STUDYING AND DISCHARGING CULTURES OF LACTIC ACID BACTERIAS, YEASTS FROM NATURAL FERMENTS TO PREPARE LOUMISS ON GOAT MILK

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This articles describes microflora of koumiss, prepared from goat milk and horse milk. Strains of the following lactic acid bacterias have been outlined: 3 aerobic 1S; 2S; 3S;, 4 anaerobic – 4S; 5S; 6S; 7S; 2 cultures of yeasts – 1Sy, 2Sy. These cultures have been studied for morphologic, physiologic, cultural, and antagonistic characteristics for Bacillusmezentericus, acid-producing activity on horse milk has been established. Ferment for receiving koumiss from lactic acid bacterias and yeasts in relation 1:1 has been composed (bacillus, coccus, yeasts). Cultures that increase collections of microorganisms have been selected in order to be used as ferments.

Keywords: starting cultures, mesophilic lactic acid bacterias, Bacillus mezentericus

Humanity has been using milk over 6 thousand years, and cultured milk beverages occupy a special place in human life. Since ancient times people have used goat, cow, horse, camel milk.

Most cultured milk products contain processes of mixed fermentation – lactic acid and spirituous.

Nomadic nations (Kazaks, Mongol, Kirghiz, Bashkir, Tatar) have been producing koumiss from horse milk since ancientry. It had different names among some nations: for example, koumiss was called «al-laban-arramaki» among Arab nations, and the Turkic called it «koumisom».

Koumiss is a cultured milk beverage that is fermented on horse milk and consists of lactic acid bacterias and yeasts. Methods of preparing koumiss have been known by ancient Scythians. In the V century B.C.E. Herodotus wrote: «the Scythians prepare koumiss from horse milk». The Scythians fermented horse milk in wooden vessels. According to Herodotus, the Scythians kept the secret of making koumiss. The first written mention on preparing koumiss, its taste and its effect upon an organism emerged in 1253 after Wilhelm Rubrikos's journey to lands of Tatar. According to some historians, koumiss's motherland is Asia, specifically its steppe part. It is considered that koumiss has been first prepared in Mongolia. We would also like to outline koumiss's significance as a medical agent that has been used since ancient times. The Kazakhs boiled kazy (horse sausage) in koumiss to treat catarrhal diseases. Koumiss with raisins was prepared for the older people and children.

Technologies of making koumiss bear urgency nowadays as they use industrial and domestic methods (facilitation of clean cultures, or stable fermenting, and using natural fermenting). This technology has a number of its advantages and disadvantages. These are: acidity, organoleptic indexes, consistency, and antagonistic activity. These cultures are necessary objects as they are starting cultures in producing koumiss.

The goal of this work is to prepare koumiss from goat milk, study and outline strains of mesophilic anaerobic and aerobic lactic acid bacterias and yeasts, grown under $t = 37°C$, define antagonistic characteristics while discharging cultures of lactic acid bacterias and yeasts.

Samples of 2 different types of milk served as the research object: goat and horse milk of morning milking, prepared in Ganyushkino village of Kurmangazinsk district of Atyrau region. Organoleptic characteristics of these samples (taste, smell, consistency, acidity according to Turner) have been proved to correspond to GOSTs.

Several objectives have been set in order to achieve the goal:

1. Ferment samples of horse milk to prepare koumiss (in domestic conditions).
2. Use the received koumiss for further fermenting of goat milk.
3. Discharge lactic acid bacterias and yeasts from the natural ferment via separation method.
4. Study and define morphologic, physiologic characteristics of these anaerobic and aerobic lactic acid bacterias and yeasts.
5. Use method of separation and sowing into liquid environment in order to receive clean cultures.
6. Define acid-forming activity according to Turner.
7. Receive koumiss from goat milk and use it as natural ferment to prepare koumiss from horse milk.
8. Define antagonistic characteristics of the discharged lactic acid bacterias and yeasts via lunula method in relation to strain Bacillus mezentericus.
9. Make a composition using cultures of lactic acid bacterias and yeasts.

