Asian Pacific Journal of Environment and Cancer
Vol 5 No S1 (2022): Special Issue: Innovative Technologies in Medicine, 19-22
Original Research

Microflora of National Dairy Products of the Aral Sea Region

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The article deals with to the study of the microflora of national dairy and lactic acid products in the Aral Sea region. The aim of the study is to separate and identify microorganism characteristic of national dairy products. Microbiological and biochemical research methods were used in the course of the study. As a result of research, it was found that the microflora of dairy products is represented by mesophilic cocci of the genus Leuconostoc and Lactococcus, thermophilic streptococci – Streptococcus termophilus and enterococci - Enterococcus faecium.

Introduction

Interesting to the study of lactic acid bacteria is due to the fact that they are of great importance in human life - they are used in baking, food preservation, ensiling, in the preparation of drinks, the polysaccharides secreted by them are widely used in medicine, the pharmaceutical, food, chemical industries, and agriculture and even in such “heavy” industries as hydrometallurgy, oil production, beneficiation of non-ferrous and rare metal ores, etc. [1]. The main property of lactic acid bacteria is the ability to form lactic acid as the main product of fermentation, therefore their role in the dairy industry is indispensable, medical and dietary products are created on the basis of their monocultures [2]. Currently, pharmacological agents are being replaced by the daily use of a set of food products containing live microorganisms or their metabolites [3]. Under the conditions of the Aral Sea ecological catastrophe, the epicenter of which is the Republic of Karakalpakstan, there is an unfavorable ecological and epidemiological situation, it is extremely necessary to develop not only dietary, but also therapeutic and prophylactic fermented milk products based on local strains of lactic acid bacteria that are most adapted to the microecology of the gastrointestinal tract of the population Karakalpakstan [4-5]. In recent years, studies have been carried out in the region on the isolation and production of pure cultures of lactic acid bacteria from dairy products, and it has been established that lactic acid bacteria living in conditions of intense solar insolation, high salinity of soils, and water differ from cultures living in other geographical regions [2, 3, 6]. However, the microflora of national dairy and lactic acid products of the Aral Sea region has been studied extremely insufficiently; there is no collection of lactic acid bacteria.

Study purpose

Segregation of lactic acid bacteria from national dairy products, their identification and creation of a collection of lactic acid bacteria strains.

Research methods and objects

The objects of research were lactic acid bacteria separated from various substrates. For the study, cultures of milk (cow, camel, goat, sheep milk), sour milk products (katyk, suzma, shubat, sour cream, etc.) taken from various points in the region (farms in the Nukus and Kungrad regions, individual owners of cows, camels in city of Nukus (village of Koskul), Karauzyak and Kegeyli

regions). In total, more than 160 cultures were studied (camel milk - 24, goat milk - 21, sheep milk - 17, cow milk - 30, shubat - 25, suzma - 18, katyk - 27, sour cream - 14, typical laboratory strains. The studies were carried out during the period 2015-2022 in the Laboratory of Microbiology of the Karakalpakstan Medical Institute.

Microbiological, biochemical and analytical research methods were used.

To isolate a pure culture of lactic acid bacteria, MRS-1,3,4 media were used (composition, g/l: dry peptone -10.0; tween 80-1.0 ml; MgSO4.7H20-0.2; MnSO4*4H20-0, 05, L-cysteine hydrochloride-0.2, K2HPO4*3H2O-2.0, ammonium citrate-2.0, sodium acetate-5.0, yeast autolysate-50 ml; glucose -20.0; liver extract-100 ml; pH-6.3±0.1. Bromocresolpurpur is added to MRS-3, sorbic acid is added to MRS-4); hydrolyzed agar according to Bogdanov (composition: hydrolyzed milk diluted 1:1, yeast autolysate-5%, sodium citrate-1%, pH -6.5); for the cultivation of bacteria of the genus Leuconostoc, modified Bogdanov's medium was used (composition, (g/l): sodium citrate - 5.0; yeast extract - 5.0; peptone - 10.0; glucose-10.0; agar-agar-18.0; medium pH-6.5±0.2) [4, p.7]; Barnes medium was used to seperate enterococci (composition, g/l: meat water - 1000; peptone - 15.0; glucose - 1.5; NaCl - 7.5; sodium azide - 0.2; agar-agar - 18.0 pH - medium -7.5); species of the genus Lactococcus were differentiated using the method of Reddy et al. [7].

