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Identification of new strains of lactic acid bacteria from south region of Kazakhstan

The article describes the process of isolation and identification of lactic acid microorganisms derived from traditional homemade dairy products of the Southern region of Kazakhstan. During the study, 10 samples were selected. Of these, 5 separate isolates were selected: 2 koumiss isolates (Al-2, Al-3) and 3 ayran isolates (Al-1, Al-4, Al-5). A microscopic observation of koumiss isolates showed that they were cocci, isolated and collected in chains, and ayran isolates were large long rods. Spores were not formed. Bacteria were gram-positive facultative anaerobes. Colonies were convex with a solid edge, opaque and not pigmented. Optimum growth temperature was 37 °C. Identification was done by the MALDI-TOF method of mass spectrometry using the Microflex device based on the Maldi Biotyper database. Mass spectra were compared using Biotyper 3.0 RTC software. Based on the results, the Score value of the analyzed strains ranged from 2.096 to 2.449. As a result, it was found that isolates Al-2, Al-3 extracted from koumiss belong to the species *Lactococcus lactis* and isolates Al-1, Al-4, Al-5 extracted from ayran to *Lactobacillus plantarum*. The data obtained by us indicate that the results of microbiological identification are consistent. Antagonistic activity of cultures was studied by 2 test strains (*S. aureus* and *E. Coli*) using agar block method. The results showed that the lysis zone around the wells with *Staphylococcus aureus* culture was in the range from 12.00±1 mm to 18.00±1 mm, and *Escherichia coli* in the range from 13.00±1 mm to 17.00±1 mm. The isolates have an inhibitory effect on the indicator test strains: *Staphylococcus aureus* and *Escherichia coli*.

Keywords: lactic acid products, lactic acid microorganisms, isolates, strain, *Lactobacillus spp*., *Lactococcus spp*., antagonistic activity, mass spectrometry.

Introduction

Preservation of biodiversity is a pressing challenge of modern time. This is reflected in the International Convention on Biological Diversity [1], which has been ratified by the Republic of Kazakhstan. The conservation and sustainable use of biological diversity is crucial to meet the food and healthcare needs, as well as other needs of the world's growing population. Thus, access to and sharing of both genetic resources and technologies are essential to handle these challenges.

Bio resource centers based on collections of microorganisms, viruses, cell cultures of plant and animal tissues, are the basis for the development of biotechnology. Interest in the collections has particularly risen since the second half of XX century. When, with the establishment of new biotechnological production facilities, the need for strains of microorganisms with certain properties has sharply increased, which has intensified work on obtaining highly active producers, a deeper study of supported microbial collections and the creation of data banks [2–4].

The effective use of modern biotechnologies in health care, agriculture, pharmacy, processing and food industry is an important prerequisite for the development and solution of problems in these sectors, as well as for environmental protection [5].

The issues of production and consumption of milk and dairy products are becoming more urgent and increasingly dependent on general trends of the world food market development.

Recently, there is an increased interest in studying lactic acid bacteria. This is largely due to the rapid development of the dairy industry worldwide, production of new fermented milk products, and the search for new strains of lactic acid bacteria suitable for use in fermentation starter.

Dairy products are an essential component of the human diet. They account for satisfaction of up to 20 % of human protein and up to 30 % of fat intake [6].

Today, from a wide range of food products, the customer often chooses those that have additional properties and advantages, such as naturality, health benefits, unusual taste, convenience and others.

Many international manufacturers strive to follow these trends and offer new solutions for people who want to improve their health. Products that help reduce fat tissue and contain pro-biotic ingredients, vitamins, minerals, dietary fiber, fatty acids, etc. are becoming increasingly popular.

Thus, the variety of dairy products is due to the use of bacterial ferments, the composition of which is represented by different types of lactic acid bacteria. The specific taste, consistency and several other properties of milk products depend on the strains that make up the bacterial starters.

The purpose of this project was to isolate and identify lactic acid bacteria from various traditional dairy products of the Southern region of Kazakhstan.

