Microflora Study of Koumiss

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***Abstract*—**In Kazakhstan, a very wide variety of dairy products, including milk beverages from national mare and camel milk. This is due to the use of bacterial starter cultures, which structure is represented by different species of lactic acid bacteria. Specificity of taste, texture and other properties of a number of dairy products depend on the strains belonging to the bacterial culture. In this regard, in recent years, attention is directed to the domestic microbiologists isolation of pure cultures of microorganisms of the national dairy products. As a result of this work, isolated local strains of crops can be further used for the production of leaven in the preparation of a variety of dairy products from mare's milk. Therefore, we carried out research work on the isolation and identification of lactic acid bacteria from different natural substrates, the establishment of their species, the study of the biological characteristics and the most important production and valuable properties. The object of the study were selected mare from the southern regions of Kazakhstan. As a result of the selection of microorganisms with the use of special methods of selection, it is possible to obtain strains of bacteria that have a special biotechnological complex of properties that allows you to design and create new functional dairy products with directional composition of lactic microflora.

***Keywords*—**Mare milk, bacterial starter cultures, koumiss.

I. Introduction

THE peoples of Central Asia, in its national diet consume dairy drinks - koumiss, which is a fermented milk product from mare's milk. With daily use of mare's milk has on the body regulating and normalizing effect.

Currently in the world there are several major regions of production and consumption of koumiss. These are: Kazakhstan, Russia, Tatarstan, Bashkiria, the South Urals, Mongolia, Kyrgyzstan, Europe.

II.. Materials And Methods

For the separation of pure cultures of lactic acid bacteria from domestic koumiss must be selected samples to plant in the liquid medium for the enrichment of the lactic microflora sow on solid medium for the isolation of a pure culture, then reseeded pure culture in sterile milk. Study of biological properties of strains isolated from koumiss necessary for their identification and production values.

Thus, for the isolation of lactic acid microorganisms from koumiss test material was inoculated into a solid powder MRS medium MIA (meat infusion agar), MPB (meat-peptone broth) used for culturing lactic acid bacteria and in parallel did seeding onto medium Sabouraud for growing yeast /1/.

After visual identification of colonies of lactic acid and microscopic organisms selectively subcultured onto nutrient pure selective medium. Repeat this process to complete microbiological purity of colonies of lactic acid microorganisms. The morphology of the strains studied and microbiota koumiss studied traditional methods with an electron microscope Motic BA310.

Study of the microbiological purity of the preparation was determined by microscopic control and seeding a number of nutrient media (MIA, MPB) / 2 /. Cultured at 37 ° C for 24-36 hours. To check for sterility using agar medium MIA and MPB. The prepared medium was dispensed into flasks and autoclaved for 30 minutes at a pressure of 1.5 atm. Thereafter tested colonies replated in selective medium ready.

Microflora study of koumiss has 9 active strains of lactic acid microorganisms were identified that were attributed to the genera Lactobacillus and Lactococcus. The isolated strains were arbitrarily designated by us: LB-1, LB-2, LC-1, LB-3, LC-2, LC-3, LC-4, LC-5, LC-6.

Further isolation of pure cultures of lactic acid microorganisms perebuvali in sterile milk.

III. Results And Discussion

Physiological and biochemical properties of the most active selected lactobacilli strains was assessed by the intake of carbohydrates, growth factors needs, the level of their inhibitory activity, spectrum of action. In studying enzyme activity it has been used a number of carbohydrates, including sugars, sugar alcohols, polysaccharides. The amount of lactic acid was determined by titration.

Antibiotic sensitivity and antagonistic activity of selected strains of lactic acid bacteria (see Table 1 and 2) were studied. (appendix 1)

Lactic bacteria strain LB-3 showed no sensitivity to streptomycin, and strains LC-LC-4 and 5 were sensitive to tetracycline and streptomycin action. As LB-1 strain was not sensitive towards chloramphenicol. LC-6 strain showed resistance to penicillin.

In the course of the experiments we have found / 3 / that all test - cultures antagonistichekuyu Activity showed only

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strains LB-1, LB-3 and LC-6. A strain LC-1, LC-3 with respect to S. Aureus, LC-3 and LC-4 with respect to E.coli, LC-1 and LC-2 with respect to P. Aeruginosa, LB-2, LC- 3, LC-LC-4 and 5 with respect to S. typhimurium showed no antagonism.

The rate of acidification of the culture medium is determined by the time of clot formation. To this culture was inoculated investigated in an amount of 0.1 ml per 10 ml of milk and placed in an incubator at a temperature of 37^0C. To determine the activity of the culture to acidify the clotting time recorded. The study analyzed for crop strains except LC-3, LC-LC-4 and 5 set the speed of acidification of culture media: by lowering the pH> 5.5 times showed more than 5 hours. These strains during acidification culture media showed more than 7 hours.