For comparing the main properties of isolated cultures of lactic acid bacteria, typical strains of lactic acid bacteria were used.

Bacteria were identified using the Bergey's Manual of systematic Bacteriology [8].

Statistical processing of the obtained data was carried out using the Microsoft Excel computer program using generally accepted data criteria.

Results and Discussion

As a result of studying 166 cultures, we isolated 45 cultures of lactic acid bacteria. At the same time, the substrate specificity is distributed as follows: from cow's milk -11, camel's milk -6, goat's milk -5, sheep's milk -3, sour cream -4, katyk -6, shubat -5, suzma -5 cultures. Selected cultures are numbered with Arabic numerals combined with Latin letters.

In the course of experimental work, we developed a method for obtaining an enrichment culture with a high content of lactic acid bacteria [2]. In this case, two main points were taken into account: on the one hand, the presence of growth factors (amino acids, vitamins, salts, etc.) in the nutrient medium, on the other hand, the presence of concomitant microflora, primarily spore bacteria, micrococci, yeast, etc.

The main biological property of lactic acid bacteria is acid-forming ability, in connection with this, the rate of milk fermentation under various temperature conditions (30, 37, 48ºC), acid accumulation (total titratable acidity, ºT), the nature of the fermented milk clot, structure, consistency and organoleptic properties (Table 1).

	Source of cumulative	Milk clotting time, h				Total titratable acidity	
N	culture	30°C	37°C	48°C	30°C	37°C	48°C
1	Cow's milk	48	20	18	157	127	90
2	Camel milk	48	20	18	163	125	84
3	Goat milk	48	20	18	154	134	63
4	Sheep milk	48	20	18	131	122	90
5	Suzma	48	20	18	152	132	93

6	Katyk	48	20	18	157	130	92
7	Shubat	48	20	18	140	136	105
	M±m				147±3,025	128±2,32	88±9,0

Table 1. Milk-clotting Activity of Cultures.

During the separation of the culture of lactic acid bacteria, an associative culture was often detected in the culture, in particular, spore forms of bacteria. Ethanol (8-10%), phosphoric acid (0.003-0.005%), sodium benzoate (0.01-0.15%) were added to the medium to inhibit the growth of foreign microflora, while growth activators were added to stimulate the growth of lactic acid bacteria. (peptone, glucose, hydrolyzed milk, yeast autolysate, sodium chloride, decoctions of alfalfa, camel thorn, licorice). With the addition of growth activators, the total number of lactic acid bacteria increased by 50%. It has been detected that the activity of acid formation, manifested in the rate of fermentation and the intensity of accumulation of organic acids, largely depends on the temperature conditions of fermentation. When cultivating in a temperature regime of 30°C, the milk coagulation time is extended to 48 hours with a high level of acid accumulation (from 131 to 163°T), when the temperature rises to 37^0C, the fermentation of milk is accelerated up to 20 hours, however, the total titratable acidity does not exceed 122- 136°T, and at a high cultivation temperature up to 48°C, a decrease in the process of acid formation to 63-105°T is observed. From this it follows that for most cultures the optimum growth temperature is 37°C.

While studying the nature of the formed clot and the consistency of the fermented product, a sharply distinctive picture was observed. Microorganisms contained in all cultures of the dairy product coagulate milk with the formation of a dense clot without gas formation, with the exception of the culture isolated from the suzma - the clot is loose, but homogeneous. The consistency of fermented milk has a different character: when inoculating cultures from cow’s milk and katyk, it is structured, from camel’s milk and shubat - creamy, goat, sheep and suzma – viscous. Accordingly, the organoleptic properties of the products are also different. If fermented milk when sowing cow’s, camel’s milk has a pure sour-milk taste, then from sheep’s and goat’s milk - sour-milk with a specific smell, the clot is dense, oily. When sowing cultures from katyk, a product with a sour-milk taste and a dense prickly clot is obtained, shubat - sour-milk with a stinging and sweetish taste.