The mixed products, presented from natural ferment of the prepared horse milk koumiss (50 ml of koumiss + 50 ml of goat milk) have been placed into thermostat for 24 hours under the temperature of 37 °C. Acidity according to Turner equaled 32 °T for the received one-day cultures milk products of mixed type, their consistency and smell – friable clot with the smell of horse milk. Cultures of lactic acid bacterias have been discharged in a dense environment of Bogdanov under $t = 37$ °C, and yeasts have been discharged in a dense nutritious environment (Sabura). Growth of lactic acid bacterias has been defined according to emergence of colonies, grown on the nutritious environment. 9 cultures of lactic acid bacterias have been discharged from ferment on goat milk, among those: 3 aerobic strains, 4 anaerobic strains, 2 strains of yeasts. All strains have been grown in liquid environments: cultures of lactic acid bacterias have been sowed into hydrolized milk, 2 cultures of yeasts and peptone have been sowed into yeasts environment with glucose.

All cultures have been inspected for catalase, and been established as catalse-negative, unable to produce catalase. Morphology of all strains has been studied according to the method of Gramm. The following cultures have been established according to morphological characteristics: 3 cultures of coccus (1S; 3S; 6S), 4 cultures of baccilus (2S; 4S; 5S; 7S), among those 5 strains are – gram-positive (G+) (1S; 2S; 4S; 5S; 7S), 2 cultures are gram-negative (G-) according to Gramm. We have also studied morphological features of yeasts, they have an oval shape.

We have defined antagonistic activity of the 9 outlined cultures in nutritious environment via method of lunulain relation to test culture Bacillus mezentericus. As a result, we have revealed an antagonistic ability to oppress growth of Bacillus mezentericus among the discharged cultures. The results are provided in Table 1.

Table 1 shows that maximum areas among aerobic cultures refer to 1S (11 mm), and minimal areas refer to 2S and 3S (7 mm). Maximum areas among anaerobic cultures refer to 4S (15 mm), minimal areas refer to 5S, 6S, 7S (7–11 mm).

Table 2 shows us maximum areas of growth – 6mm (2Sy) and minimal area – 5 mm (2Sy).

Acidity has been studied among the 9 discharged aerobic and anaerobic cultures and lactic acid bacterias and yeasts while sowing them on horse milk during different periods. The basic feature of lactic acid bacterias and yeasts is their ability to accumulate lactic acid. This characteristic is important for producing koumiss from the practical point of view. Dynamics of acid formation has been studied on hourly basis during the first day of growth. The data is presented in Table 3.

Table 1

Antagonistic activity of lactic acid bacterias that have been grown on milk hydrolyzate in relation to Bacillus mezentericus

Aerobic strains of lactic acid bacterias	Growth areas (mm)
Control	0 mm
1S (Lactococcus)	11 ± 0,3
2S (Lactobacillus)	7 ± 02
3S (Lactococcus)	7 ± 02
Anaerobic strains of lactic acid bacterias	Growth areas (mm)
Control	0 mm
4S (Lactobacillus)	15 ± 0,6
5S (Lactobacillus)	11 ± 0,4
6S (Lactococcus)	7 ± 0,2
7S (Lactobacillus)	11 ± 0,3

Table 2

Antagonistic activity among the discharged yeasts, grown in peptone – yeast environment with glucose in relation to Bacillus mezentericus

Yeasts	Growth areas (mm)
Control	0 mm
1Sy	6 ± 0,3
2Sy	5 ± 0,2

From Table 3 we can see that strains form a moderate acidity within 3 hours, among them – 1S; 2S (10–12 °T); low acidity is formed by 3S (9 °T). Within 24 hours these cultures differ in acidity: 1S and 2S have a higher acidity (49–48 °T), 3S shows a moderate acidity (40 °T).

We have used the sample of fresh horse milk under an active acidity for 4 aerobic cultures. The table shows that strains 4S and 5S form low acidity (10–12 °T) within 3 hours. Moderate acidity is formed by strains 6S and 7S (13–130 °T). Strains 6S and 7S have shown high acidity (55–57 °T) within 24 hours. Moderate acidity was shown by strains 4S and 5S (40–45 °T). All cultures have been grown on horse milk in order to define acidity level.

