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Some enzymatic properties of lactic acid bacteria isolated from dairy products

This article presents data on the study of physiological and biochemical properties, antagonistic and enzymatic activity of lactic acid bacteria isolated from dairy products. 9 types of lactic acid bacteria were studied: *Lactobacillus bulgaricus* GM – 08, *Lactobacillus bulgaricus* KZh – 01, *Lactobacillus bulgaricus* GS – 03, *Lactococcus cremoris* – 6, *Lactococcus cremoris* – 17, *Lactococcus cremoris* – 26, *Lactococcus lactis* – 1, *Lactococcus lactis* – 15, *Lactococcus lactis* – 23. These strains were found to have resistance to 2% and 4%-vertical NaCl concentrations, bile and phenol. In addition, the antagonistic activity of Gram-positive and Gram-negative microorganisms in relation to test cultures of *Staphylococcus aureus, Salmonella dublin, Escherichia coli, Bacillus subtilis, Sarcina flava* was studied. All studied lactic acid bacteria showed activity in Test cultures with different inhibition zones. The *Lactococcus lactis* – 23 strains showed high activity for all cultures, with an inhibition zone of 17-25 mm. Further, 5 strains were selected from these strains and their aroma-forming properties, the formation of diacetyl and ammonia from arginine, hemolytic and lecithinase activity were studied. Compositions were compiled from these strains to make yeast. The compatibility of strains of lactic acid bacteria was checked with each strain individually and with the duration of milk clotting according to organoleptic indicators compared to the duration of milk clotting. Thus, the most active clot formation was obtained by the *Lactococcus lactis* – 23 strain of the selected combinations.

Keywords: microorganisms, strain, antagonistic activity, enzymatic activity, aroma formation, acid formation, yeast, *Lactococcus sgemogis, Lactobacillus bulgaricus*.

Introduction

There is a need for compact and efficient processing of raw materials produced in agriculture for special food purposes in order to maximize the use of the capabilities of Biotechnology in many countries as well as in Kazakhstan. To achieve the goals such tasks were implemented as the importance of metabolites of microorganisms with a wide range of areas of influence on the body and prevention of various diseases is growing in the preparation and implementation of lactic acid products.

Currently, the production of new types of lactic acid products with the maximum use of probiotics and biologically active microorganisms has become the main directions of Biotechnology Science [1, 2].

One of the priority areas in agricultural production is the effective and rational use of the gene pool of microorganisms. In this context, research on the extraction of new strains of microorganisms and the preservation of existing and used strains of microorganisms in the practice of agricultural biotechnology and the creation of biopreparations based on them in the agro-industrial complex is interesting.

A special place in the gene pool of microorganisms is occupied by prokaryotes including lactic acid bacteria [3].

The ability of lactic acid bacteria to form antibiotic substances and to have a bactericidal, bacteriostatic effect on other microflora is widely used in agricultural biotechnology.

When sorting industrial strains of lactic acid bacteria, many of their biological properties are taken into account. Previously, we studied antagonistic activity; pH range capable of increasing strains; resistance to bile, phenol, increased concentration of NaCl, antibiotic resistance; adhesive activity [4, 5].

Experimental

New strains of lactic acid bacteria were isolated from home-made dairy products: kefir, kumys, shubat, cow's and camel's milk. For inoculation and long-term storage of newly extracted lactic acid bacteria, the following nutrient media were used:

1. MRS nutrient medium: yeast autolysate – 5 ml, peptone – 10 g, glucose – 20 g, ammonium citric acid – 2 g, sodium acetic acid – 5 g, $MgSO_4$ x $7H_2O$ – 200 g, $MnSO_4$ x H_2O – 50 mg, K_2PO_4 – 2 g, twin-1 ml, agar – agar – 20 g, water – 1000 mL, pH medium – 6.2-6.6.

2. Hydrolyzed milk nutrient medium according to Bogdanov: 1 L of boiled, cooled, sterilized milk without fat, the pH medium was 7.4 – 7.6; pancreatin – 1 g, chloroform – 5 ml. The container is tightly closed and placed on the thermostat at 40°C, 72 hours. The resulting hydrolyzes were filtered, the pH medium was set to 7.0-7.2, decontaminated for 10 minutes in 1 atmosphere [6].