One of the diagnostic signs is the reducing properties of bacteria in relation to litmus milk and milk with methylene blue. The data obtained show that lactic acid bacteria in the composition of enrichment cultures actively reduce these indicators (Table 2).

	Substrates	Relation to litmus milk		Relation to methylene milk (0.1%)	
#		Clotting time, h	Character Growth*	Clotting time, h	Character Growth*
1	Cow's milk	20	BC	18	BC
2	Camel milk	20	BC	18	BC
3	Goat milk	20	BC	18	BC
4	Sheep milk	20	BC	18	BC
5	Suzma	20	BC	18	BC
6	Katyk	20	BC	18	BC
7	Shubat	48	BC	48	BC

Table 2. The Reducing Ability of Microorganisms Contained in Various Substrates.

Note, *BC - recovery to white with the formation of a dense even clot

From the obtained data it follows that all the studied cultures ferment and restore milk with methylene blue at a concentration of 0.1% for 18 hours, ferment litmus milk and restore within 20

hours. The cultures isolated from shubat differed sharply, in which the reduction of litmus and methylene milk occurs within 48 hours, which is apparently associated with the specificity of the biochemical and microbiological composition of shubat. As a result of studying the morphological and cultural properties of the isolated cultures, it was found that the type of colonies on agar media and cell morphology depend on the components of nutrient media, cultivation conditions, and fermentation temperatures. At 48°C growing in the depth of the agar, small lenticular or round and very rarely cotton-like colonies grow. Superficial - smooth, small in size (no more than 0.5-0.6 mm or up to 1 mm), white, or the color of agar with a rough surface, the cells are mainly small diplococci, less often chains of 3-4 cells .

There are large lactic bacilli in the form of single or paired thick rods containing metachromatin grains.

There are bacilli similar to the Bulgarian stick, long, with metachromatin grains. At a lower temperature (30-37°C), the appearance of various types of both deep and surface colonies is observed. We have described 4 types of surface colonies. Lactic acid streptococci do not differ in the variety of forms of colonies: deep - boat-shaped, surface round with clearly defined edges, smooth, shiny, white in size from 1 to 2 mm.

The morphology of colonies and cells of lactobacilli is more diverse. Deep in the form of lentils, round, cotton-like, near-bottom in the form of round or flat white colonies. Surface colonies are diverse: white round, convex, shiny, flat with a rough surface of a bluish color or agar color, as well as in the form of snowflakes.

Heat-resistant sticks - thick in width, found singly or in pairs; Bulgarian stick is presented in the form of thick long sticks containing metachromatin grains; acidophilus sticks - long sticks, often fragmenting.

We found that different cultures of milk and dairy products contain different amounts of lactic acid bacteria: in camel, goat and sheep milk, the concentration of bacteria is 1.1-1.6x106 in 1 ml, in cow’s milk - 1.4x107, in suzma, katyk and shubat, the largest number of bacteria was revealed - 1.2-1.8x108 in 1 ml.

The taxonomic position of isolated strains of lactic acid bacteria was determined. As a result of studying the morphological, biochemical, cultural, physiological, genetic properties of the isolated cultures, they are divided into 3 large groups: mesophilic streptococci, thermophilic streptococci and enterococci.

22 cultures are represented by mesophilic streptococci of the genera Lactococcus and Leuconostoc. Lactococci are isolated from shubat, sour cream and goat milk. They differed from the typical cultures in greater salt tolerance; in other characteristics (growth temperature, bile resistance, reducing ability, in the formation of diacetyl, the absence of reduction of triphenyltetrazolium), they did not differ from the typical cultures. Acid accumulation (total titratable acidity 92.7 ± 2.480T) did not differ from the typical ones (95.7 ± 3.35).

