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[1]C.A. Nadirova*, [2]Yu.A. Sinyavskiy, [3]R.S. Utegalieva, [1]E.Zh. Gabdullina, [4]S.N. Abdreshov

[1]Almaty Technological University, Kazakhstan, Almaty
[2]Kazakh Academy of Nutrition, Kazakhstan, Алматы
[3]Kazakh National Women's Pedagogical University, Kazakhstan, Алматы
[4]Republican State enterprise on the Right of economic management "Institute of genetics and physiology" of the Committee of science of the Ministry of education and science of the Republic of Kazakhstan, Kazakhstan, Алматы
*e-mail: sanama_777@mail.ru

MOLECULAR GENETIC IDENTIFICATION OF BACTERIA ISOLATED FROM GOAT MILK

In recent years there has been a steady trend towards an increase in the number of studies on the study of probiotics, which have a beneficial effect on the body: improve the intestinal microflora; contribute to the reduction of pathogenic bacteria; have the ability to produce substances with antimicrobial activity. In order to obtain probiotics from domestic raw materials, molecular genetic identification of bacterial strains was carried out. The object for the isolation of lactic acid bacteria was natural goat milk from the Almaty region. Identification of bacterial strains was carried out on an ABI 3500 xL genetic analyzer (Applied Biosystems) using 16S primers 8F and 806R at the Scientific and Practical Center for Microbiology and Virology (Almaty). Phylogenetic analysis was performed using MEGA 6 software. Nucleotide sequence alignment was performed using the ClustalW algorithm. The results were obtained using the method of determining the direct nucleotide sequence of the 16S rRNA gene fragment, followed by comparison of the nucleotide identity with the sequences deposited in the international GenBank database. According to the results of the studies, the isolated strains of lactic acid bacteria according to the GenBank database were assigned to Lactobacillus fermentum (identity above 99.73%). These strains of microorganisms isolated from goat's milk may be promising for the production of domestic food products. On their basis it is proposed to create fermented milk products for preventive purposes.

Key words: molecular genetic identification; phylogenetic analysis; goat milk; lactic acid bacteria; strains; probiotics; dairy products.

[1]С.А. Надирова*, [2]Ю.А.Синявский, [3]Р.С. Утегалиева, [1]Е.Ж.Габдуллина, [4]С.Н. Абдрешов

[1]Алматы технологиялық университеті, Қазақстан, Алматы қ.
[2]Қазақ тағамтану академиясы, Қазақстан, Алматы қ.
[3]Қазақ ұлттық қыздар педагогикалық университеті, Қазақстан, Алматы қ.
[4] Қазақстан Республикасы білім және ғылым министрілігі ғылым комитетінің «Генетика және физиология институты» ШЖҚ РМК, Қазақстан, Алматы
*e-mail: sanama_777@mail.ru

Ешкі сүтінен бөлінген бактериялардың молекулалық-генетикалық идентификациясы

Соңғы жылдары ағзаға пайдалы әсер ететін пробиотиктер бойынша зерттеулерді арттырудың тұрақты үрдісі байқалады: ішек микрофлорасын жақсарту; патогендік бактериялардың азаюына ықпал ету; микробқа қарсы белсенділігі бар заттарды шығару қабілеті бар. Отандық шикізаттан пробиотиктерді алу үшін бактерия штаммдарының молекулалық-генетикалық идентификациясы жүргізілді. Сүт қышқылды бактерияларды бөліп алу объектісі Алматы облысынан табиғи ешкі сүті болды. Бактерия штаммдарын анықтау Микробиология және вирусология ғылыми-практикалық орталығында (Алматы) 8F және 806R 16S праймерлерін қолдану арқылы ABI 3500 xL генетикалық анализаторында (Applied Biosystems) жүргізілді. Филогенетикалық талдау MEGA 6 бағдарламалық құралының көмегімен жүргізілді. Нуклеотидтер тізбегін туралау ClustalW алгоритмі арқылы орындалды. Нәтижелер 16S рРНҚ генінің фрагментінің тікелей нуклеотидтер тізбегін анықтау әдісін қолдану арқылы алынды, содан кейін нуклеотидтердің сәйкестігін халықаралық GenBank дерекқорында сақталған тізбектермен салыстыру. Зерттеу нәтижелері бойынша GenBank деректер базасына сәйкес сүт қышқылы бактерияларының оқшауланған штаммдары Lactobacillus fermentum (идентификатор 99,73%-дан жоғары) болып тағайындалды. Ешкі сүтінен бөлініп алынған микроағзалардың бұл штамдары отандық азық-түлік өнімдерін өндіру үшін перспек-

тивалы болуы мүмкін. Олардың негізінде профилактикалық мақсатта ашытылған сүт өнімдерін жасау ұсынылады.

Түйін сөздер: молекулалық-генетикалық идентификация; филогенетикалық талдау; ешкі сүті; сүт қышқылы бактериялары; штаммдар; пробиотиктер; сүт өнімдері.

