FORMATION AND STUDY OF SYMBIOTIC CONSORTIUM OF LACTOBACILLI TO RECEIVE A DIRECT APPLICATION STARTER

S. A. Sukhikh[a,*], V. Yu. Krumlikov[a], A. O. Evsukova[b], and L. K. Asyakina[a]

[a] Kemerovo Institute of Food Science and Technology (University), Stroiteley blvd. 47, Kemerovo, 650056, Russian Federation

[b] All-Russia Scientific Research Institute of Technology of Canning, Shkol'naya Str. 78, Moscow region, Vidnoe, 142703, Russian Federation

* e-mail: stas-asp@mail.ru

Received April 10, 2017; Accepted in revised form May 30, 2017; Published June 29, 2017

Abstract: One of the ways of formation of innovative products is the design of new types of direct application starters on the basis of viable microorganisms for their further use in the dairy industry. The purpose of the study was the isolation of lactobacilli activators of lactic fermentation made of national Kazakh drinks (ayran, koumiss, chegen and kurunga) for the formation of symbiotic consortium to receive a direct application starter. The authors have isolated and studied the following strains of microorganisms: *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus thermophiles*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. lactis biovar diacetilactis*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Pediococcus damnosus*, *Pediococcus acidilactici*, *Lactobacillus gallinarum*. Cultural, morphological and physiological and biochemical properties have been studied, the antibiotic resistance, antagonistic activity and biocompatibility of the isolated microorganisms have been determined. It has been established that the isolated microorganisms have a stick-like shape of 0.6 x 3.5 to 6.0 x 0.7 microns in size characteristic of the sort *Lactobacillus bacteria*, and are Gram-positive and nonspore-forming. Mobility was only observed in the strains *Lactococcus lactis subsp. lactis biovar diacetilactis*, *Pediococcus damnosus* and *Pediococcus acidilactici*. The strains differed considerably from each other by the consumption of other carbohydrates, however all the studied strains ferment ribose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetyl-glucosamin, amygdalin, aesculin, salicin, cellobiose, maltose, lactose, D-sucrose which is characteristic of the representatives of the sort *Lactobacillus*. All the studied strains are sensitive to the effect of β-laktam antibiotics except *Streptococcus thermophilus*. The analysis of biocompatibility has shown that full compatibility is only characteristic of four strains – *Lactobacillus gallinarum* (kurunga), *Streptococcus thermophilus* (koumiss), *Lactococcus lactis subsp. lactis* (ayran) and *Pediococcus damnosus* (chegen). To create a symbiotic consortium such strains of microorganisms as *Lactobacillus gallinarum, Streptococcus thermophilus, Lactococcus lactis subsp. lactis and Pediococcus damnosus* and optimum conditions of cultivation have been chosen. The maximum growth of microorganisms has been noted in the following culture medium: proteose-peptone – 5.00; meat extract – 10.00; yeast extract – 5.00; glucose – 20.00; Tween-80 – 1.00; ammonium citrate – 1.00; manganese sulfate – 0.05; sodium hydrophosphate – 1.00; agar-agar – 15.00; methyl rosaniline – 0.0002; casein hydrolyzate – 12.50; papain digest of soy flour 2.50; sodium chloride – 3.00; sodium citrate – 0.50; sodium sulfite – 0.20; L-cystine – 0.05; sodium azide – 0.10; potassium phosphate dibasic – 0.50; ammonium citrate dibasic – 0.80.

Keywords: Consortium of microorganisms, lactobacilli, dairy products, microorganism isolation, direct application starter, antagonistic properties, antibiotic resistance, identification of microorganisms, symbiotic systems

DOI 10.21179/2308-4057-2017-1-51-62 *Foods and Raw Materials*, 2017, vol. 5, no. 1, pp. 51–62.

INTRODUCTION

The problems of production and consumption of dairy products become more and more relevant and increasingly dependent on the general tendencies of development of the world food market [1]. Changes in the development of dairy business which are already determined by the processes of globalization of the world economy, the changes of the social food patterns of the population which are reflected in the structure of agrofood markets, the growth of level of information and technical support, the deterioration in the ecological situation and the achievements of world science in the field [1, 2] can already be observed in practice.

According to experts, only 8-12% of the health of a nation depend on the health system while the percentage of influence on the health of social and economic conditions and mode of living is 52–55%, at the same time one of the main components is the food factor [3, 4].

