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New Probiotic Culture of *Lactococcus lactis* ssp. *lactis*: Effective Opportunities and Prospects

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Abstract

We have isolated several new strains of lactococci from raw milk of Buryatia region of Russia near Lake Baikal with wide variety of climatic and ecological niches. Physiological and biochemical features of new strains were studied and compared to the nisin-producing strain *Lactococcus lactis* ssp. *lactis* MSU. According to morphological, cultural, physiological, biochemical properties and gene sequence of 16S rRNA a novel most effective strain 194 was identified as *Lactococcus lactis* ssp. *lactis* (GenBank database DQ 255954), which has status "GRAS" (absolutely harmless for human health and animals). The strain 194 had inhibitory activity against Gram-positive, Gram-negative pathogenic bacteria and also on fungi of genera *Aspergillus, Fusarium* and *Candida*. This is unique biological property for natural strains of *Lactococcus lactis* specie. We also studied the probiotic properties of strain as resistance to HCl and bile acids, sensitivity to antibiotics and show the therapeutic effect of strain as a food additive on model mice CBRB-Rb (8,17) 1Iem chronic dermatitis.

Keywords: *Lactococcus lactis* subsp. *Lactis;* Identification; Bacteriocin; Sensitivity to antibiotics; Feed additive; Mouse model CBRB-Rb (8.17) 1Iem; Probiotic

Introduction

Probiotics are live microorganisms, which refer to a normal inhabitant of the intestines of healthy animals and human [1]. A common property of probiotic bacteria is a formation of natural antibiotic-like substances. Factors associated with the possible effect of bacteria on the microbiocenosis of intestine, may include the high antagonistic potential, especially against pathogens, synthesis of antibiotics, protease, amylase, xylanase, lipase, endoglucanases. But to define probiotic properties, microbes should be considered with the ability to provide immunomodulatory impact and allocate metabolites which stimulate the development of normal microbiocenosis [2,3]. Generally, probiotics may define as a safe and "natural" approach that helps to curb the population of bacteria that cause microbial infections. The largest group of probiotic bacteria in the intestine is lactic acid bacteria (LAB). LAB widely used in various food fermentations and has a long history. The genus Lactococcus has "GRAS" - status (absolutely harmless for human health and animals) accordingly to the European Commission [4], while the member of genera Streptococcus and Enterococcus contain some opportunistic pathogens. The LAB isolated from the natural sour milk products draw a special interest among the probiotic correctors of normal intestinal microbiota and play an important role in human ecology. Many of them are known to synthesize biologically active peptides or protein complexes, known as bacteriocins. Bacteriocins differ from each other by chemical structure and antagonistic activity. *Lactococcus lactis* produces bacteriocins as lacticins 48, 3147, several forms of nisin and lactococcins [1,5]. Nisin is a unique, nontoxic antibiotic. Nisin is the best studied compound in the latter group. It is the only antibiotic substance given the status "GRAS", which is allowed for application as food preservative under the code E234. Nisin is the low-molecular mass protein so it is easy to apart it into amino acids during digestion and does not influence microbiota of gastrointestinal tract [6,7]. One of the very important properties of nisin is the activity against Gram-positive bacteria and bacterial spores of clostridia and bacilli, other non-spore forming bacteria, as many species of pathogenic Streptococci, Staphylococci, Listeria, *Mycobacterium tuberculosis*, but it is not effective against Gram-negative enterobacteria and fungi [5,8].

Fungal spoilage of food is a common and global phenomenon. The potential production of toxins by fungi is of particular health concern. As mentioned above, *Lactococcus* spp. strains generally inhibit only Gram-positive bacteria and they are not effective against fungi. But recently it was revealed that some lactococci strains have an ability to produce antifungal substances which were determined as peptide and low-molecular phosphoglycolipid [9]. For this reason lactococci can be considered as potential producers of different antimicrobials with wider activity spectrum than nisin. In recent years, the concept of probiotic bacteria has also stimulated work on bacteriocins. In the light of the increased antibiotic resistance among pathogens, bacteriocins have attracted attention as an alternative means to prevent infection by pathogens. In fact, two lantibiotics, nisin and lacticin 3147, have been found useful in preventing dyskinesia, mastitis, dermatitis, type impetigo, ecthyma, scalded skin syndrome forms of chronic dermatitis in humans [6,10-12].