[1]С.А. Надирова*, [2]Ю.А. Синявский, [3]Р.С. Утегалиева, [1]Е.Ж. Габдуллина, [4]С.Н. Абдрешов
[1]Алматинский технологический университет, Казахстан, г. Алматы
[2]Казахская академия питания, Казахстан, г. Алматы
[3]Казахский национальный женский педагогический университет, Казахстан, г. Алматы
[4]РГП на ПХВ «Институт генетики и физиологии» КН МНВО РК, Казахстан, г. Алматы
*e-mail: sanama_777@mail.ru

Молекулярно-генетическая идентификация бактерий, выделенных из козьего молока

В последние годы имеется устойчивая тенденция к увеличению количества исследований по изучению пробиотиков, которые благотворно влияют на организм: улучшают микрофлору кишечника; способствуют уменьшению патогенных бактерий; обладают способностью продуцировать вещества с антимикробной активностью. С целью получения пробиотиков из отечественного сырья проведена молекулярно-генетическая идентификация штаммов бактерий. Объектом для выделения молочнокислых бактерий было натуральное козье молоко из Алматинской области. Идентификация штаммов бактерий проводилась на генетическом анализаторе марки ABI 3500 xL (Applied Biosystems) с использованием 16S праймеров 8F и 806R в НПЦ микробиологии и вирусологии (г. Алматы). Филогенетический анализ проводили с использованием программного обеспечения MEGA 6. Выравнивание нуклеотидных последовательностей проводили, используя алгоритм ClustalW. Результаты получены с помощью метода определения прямой нуклеотидной последовательности фрагмента гена 16S рРНК с последующим сравнением нуклеотидной идентичности с последовательностями, депонированными в международной базе данных GenBank. По результатам проведённых исследований выделенные штаммы молочнокислых бактерий по базе данных GenBank были отнесены к Lactobacillus fermentum (идентичность выше 99,73%). Данные штаммы микроорганизмов, выделенные из козьего молока, могут оказаться перспективными для производства пищевых продуктов отечественного производства. На их основе предлагается создание ки**сломолочных продуктов профилактического назначения.**

Ключевые слова: молекулярно-генетическая идентификация; филогенетический анализ; козье молоко; молочнокислые бактерии; штаммы; пробиотики; кисломолочные продукты.

Introduction

The demand of the food industry for probiotic preparations is growing every year. The search for new natural antimicrobial substances synthesized by non-pathogenic beneficial microorganisms is an urgent task. Lactic acid bacteria (LAB) are widespread in nature: they can be found in soil, on decomposing animal and plant debris, in the intestines of vertebrate animals, in milk and dairy products. Together with plants and food they enter the gastrointestinal tract of humans and animals, making up its microbiota [1-3]. The main property of LAB, by which they are grouped into a separate large group of microorganisms, is the ability to form lactic acid as the main product of fermentation. Lactic acid fermentation is carried out by bacterial organisms that are heterogeneous in morphology: bacilliform and spherical (spherical or elliptical cocci), belonging to the genera *Lactococcus*, *Lactobacillus*, *Bifodobacterium*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Vagococcus*, *Tetragenococcus*, *Carnobacterium* [4]. Since ancient times, people have used LAB in the preparation of fermented milk products. Due to the synthesis of antimicrobial metabolites, such as lactic and acetic acids, diacetyl, LAB peroxides inhibit the growth of pathogens. One of the important steps in the search and selection of a strain that is promising for use in the food industry is the determination of its taxonomic affiliation. Correct identification of the strain at the species level allows the researcher to have an idea about the origin, safety, habitat and physiological characteristics of the isolated microorganism [5]. LAB are the bacterial basis of many products, natural inhabitants of the gastrointestinal tract and can serve as bacteriocins and probiotics for use in the food industry. Once in the body, lactic acid bacteria have a detrimental effect on the putrefactive environment and help the beneficial bacteria regenerate. [6-8]. It is known that lactobacilli can have a versatile influence on the biochemical, physiological, neurohumoral and immune processes in human and animal organisms [9, 10]. These bacteria normalize

the content of carbohydrates, cholesterol, bile acids, carry out the synthesis of vitamins and other biologically active compounds. A characteristic property of lactic acid bacteria is their ability to produce substances with antibiotic activity (bacteriocins). The main requirement for probiotic LAB strains is the presence of pronounced antimicrobial activity [11-14]. This allows the use of probiotics to enhance or correct the effects of antibiotics, and in some cases as their alternative. On the basis of a large number of clinical observations it is possible to believe that probiotics are practically irreplaceable in therapy of dysbiotic conditions, Crohn's disease, gastric and duodenal ulcer [15-17].

Yu et al. studied the composition of IBCs of traditional Russian sour-milk products. In their work, they cultured lactic acid bacteria isolated from fermented mare's and cow's milk, sour cream, and cheese collected in Buryatia, Kalmykia, and Tuva. Seven species of lactobacilli and the genus Bifidobacterium were identified by quantitative PCR. 599 LAB strains were obtained from these samples using de Mane, Rogoza, Sharpe, and M17 agars. The scientists' results showed that traditional fermented dairy products from different regions of Russia have a diverse species composition of LAB, which may be related to the type of fermented dairy product, geographic origin, and peculiarities of the production process [18]. In addition, lactic acid bacteria are known to produce antimicrobial compounds that are considered to be natural bioconservatives against foodborne pathogens (Paul et al., 2004). Many strains of Lactobacillus have been isolated from the milk of various animals and used as probiotics in food production (19, 20). N.S. Sowmya et al. isolated nine lactic acid bacteria (LAB) from food and fruits and vegetables (beet, mango, banana, pineapple, radish, tomato, milk and watermelon) on de Man Rogoz and Sharp (MRS) agar medium [21]. These bacteria were further identified as L. fermentum (3 isolates), L. buchneri (4 isolates), L. brevis (1 isolate), and Weisella ciberia (1 isolate) by the 16S rRNA gene sequence. The genetic diversity of Lactobacillus species was analyzed by random amplification of polymorphic DNA (RAPD), and diversity analysis showed differences between Lactobacillus species isolated from different sources.