3. Hydrolyzed milk containing agar-agar. To prepare agar medium, 2 times water and 2% agar-agar were added to hydrolyzed milk.

4. Wort-Agar: toxic wort – 500 ml, agar-agar – 20 g, water – 1000 ml, ph medium – 6.5-6.7 should be. It is disinfected at 116°C for 30 minutes.

5. The common diagnostic media used by everyone are: ammonia formation medium from arginine, 2% and 4%-vertical NaCl milk hydrolyzate.

Physiological and biochemical properties of lactic acid bacteria resistance to table salt, bile, phenol, ability to form flavoring substances from the composition, acid formation energy, milk clotting time, ammonia formation properties from arginine, diacetyl formation abilities were evaluated according to the generally accepted methods.

By titration with 0.1 N NaOH, the toxicity was checked and measured in Turner degrees.

Pre-decontaminated milk is added at a certain temperature, one of the strains under study, or yeast grown for 18 hours. The milk is simply mixed and kept in the thermostat at a temperature typical of this strain until thick. Later, the paste is left at room temperature for about 1-2 hours, setting the duration of its appearance. Then it was placed to the refrigerator from +3 till +5°C. A day later, during the tasting, an assessment of its taste, smell and consistency, thickening time was made.

The ability of cultures to form flavoring substances from their composition was determined by a qualitative reaction. The property of acetoin formation is established by an alkaline sample. To do this, two drops of the studied cultures are applied to a white porcelain plate, then 40% Kon and 0.04% creatine solutions are added and mixed well. The time is set by tracing until the pink color appears within 20-25 minutes. The volume of formed flavoring substances was measured based on the speed and intensity of color formation.

The ability to form diacetyl was determined by the method of A.G. Grinevich. The test cultures were sprayed on a nutrient agar medium (composition: hydrolyzed milk – 1000 mL, potassium citric acid – 10 g, glucose – 10 g, agar – 25 g, ph medium – 6.8-7.0). Cultures sprinkled with milk hydrolyzate with beveled Agar were kept in a thermostat for 24 hours at a temperature of 30°C, after which a mixture of the following reagents was added: 5 mL of 20%-vertical hydrochloric acid hydroxyl amine and 1 mL of 10%-vertical chlorine nickel. All reagents were prepared before the experiment. The prepared test tubes were placed in a horizontal position on the thermostat for about 2-4 hours.The diacetyl reaction was determined by the formation of red colored crystals of nickel dimethylglioxima. The amount of formed diacetyl was indicated according to the degree of speed of the dye [7-9].

The antagonistic activity of lactic acid bacteria has been studied due to the diffusion of Agar. Gram-negative bacteria are *Escherichia coli, Salmonella dublin,* Gram-positive bacteria are *Bacillus subtilis, Staphylococcus aureus, Sarcina flava* were used as test cultures [10].

Results and Discussion

This paper examines the enzymatic activity of lactic acid bacteria; aroma formation, diacetyl and ammonia formation from arginine, proteolytic, hemolytic and lecithinase activity.

The formation of aroma is the result of the vital activity of a special group of lactic acid bacteria, which is in addition to lactic acid, form diacetyl, acetoin, volatile acids, carbon dioxide, alcohols and esters. Among

these compounds, diacetyl plays a leading role in the formation of aromas. Aromatic lactic acid bacteria are a necessary component of primary yeast for the production of a number of dairy products.

The ability of lactic acid bacteria to proteolysis is widely used in cheese making based on the processes of fermentation of milk components: lactose, protein and fat. In addition, the quality of cheese is greatly influenced by the proteolytic processes present in it, which lead to the formation of flavoring compounds (peptides, free aminoacids, amides, etc.) [8].

In this regard, we studied the properties of lactic acid bacteria, a selection of the most active and compatible strains was carried out.

Previously, we studied some physiological and biochemical properties and antagonistic activity of the studied strains of lactic acid bacteria (Table 1, 2).