At present, the ways of targeted synthesis of antimicrobial substances by lactococci are studied in order to obtain new ones with more valuable properties for application them as probiotics. The interest in the use of bacteriocin producer cultures as probiotics has increased tremendously. Screening of effective strains from raw milk products from Buryatia - North Asiatic region of Lake Baikal, with wide variety of climatic and ecological niches, which creates conditions for a variety of LAB and their unique properties are of great scientific and practical interest.

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The aim of this investigation was to isolate and identify the new lactococci strains from Buryatia with bactericidal and fungicidal activity and study of their probiotic properties.

Materials and Methods

Media and conditions for isolaton of natural lactococci strains

Raw cow's milk obtained from the milk farm of Buryatia (Russia) was used in the work. The tubes with investigated milk were left for spontaneous self-fermentation at 28°C in steady-state conditions for 17 h. Then bacteria from the tubes, where a dense milk clot formed, were subjected upon several passages in skimmed milk over 10–17 h. These conditions are allowed to propagate the homo-fermentative lactococci, which then were inoculated into the agar milk hydrolysate with bromocresol purple indicator. The individual acid-producing colonies from the surface of the agar medium were transferred onto the lawn with the test culture for nisin determination activity - *Bacillus coagulans* 429, a thermophilic spore-forming, acid-resistant bacterium, using a sterile replicator and, in parallel, to the same solid medium without test culture. The clones forming the largest growth inhibition zones of test organism were selected as active nisin-producing strains. To obtain the lawn of the test culture, a 24 h-old culture of *B. coagulans,* grown at 55°C on the agar organic medium (g/l): glucose: 10.0, peptone: 5.0, NaCl: 5.0, agar: 20.0 with an addition of Hottinger's broth (at a concentration of 28 mg% according to ammonium nitrogen) at pH 7.0 was plated in Petri dishes.

Cultural properties of the isolated strains were examined by classical microbiological methods of identification and determination of the culture [13,14].

The identification of the isolated strains

The phylogenetic analysis using the sequences of the 16S rRNA genes was performed using the programs Vector NTI: ContigExpress and AlignX. A comparative analysis and search for homologous sequences were performed using the NCBI database (http://www.ncbi.nlm.nih.gov/blast). Multiple alignments of the sequences were obtained by the program ClustalX; the alignments were constructed and the genetic distances between the strains compared were computed by the program Mega2.

The strains were compared according to their cultural, physiological, and biochemical properties. The physiological and biochemical properties of strains displaying the most pronounced antibiotic activity were assessed according to the fermentation of carbohydrates, the demand for growth factors, the level of inhibitory activity, and the range of antimicrobial action. We used the series of carbohydrates: D-arabinose, D-xylose, D-ribose, D-glucose, L-rhamnose, D-maltose, D-sucrose, D-mannose, D-lactose, D-galactose, raffinose, D-fructose, D-sorbitol, dulcitol, mannitol, dextrin, glycerol and starch, which were added to the base fermentation medium in amounts of 1.0% each. For growth factors DL-valine, DL-threonine, DL-leucine, L-serine, L-lysine, DL-cystine, L-glutamate, L-aspartate, L-isoleucine, L-methionine, and L-cysteine were used. Each amino acid was introduced into the base fermentation medium in the amount of 0.01%.

