Microbial Profile of Kurungua and Koumiss (Review Paper)

T N Zandanova, K V Ivanova, Yu E Losorova, T P Myryanova

1Yakutsk State Agricultural Academy

E-mail: tuyana35@mail.ru

Abstract. The review outlines the achievements in the field of research and identification of the kurungua and koumiss microflora. These are mixed fermentation sour milk products with similar healing properties. The paper represents information on the structure of spontaneously formed natural communities of the drink microflora and the probiotic properties of the cultures isolated. It has been found that the cultures isolated are bile- and acid-tolerant, exhibit high exopolysaccharide synthesizing activity, as well as cholesterol-degrading and antibiotic activity. The koumiss' ability to inhibit angiotensin-converting enzyme is shown.

1. Introduction

Under current conditions, the preservation and strengthening of human health is a relevant issue of society. Nutrition is one of the factors strengthening human health, which means the search and creation of adequate food meeting the evolutionarily arisen body’s needs. From olden times, mixed fermentation sour milk products such as kurungua and koumiss have taken one of the main places in the traditional food system of various peoples.

Unlike koumiss, kurungua is a less well-known drink being on a par with the first one by its healing properties. Kurungua is widespread in Buryatia, Khakassia, Tuva, and Mongolia. This is a bubbling and biting drink made of cow milk with a liquid lumpy consistency, a sour-milk taste, and an alcoholic fermentation flavor. The healing properties of kurungua are determined by a complex microbiological composition including lactic acid bacteria, yeast, and aceto bacter.

Studying the patterns of formation and functioning of such microbial communities as koumiss and kurungua’s microflora allow obtaining new knowledge in the formation of probiotic properties of microbial communities and individual strains.

This review is devoted to the study of the species composition of the kurungua and koumiss microflora, a modern concept testifying to the integral microbial population nature, and identification of the specifics of the biochemical activity of the cultures isolated.

Natural microbial communities have always been sources of strains with high biochemical activity. The microflora of the mixed fermentation sour milk products such as kurungua and koumiss is represented by an associative spontaneously formed population of lactobacilli and yeast.

The composition and properties of the kurungua’s culture microflora have been studied since the 40s of the last century. The first information about the kurungua’s microflora indicates the presence of long Lactobacillus casei – 82 %, short Acetobacter rods – 71 %, and the Torula yeast – 11 % (Khundanov, 1975).

For the first time, the microflora was studied in more detail by Z.P. Chuzhova (1966). She has found that the species composition of the kurungua microflora is very diverse, the most numerous...
representatives are Lactobacillus casei and Lactobacillus acidophilus, and lactic acid streptococci such as Streptococcus lactis, Streptococcus diacetilactis, Streptococcus faecalis, and Acetobacter aceti also present there. The Mycoderma, Torulopsis, and Candida pseudotropicalis var lactose yeasts have also been isolated. The ratio of lactic acid bacteria and yeast in kurunga has been estimated as symbiotic.

G.B. Lev (1977) has discovered the presence of lactic acid bacteria such as Streptococcus lactis, Streptococcus thermophilus, Lactobacillus delbruecki subsp. bulgaricus, and Lactobacillus acidophilus and yeast such as Candida u Torulopsis in the home-made kurunga samples from different regions of Buryatia and the Irkutsk region.

When studying a microbial consortium of home-made kurunga, G.G. Gonchikov et al. (1992) have determined the below species and quantitative composition of kurunga, in CFU/cm²: mesophilic lactobacilli (L. casei, L. plantarum, L. fermentum, L. breve) – 10⁴; thermophilic lactobacilli (L. delbruecki subsp. bulgaricus, L. acidophilus) – (10⁷-10⁹); lactose-fermenting yeast (Saccharomyces cartilaginosus) – 10⁸; lactose yeast (Saccharomyces lactis) – (10³-10⁴); acetic acid microorganisms (acetobacter – (10⁴-10⁵)). It is noted that the kurunga’s microbial consortium has a naturally formed population ratio of microorganisms in the community, which is characterized by a stable predominance of the cells of mesophilic, syntrophic in the consumption of lactose microorganisms such as mesophilic lactobacilli and non-lactose fermenting yeast: if the association contains the population-unstable (labile depending on ambient conditions) number of cells of accompanying microorganisms such as thermophilic lactobacilli, non-lactose fermenting yeast, and acetobacter.

In the Mongolian kurunga (Mongolian khoormog), Lactococcus. lactis, Lactobacillus casei, Saccharomyces lactis, Saccharomyces fragilis, and acetic and propionic acid rods have been found (Damdinsuren, 2010).