Identification of Lactobacillus species can be performed by morphological and biochemical features, but the molecular method (16S rRNA gene sequence) proved to be more convenient to use and useful for microorganism identification. The aim of this study was the molecular genetic identification of microorganisms isolated from goat milk of Almaty region, capable of exhibiting antagonistic activity by analyzing the nucleotide sequence of the 16S rRNA gene for the development of technology of dairy products of domestic production.

Materials and methods

Genomic DNA was isolated from daily cultures of bacteria isolated from the milk of locally produced Zaan goats using the PureLink Genomic DNA Kit according to the manufacturer's protocol (Invitrogen, Carlsbad, USA). DNA concentration in the test samples was determined on a Qubit® 2.0 fluorimeter using the QubitTM dsDNA HS Assay Kit (Life Technologies, Oregon, USA). The product was purified using CleanSweep™ PCR Purification reagent (Life Technologies, Carlsbad, CA). 16S rRNA gene fragments were sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) according to the manufacturer's protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol Applied Biosystems, USA), followed by fragment separation on a 3500 DNA Genetic Analyzer (Applide Biosystems, Hitachi, Tokyo Japan).

The sequencing results were processed using the SeqA program (Applied Biosystems); the search for homologous nucleotide sequences of 16S rRNA genes was performed using the BLAST (Basic Local Alignment Search Tool) program in the International Gene Bank database (US National Center for Biotechnology Information) [22]. The phylogenetic tree was constructed using the MEGA 6 program [23]. Nucleotide sequences were aligned using the ClustalW algorithm. The BLASTN Neiighbor-Joining (NJ) method was used to construct phylogenetic trees [24].

Results

The DNA concentration according to the readings of the Quibit fluorimeter is demostrated in Table 1.

Table 1 – The DNA concentration according to the readings of the Quibit fluorometer

№ п/п	Sample name	Concentration, ng/µl
1	**2**	44,0
2	**4**	81,2
3	**5**	69,0
4	**8**	78,0
5	**10**	66,4
6	**14**	48,8
7	**16**	16,0

A fragment of the 16S rRNA gene, about 600 bp in size, was amplified from the obtained DNA by PCR. The results of sample amplification are demonstrated in Figure 1.

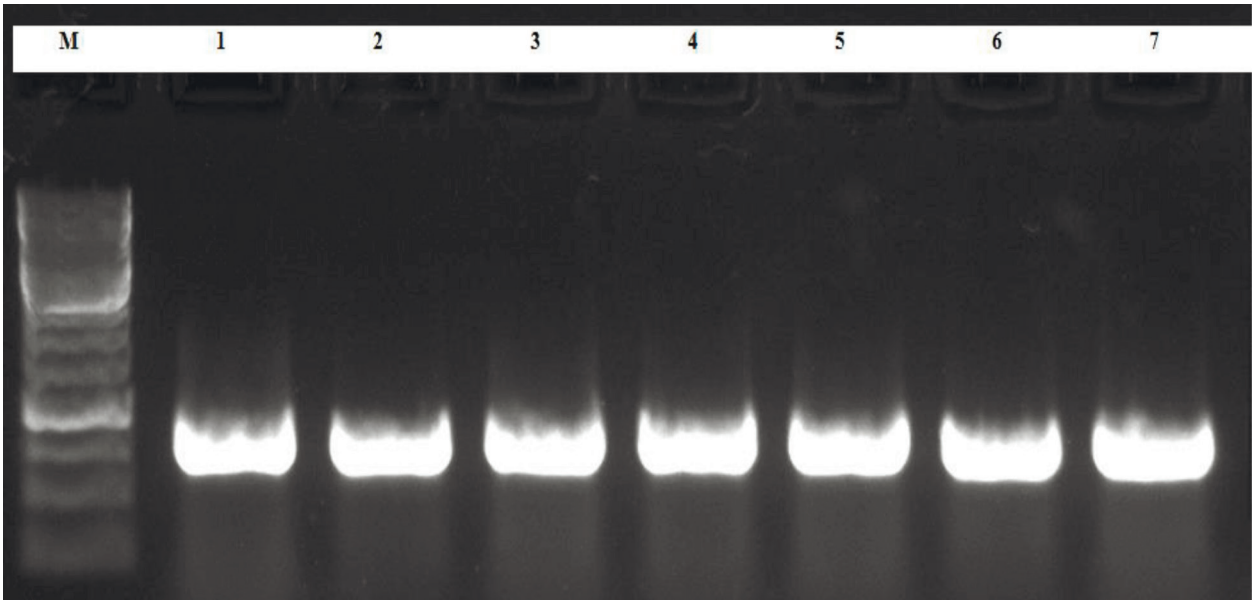

Figure 1 – PCR – product obtained with universal primers to the 16S rRNA region of the gene
Note: 1-7 samples; M – O'GeneRuler 1 kb DNA Ladder length marker.

After the sequencing reaction, the PCR product was purified a second time with the BigDye XTerminator Purification Kit and loaded into an ABI 3500 genetic analyzer for capillary phoresis.