Improper feeding is the frequent reason of the development of malfunction of a lot of organs and

systems. It is possible to influence metabolism and the adaptive and compensatory abilities of an organism by changing feeding habits. Now the developments of healthy food products that meet the evidence-based recommendations about the balanced diet of the population [4] are being performed. The main orientation of these studies is the improvement of metabolism and the increase in the immune properties of an organism by the correction of the protein, fat and carbohydrate component of the product.

Sour-milk products make a sizable group of healthy food products. The useful properties of sour-milk products are, first of all, that they improve metabolism, stimulate the exudation of gastrointestinal juice and are the source of highly digestible calcium. The presence of the microorganisms in their structure capable to survive in intestines and to supress putrefactive microflora provides the inhibition of putrefactive processes and the termination of formation of poisonous proteolysis products flowing into human blood [5, 6].

Starters are pure cultures or the mix of pure cultures of the lactic bacteria added into milk to obtain high-quality sour-milk products by means of lactic and spirit fermentation.

Starters consisting of different strains and often of different types and sorts of microorganisms, as well, are applied to produce sour-milk products [7]. It allows to improve the starter resistant to adverse effects [8].

Starters are usually prepared on the basis of monocultures or of the mechanical mix of several strains. The most often applied lactobacilli which are the causative agents of lactic fermentation are: *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus, Lactobacillus ptantarum*., *Lactobacillus brevis* and *Lactobacillus helveticus* [9, 10].

However such starter cultures are often insufficiently effective. The abstract concepts allow to believe that it is preferable to use symbiotic cultures of microorganisms. The elaboration of combined direct starters is due to a new approach – the creation of the artificial symbiotic systems that have a wide range of necessary characteristics [11].

In this regard, the development of the evidence-based approaches based on the study of physiological and biochemical and industrially valuable properties of lactic microorganisms of various groups is relevant when making combined starters of direct application with a high activity.

The purpose of the work is the formation and study of symbiotic consortium of lactobacilli to obtain a direct application starter.

OBJECTS AND METHODS OF STUDY

The strains of microorganisms isolated from national Kazakh drinks became the object of scientific research:

– ayran in accordance with GOST 31702-2013 [12];

– koumiss in accordance with GOST R 52974-2008 [13];

– chegen (imported);

– kurunga in accordance with GOST 10382-85 [14].

The isolation of microorganisms from national Kazakh drinks was performed as follows: 1 ml of each of sour-milk drinks (ayran, kurunga, koumiss and chegen) was taken, culture media were added and plates and test tubes were incubated statically at a temperature of 37°C in a carbon dioxide medium for 1–5 days.

For primary isolation a lactic medium – sterile skim milk; MRS broth, brain heart infusion broth; milk agar – milk with agar of 3% in the ratio of 1 : 1; fish peptone agar and MRS agar were used.

Exhaustive sowings with the use of plates were performed from the test tubes with the visible growth of microorganisms (turbidity or the presence of a dairy clot) and from the combined lawns. The isolated microorganisms were cultivated on the agarized media (fish peptone agar, MRS agar and milk agar), cloned to pure cultures and their genetic identification and the analysis of antimicrobic properties were performed.

The study of phenotypical and morphological properties of microorganisms was performed in a dense medium – MRS agar, g/l: bacto-peptone – 10.0; meat extract – 10.0; yeast extract – 5.0; glucose – 20.0; Tween – 1.0; ammonium citrate – 2.0; sodium acetate – 5.0; sodium hydrophosphate – 2.0; magnesium sulphate 7-water – 0.1; manganese sulfate 5-water – 0.05; agar – 20.0.

In the course of study the diameter of colonies, color, their form, consistence, structure, surface and edge pattern were determined.

The main method to study the morphology of bacteria is the microscopy of the fixed stained medicines. The microscopic examination was performed with the use of the biological microscope Axio Scope A1 ("Carl Zeiss", Germany). The determination of sizes of cells of the studied cultures of microorganisms was performed with the use of an ocular ruler and an object micrometer.

The differentiation of bacteria by the biochemical properties of their cellular wall was performed according to Gram with the use of a set for Gram staining ("Lab-Biomed", Moscow). The essence of the method is that the cellular wall of Gram-positive bacteria tightly fixes gentian violet, does not become colourless after using ethanol and therefore does not perceive additional dye (fuchsin). Gentian violet is easily washed away by ethanol from the cell in Gram-negative microbes, and they are stained by additional dye.