To obtain an inoculum, the culture from skimmed milk was re-inoculated into the inoculation medium, which contained 1% glucose, yeast autolysate (35 mg% of ammonium nitrogen), and tap water (pH 6.8-7.0). The culture was grown under steady-state condition at 28°C. Thereafter, the inoculum (OD_{540} 0.14-0.19) was introduced in an amount of 5 vol.% into the base fermentation medium, which contained (g/l): KH_2PO_4: 20.0; glucose: 10.0; NaCl: 1.0; $MgSO_4$: 0.2 and yeast autolysate (35 mg% ammonium nitrogen), pH 6.8-7.0 and culture was allow to grow at 28°C.

Antimicrobial testing

The antimicrobial activity of lactic acid bacteria was determined by the diffusion into agar by measuring of the growth inhibition zone of test cultures in mm [14].

The spectrum of inhibitory effects of the strains was studied by culturing strains under steady-state conditions in the fermentation medium of the above composition. The microorganisms used in these experiments were from the Collection of Microbes of the Department of Microbiology of Moscow State University: 6 strains of Gram-positive bacteria (*Micrococcus luteus* 128, *Bacillus mycoides* 32, *B. subtilis* 2, *B. coagulans* 429, *B. cereus* 112 and *Staphylococcus aureus* 144); 6 strains of Gram-negative bacteria (*Alcaligenes faecalis* 82, *Escherichia coli* 52, *Proteus vulgaris* 206, *Pseudomonas aeruginosa* 54, *Salmonella gallinarum*); 3 strains of fungi (*Fusarium oxysporum* 61, *Penicillium chrysogenum* 37, *Aspergillus niger* 369); and two yeast strains (*Candida guilliermondii* 17 and *Rhodotorula aurantiaca* 226).

The bacilli were grown on an organic medium containing (g/l): glucose: 10.0, peptone: 5.0, NaCl: 5.0 and agar: 25.0; the medium was supplemented by Hottinger's broth (25 mg% amine nitrogen) and had a pH of 7.0. The yeasts were grown in a wort (6-8°B) medium supplemented with 2.5% agar (pH 6.8). The medium for the fungi was similar (wort 3-4°B with 2.0% agar; pH 6.0).

The bacteria were cultured at 28-:-55°C: the temperature used in the case of bacilli, staphylococci, and micrococci was 37°C; *E. coli* was grown at 42°C; *B. coagulans* at 55°C, and yeasts and fungi, at 28°C. Petri dishes were inoculated with 1-day cultures of test microorganisms (0.1 ml cell suspension in physiological saline per dish; the concentrations were adjusted to 10^9 cells per 1 ml using a bacterial turbidity standard).

The bacteriocin-synthesizing activity was assessed as nisin production judged by the suppression of growth of the indicator culture *B. coagulans*, which s was introduced into the agar medium as a suspension with a density of 10^9 cell/ml. Antibiotic substances were extracted from the cells and culture liquid using a 4:1:5 mixture of acetone, acetic acid, and water (55°C, 1.5 h).

Quantitative determination of the antimicrobial activity was performed by measuring the zones of growth suppression with subsequent calculation involving a calibration plot for standard nisin solutions. Solutions of the preparations served as the standards: "Nisaplin" (activity 1 000 000 IU/g, "Aplin & Barrett, Ltd" Co., UK) – for Gram-positives; chloramphenicol (HiMedia Laboratories Ltd, Mumbai) - for Gram-negative bacteria; nystatin (4670 U/mg, Sigma) – for fungi. The fungicidal activity was assessed with indicator culture *Aspergillus niger* 369 [14].

Physiological and biochemical features of new strains were studied and compared to the nisin-producing strain *Lactococcus lactis* ssp. *lactis* MSU. This strain was a reference strain which produces nisin A identical to that contained in the commercial preparation Nisaplin (Aplin & Barrett Ltd., UK).The strain MSU was stored as lyophilized in a household refrigerator at 4°C. The lyophilized culture was reconstituted by sterile non-fat (skimmed) milk.

The dynamics of growth and bacteriocin accumulation in the culture liquid of strains were followed for 24 h. The amount of the biomass was determined by optical density (540 nm).