Materials and research methods

Samples of home-made dairy products (ayran, koumiss) selected in the southern region of Kazakhstan were used as study materials.

In order to obtain the accumulation culture of lactic acid strains, the following nutrient medium was used: skimmed milk powder (87 g/L); yeast autolysate (3 ml). Cultivation was carried out at 37 °C, during 16–24 hours.

Pure cultures were isolated by tenfold dilution followed by inoculation on Petri dishes with MRS agar medium. Grown isolated colonies were transferred with a loop to slant agar in tubes and cultivated at 37 °C for 48 hours. Culture purity was observed for absence of extraneous growth in the beef-extract broth.

The cultures of isolates were visually observed using a phase contrast microscope.

Mass spectrometric identification of lactic acid bacteria was performed by the MALDI-TOF mass spectrometry method using the Microflex device based on the Maldi Biotyper database (Bruker Daltonics, Germany).

Antagonistic activity of cultures was studied by 2 test strains: *Staphylococcus aureus* and *Escherichia coli* using agar block method [7].

Results and discussion

During the study 10 samples were taken from traditional homemade dairy products. Of these, 5 separate isolates were obtained, including: 2 koumiss isolates (Al-2, Al-3) and 3 ayran isolates (Al-1, Al-4, Al-5).

Identification of the isolates was made on the basis of cultural, morphological and physiological features using "Bergey's manual". A microscopic observation of koumiss isolates showed that they were cocci, isolated and collected in chains, and ayran isolates were large long rods. Spores were not formed. Bacteria were gram-positive facultative anaerobes. Colonies were convex with a solid edge, opaque and not pigmented. Optimum growth temperature was 37 °C [8].

As a result of the study of cultural and morphological properties, the isolated bacteria were identified as representatives of the genus *Lactobacillus spp*. and *Lactococcus spp*.

Proteomics using matrix-assisted laser desorption/ionization and time-of-flight mass-spectrometry (MALDI-TOF) has been rapidly developing in microbiology in recent years. The basic principle of MALDI-TOF technology is the use of a matrix solution which crystallizes from the sample and matrix substrate after evaporation of the solvent. The formation of the matrix-sample will absorb energy when exposed to laser and transfer ion charge from matrix to sample. When charged samples enter the vacuum tube and the accelerating electric field of the system, fragments of charged samples will be separated based on their mass-to-charge ratio and the flight detector will analyze this separation based on mass and charge and generate what is known as mass spectra. Using software and algorithmic analysis, the mass spectra for a sample are compared with the mass spectra for known species contained in the system database [9].

Identification by the MALDI-TOF method of mass spectrometry has been carried out using the Microflex instrument based on the Maldi Biotyper database (Bruker Daltonics, Germany). As a matrix, a α-cyano-4-hydroxycoric acid in 50 % acetonitrile with addition of 2.5 % trifluoroacetic acid was used. For identification, microbial colonies were used after primary inoculation. The colony was applied to a metal target, covered with a matrix solution, and after drying, microorganisms were identified in a mass spectrometer by ribosomal protein spectra. The results are presented in Table 1.

The mass spectra were compared using the Biotyper 3.0 RTC program (Bruker Daltonics, Germany). The degree of identification reliability was assessed using the obtained Score values, comparing the spectra data from the Biotyper 3.0 reference database. Cases with Score <1.7 were considered as unreliable and were not considered as cases of successful determination of taxonomic properties of an isolate [10].

The results presented in Table 1 show that the Score value of the analyzed strains varies from 2.096 to 2.449.

Table 1

Mass spectrometric identification results

№	Sample	Homologous bacteria	Score value	Quality	Number from NCBI database
1	AL-1	*Lactobacillus plantarum* DSM 2601 DSM	2.19	++	1590
2	AL-2	*Lactococcus lactis ssp. lactis* DSM 20661 DSM	2.449	+++	1360
3	AL-3	*Lactococcus lactis* DSM 4366 DSM	2.096	++	1358
4	AL-4	*Lactobacillus plantarum* DSM 2601 DSM	2.152	++	1590
5	AL-5	*Lactobacillus plantarum* DSM 1055 DSM	2.206	++	1590

Notes. 1 "+" identification of the genus (1.700...1.999); 2 "++" genus identification, identification of probable species (2.000...2.999); 3 "+++" species identification (2.300...3.000).