The staining of bacteria spores was performed according to Schäffer-Fulton. The essence of the method is the combined effect of concentrated solution of ethyl green dye and temperature on the low-permeable spore exine with the further decoloration of cytoplasm of a vegetative cell and its contrast finishing dyeing using safranin. Spores are stained in green and cells in red during the microscopic examination.

The existence of flagella is established by the study of cultures for vagility in "the crushed drop" medicines.

The determination of physiological and biochemical properties of strains of microorganisms was performed with the use of microtests – the API-50

test systems (Promix, Russia) or the indicator paper systems which are a set of plastic holes with indicators.

The antibiotic resistance of the considered lactic bacteria was analyzed by means of a set of the disks impregnated with different types of antibiotics. The antibiotic disks were plated on a dense culture medium sowed by the culture of the studied strain and were cultivated for a day at a temperature of 37°C. Then the diameter of inhibition zone around the disks was measured.

The antagonistic activity was studied with the use of the following test strains: *Escherichia coli* B-6954, *Bacillus fastidiosus* B-5651, *Pseudomonas fluorescens* B-3502, *Pseudomonas aeruginosa* ATCC 9027, *Leuconostoc mesenteroides* B-8404, *Candida albicans* ATCC 885-653 and *Staphylococcus aureus* ATCC 25923 based on a dense culture medium using a diffusive method. In this regard, the test strain was plated on an agarized culture medium (fish peptone agar) in the form of lawn and the lawn at the same time was covered with the paper discs impregnated with lactobacillus metabolites (10 µl per disc). A disk with the MRS medium was used as a control sample, and disks with the antibiotics ciprofloxacin and gentamycin from a standard set were used as comparison medicines. The plates were incubated at 37°C for 24 hours. The results were taken into account by the presence and size (in mm) of the transparent zone of non-growth of microorganisms around the disc.

The study of biocompatibility of microorganisms was performed using the method of co-culture on a dense MRS culture medium. The diurnal culture grown up on a liquid culture medium and standardized according to the standard of turbidity was put on the surface of dense culture medium in the form of a bacteriological loop with a diameter of 3 mm. A drop of other tested culture which covered approximately half of the first drop when spreading was put on the surface of the same medium with the same volume after the absorption of the drop, having receded 1–2 mm from its edge.

In the superimposed part the cultures develop with a mutual presence (co-culture) competing with each other. After drying of the second drop the plate with crops was turned upside down and incubated at 28–32°C in the air medium. Each experiment was made with two repetitions, changing the position of cultures (to exclude the influence of sequence of stratification of drops of cultures on the nature of growth in the co-culture zone). The drops of the same culture layered on each other according to the technique described above were the control sample.

The account of results was performed in 24 and 48 h after the beginning of incubation. With the growth inhibition of one of the studied cultures the relations between them were considered as antagonistic, and the cultures themselves were assigned to the category of bioincompatible ones. The cultures were considered biocompatible in case of revelation of full merge of spots or an increase in the growth of the studied strains in the co-culture zone (mutualism, synergysm and satellism). If one of the cultures goes upward in the co-culture zone suppressing the growth of the second culture irrespective of the sequence of their application, such a variant was regarded as poor antagonism. The existence of a distinct oppression (growth inhibition) zone of one culture along the periphery of stain of the other tested culture was regarded as a sign of high antagonism.

The choice of optimum composition of culture medium for the cultivation of consortium of microorganisms was performed by means of variation of the ratio of components and the assessment of viability of consortium of microorganisms. The cultivation was performed at a temperature of 37.0 ± 2.0^{o}C, with the active acidity of 6.8 ± 0.2 and duration of 24 h.

RESULTS AND DISCUSSION

Table 1 provides the pure cultures of microorganisms isolated from dairy drinks.

The morphological properties of microorganisms are studied in stained medicines for the purpose of their differentiation, analyzing their geometry, size, Gram staining, the existence of spores, capsules and flagella [15]. The shape and size of bacteria are influenced by the composition of culture medium and the cultural variability of microorganisms [16].

Table 2 provides the results of study of cultural and morphological properties of the isolated strains of microorganisms.