Table 1

Physiological and biochemical properties of lactic acid bacteria

Types of strains	Stability to NaCl		Bile resistance		Resistance to Phenol
	2%	4%	30%	40%	
Lactobacillus bulgaricus GM – 08	+	-	+	-	-
Lactobacillus bulgaricus KZh – 01	+	-	+	-	-
Lactobacillus bulgaricus GS – 03	+	+-	+	+	+-
Lactococcus cremoris - 6	+	-	+-	-	-
Lactococcus cremoris - 17	+-	-	+	+-	-
Lactococcus cremoris – 26	+	-	+	+	+-
Lactococcus lactis – 1	+	-	+	-	-
Lactococcus lactis– 15	+	+-	+	+-	-
Lactococcus lactis – 23	+	+	+	+	+
Note: "+ "– the presence of growth; " - "- the absence of growth; "+ - " - weak growth					

Table 2

Antagonistic activity of lactic acid bacteria

Types of microorganisms	*Lactobacillus bulgaricus* GM – 08	*Lactobacillus bulgaricus* KZh-01	*Lactobacillus bulgaricus* GS – 03	*Lactococcus cremoris*-6	*Lactococcu scremoris*-17	*Lactococcus cremoris*-26	*Lactococcus lactis* – 1	*Lactococcus lactis*– 15	*Lactococcus lactis* – 23
Staphylococcus aureus	19	21	11	13	13	15	13	11	23
Salmonella dublin	21	13	11	7	0	19	0	7	19
Escherichia coli	19	17	21	3	0	17	0	11	17
Bacillus subtilis	21	13	19	9	9	21	13	9	25
Sarcina flava	17	19	17	7	3	19	0	0	21
Note: numbers – test-areas of inhibition of the growth of microorganisms (mm)									

As a result of the study, the following active strains were selected to determine enzymatic activity based on these physiological and biochemical properties: *Lactobacillus bulgaricus* – strain 3, *Lactococcus lactis* – strain 1, *Lactococcus cremoris* – strain 1.

From Table 2, it can be seen that the study significantly inhibited the growth of *Lactococcus cremoris* – 26, *Bacillus subtilis*, *Sarcina flava*, *Salmonella dublin*, and less inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. Strains *of Lactococcus cremoris* – 17, *Lactococcus lactis* – 1 showed little activity on all test cultures (3-13 mm). The *Lactococcus lactis* – 23 strain has a high inhibition zone for all cultures (17-25 mm). In addition, the activity range of strains of *Lactobacillus bulgaricus* GM – 08, *Lactobacillus bulgaricus* KZh-01, and *Lactobacillus bulgaricus* GS – 03 was around 11-21 mm.

Further, 5 strains selected from these lactic acid bacteria are examined for flavoring properties, the formation of diacetyl and ammonia from arginine, hemolytic and lecithinase activity, and the indicators are given in Table 3.

Table 3

Enzymatic activity of lactic acid bacteria

Enzymatic properties	Strains				
	Lactobacillus bulgaricus GM – 08	*Lactobacillus bulgaricus* GS – 03	*Lactobacillus bulgaricus* KZh – 01	*Lactococcus lactis* – 23	*Lactococcus cremoris* – 26
Diacetyl formation	+	+	+	+	+
Aroma formation	+-	+	+	+	+
Ammonia formation from arginine	-	-	+-	+	-
Hemolytic activity	+	-	-	+	-
Lecithinase activity	-	-	-	-	-
Proteolytic activity	+	+	-	+	-

As a result of the study, it was found that all strains of aromatic substances, diacetyl and two strains of Lactobacillus bulgaricus CJ – 01 and Lactococcus lactis – 23 form ammonia from arginine; hemolytic activity was shown by Lactobacillus bulgaricus GM – 08 and Lactococcus lactis – 23; no single strain showed lecithinase activity.

Hemolysis can be of three types: B – hemolysis (a transparent, colorless area is formed around the colonies, the width of which depends on the hemolytic activity of the microorganism); B–hemolysis (a greenish area is formed around the colonies due to the formation of methemoglobin from the hemoglobin of partially lysed red blood cells) and y-hemolysis (near the colony there is a narrow cloudy area of partial hemolysis, and then a transparent area of full hemolysis) [11, 12].