As a result, it was found that isolates Al-2, Al-3 derived from koumiss belong to the species *Lactococcus lactis* and isolates Al-1, Al-4, Al-5 resulted from ayran to *Lactobacillus plantarum*. The data we have obtained indicate that the results of microbiological identification are consistent with the following.

When creating products based on several strains, an attention should be given to studying antagonistic properties. The antagonism of Lactic acid bacteria (LAB) is caused by the production of lactic acid, which has a certain bactericidal effect and, besides, causes the pH of the medium to drop to values unfavorable for many microorganisms [11].

Antagonistic activity of cultures was studied by 2 test strains: *S. aureus* and *E. coli* by agar blocks method. The test strains were obtained from the collection of microorganisms of the Branch of RSE "National Center of Biotechnology" of the Academy of Sciences of the Republic of Kazakhstan in Stepnogorsk.

Inoculants of *Staphylococcus aureus* and *Escherichia coli* cultures were mixed with heated and cooled to 50 °C agar and poured into Petri dishes. The dishes were cultivated at 37 °C for 20 min. Symmetrically arranged discs with a diameter of 10 mm were cut out of the agar plates obtained by the deep method, and a suspension of selected cultures was introduced into the wells. After the suspension was introduced into wells, the tested plates were incubated at 37 °C for 24 hours. Antagonistic activity was judged by the absence of zone of test strains growth around the colony of the tested isolate. The antagonistic activity was differentiated by 4 degrees: zero in the zone of no growth up to 1.0 mm, low 1.1 4.9 mm, average 5.0 8.9 mm, high 9.0 mm and more (Table 2).

Table 2

Antagonistic activity assessment results

Sample	*Staphylococcus aureus*	*Escherichia coli*
	Zone diameter, mm	
Lactobacillus plantarum AL-1	14,0±1	13,0±1
Lactococcus lactis AL-2	13,0±1	12,0±1
Lactococcus lactis AL-3	12,0±1	13,0±1
Lactobacillus plantarum AL-4	15,0±1	14,0±1
Lactobacillus plantarum AL-5	18,0±1	17,0±1

The results showed that the lysis zone around the wells with *Staphylococcus aureus* culture is in the range from 12.00±1 mm to 18.00±1 mm, and *Escherichia coli* is in the range from 13.00±1 mm to 17.00±1 mm. The results of the study of antagonistic activity showed that the studied isolates have an inhibitory effect on the indicator test strains: *Staphylococcus aureus* and *Escherichia coli*.

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Оңтүстік Қазақстан өңірінен алынған сүтқышқылды бактериялардың жаңа штамдарын идентификациялау