The nucleotide sequences of the 16S rRNA gene of the identified strains were analyzed and combined into a common sequence in the SeqaA software (Appllied Biosystems). After obtaining a nucleotide sequence of about 600 bp, identification was carried out in the GeneBank International Database using the BLAST algorithm.

Nucleotide sequences and the results of phylogenetic analysis of the 16S rRNA gene sequences of the studied strains are presented as trees constructed

in the MEGA6 program using the Neiighbor-Joining cluster method for calculating genetic distances.

Lactic acid bacteria:

2 – Lactobacillus fermentum

Nucleotide sequence:

TGCTTGCACCTGATTGATTTTGGTCGCC
AACGAGTGGCGGACGGGTGAGTAACACGT
AGGTAACCTGCCCAGAAGCGGGGGACAAC
ATTTGGAAACAGATGCTAATACCGCATAAC
AACGTTGTTCGCATGAACAACGCTTAAAAG
ATGGCTTCTCGCTATCACTTCTGGATGGAC
CTGCGGTGCATTAGCTTGTTGGTGGGGTAA
CGGCCTACCAAGGCGATGATGCATAGCCGA
GTTGAGAGACTGATCGGCCACAATGGGACT
GAGACACGGCCCATACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCCACAATGGGCGCA
AGCCTGATGGAGCAACACCGCGTGAGTGA
AGAAGGGTTTCGGCTCGTAAAGCTCTGTTG
TTAAAGAAGAACACGTATGAGAGTAACTGT
TCATACGTTGACGGTATTTAACCAGAAAGT
CACGGCTAACTACGTGCCAGCAGCCGCGGT
AATACGTAGGTGGCAAGCGTTATCCGGATT
TATTGGGCGTAAAGAGAGTGCAGGCGGTTT
TCTAAGTCTGATGTGAAAGCCTTCGGCTTA
ACCGGAGAAGTGCATCGGAAACTGGATAA
CTTGAGTGCAGAAGAGGGTAGTGGAACTCC
ATGTGTAGCGGTGGAATGCGTAGATATATG
GAAGAACACCAGTGGCGAAGGCGGCTACC
TGGTCTGCAACTGACGCTGAGACTCGAAAG
CATGGGTAGCGAACAGGA

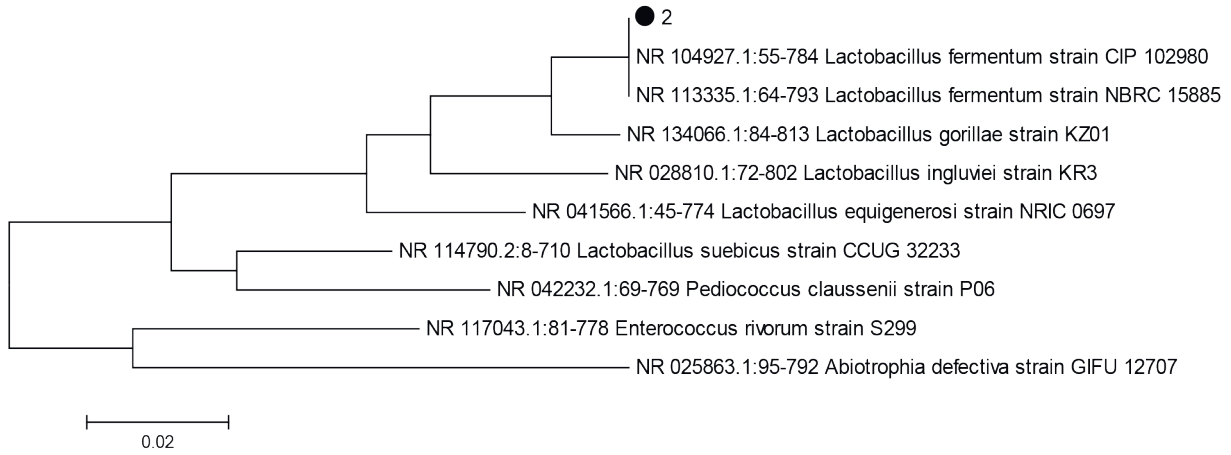

Figure 2 – Phylogenetic tree constructed based on the analysis of the 16S rRNA gene fragment showing the relationship of *Lactobacillus fermentum 2* to *Lactobacillus fermentum strain CIP 102980*

4 – *Lactobacillus fermentum*

Nucleotide sequence:

TGCTTGCACCTGATTGATTTTGGTC-
GCCAACGAGTGGCGGACGGGTGAG-
TAACACGTAGGTAACCTGCCCAGAAGC-
GGGGGACAACATTTGGAAACAGAT-
GCTAATACCGCATAACAACGTTGTTC-
GCATGAACAACGCTTAAAAGATGG-
CTTCTCGCTATCACTTCTGGATGGACCT-
GCGGTGCATTAGCTTGTTGGTGGGG-
TAACGGCCTACCAAGGCGATGATG-
CATAGCCGAGTTGAGAGACTGATC-
GGCCACAATGGGACTGAGACACG-
GCCCATACTCCTACGGGAGGCAG-
CAGTAGGGAATCTTCCACAATGGGCG-
CAAGCCTGATGGAGCAACACCGCGTGAGT-
GAAGAAGGGTTTCGGCTCGTAAAGCTC-
TATTGTTAAAGAAGAACACGTATGAGA-
GTAACTGTTCATACGTTGACGGTATT-
TAACCAGAAAGTCACGGCTAACTACGT-
GCCAGCAGCCGCGGTAATACGTAGGTG-
GCAAGCGTTATCCGGATTTATTGGGCGTA-
AAGAGAGTGCAGGCGGTTTTCTAAGTCT-
GATGTGAAAGCCTTCGGCTTAACCGGAGA-
AGTGCATCGGAAACTGGATAACTTGAGT-
GCAGAAGAGGGTAGTGGAACTCCATGT-
GTAGCGGTGGAATGCGTAGATATATG-
GAAGAACACCAGTGGCGAAAGGCGGC-
TACCTGGTCTGCAACTGACGCTGAGACTC-
GAAAGCATGGGTAGCGAACAGGA