The sensitivity experiments to the antibiotics: ampicillin, erythromycin, tetracycline, lincomycin, kanamycin, streptomycin, rifampicin, neomycin and oleandomycin was performed by disk diffusion method.

Testing the *Lactococcus lactis* ssp. *lactis* strain 194 as probiotic

In our experiments the isolated lactococci were studied as probiotic cultures. It was conducted in model experiments of the effects of adverse conditions of the gastrointestinal tract by the action of high concentrations of bile acids (0-50%) and hydrochloric acid (0 to 1.0%), added to fermentative medium, on the viability of strains during the incubation for 1–3 h.

We checked the effect of culture of novel strain 194 on the symptoms of dermatoses on male mice CBRB-Rb (8.17) 1Iem at the age of 23,0 ± 1,3 weeks, as an adequate model of spontaneous chronic skin dermatitis, type impetigo, ecthyma and scalded skin syndrome. Animals were fed by complete pelleted feed. As a standard feed used cereal grains (5 g per mouse per day) with the addition of sunflower oil and vitamins A, E, D, F; water was unrestricted. Males mice of the experimental group (n=17) were added daily for two weeks with the culture of *Lactococcus* in feed by 194 to 300 mkl per mouse per day. Assessment of symptoms of dermatitis conducted a double-blind study once a week in all mice individually. The following information was recorded: 1) the degree of ulceration or manifestation of alopecia of the skin on their backs by the 7-point scale; 2) the area of the affected area of the back, in mm^2; 3) weight gain. The experiment comprised three series of triplicate measurements each and the results were statistically processed.

Results and Discussion

Screening for the strains and their isolation

Screening for the effective bacteriocins-synthesizing strains of *Lactococcus lactis* was performed. Lactococci were isolated from raw milk from Ulan-Ude city area (Buryatia, Russia).

The lactococcal colonies were selected in the media with the indicator bromocresol purple, which changed the color of the medium from violet to yellow indicating the acidification degree. It is known that the major product of homolactic acid fermentation is lactic acid. Overall, 18 colonies of mesophilic acid-producing bacteria were selected from which 5 isolated colonies inhibited the growth of test-microorganism *B. coagulans*, 2 of them displayed the highest activity. The best strain named 194 was chosen for further study. The morphology and cultural properties of it were compared to the nisin-producing strain *Lactococcus lactis* ssp. *lactis* MSU.

The morphology of the isolated strains demonstrated that the cultures were represented by cocci assembled in pairs or short chains of various lengths: two, four or 12 cells. in compared to strain *L. lactis* ssp. *lactis* MSU (Figure 1).

The bacteria were non-motile and Gram-positive. They did not differ in their cultural features from the bacteria belonging to the genus *Lactococcus* (Table 1).

Growth was absent in the meat–peptone broth containing 6.5% NaCl and at pH 9.6. The pH from 6.6 to 7.2 was optimal for the growth and development of these strains. The optimal incubation temperature was 28°C; at 10°C the growth was minimal and was completely absent at 45°C. Uniform growth of bacteria along the entire inoculum's length in a stab of an agar medium is characteristic of facultative anaerobes [11].

Biochemical testing of isolates

Ability to consume of various carbohydrates, including sugars, alcohols and organic acids is the basis of the distinguishing features in the identification of lactic acid bacteria. Studies have found that all strains utilized glucose, sucrose, lactose, galactose, maltose, mannose, fructose, and did not utilize - rhamnose, the trisaccharide raffinose and did not hydrolyze starch

Strain 194 utilize not only arabinose and xylose, but also the alcohols: dulcitol, mannitol and sorbitol.

The hallmark of lactic acid bacteria is a great need for complex nutrients: purines, pyrimidines, amino acids and vitamins, especially of group B. Amino acids are essential for the construction of the cell and for the bacteriocin formation. It is known that threonine, serine, cysteine, lysine, and aspartic acid are precursors of lanthionine and methyl-lanthionine and part of the nisin molecules [5].