To make yeast, it is necessary to study the compatibility of strains. For this, compositions of different strains were selected:

Option 1: *Lactobacillus bulgaricus* GM-08 + *Lactobacillus bulgaricus* KZh-01 + *Lactobacillus bulgaricus* GS-03;

Option 2: *Lactococcus cremoris* – 26+ *Lactococcus lactis* – 23;

Option 3: each strain is separate, it is part of the above compositions.

The compatibility of strains of lactic acid bacteria is checked with each strain individually and with the duration of milk clotting according to organoleptic indicators compared to the duration of milk clotting. When yeast is selected, strains with similar acid formation activity are combined.

Combinations are selected that coagulate milk at the level of the most active strain or even faster, as well as with a good sour-milk taste and aroma [9].

As a result, the following indicators were obtained:

1st composition: sour milk taste, pleasant, aromatic, without external taste, dense in consistency, milky in color, smooth throughout the mass.

2nd composition: the taste is delicious, without external taste, fragrant, the consistency is compacting, with small grains.

3rd composition:

3.1. *Lactobacillus bulgaricus* GM-08 – the taste is pleasant, aromatic, without external taste, the consistency is dense, the color is milky, smooth throughout the mass;

3.2. *Lactobacillus bulgaricus* GS-03 – the taste is sour-milky, pleasant, the consistency is dense, the color is milky, with small grains;

3.4. *Lactococcus lactis-23* – the taste is sour milk and pleasant, the consistency is viscous, the color is caramelized;

3.5. *Lactococcus cremoris – 26* is fermented milk with a bitter taste, dense in consistency with small granules, milky in color.

As follows from Table 4, as a result of the study, it was found that the selected combinations have a high activity of acid formation and clot formation at the level of the most active *Lactococcus lactis* – 23 stam (Table 4).

Table 4

Acid formation and clotting activity of lactic acid bacteria

Properties	Types of strains						
	Lactobacillus bulgaricus GM - 08	*Lactobacillus bulgaricus* GS - 03	*Lactobacillus bulgaricus* KZh – 01	*Lactococcus cremoris* – 26	*Lactococcus lactis*– 23	1 composition	2 composition
Formation of clots (hours)	6	6,5	8	9	5,5	5,5	6
Acid formation ^{0}T	230	280	265	200	280	280	220

Conclusion

Worldwide, the use of dairy and sour milk products has increased significantly in recent years. These are often used for the treatment of various diseases, for therapeutic purposes. This is primarily due to the fact that enzymatic processes in milk, which have long been known, occur with the help of microorganisms, which are characteristic for a long time. Modern technology has its own specifics for this purpose, using dairy microorganisms. This makes it possible to carry out fermentation work which has its own specifics under certain agreed conditions. As a result, it opened the way for the preparation of fermented milk products with very high nutritional quality, physical and chemical, sanitary and healing properties. And lactic acid bacteria significantly affect the absorption of such food in the body [13].

The physiological, biochemical properties of isolated lactic acid bacteria, including acid-forming, antagonistic activity and enzymatic activity were studied. Lactic acid bacteria were not the same in that they showed antagonistic activity to the test cultures tested, depending on the cultivation conditions. When grown in the environment of MRS, their suppression of cultures of lactococci and lactobacilli was of the highest degree. In particular, the *Lactococcus lactis* – 23 strain showed test culture with a inhibition zone of 17-25 mm.

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Г.Б. Адманова, Ж.І. Қуанбай, Р. Изимова, Г.О. Кеубасова, Л.С. Кожамжарова

Сүт өнімдерінен бөлініп алынған сүтқышқылы бактерияларының кейбір ферментативті қасиеттері