The Japan Milk and Dairy Products Research Center jointly with the Mongolian State University of Science and Technology and the State Research Institute of Genetics and Selection of Industrial Microorganisms of Russia studied the microflora of self-fermented sour-milk product khoormog from various Mongolian aimaks and isolated 420 strains of lactic acid bacteria and yeast. Most of the strains isolated have been assigned to the Streptococcus thermophilus, Lactobacillus delbruecki subsp bulgaricus, Lactobacillus fermentum, and Lactobacillus helveticus taxonomic groups. Studies of the probiotic properties of the cultures isolated have shown that Lactobacillus plantarum and Lactobacillus paracasei ssp paracasei exhibit the highest tolerance to gastric juice and bile acids, as well as high adhesion to CaCo-2 cells (Tseng-Ayush, 2013).

A.B. Shevtsov et al. (2011) established the presence of bacteria belonging to the Lactobacillus group such as L. brevis, L. buchneri, L. diolivorans, and L. parabuchneri in kurunga based on the analysis of the 16S rRNA gene sequence.

L.G. Stoyanova (2017) isolated strains of Lactococcus lactis subsp. Lactis in kurunga; in skim milk, they formed a cluster of a creamy consistency and fermented fructose, sucrose, maltose, and glucose. It has been found that to grow, bacteria needs such growth substances as alanine, asparagin, aspartic acid, valine, glycine, thiamine, and uracil. The cultures isolated were distinguished by a high level of antibiotic activity with the inhibition zone diameter of 12-23 mm to a wide range of gram-positive and gram-negative microflora: Bacillus subtilis, Micrococcus luteus, Bacillus coagulans, Staphylococcus aureus, Alcaligenes faecalis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Pseudomonas fluorescens. Also, they showed a fungicidal effect, i.e. suppressed the growth of filamentous fungi and yeasts such as Aspergillus niger, Penicillium chrysogenum, Fusarium oxysporum, Candida guilliermondii, and Rhodotorula aurantiaca.

Koumiss is known for its healing properties determined by the microflora composition. Table 1 represents data on the microflora species composition of the koumiss from different countries.
Table 1. Species Composition of the Koumiss Microflora.

| Microorganisms                                                                 | Country of origin                        |
|--------------------------------------------------------------------------------|------------------------------------------|
| Enterococcus durans, Enterococcus faecalis, Enterococcus faecium, Enterococcus  | Colombia (Serioa et al., 2011)           |
| garviae, Vagococcus penaei, Lactobacillus fermentum, Enterococcus faecalis,     | Turkey (Ispirli et al., 2017)            |
| Enterococcus durans, and Lactobacillus helveticus                            |                                          |
| Enterococcus faecium and Enterococcus durans, Leuconostoc mesenteroides,       | Kazakhstan (Baubekova et al., 2014)      |
| Leuconostoc pseudomesenteroides, Lactococcus lactis, Saccharomyces lactis,     |                                          |
| Saccharomyces fragilis, Torulopsis kefir.                                    |                                          |
| Propionibacterium acidopropioni, Lactobacillus acidophilus, Lactobacillus     | Kazakhstan (Ermolaeva et al., 2013)      |
| plantarum, Lactococcus lactis                                                | Japan (Akuzava et al., 2011)             |
| Lactococcus lactis subsp.lactis                                              |                                          |
| Schizosaccharomyces, Sehizoblastosporion, Torulopsis, Saccharomyces, Kloeceha  | China (Jing et al., 2012)                |
| Klyuyveromycies, Dekker, Trichosporon, Hansenuila and Brettanomyces, Klyuyveromycies marxianus |                      |
| Lactobacillus casei, Lactobacillus helveticus and Lactobacillus plantarium,   | China (Wang et al., 2015, Sun, 2009)     |
| Lactococcus coryniformis subsp. coryniformis, Lactobacillus paracasei,        |                                          |
| Lactobacillus kefiranofaciens, Lactobacillus lactis, Lactobacillus                |                                          |
| acidophilus, Lactococcus curvatus, Lactobacillus fermentum, and Weissella.     |                                          |
| kandleri.                                                                    |                                          |
| Lactobacillus helveticus, Streptococcus parauberis, Acetobacter pasteurianus  | China (Wurihan et al., 2019)             |
| Lactobacillus helveticus, Lactobacillus kefiranofaciens, Lactococcus lactis,   | China (Tang et al., 2020).               |
| Lactococcus raffinolactis, Citrobacter freundi, Dekkera anomala, Kazachstania |                                          |
| uninispora, Meyerozyma caribbica, Pichia sp.BZ159, Klyuyveromycies marxianus,   |                                          |
| Guehomyces L. casei Zhang, L. helveticus ZL12-1, and L. plantarum BX6-6.       | China (Wu et al., 2009)                  |
| Candida pararugosa, Dekkera anomala, Geotrichum sp., Issatchenka orientalis,  | China (Mu et al., 2012)                  |
| Kazachstania uninispora, Klyuyveromycies marxianus, Pichia deserticola, Pichia |
| fermentans, Pichia manshurica, Pichia membranaefaciens, Saccharomyces cerevisiae |                                          |
| and Torulaspora delbrueckii. Klyuyveromycies marxianus, Kazachstania uninispora, |                                          |
| Saccharomyces cerevisiae Lactobacillus salivarius, Lactobacillus buchneri, Lactobacillus |                                          |
| plantarum                                                                     | Bulgaria (Svenlova et al., 2005)         |
| Lactobacillus helveticus, Lactobacillus acidophilus, Lactobacillus plantarum, and Lactobacillus casei | China (Chun-Feng et al., 2015)          |