Table 1. Results of isolation of microorganisms from national Kazakh sour-milk drinks

№	Name of the microorganism	Source of isolation
1	*Lactobacillus delbrueckii subsp. bulgaricus*	Koumiss, ayran
2	*Lactobacillus acidophilus*	Koumiss
3	*Streptococcus thermophilus*	Koumiss, ayran
4	*Lactococcus lactis subsp. lactis*	Ayran
5	*Lactococcus lactis subsp. Lactis biovar diacetilactis*	Ayran
6	*Lactobacillus paracasei*	Chegen
7	*Lactobacillus fermentum*	Chegen
8	*Pediococcus damnosus*	Chegen
9	*Pediococcus acidilactici*	Chegen
10	*Lactobacillus gallinarum*	Kurunga

ISSN 2310-9599. Foods and Raw Materials, 2017, vol. 5, no. 1

Table 2. Characteristic of the cultural and morphological properties of the strains of microorganisms isolated from national Kazakh sour-milk drinks

Parameters	Name of the microbial strain									
	Lactobacillus delbrueckii subsp. bulgaricus	*Lactobacillus acidophilus*	*Streptococcus thermophilus*	*Lactococcus lactis subsp. lactis*	*Lactococcus lactis subsp. lactis biovar diacetilactis*	*Lactobacillus paracasei*	*Lactobacillus fermentum*	*Pediococcus damnosus*	*Pediococcus acidilactici*	*Lactobacillus gallinarum*
Spore formation	–	–	–	–	–	–	–	–	–	–
Vagility	–	–	–	–	+	–	–	+	+	–
Size of bacteria (microns)	0.6 x 3.5	0.9 x 5.3	0.7 x 1.0	0.7 x 4.3	0.6 x 1.0	0.9 x 2.6	0.9 x 1.0	0.8 x 1.0	0.6 x 1.0	6.0 x 0.7
Shape	stick-like	stick-like	spherical	stick-like	spherical	stick-like	stick-like	spherical	spheri cal	stick-like
Edge pattern	fringed	wave-shaped	straight	fringed	straight	wave-shaped	wave-shaped	straight	straight	indistinct
Profile	flat	flat	convex	convex	convex	flat	convex	convex	convex	convex
Surface	smooth	rough	smooth	smooth	rough	smooth	rough	rough	rough	smooth
Color	creamy white	white	white	skin-colored	white	creamy white	skin-colored	skin-colored	skin-colored	grayish
Structure	homogeneous	homogeneous	homogeneo us	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous	homogeneous
Consistence	dense	soft	soft	soft	soft	dense	dense	soft	soft	soft
Transparence	mat	mat	non-transparent	mat	non-transparent	mat	mat	non-transparent	non-transparent	mat
Gram-color staining	+	+	+	+	+	+	+	+	+	+

The results of studies of cultural and morphological properties of the microorganisms isolated from national Kazakh sour-milk drinks testify that the strains *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactococcus lactis subsp. lactisbiovar diacetilactis*, *Lactobacillus paracasei*, *Lactobacillus fermentum* and *Lactobacillus gallinarum* have a stick-like shape from 0.6 x 3.5 to 6.0 x 0.7 microns in size characteristic of bacteria of the sort *Lactobacillus*. The microorganisms are Gram-positive, nonspore-forming, vagility is only observed in the strains *Lactococcus lactis subsp. Lactis biovar diacetilactis*, *Pediococcus damnosus* and *Pediococcus acidilactici*.

Bacteria have a homogeneous structure soft in consistence. The consistence of colonies is only dense in *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus paracasei* and *Lactobacillus fermentum*. The colonies of the studied microorganisms *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Lactococcus lactis subsp. lactis*, *Lactobacillus paracasei*, *Lactobacillus fermentum* and *Lactobacillus gallinarum* are mat, and the colonies of the strains *Streptococcus thermophilus*, *Lactococcus lactis subsp. lactis biovar diacetilactis*, *Pediococcus damnosus* and *Pediococcus acidilactici* are non-transparent.

The profile of colonies is various, too, thus, a flat profile of colonies was observed during the study in *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus* and *Lactobacillus paracasei*, and a convex profile of colonies in *Streptococcus thermophilus*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. lactis biovar diacetilactis*, *Lactobacillus fermentum*, *Pediococcusdamnosus* and *Pediococcus acidilactici*. At the same time the edge pattern in *Lactobacillus delbrueckii subsp. bulgaricus* and *Lactococcus lactis subsp. lactis* is fringed, and it is straight in *Streptococcus thermophilus*. *Lactobacillus gallinarum* is characterized by an indistinct edge of the colony while the edge pattern of the colony in *Lactobacillus acidophilus*, *Lactobacillus paracasei* and *Lactobacillus fermentum* is wave-shaped.