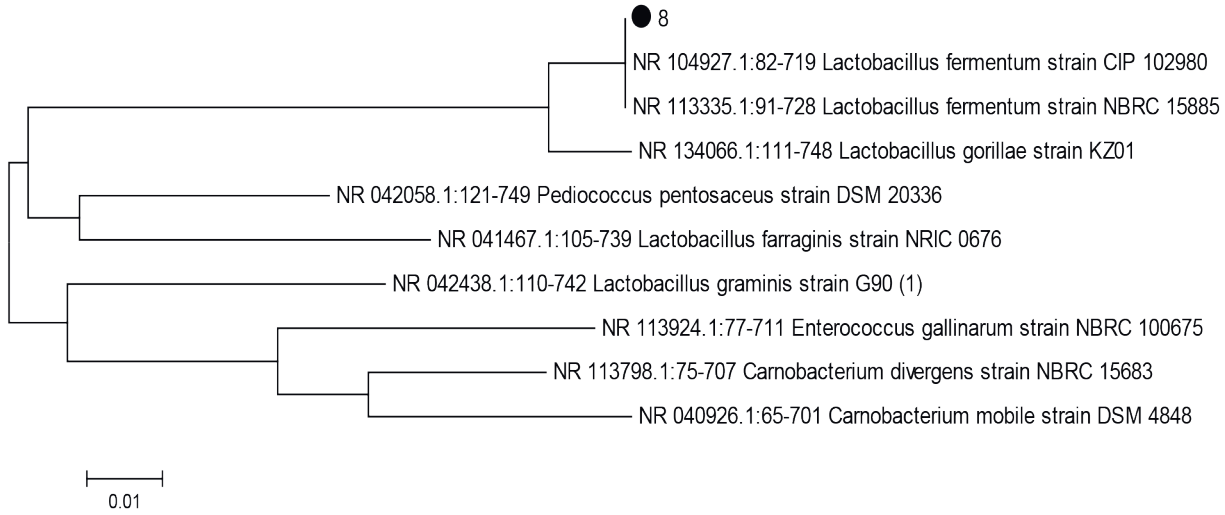

Figure 3 – Phylogenetic tree built on the basis of the analysis of the 16S rRNA gene fragment showing the relationship of *Lactobacillus fermentum 4* with *Lactobacillus fermentum strain CIP 102980*

8 – *Lactobacillus fermentum*

Nucleotide sequence:

CAACGAGTGGCGGACGGGTGAG-
TAACACGTAGGTAACCTGCCCAGAAGC-
GGGGGACAACATTTGGAAACAGATGC-
TAATACCGCATAACAACGTTGTTCGCAT-
GAACAACGCTTAAAAGATGGCTTCTC-
GCTATCACTTCTGGATGGACCTGCGGTG-
CATTAGCTTGTTGGTGGGGTAACGGCCTAC-
CAAGGCGATGATGCATAGCCGAGTTGAGA-
GACTGATCGGCCACAATGGGACTGAGA-
CACGGCCCATACTCCTACGGGAGGCAG-
CAGTAGGGAATCTTCCACAATGGGCG-
CAAGCCTGATGGAGCAACACCGCGTGAGT-
GAAGAAGGGTTTCGGCTCGTAAAGCTCT-
GTTGTTAAAGAAGAACACGTATGAGAG-
TAACTGTTCATACGTTGACGGTATTTAAC-
CAGAAAGTCACGGCTAACTACGTGCCAG-
CAGCCGCGGTAATACGTAGGTGGCAAGC-
GTTATCCGGATTTATTGGGCGTAAAGAGA-
GTGCAGGCGGTTTTCTAAGTCTGATGT-
GAAAGCCTTCGGCTTAACCGGAGAAGT-
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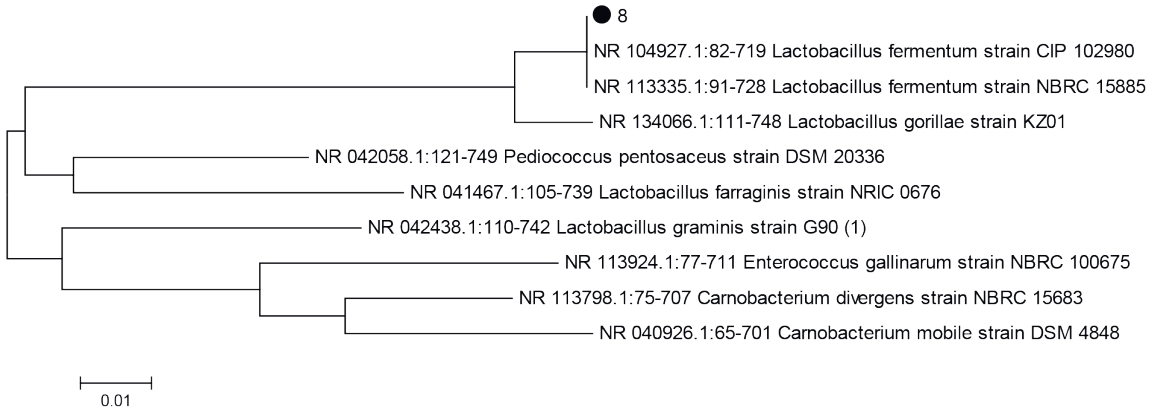