According to many scholars, the dominant koumiss microflora is represented by Lactobacillus (Akuzava et al., 2011, Wang et al., 2015, Zhihong Sun, 2009, Chun-Feng Guo et al., 2015, Serioa et al., 2011)

Studying the microbial community of koumiss from the Kerkin province, Inner Mongolia (China), has revealed the distribution of microflora in 4 genera: Lactobacillus (84.08 %), Acetobacter (9.83 %), Lactococcus (2.41 %), and Streptococcus (2.18 %) with the dominant Lactobacillus genus (Wu et al., 2019)
In various samples of home-made koumiss, the dominant Lactobacillus species were *Lactobacillus helveticus* (Wurihan et al., 2019), *Lactobacillus casei*, *Lactobacillus plantarum* (Wang et al., 2015, Sun, 2009), *Lactobacillus acidophilus*, *Lactobacillus plantarum* (Ermolaeva et al., 2013), *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus kefiranofaciens*, and *Lactobacillus lactis* (Akuzava et al., 2011)

Bacteria with a low isolation frequency include the following *Lactobacillus*: *L. coryniformis* subsp. *coryniformis*, *L. paracasei*, *L. kefiranofaciens*, *L. curvatus*, *L. fermentum* (Wang et al., 2015, Sun, 2009, Tang et al., 2020)

The strains isolated from koumiss were characterized by tolerance to media with low active acidity; the optimal pH for the development of these bacteria was within 3 to 4 (Wang et al., 2015, Sun, 2009, Svenlova et al., 2005).

They show fermentation activity mainly to mono- and disaccharides (Svenlova et al., 2005), lactose, cellubiose, fructose, maltose, mannose, raffinose, and sucrose, but do not utilize arabione, xylose, mannitol, and starch. The degree of converting glucose into lactic acid by the isolated strains ranged within 47-79% (Ermolaeva et al., 2013).

The optimal temperature for the development of the cultures isolated was 37 °C (Ermolaeva et al., 2013), and they also showed long-term viability at a temperature of 4 °C (Svenlova et al., 2005, Wu, 2009)

*Lactobacillus* isolated from koumiss showed tolerance to bile and salt and high adhesive activity (Wu, 2009, Rong et al., 2015, Chun-Feng et al., 2015, Rong et al., 2015, Menghe et al., 2009).

The conditions for the development of the associative starter culture of koumiss form the specific probiotic properties of *Lactobacillus*. In koumiss, inhibitors of the angiotensin-converting enzyme (ACE), which is the central component of the renin-angiotensin system, have been found. ACE converts the angiotensin hormone to the active vasoconstrictor angiotensin II, thereby indirectly increasing blood pressure. 4 inhibitory-type peptides (PI, PK, PM, and PP) have been isolated. The analysis of the amino acid composition sequence has shown that the PI peptide is part of the β-casein protein of mare's milk. The authors suggest that koumiss is rich in similar ACE-inhibiting peptides. An indirect indicator of this functional property of koumiss is its positive effect on the human cardiovascular system (Chen et al., 2009, Sun et al., 2009). Also, in supernatants, gamma-aminobutyric acid (GABA) was found. GABA is considered an inhibitory neurotransmitter since it blocks or inhibits certain brain signals and reduces the nervous system activity (Sun et al., 2009).

Proteomic analysis of the strain proteins expressed by *Lactobacillus casei* Zhang isolated from koumiss in exponential and stationary phases has shown that these are mainly stress-response proteins and key components of central and intermediate metabolism. This indicates that these proteins may play a potentially important role in adaptation to the environment, especially the accumulation of lactic acid during growth and physiological processes in bacterial cells (Wu et al., 2009).