The smooth surface of the colony is observed in five strains of microorganisms (*Lactobacillus delbrueckii subsp. bulgaricus*, *Streptococcus thermophilus*, *Lactococcus lactis subsp. lactis* and *Lactobacillus paracasei* and *Lactobacillus gallinarum*), and it is rough in the others. According to the results of the study, the color of the studied microorganisms varies from white to skin and grayish. The color of the colony in *Lactobacillus gallinarum* is grayish, and the skin color is observed in the strains *Lactococcus lactis subsp. lactis* and *Lactobacillus fermentum*. Colonies of white color are formed by the microorganisms of *Lactobacillus acidophilus* and *Streptococcus thermophilus*.

For the purpose of deeper identification of bacteria a microscopic examination was performed using the inverted microscope AxioVert.A1 (Carl Zeiss, Germany). In the course of microscopic examination the size of particles, the area and number of colonies were determined, besides, photographing of the studied samples the results of which are presented in Fig. 1 was performed.

Then, the physiological and biochemical properties of the strains of microorganisms isolated from national Kazakh sour-milk drinks were studied. The identification of microorganisms only by cultural and morphological features is difficult due to the simplicity of geometry of microorganisms and the same structure of eukaryotic cell [17]. Besides, the physiological and biochemical properties allow to judge about the fitness of microorganisms to various external and internal factors of the environment and to increase the biomass of cultural cells to the maximum.

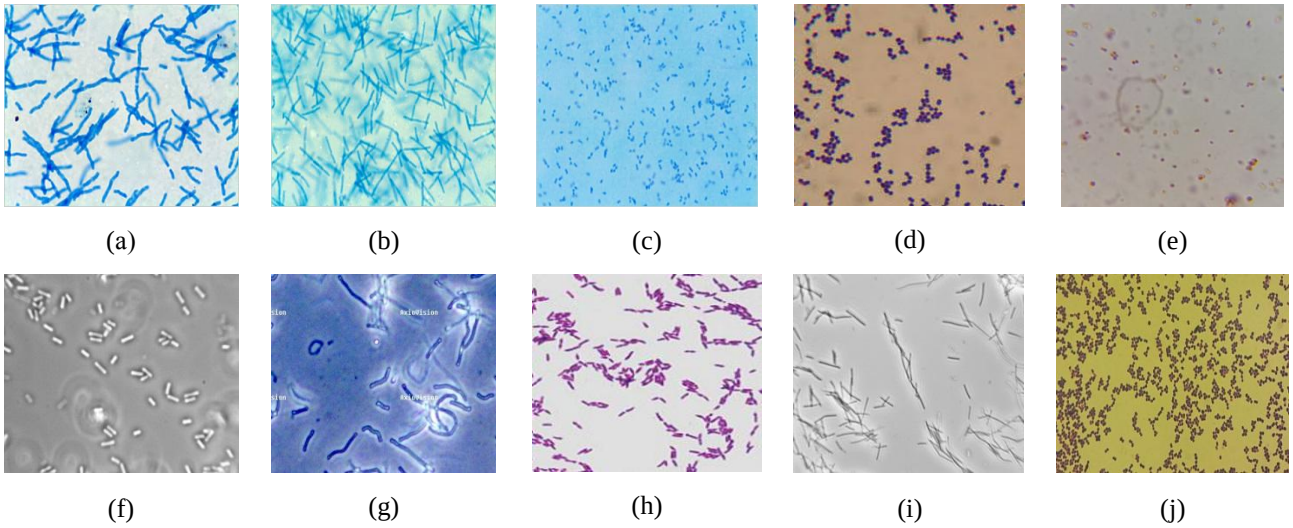

Fig. 1. Micrographs of native dabs of the strains of microorganisms isolated from the national Kazakh sour-milk drinks: (a) *Lactobacillus delbrueckii subsp. bulgaricus*; (b) *Lactobacillus acidophilus*; (c) *Streptococcus thermophilus*; (d) *Lactococcus lactis subsp. lactis biovar diacetilactis*; (e) *Pediococcus damnosus*; (f) *Lactobacillus fermentum;* (g) *Lactobacillus gallinarum*; (h) *Lactococcus lactis subsp. lactis*; (i) *Lactobacillus paracasei;* (j) *Pediococcus acidilactici.*

It is known that microorganisms differ from each other in biochemical properties – the ability to metabolize nutrients, antibiotic substances, various oxygen-containing organic compounds, such as carbohydrates, sugar, alcohols and organic acids, to synthesize enzymes, proteins, amino acids and vitamins [18].