Bacteria of the genus Leuconostoc have been isolated from cow’s and goat’s milk, from sour cream, and katyk. Two strains of Leuconostoc cremoris (Leu 15 and Leu-9) were isolated from shubat and sour cream, respectively. The cultures are weak acid formers: after 24 hours the total titratable acidity was (63.6±4.2)0T, after 48h it was (79±2.1) 0T, the cultures did not hydrolyze arginine, formed dextran and diacetyl.

Leuconostoc cremoris, unlike other leuconostocs, formed yellow colonies with a transparent zone of chalk dissolution on Reddy medium.

12 cultures are represented by thermophilic streptococci of the genus Streptococcus, they are

mainly isolated from fermented milk products: from katyk-6, from shubat-3, sour cream-3 and are designated Tst-1-12. In terms of acid production, these local strains lagged behind the typical ones, the total titratable acidity of local cultures was 91.8±1.8°T, the titratable acidity of typical cultures was 104.2±4.6°T, the difference in indicators is significant. Cultures reduced methylene blue in milk (0.1%), only in local strains the recovery process occurred within 36-48 hours, in typical strains - only after 72 hours. Local strains of thermophilic streptococci are resistant to 4 and 6.5% NaCl in the medium when standard cultures did not grow; local strains are resistant to 20% bile in the medium, typical strains are not. Biochemical activity also varies: local strains Tst-1,2,8-10) do not ferment sucrose, typical ones ferment everything; type strains do not ferment maltose, while among the local ones there are cultures that ferment maltose (strains Tst-1, Tst-2, Tst-3, Tst-9, Tst-10), do not hydrolyze arginine; local strains, in contrast to typical strains, weakly reduced triphenyltetrazolium chloride salts in Barnes' medium (colonies of slightly pink color), therefore, genetic tests for the presence of the plasmid and sensitivity to the SF-1 phage were introduced. Cultures contained one large plasmid (except for 4 strains), not sensitive to phage; in addition, they have β-galactosidase and urease activity.

It was established that the microflora of cow's milk is mainly represented by enterococci of the genus Enterococcus, 11 cultures were isolated (Ecm-12-22). In terms of acid formation, local strains of enterococci are not inferior in acid formation to typical industrial strains. Thus, the total titratable acidity in local strains is 76±1.79°T, in typical strains -80.2±3.3°T. All local strains, as well as typical ones, hydrolyze arginine, reduce salts of triphenyltetrazolium chloride on Barnes' medium with the formation of pink colonies, and are sensitive to SF-1 phage.

In terms of enzymatic properties, isolated local strains differ significantly from typical enterococcus cultures: out of 11 strains, 4 cultures ferment ribose (typical ones do not ferment); variable in sucrose fermentation (7 cultures are fermented, 4 are not active), in melibiosis (9 cultures are fermented, 2 are not fermented), in mannitol (4 cultures have a pronounced enzymatic activity, 4 are weakly active; 3 cultures are not fermented). It is known that melibiose and mannitol are diagnostically important sugars in the differentiation of thermophilic streptococcus and enterococcus.

It has been shown that a distinctive feature of local strains of lactic acid bacteria is resistance to high concentrations of bile in the medium. An exception is the culture of Lactococcus lactis subsp cremoris (strain Lac-12), which is sensitive to 20% bile. Lactic acid bacteria in the process of acid formation at low pH can precipitate bile salts [9]. Despite the ambiguity of the mechanism of bile resistance of local strains of microorganisms, it can be assumed that this physiological trait may be useful in the selection of cultures for industrial use.

In conclusions, we found that in the conditions of the Republic of Karakalpakstan, the microflora of national dairy and lactic acid products is very rich, their biological diversity is mainly represented by thermophilic streptococci, mesophilic representatives of the genera Leuconostoc and Lactococcus, and enterococci.

It was revealed that local strains of lactic acid bacteria differ from typical cultures in physiological, biochemical and genetic properties, they have high thermal resistance, antagonism to pathogenic microorganisms, increased salt resistance, high bile resistance, phage resistance and the presence of mega-plasmids in thermophilic streptococci. In the future, these cultures, especially thermophilic lactic acid bacteria, can be widely used as probiotics for the production of dietary lactic acid products.

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