The results of an experiment to determine the needs of strains in amino acids and other growth components revealed some features of the isolates (Table 2). Strain 194 showed good growth in media, indicating the specific needs in glutamine, asparagine, uracil, aspartic acid and arginine. Strain 122 does not require adenine and phenylalanine, as strain 105 did not require methionine, threonine, and diaminopimelic acid. Strain 205 did not need the following growth components:

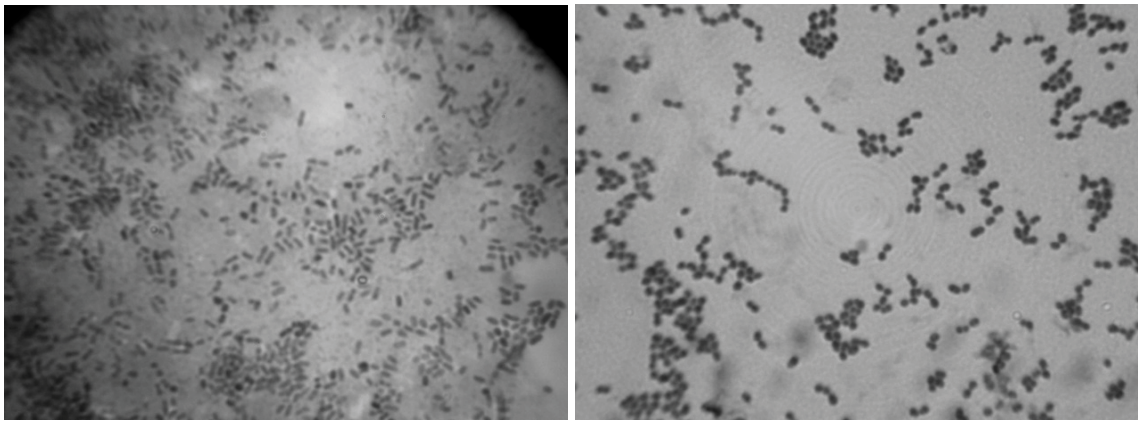


Figure 1: Morphology of perspective strains *Lactococcus lactis* subsp. *lactis*: A – strain MSU, B - strain 194.

Properties	*L. lactis* subsp. *lactis**	Strain MSU	Strain 194	Strain 105	Strain 122	Strain 205
The preferential location of the most typical cells	short chains	chains (to 4-7 cocci)	diplo-cocci and long chains	chains to 7 cocci	diplo-cocci	chains to 7 cocci
Mobility	-	-	-	-	-	-
Growth at 10°C	+	+	+	+	+	+
Growth at 45°C	-	-	-	-	-	-
pH 9.6	-	-	-	-	-	-
Growth in the presence of 4% NaCl	+	+	+	+	+	+
Growth in the presence of 6.5% NaCl	-	-	-	-	-	-
Sensitivity to oxygen	All are facultative anaerobes					
The ability to consume carbohydrates						
Xylose	-	-	+_	-	-	-
Fructose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Raffinose	-	-	-	-	-	-
Starch	-	-	-	-	-	-
Mannitol	-	-	+	-	-	+

Note: **-** (negative); **+** (positive)

Table 1: Differentiating features of isolated lactococcal strains.