It has been found that *Lactobacillus casei*, *Lactobacillus helveticus*, and *Lactobacillus fermentum* have the highest exopolysaccharide-producing activity as compared with other lactobacilli isolated from koumiss. The impressive adhesive capacity of the cultures isolated indicates that adherent NS8 cells may potentially form protective biofilm-like communities by auto aggregating on the intestinal mucosa. Such close interaction with the host’s intestinal mucosa may lead to the competitive exclusion of pathogenic microorganisms. The specific probiotic characteristics of lactobacilli are associated with the presence of certain surface molecules or structures such as peptidoglycan, teichoic acids, exopolysaccharides, and surface proteins (Tang Xue Mei et al., 2012, Wurihan et al., 2019, (Rong et al., 2015, Menghe et al., 2009, Yang et al., 2016).

*L casei* Zhang, *L. helveticus* ZL12-1, *L. fermentum* SM-7, and *L. plantarum* BX6-6 isolated from koumiss show high antibiotic activity against pathogenic and putrefactive microflora (Wu et al., 2009, Dong, et al., 2011, Wang, et al., 2011, Man et al., 2019, Wurihan et al., 2019))

*Lactobacillus plantarum* LB-B1 isolated from koumiss produces bacteriocin, which is active against strains of *Listeria*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Pediococcus Escherichia*
Botrytis cinerea, Alternaria solani, Phytophthora drechsleri Tucker, Fusarium oxysporum, and Glomerella cingulata (Xie et al., 2011, Wang, et al., 2011).

It has been found that the highest concentration of antibacterial substances is reached in the middle of the stationary phase in 24 hours; in the presence of enzymes (pepsin, chymotrypsin, trypsin, proteinase), the activity decreases. Studying the chemical composition of antibiotic substances has established their protein origin. A decrease in temperature increases the duration of bacteriocin activity at pH7 (Man et al., 2019).

Along with a high tolerance to bile and adhesion to HT-29 cells, Lactobacillus helveticus, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, and Lactobacillus fermentum isolated from home-made koumiss can reduce the concentration of low-density lipoprotein cholesterol. According to the authors, koumiss is the best source of probiotics lowering the cholesterol level (Chun-Feng et al., 2015, Dong et al., 2011).

Along with Lactobacillus, the Acetobacter genus bacteria such as A. Pasteurianus can be attributed to the ever-present koumiss microflora (Wurihan et al., 2019, Wu Rihan et al., 2019).

The ever-present koumiss microflora includes lactose and non-lactose fermenting yeast; their density is $10^5$–$10^7$ CFU/cm$^3$ (Mu et al., 2012, Jing et al., 2012, Baubekova et al., 2014).

In koumiss, yeast was found that can be attributed to opportunistic pathogenic microflora such as P. fermentans, P. deserticola, C. pararugosa, Geotrichum sp., etc. (Mu et al., 2012).

Thus, P. fermentans is a biofilm-forming yeast that undergoes a dimorphic transition. Having a yeast-like morphology, it inhibits the development of brown rot caused by Monilia spp. on an apple by forming a biofilm that protects the fruit. However, being in pseudo-hyphal form, it exhibits pathogenic behavior on a peach. According to the author, such a transition depends on the nitrogen source (Maria Lina Sanna et al., 2012).

P. deserticola is a representative of the human necrotic tissue microflora (Cacti Herman et al., 1985), and the Candida yeast is the candidiasis agent. A sharp increase in their number causes disease (Guy et al., 2017). Herewith, the isolated yeast strains exhibit antibacterial properties to Escherichia coli O8 (Chen, et al., 2019).

Until recently, enterococci have been considered a normal intestinal microflora of humans and animals and a foreign microflora in dairy products. According to the present-day publications, in dairy products, various enterococci may be found. According to some scholars, they can even be attributed to the dominant koumiss microflora. Thus, in koumiss from various countries, Enterococci have been detected such as E. faecium, E. durans, E. faecalis, E. garviae, and Vagococcus penaei (Serioa et al., 2011, Hümeyard Ispirli et al., 2017). 5 of the isolated strains had no virulence signs, and E. faecalis KE09 strain had a high antibiotic, proteolytic, and acid-forming activity (Serioa et al., 2011).

2. Conclusion
Related mixed fermentation sour milk products kurunga and koumiss contain a spontaneously formed population of microorganisms including mainly Lactobacillus and yeast. It has been found that koumiss contains opportunistic pathogenic enterococci and yeasts, which are unusual for dairy products and exhibit antibiotic properties under the associative fermentation conditions. Lactobacillus culture strains isolated from koumiss have high probiotic properties. According to the analysis performed, despite its historical age, kurunga has not been sufficiently studied, therefore, studying the microflora of this drink and the mechanism of forming a microbial population with high probiotic activity is a promising area of biotechnology and the production of functional food products.

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