Мақалада Оңтүстік Қазақстан өңірінің түрлі дәстүрлі сүтқышқылды өнімдерінен бөлініп алынған сүтқышқылды микроорганизмдердің бөлінуі мен идентификациясы сипатталған. Жұмысты орындау барысында 10 үлгі іріктеліпп алынған. Олардан 5 жекеленген изоляттар бөлінді, оның ішінде: 2 изолят (Al-2, Al-3) қымыздан және 3 изолят (Al-1, Al-4, Al-5) айраннан. Қымыз изоляттарын микроскопиялық бақылау нәтижесінде, олардың бір-бірімен жеке және тізбектеп орналастырылған коктар, ал айраннан жасалған изоляттар үлкен ұзын таяқшалар екенін көрсетті. Спора пайда болған жоқ. Факультативті анаэробтар. Грамоң. Колониялар тұтас шеті дөңес, мөлдір емес және пигменттелмеген. Оңтайлы өсу температурасы 37 °С. Maldi-TOF масс-спектрометрия әдісімен, Maldi Biotyper деректер базасы негізінде Microflex құралын қолдана отырып, идентификациялау жүргізілген. Масс-спектрлерді салыстыру Biotyper 3.0 RTC бағдарламасының көмегімен жүргізілді. Нәтиже бойынша талдау жасалған штамдардың Score мәні 2.096-ден 2.449-ға дейін өзгерді. Жүргізілген талдаулар нәтижесінде, қымыздан алынған Al-2, Al-3 изоляттар *Lactococcus lactis,* ал айраннан алынған Al-1, Al-4, Al-5 изоляттары *Lactobacillus plantarum* түріне жататындығы анықталды. Алынған мәліметтер микробиологиялық сәйкестендіру нәтижелерінің сәйкестігін растады. Өсіріндінің антагонистік белсенділігі *S. aureus* және *E. coli* тест-штаммдарына блокты агар әдісімен зерттелген. Нәтижесінде, *S. aureus* өсіріндісімен шұңқырдың айналасындағы лизис аймағы 11,00±1 мм-ден 18,00±1 мм-ге дейінгі диапазонда, ал *E. coli* 11,00±1 мм-ден 17,00±1 мм-ге дейінгі диапазонда екенін көрсетті. Зерттелінген изоляттар *Staphylococcus aureus* және *Escherichia coli* индикаторлық тест-штамдарға қатысты тежеуші әсерге ие екенін көрсетті.

Кілт сөздер: сүтқышқылды өнімдер, сүтқышқылды микроағзалар, изоляттар, штамм, *Lactobacillus spp.*, *Lactococcus spp.*, антагонистік белсенділік, масс-спектрометрия.

Л.Ж. Байрон, А.О. Исенова, Н.К. Жаппар, В.М. Шайхутдинов

Идентификация новых штаммов молочнокислых бактерий из Южного региона Казахстана

В статье описан процесс выделения и идентификации молочнокислых микроорганизмов, полученных из традиционных молочнокислых продуктов домашнего приготовления Южного региона Казахстана. В ходе выполнения работы было отобрано 10 образцов. Из них было выделено 5 отдельных изолята, в том числе 2 изолята из кумыса (Al-2, Al-3) и 3 изолята из айрана (Al-1, Al-4, Al-5). Микроскопическое наблюдение изолятов из кумыса показало, что они представляют собой кокки, расположенные единично и собранные в цепочки, а изоляты из айрана — крупные длинные палочки. Спор не

образовывали. Факультативные анаэробы. Грамположительные. Колонии выпуклые, с цельным краем, непрозрачные и не пигментированы. Оптимальная температура роста — 37 °С. Проведена идентификация методом MALDI-TOF масс-спектрометрии с применением прибора Microflex на основе базы данных Maldi Biotyper. Сравнение масс-спектров проводилось с помощью программы Biotyper 3.0 RTC. По результатам значения Score проанализированных штаммов варьируются от 2,096 до 2,449. Было установлено, что изоляты Al-2, Al-3, выделенные из кумыса, относятся к виду *Lactococcus lactis*, а изоляты Al-1, Al-4, Al-5, выделенные из айрана, — к *Lactobacillus plantarum.* Полученные данные свидетельствуют о соответствии результатов микробиологической идентификации. Антагонистическую активность культур исследовали к 2 тест-штаммам: *S. aureus* и *E. coli* методом агаровых блоков. Результаты показали, что зона лизиса вокруг лунок с культурой *Staphylococcus aureus* находится в диапазоне от 12,00±1 мм до18,00±1 мм, а *Escherichia coli* — в пределах от 13,00±1 мм до 17,00±1 мм. Исследуемые изоляты обладают ингибирующим действием в отношении индикаторных тест-штаммов: *Staphylococcus aureus* и *Escherichia coli*.

Ключевые слова: молочнокислые продукты, молочнокислые микроорганизмы, изоляты, штамм, *Lactobacillus spp., Lactococcus spp.*, антагонистическая активность, масс-спектрометрия.

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