraine)
- Fernandez MF, Boris S, Barbes C (2003) Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J Appl Microbiol* 94:449–455. doi:[10.1046/j.1365-2672.2003.01850.x](https://doi.org/10.1046/j.1365-2672.2003.01850.x)
- Gao J, Gu F, Abdella N, Ruan H, He G (2012) Investigation on culturable microflora in Tibetan kefir grains from different areas of China. *J Food Sci* 77:425–433. doi:[10.1111/j.1750-3841.2012.02805.x](https://doi.org/10.1111/j.1750-3841.2012.02805.x)
- Gao J, Gu F, Ruan H et al (2013) Induction of apoptosis of gastric cancer cells SGC7901 in vitro by a cell-free fraction of Tibetan kefir. *Int Dairy J* 30:14–18. doi:[10.1016/j.idairyj.2012.11.011](https://doi.org/10.1016/j.idairyj.2012.11.011)
- Guzel-Seydim ZB, Seydim AC, Greene AK (2003) Comparison of amino acid profiles of milk, yoghurt and Turkish kefir. *Milch-wissenschaft* 58:158–160
- Hadisaputro S, Djokomoeljanto RRJ et al (2012) The effects of oral plain kefir supplementation on proinflammatory cytokine properties of the hyperglycemia Wistar rats induced by streptozotocin. *Acta Med Indones* 44:100–104
- Hertzler SR, Clancy SM (2003) Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *J Am Diet Assoc* 103:582–587. doi:[10.1053/jada.2003.50111](https://doi.org/10.1053/jada.2003.50111)
- Jianzhong Z, Xiaoli L, Hanhu J, Mingsheng D (2009) Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis. *Food Microbiol* 26:770–775. doi:[10.1016/j.fm.2009.04.009](https://doi.org/10.1016/j.fm.2009.04.009)
- Kabak B, Dobson A (2011) An introduction to the traditional fermented foods and beverages of Turkey. *Crit Rev Food Sci Nutr* 51:248–260. doi:[10.1080/10408390903569640](https://doi.org/10.1080/10408390903569640)
- Kukhtyn M, Vichko O, Berhilevych O, Horyuk Y, Horyuk V (2016) Main microbiological and biological properties of microbial associations of *Lactomyces tibeticus*. *Res J Pharm Biol Chem Sci* 6:1266–1272
- Lee M, Ahn K, Kwon O et al (2007) Anti-inflammatory and anti-allergic effects of kefir in a mouse asthma model. *Immunobiology* 212:647–654. doi:[10.1016/j.imbio.2007.05.004](https://doi.org/10.1016/j.imbio.2007.05.004)
- Lourens-Hattingh A, Viljoen BC (2001) Yogurt as probiotic carrier food. *Int Dairy J* 11:1–17. doi:[10.1016/S0958-6946\(01\)00036-X](https://doi.org/10.1016/S0958-6946(01)00036-X)
- Maeda H, Zhu X, Omura K et al (2004) Effects of an exopolysaccharide (kefir) on lipids, blood pressure, blood glucose, and constipation. *BioFactors* 22:197–200. doi:[10.1002/biof.5520220141](https://doi.org/10.1002/biof.5520220141)
- Mishra V, Prasad DN (2005) Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics. *Int J Food Microbiol* 103:109–115. doi:[10.1016/j.ijfoodmicro.2004.10.047](https://doi.org/10.1016/j.ijfoodmicro.2004.10.047)
- Order of the Ministry of Healthcare of Ukraine No. 167 of April 05, 2007 (2007) On approval of guidelines for determining the sensitivity of microorganisms to antibiotics. Kyiv Ministry of Healthcare of Ukraine, p 63
- Prado M, Blandón L, Vandenbergh L, Rodrigues C, Castro G, Thomaz-Soccol V, Soccol C (2015) Milk kefir: composition, microbial cultures, biological activities, and related products. *Front Microbiol* 6:1–10. doi:[10.3389/fmicb.2015.01177](https://doi.org/10.3389/fmicb.2015.01177)
- Rodrigues KL, Caputo LRG, Carvalho JCT et al (2005) Antimicrobial and healing activity of kefir and kefir extract. *Int J Antimicrob Agents* 25:404–408. doi:[10.1016/j.ijantimicag.2004.09.020](https://doi.org/10.1016/j.ijantimicag.2004.09.020)
- Rosa DD, Dias MM, Grześkowiak ŁM, Reis SA, Conceição LL, Maria do Carmo GP (2017) Milk kefir: nutritional, microbiological and health benefits. *Nutr Res Rev*. doi:[10.1017/S0954422416000275](https://doi.org/10.1017/S0954422416000275)
- Shah NP (1997) Isolation and enumeration of bifidobacteria in fermented milk products: a review. *Milchwissenschaft* 52:72–76
- Taylor GRJ, Williams CM (1998) Effects of probiotics and prebiotics on blood lipids. *Br Food J* 80:S225–S230
- Tsed EA, Koroleva LM (2007) New fermented alcohol free drink based on rice fungus *Oryzomyces indicus*. *Drinks Beverages* 2:48–50
- Vichko O, Chervetsova V, Novikov V (2013) Microbiological characteristics of sour-milk feed supplements and their influence on intestinal micro-biocenosis of piglets. *Res J Pharm Biol Chem Sci* 4:1404–1410
- Vinderola CG, Reinheimer JA (2003) Lactic acid starter and probiotic bacteria: a comparative “in vitro” study of probiotic characteristics and biological barrier resistance. *Food Res Int* 36:895–904. doi:[10.1016/S0963-9969\(03\)00098-X](https://doi.org/10.1016/S0963-9969(03)00098-X)
- Yelinov NP, Larina OG (1999) Mykrobryota of natural association *Lactomyces tibeticus*. *Probl Med Mycol* 1:51–56