Figure 4 – Phylogenetic tree built on the basis of the analysis of the 16S rRNA gene fragment showing the relationship of *Lactobacillus fermentum 8* with *Lactobacillus fermentum strain CIP 102980*

14 – ***Lactobacillus fermentum***

Nucleotide sequence:

ACGAGTGGCGGACGGGTGAGTAACACGTAGGTAACCTGCCCAGAAGCGGGGGACAACATTTGGAAACAGATGCTAATACCGCATAACAACGTTGTTCGCATGAACAACGCTTAAAAGATGGCTTCTCGCTATCACTTCTGGATGGACCTGCGGTGCATTAGCTTGTTGGTGGGGTAATGGCCTACCAAGGCGATGATGCATAGCCGAGTTGAGAGACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGGCGCAAGCCTGATGGAGCAACACCGCGTGAGTGAAGAAGGGTTTCGGCTCGTAAAGCTCTGTTGTTAAAGAAGAACACGTATGAGAGTAACTGTTCATACGTTGACGGTATTTAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGATTTATTGGGCGTAAAGAGAGTGCAGGCGGTTTTCTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGGATAACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTGTAGCGGTGGAATGCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGGCTACCTGGTCTGCAACTGACGCTGAGACTCGAAAGCATGGGTAGCGAAC

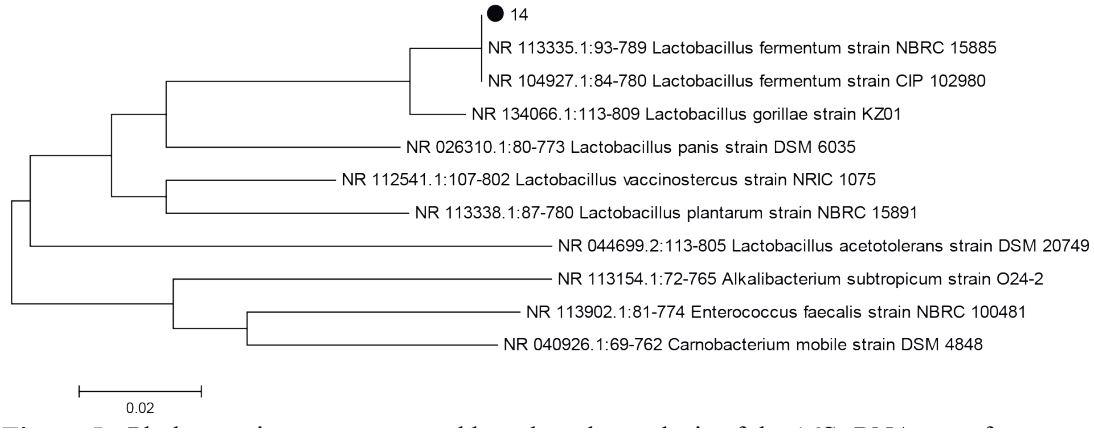

Figure 5 – Phylogenetic tree constructed based on the analysis of the 16S rRNA gene fragment showing the relationship of *Lactobacillus fermentum 14* to *Lactobacillus fermentum strain NBRC 15885*

5 – ***Enterococcus faecalis***

Nucleotide sequence:

CCGAGTGCTTGCACTCAATTGGAAAGAGGAGTGGCGGACGGGTGAGTAACACGTGGGTAACCTACCCATCAGAGGGGGATAACACTTGGAAACAGGTGCTAATACCGCATAACAGTTTATGCCGCATGGCATAAGAGTGAAAGGCGCTTTCGGGTGTCGCTGATGGATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGAAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGAGAAGAACAAGGACGTTAGTAACTGAACGTCCCCTGACGGTATCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGA

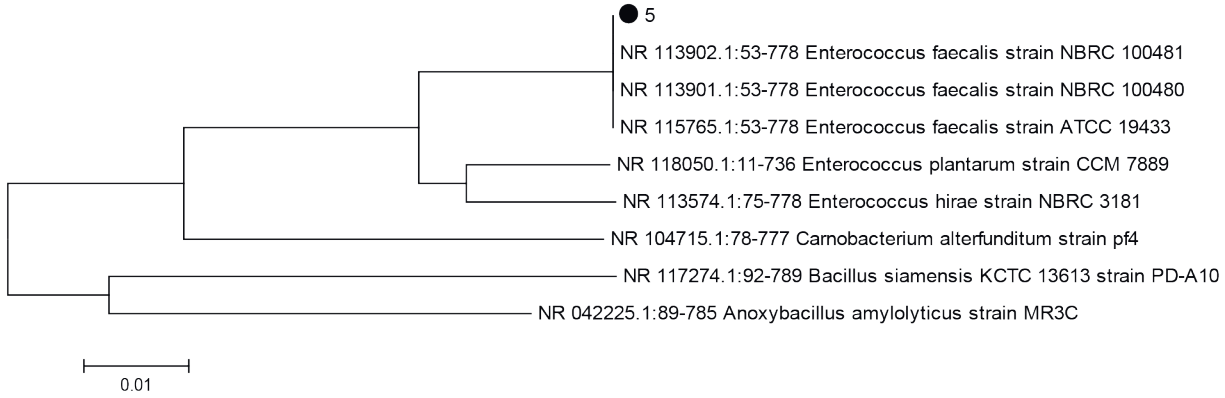

Figure 6 – Phylogenetic tree constructed based on the analysis of the 16S rRNA gene fragment of *Enterococcus faecalis*

The degree of homology with the nearest strain *NR 113902.1:53-778 Enterococcus faecalis* strain *NBRC 100481* was 100.00%.

Catalase positive bacteria:

10 – *Staphylococcus warneri*

Nucleotide sequence:

TGCAGTCGAGCGAACAGATA-
AGGAGCTTGCTCCTTTGACGTTAGCG-
GCGGACGGGTGAGTAACACGTGGATA-
ACCTACCTATAAGACTGGGATAACTTC-
GGGAAACCGGAGCTAATACCGGATAA-
CATATTGAACCGCATGGTTCAATAG-
TGAAAGGCGGCTTTGCTGTCACTTATAG-
ATGGATCCGCGCCGTATTAGCTAGTTG-
GTAAGGTAACGGCTTACCAAGGCAAC-
GATACGTAGCCGACCTGAGAGGGT-
GATCGGCCACACTGGAACTGAGACAC-
GGTCCAGACTCCTACGGGAGGCAG-
CAGTAGGGAATCTTCCGCAATGGGC-
GAAAGCCTGACGGAGCAACGCCGCGT-
GAGTGATGAAGGTCTTCGGATCGTA-
AAACTCTGTTATCAGGGAAGAACAAAT-
GTGTAAGTAACTGTGCACATCTTGACGG-
TACCTGATCAGAAAGCCACGGCTAACTAC-
GTGCCAGCAGCCGCGGTAATACGTAGGTG-
GCAAGCGTTATCCGGAATTATTGGGCGTA-
AAGCGCGCGTAGGCGGTTTTTTAAGTCT-
GATGTGAAAGCCCACGGCTCAACCGTG-
GAGGGTCATTGGAAACTGGAAAACTT-
GAGTGCAGAAGAGGAAAGTGGAATTC-
CATGTGTAGCGGTGAAATGCGCAGAGA-
TATGGAGGAACACCAGTGGCGAAGGC-
GACTTTCTGGTCTGTAACTGACGCTGATGT-
GCGAAAGCGTGGGGGATCAAACAGGA

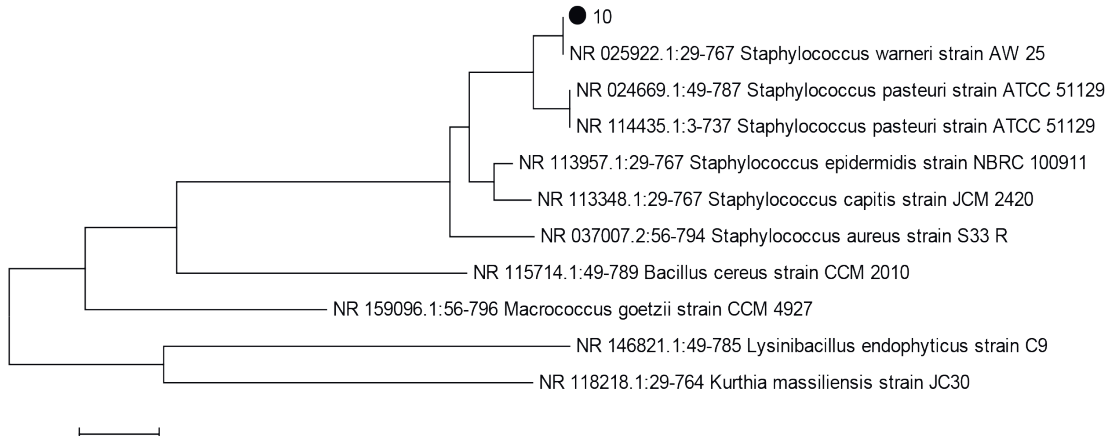

Figure 7 – Phylogenetic tree constructed based on the analysis of the 16S rRNA gene fragment of *Staphylococcus warneri 10*

The degree of homology with the nearest strain NR 025922.1:29-767 *Staphylococcus warneri* strain AW 25 was 99.73%.