Growth components of strain	Strain 194	Strain 105	Strain 122	Strain 205	Strain MSU
Alanine	+	-	+	+	-
Arginine	+	+	+	+	+
Asparagine	+	+	-	+	-
Aspartic acid	+	+	+	+	+
Glycine	+	-	-	-	+
Glutamine	+	-	-	+	+
Glutamine	-	+	-	-	+
Thymine	+	-	+	+	-
Uracil	+	+	+	+	+

Note: **-** (negative); **+** (positive)

Table 2: The need for growth components of isolated strains lactococci.

thymine, valine, proline, glycine. Rapid growth of the strain MSU was detected in the media, which included histidine, glutamine, pyridoxine, isoleucine, uracil, biotin, valine, arginine, alanine. In medium containing glutamine, asparagine, uracil, aspartic acid, arginine, and which is a blend of vitamins to the amino acid alanine, grew all strains, indicating their need for uracil, arginine and vitamins. Studied lactococci grew equally well in media containing a mixture of amino acids in combination with vitamin B_1 and biotin, as well as with uracil. In control experiments in the absence of growth factors in the environment lactococci did not grow, confirming that they belong to auxotrophic microorganisms. Strains 194 and MSU need the presence of glycine in the medium. The absence of a serine or glycine had no effect on the growth of strains, except strain 194. Strain 105 has a very limited need for growth components, but proline and uracil stimulated its growth. All strains were in need of arginine.

Taxonomic description of isolates

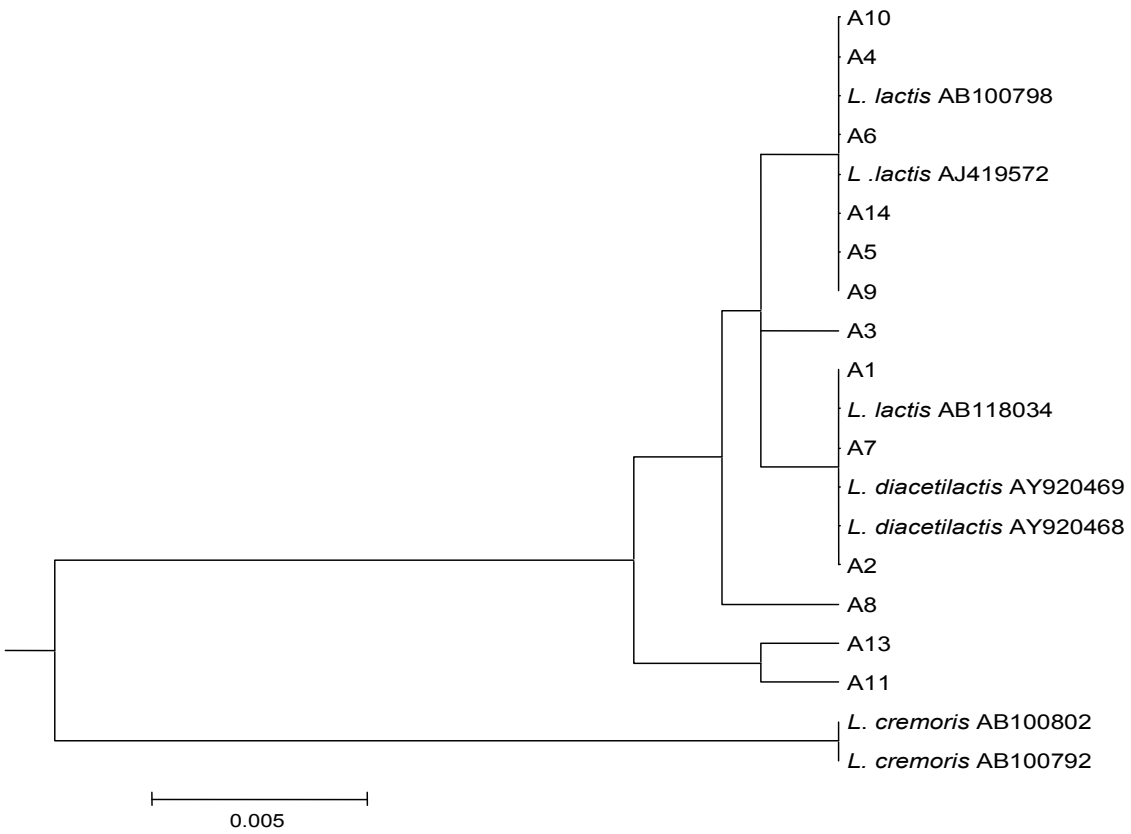
To confirm the taxonomic status were conducted molecular genetic studies. Computer processing of the results of 16S rRNA gene sequencing and comparative analysis of our data with the sequences of the type strains revealed high similarity between them. Based on the phylogenetic distance, reflecting the differences between strains in the number of nucleotide substitutions per 100 base pairs (bp), 16S rRNA homology genes were calculated. All strains show a high degree of DNA homology 98.9-100%) relative to the reference strain of *L. lactis* subsp. *lactis*. The level of genetic similarity (in %%) of all the strains studied in relation to closely related strains *L. lactis* subsp. *cremoris* was 95.4-96.6% (Figure 2).