Acid isolated cultures (Cultural activity) is determined by the rate of formation of lactic acid. To this culture was inoculated in an amount of 0.1 ml per 10 ml of milk and placed in an incubator at a temperature of 37^0C. After 24 hours, titratable acidity is determined by the method of Turner. According to the procedure 10 ml of the sample was diluted with 20 ml of distilled water and added 1-2 drops of phenolphthalein indicator. Titration is carried out with 0.1 N NaOH solution until a stable pink color.

To determine aroma-producing cultures (Table 3), we have mare skim milk, pH 5.0, and cultured at 25 ^{0}C for 16-24 hours / 4 /. As a result, there was a light spicy flavor of milk and a peculiar odor.

To obtain reliable results, all tests were carried out 3-5 times. (see Table 3).

IV. CONCLUSION

Obtained 3 active strain of lactic acid microorganisms from domestic koumiss identified at the genus level as Lactobacillus and Lactococcus.

During research and antibiotic capacity and antagonistic activity of selected lactobacilli and bifidobacteria strains, showed the following results. Strains LC-LC-4 and 5 showed high sensitivity to antibiotics than the rest (10-15 mm). A strain LB-3 relative to streptomycin, as well LB-1 strain towards chloramphenicol, and a strain LC-6 towards were resistant to penicillin.

Antagonistic activity all test - cultures showed strain LB-1, LB-3 and LC-6. A strain LC-1, LC-3 with respect to S. Aureus, LC-3 and LC-4 with respect to E.coli, LC-1 and LC-2 with respect to P. Aeruginosa, LB-2, LC- 3, LC-LC-4 and 5 with respect to S. typhimurium showed no antagonism.

Study cultures activity except acidify strains LC-3, LC-LC-4 and 5 during acidification culture media by lowering the pH> 5.5 over time showed 5 hours. As a result, the definition of aromatoobrazovaniya cultures observed a light spicy flavor of milk and a peculiar odor.

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APPENDIX I

TABLE I

ANTIBIOTIC SUSCEPTIBILITY OF LACTIC ACID BACTERIA

Antibiotics	Inhibition zone diameter, mm								
	LB-1	*LB-2*	*LC-1*	*LB-3*	*LC-2*	*LC-3*	*LC-4*	*LC-5*	*LC-6*
Streptomycin	2,0±1,0	14±0,9	13±1,0	0	12±1,0	12±1,0	22±1,0	21±1,0	3,0±0,8
Tetracycline	5,0±1,0	10±1,0	15±1,0	4,0±1,0	19±1,0	29±1,0	17±1,0	21±0,8	3,0±0,9
Laevomycetin	0	5±0,9	7±0,8	1,0±1,0	12±1,0	13±0,8	15±1,0	13±1,0	5,0±1,0
Penicillin	1,0±1,0	17±1,0	18±1,0	5,0±1,0	16±1,0	12±1,0	16±0,8	12±1,0	0

TABLE II

ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA

Antibiotics	Inhibition zone diameter, mm								
	LB-1	*LB-2*	*LC-1*	*LB-3*	*LC-2*	*LC-3*	*LC-4*	*LC-5*	*LC-6*
S. aureus	18±0,4	3±0,9	-	16±0,9	2±1,0	-	1±1,0	1±1,0	3±1,0
E.coli	21±0,7	2±1,0	2±1,0	19±0,5	2±1,0	-	-	2±0,8	10±0,4
P. aerugi-nosa	20±0,7	4±0,9	-	23±0,5	-	3±0,8	2±1,0	3±1,0	8±1,0
S. typhimu-rium	15±0,6	-	1±1,0	13±1,0	1±1,0	-	-	-	2±0,2

TABLE III

THE RATE OF ACIDIFICATION OF THE CULTURE MEDIUM OF ACID, AROMATOOBRAZOVANIE ISOLATED STRAINS OF LACTIC ACID CULTURES

Antibiotics	Inhibition zone diameter, mm								
	LB-1	*LB-2*	*LC-1*	*LB-3*	*LC-2*	*LC-3*	*LC-4*	*LC-5*	*LC-6*
The rate of acidification of the culture medium, hour	свыше 5 ч	свыше 5 ч	свыше 5 ч	свыше 5 ч	свыше 5 ч	свыше 7 ч	свыше 7 ч	свыше 7 ч	свыше 5 ч
Acid isolates, °T	76±3	70±3	60±5	94±2	61±5	62±6	61±5	60±5	62±5
aroma-producing	+	+	+	+	+	+	+	+	+