16 – *Staphylococcus aureus*

Nucleotide sequence:

ACACGTGGATAACCTACCTATA-
AGACTGGGATAACTTCGGGAAACCG-
GAGCTAATACCGGATAATATTTTGAACC-
GCATGGTTCAAAAGTGAAAGACGGTCTT-
GCTGTCACTTATAGATGGATCCGCGCT-
GCATTAGCTAGTTGGTAAGGTAACG-
GCTTACCAAGGCAACGATGCATAGCC-
GACCTGAGAGGGTGATCGGCCACACTG-
GAACTGAGACACGGTCCAGACTCCTAC-
GGGAGGCAGCAGTAGGGAATCTTC-
CGCAATGGGCGAAAGCCTGACG-
GAGCAACGCCGCGTGAGTGAT-
GAAGGTCTTCGGATCGTAAAACTCTGT-
TATTAGGGAAGAACATATGTGTAAGTA-
ACTGTGCACATCTTGACGGTACCTAAT-
CAGAAAGCCACGGCTAACTACGTGCCAG-
CAGCCGCGGTAATACGTAGGTGGCAAGC-
GTTATCCGGAATTATTGGGCGTAAAGC-
GCGCGTAGGCGGTTTTTTAAGTCTGATGT-
GAAAGCCCACGGCTCAACCGTGGAGGGT-
CATTGGAAACTGGAAAACTTGAGTG-
CAGAAGAGGAAAGTGGAATTCCATGTG-
TAGCGGTGAAATGCGCAGAGATATGGAG-
GAACACCAGTGGCGAAGGCGACTTTCTG-
GTCTGTACTGACGCTGATGTGCGAAAGC-
GTGGGG

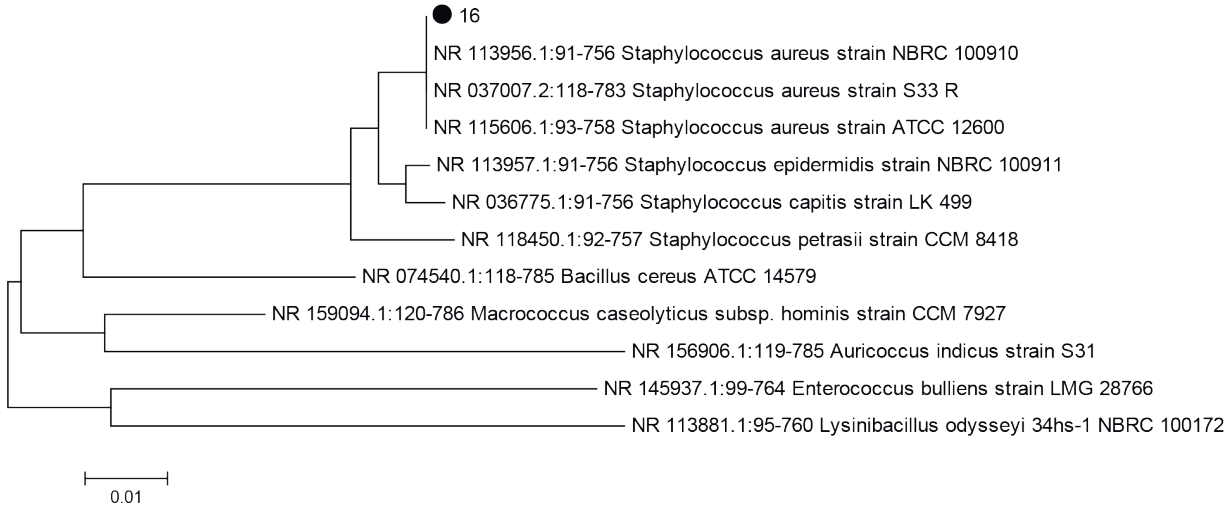

Figure 8 – Phylogenetic tree constructed based on the analysis of the 16S rRNA gene fragment of *Staphylococcus warneri 16*

The degree of homology with the nearest strain NR 113956.1:91-756 *Staphylococcus aureus* strain NBRC 100910 was 99.85%.

These identified strains of staphylococci were used as test cultures for the selection of antagonistically active lactic acid bacteria.

Results and discussion

As a result of molecular genetic identification of the studied strains, isolated for the first time from goat milk of the Almaty region, based on the analysis of nucleotide sequences

16S rRNA gene values were determined for bacterial strains: for the *Lactobacillus fermentum* 14 strain, the identity with the NR 113335.1:93-789 strain *Lactobacillus fermentum* strain NBRC 15885 was 99.86%; for the strain *Lactobacillus fermentum* 8, the identity with the strain NR 104927.1:82-719 *Lactobacillus fermentum* strain CIP 102980 was 100.00%; for the strain Lactobacillus fermentum 4, the degree of homology with the nearest strain NR 104927.1:55-784 Lactobacillus fermentum strain CIP 102980 was 99.73%; for strain Lactobacillus fermentum 2, the degree of homology with the nearest strain NR 104927.1:55-784 *Lactobacillus fermentum* strain CIP 102980 was 100.00%.

Thus, the isolation of lactic acid bacteria from local raw milk will increase the creation of starter culture for the dairy industry of domestic production.

Conclusion

Thus, this study presents the results of molecular genetic identification of bacteria based on the analysis of the nucleotide sequences of the 16S rRNA

gene isolated from raw goat milk of the Zaanen breed of the Almaty region. All bacterial strains were sequenced and analyzed. A comparative analysis of the nucleotide sequences of both strains of lactic acid bacteria showed their high identity. A passport was drawn up for a strain of *Lactobacillus fermentum 14* isolated from goat milk of the Almaty region. *Lactobacillus fermentum 14* was deposited in the Republican Collection of Microorganisms (Astana) as a promising strain for inclusion in the composition of bacterial starter cultures in the production of fermented dairy products [25].

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