The nucleotide sequences of the 16S rRNA genes of novel strains were deposited to the GenBank database under following accession numbers: DQ255952 - strain MGU and DQ255954 - strain 194.

Antimicrobial activity testing

Only four of the selected strains expressed a broad spectrum of activity against pathogens: *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella* and fungi of *Aspergillus, Fusarium, Penicillium* genera, as well as against yeasts *Rhodotorula aurantiaca* and *Candida guilliermondii*.

The results of our study of the spectra of antibiotic activity of culture liquids of the strains of *L. lactis* subsp. *lactis*, grown in the fermentation medium, are summarized in Table 3. The strain MSU suppressed the growth of Gram-positive bacilli and micrococci in a manner similar to that of nisin. The strain 194 exhibited



Isolated strains A: 1 - 109; 2 – 129; 3 - 116; 4 – 105; 5 – strain MSU; 6 - 95; 7 - 116; 8 – 122; 9 – 220; 10 – 112; 11 – 122; 13 – 194; 14 – 805.
Reference strains: *L. lactis* ssp. *lactis:* AB100798, AJ419572, AB118034;
L. lactis bv. *diacetylactis:* AY920469, AY9204684; *L. cremoris:* AB100802 and AB100792.

Figure 2: Relationships of new isolated lactococci strains on the base of gene sequence of 16S rRNA.

the highest antibiotic activity and exerted broad-spectrum of antibacterial and fungicidal effects, which has not been reported so far for this specie. The study of the antimicrobial spectrum of action showed that the strains suppressed the growth of Gram-positive bacteria, including *Bacillus coagulans*, *Staphylococcus aureus*; Gram-negative, such as: *Alcaligenes faecalis, Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and also possessed fungicidal action - suppressed the growth of microscopic fungi: *Fusarium oxysporum, Penicillium chryzogenum, Aspergillus niger,* what is rare biological property for the natural strains of lactococci. The strain 194 was more effective against *Rhodotorula aurantiaca* and *Candida guilliermondii*.

It has been revealed, that the strain 194 produced antibiotic complex which differed from nizin.

The strain 194 had higher antimicrobial productivity, up 3600 IU/ml (with test-culture *B. coagulans*) as compared with strain MSU (2000 IU/ml). The inhibition activity against Gram-negative bacteria consisted of 370 U/ml (was detected with chloramphenicol), and its antifungal activity (with nistatin as a standard and test-culture .*A. niger*) was 1700 u/ml. The strain MSU did not inhibited the growth of Gram-negative bacteria and fungi.

Sensitivity to antibiotics and other probiotic properties

Accordingly to the results of the study of the sensitivity of isolated strains to antibiotics, they were sensitive to: ampicillin, erythromycin, tetracycline, lincomycin, and to a lesser degree to aminoglucoside antibiotics - kanamycin, streptomycin, to the macrolactam antibiotic - rifampicin, but they are resistant to neomycin and oleandomycin.