Мақалада сүт өнімдерінен бөлініп алынған сүт қышқылды бактериялардың физиологиялық және биохимиялық қасиеттері, антагонистік және ферментативтік белсенділіктері зерттелген мәліметтер көрсетілген. Зерттеуге 9 түрлі сүт қышқылы бактериялары алынды. Олар: *Lactobacillus bulgaricus* ГМ — 08, *Lactobacillus bulgaricus* КЖ — 01, *Lactobacillus bulgaricus* ГС — 03, *Lactococcus cremoris* — 6, *Lactococcus cremoris* — 17, *Lactococcus cremoris* — 26, *Lactococcus lactis* — 1, *Lactococcus lactis* — 15, *Lactococcus lactis* — 23. Осы штамдардың 2% және 4%-дық NaCl концентрациясына, өтке және фенолға төзімділігі анықталды. Сонымен қатар, грамоң және грамтеріс микроорганизмдердің *Staphylococcus aureus, Salmonella dublin, Escherichia coli, Bacillus subtilis, Sarcina flava* өсіндісінің сынамаға қатысты антагонистік белсенділігі зерттелді. Зерттеліп отырған барлық сүт қышқылды бактерияларының сынама-өсінділері тежелу аймағында әртүрлі мөлшерде белсенділік танытты. Соның ішінде *Lactococcus lactis* — 23 штамы барлық өсіндіде жоғары белсенділік көрсетті, тежелу аймағы 17-25 мм. Ары қарай осы штамдардың ішінен 5 штамм таңдалып, олардың хош иіс түзу қасиеттері, аргининнен диацетил мен аммиактың түзілуі, гемолитикалық және лецитиназдық белсенділігі зерттелді. Ашытқы жасау үшін осы штамдардан композициялар құрастырылды. Сүт қышқылы бактериялары штамдарының үйлесімділігі сүттің ұю ұзақтығымен салыстырғанда әрбір штаммен жеке-жеке және органолептикалық көрсеткіштер бойынша сүттің ұю ұзақтығымен тексерілді. Осылайша, таңдалған комбинациялардың ішінен ең белсенді ұйытқы түзуге *Lactococcuslactis* — 23 штамы ие болды.

Кілт сөздер: микроорганизмдер, штамм, антагонистік белсенділік, ферментативтік белсенділік, хош иіс түзу, қышқыл түзу, ашытқы, *Lactococcus cremoris, Lactobacillus bulgaricus*.

Г.Б. Адманова, Ж.И. Куанбай, Р. Изимова, Г.О. Кеубасова, Л.С. Кожамжарова

Некоторые ферментативные свойства молочнокислых бактерий, выделенных из молочных продуктов

В статье представлены данные об изучении физиологических и биохимических свойств, антагонистической и ферментативной активности молочнокислых бактерий, выделяемых из молочных продуктов. Для исследования были взяты 9 различных молочнокислых бактерий: *Lactobacillus bulgaricus* ГМ–08, *Lactobacillus bulgaricus* КЖ-01, *Lactobacillus bulgaricus* ГС–03, *Lactococcus cremoris* — 6, *Lactococcus cremoris* — 17, *Lactococcus cremoris* — 26, *Lactococcus lactis* — 1, *Lactococcus lactis* — 15, *Lactococcus lactis* — 23. Установлено, что эти штаммы устойчивы ко 2 и 4%-ной концентрациям NaCl, желчи и фенолу. Кроме того, изучена антагонистическая активность грамположительных и грамотрицательных микроорганизмов в отношении тестовых культур *Staphylococcus aureus, Salmonella dublin, Escherichia coli, Bacillus subtilis, Sarcina flava*. Все исследуемые молочнокислые бактерии проявляли активность в различных количествах в зоне ингибирования тест-культур. Среди них *штамм 23* показал высокую активность на всех культурах, зона роста 17–25 мм. В дальнейшем из этих штаммов было выбрано 5, изучены их ароматические свойства, образование диацетила и аммиака из аргинина, гемолитическая и лецитиназная активность, а также были составлены композиции для изготовления заквасок. Сочетаемость штаммов молочнокислых бактерий была проверена по продолжительности свертывания молока по сравнению с продолжительностью свертывания молока каждым штаммом в отдельности и по органолептическим показателям. Таким образом, из выбранных комбинаций наиболее активным образованием закваски обладал штамм *Lactococcus lactis* — 23.

Ключевые слова: микроорганизмы, штамм, антагонистическая активность, ферментативная активность, ароматообразование, кислотообразование, закваска, *Lactococcus cremoris, Lactobacillus bulgaricus.*

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