Table 4 shows that yeasts form low acidity within 3 hours: 1Sy, 2Sy (10–9 °T). Within 25 hours these cultures begin to differ in acidity. 1Sy and 2Sy have moderate acidity (34–36 °T). All yeasts have been grown on hors milk in order to define acidity level. Thus, active accumulation of acidity has been registered for culture 1Sy of lactic acid bacteria.

Table 3

Dynamics of acid formation among the discharged cultures of lactic acid bacterias during 24 hours (1 day) and 3 hours.

Aerobic strains	3 hours	acidity, °T	24 hours	acidity, °T
Control	–	–	–	–
1S Lactococcus	++	10 ± 1,4	++ +	49 ± 3,7
2S Lactobacillus	++	12 ± 2,8	++ +	48 ± 3,6
3S Lactococcus	+	9 ± 1,0	++	40 ± 3,5
Anaerobic strains	3 hours	acidity,0T	24 hours	acidity,0T
Control	–	–	–	–
4S Lactococcus	+	10 ± 1,4	++	40 ± 3,5
5S Lactobacillus	+	12 ± 2,8	++	45 ± 3,5
6S Lactobacillus	++	13 ± 3,0	+++	55 ± 3,8
7S Lactobacillus	++	13 ± 3,0	+++	57 ± 4,0

Table 4

Dynamics of forming acidity among the discharged yeasts

Yeasts	3 hours	Acidity, °T	24 hours	Acidity, °T
Control	–	–	–	–
1Sy	+	10 ± 1,1	++	34 ± 3,0
2Sy	+	9 ± 1,0	++	36 ± 3,1

After defining antagonistic features and acidity according to Turner, we have formed compositions from ferments, taking 2 ml of liquid nutritious environment from each culture and 8 ml of horse milk. The ferment compositions looked as follows: (1:1:1) – 5S (anaerobic culture) – baccilus, 1S (aerobic culture) – coccus, 1Sy – yeasts on horse milk. Thermostat under $t = 37°C$ has been used to grow cultures of lactic acid bacteriasand yeasts on horse milk during 24 hours.

Acidity according to Turner and organoleptic characteristics have been studied among the received one-day lactic acid products of mixed type. Acidity of the formed composition equaled 20°T. After defining acidity, all cultures, placed in 10 ml test tubes, have been mixed, and 30 ml mixture has been received. This mixture has been added to 70 ml of horse milk and then placed to thermostat under temperature of 37°C for 24 hours. Acidity according to Turner and organoleptic features have been defined once again in a day. The prepared lactic acid product had a non-thick sediment at the vessel bottom and gas bubbles on its surface. Acidity according to Turner equaled 47°T. The received 100 ml mixture has been added to 200 ml of horse milk once again. The total mass of 300 ml has been placed into thermostat under the temperature of 37°T for 2 days. Acidity according to Turner and organoleptic characteristics have been measured in 2 days once again. Acidityaccordingto Turnerequaled 110°T. This mixture has been used as a ferment in relation 1:2, lactic acid ferment and milk correspondingly. Goat milk can be used to increase ferment mass.

Resume

1. Samples of horse and goat milk of Atyrau region, their organoleptic characteristics and acidity according to Turner have been studied.

2. 9 active cultures have been discharged from natural ferment, among those 4 anaerobic, 3 aerobic lactic acid bacterias and 2 yeast cultures.

4. Nutritious environments of Bogdanov, Sabur, milk hydrolyzate, peptone-yeast environment with glucose has been used to discharge and study cultures of lactic acid bacterias and yeasts.

5. Morphological and cultural antagonistic characteristics have been revealed and studied among these 9 discharged cultures.

6. Active strong acid formers of have been determined among lactic acid bacterias– 1S, 2S, 6S, 7S and 1 – among yeast cultures – 1Sy.

7. Composition of preparing koumiss in relation 1:2 has been composed (ferment and milk correspondingly).

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