In our experiments isolated lactococci were studied for its probiotic properties. The food entering the stomach is exposed to the gastric juice. Pure gastric juice has hydrochloric acid concentration of about 0.3-0.5%, this corresponds to a pH of 1 to 3 in the healthy stomach. It was revealed that addition of 0.2% of hydrochloric acid to the cultural medium the growth rates were reduced by 30% to 49% after 3 hours of incubation. But the strain is resistant to high concentrations of hydrochloric acid (0.3%-0.5%). The survival rate of strain 194 was 96% after 1 h of exposure, 94% - after 2 h and after 3 h - 92%.

In the investigations on the influence of culture broth (lived lactococci with metabolites) of strain 194 on symptoms of chronic dermatitis on model mice CBRB-Rb (8,17) 1Iem during two weeks of feeding (used daily as food additive and twice application on the skin of the back) showed the degree of ulceration and the square of damaged skin of the back of treated mice decreased by 21% after one week of culture application and the weight of the animals increased (by 4.5%) to the end of experiment. Thus, we showed that use of 194 strain as food additive clearly reduced the severity of dermatitis symptoms.

Conclusion

Screening for the effective strains from raw milk of Buryatia in accordance with its geographical and natural characteristics of this

Test	Strains								
	119	222	229	116	205	115	194	МГУ	Nisaplin 3000 IU/ml
	Diameter of inhibition zone, mm								
Bacillus mycoides	20.0	10.0	16.0	19.5	16.0	11.0	**24.0**	15.0	17.0
Bacillus subtilis	12.0	10.0	15.0	22.0	20.0	11.0	**24.0**	16.0	18.0
Bacillus coagulans	17.0	8.0	15.0	18.0	18.0	20.0	**23.0**	18.0	21.0
Bacillus cereus	18.5	10.0	14.0	21.0	19.0	15.0	**12.0**	16.0	18.0
Micrococcus luteus	21.5	13.0	20.0	22.5	16.5	21.5	**19.0**	19.0	25.0
Staphylococcus aureus	16.0	0	16.0	20.0	17.0	17.0	**16.0**	12.0	15.0
Alcaligenes faecalis	0	9.0	10.0	12.5	12.5	15.0	**12.0**	0	0
Escherichia coli	0	11.0	0	15.0	14.0	19.0	**12.0**	0	0
Proteus vulgaris	0	9.0	0	16.5	16.0	14.0	**16.0**	0	0
Pseudomonas aeruginosa	0	0	0	16.5	15.5	12.0	**12.0**	0	0
Fusarium oxysporum	0	0	0	16.5	10.0	12.0	**10.0**	0	0
Penicillium chryzogenum	0	0	0	17.5	10.5	12.0	**16.0**	0	0
Aspergillus niger	0	0	0	21.0	10.0	14.0	**15.0**	0	0
Rhodotorula aurantiaca	0	0	0	19.5	10.0	13.0	**14.0**	0	0
Candida guellermondii	0	0	0	11.0	12.0	10.0	**16.0**	0	0

Table 3: Antimicrobial action of isolated strains of *Lactococcus lactis* ssp. *lactis* in comparison with Nisaplin.

North Asiatic region of lake Baikal, revealed the isolation of new lactococci cultures. This region includes a wide variety of climatic and ecological niches, thus creating conditions for a variety of new LAB with their unique properties. Thus, our studies are of great scientific and practical interest for the operation. On the basis of morphological, cultural, physiological, biochemical properties and accordingly to 16S rRNA gene sequence of isolates they were identified as *Lactococcus lactis* subsp. *lactis*. The most promising strain was 194 (accession number in GenBank is DQ255954), which synthesized antibiotic complex with a wide spectrum of antimicrobial activity, effective against pathogens. This strain have "GRAS" status. Thus, the properties of these strains with the wide spectrum of bactericidal and fungicidal action to the pathogens, the absence of toxicity and medical effect in a mouse model of chronic dermatitis enable to recommend the strain *L. lactis* ssp. *lactis* 194 for using as probiotic culture or as potential perspective biopreservative for preventing fungal spoilage of foodstuffs and edible raw materials with probiotic effect.

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