The study of physiological and biochemical properties of bacteria allows to divide them by means of special culture media, to reveal their pertaining to a species and to divide them by strain. It is relevant for pure cultures, lactic and other kinds of bacteria used in the food industry [19].

Using the culture conditions and the composition of the medium, it is possible to determine the distinctive features of a microorganism used in taxonomy, the ecological niche and to find out a possibility of its use to solve practical tasks [17]. At last, the control of growth of cultures is the basis of biotechnological processes. All this emphasizes the importance of study of nature of growth and nutritious requirements of bacterial cultures. Table 3 provides the results of study of the physiological and biochemical properties of the strains isolated from national Kazakh sour-milk drinks.

The methods of study of physiological and biochemical strains of the microorganisms isolated from national Kazakh sour-milk drinks come down to the use of the microtests or the indicator paper systems which are a set of plastic holes with indicators. Some microorganisms can be identified by the ability to form stained compounds [20, 21].

The analysis of data of Table 3 allows to draw a conclusion that all the studied strains ferment ribose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetyl-glucosamin, amygdalin, aesculin, salicin, cellobiose, maltose, lactose and D-sucrose which is characteristic of the representatives of the sort *Lactobacillus*. Strains considerably differ from each other in the consumption of other carbohydrates.

The research of antibiotic resistance of the strains of microorganisms isolated from national Kazakh sour-milk drinks is an important component in the course of complex study of microorganisms. A lot of microorganisms show resistance to the effect of antibiotics for several reasons. First, the resistance to the effect of antibiotic emerges with a change of genome as a result of multiple or single mutations. The acquired resistance descends further from one microorganism to another. As a result, a new population of the microorganisms resistant to the effect of antibiotics emerges. Thus, there is a selection of bacterial cells. Secondly, microorganisms are capable to adapt in adverse conditions, in particular to the effect of antibiotic substances. As a result, the replacement of the metabolitic links of a microorganism, the natural course of which is broken by an antibiotic, with other metabolitic reactions resistant to the effect of the medicine occurs. Thirdly, microorganisms are capable to provide the synthesis of the substances destroying an antibiotic molecule in adverse conditions [22, 23].

Table 4 provides the results of the studies of antibiotic resistance of the strains of microorganisms isolated from national Kazakh sour-milk drinks.

The analysis of the empirical data provided in Table 4 testifies that bacterial cells are sensitive to the effect of the antibiotics that inhibit protein synthesis: laevomycetin, tetracycline and doxycycline (the diameter of the zone of inhibition is at the level from 18.4 to 34.0 mm) and are to a lower degree sensitive to the effect of the aminoglycoside antibiotic substances that impede both protein synthesis and the processes of replication of a cell genome. Neomycin, sizomicin, kanamycin and streptomycin belong to such antibiotics (the width of zone of inhibition is at the level from 0 to 10.5 mm) [24].

These data confirm the belonging of the considered microorganisms to the group of Gram-positive bacteria. Besides, the results presented in Table 4 testify that all the studied strains are sensitive to the effect of β-lactam antibiotics which inhibit the synthesis of a cellular wall (ampicillin), except *Streptococcus thermophilus*.

The study of antagonistic properties of the strains of microorganisms isolated from national Kazakh sour-milk drinks is an important component of scientific research of the process of development of technology of a direct application starter as the antagonistic properties of microorganisms are the mechanism of functioning of microbiocenoses. In case of antagonism, some microorganisms oppress the development of other species, and sometimes they completely destroy it. The antagonistic properties are very widespread among microorganisms. Various populations of microorganisms have developed these or those methods of fight against their competitors. The antagonistic properties may consist in very fast reproduction, at the same time the microorganisms that grow fast push out and slow down the other microorganisms. And they can also relate to the synthesis of specific and nonspecific metabolites which can be formed not only during the cultivation of monocultures, but also in the presence of heterogeneous cultures. At the same time, co-culture provides the amplification of antagonistic properties of bacteria [25]. A method of co-culture on dense culture media was used to define the antagonistic activity of the strains of microorganisms isolated from national Kazakh sour-milk drinks. In the course of work, antagonistic properties of the strains of microorganisms isolated from national Kazakh sour-milk drinks using a diffusive method on a solid culture medium with the use of the following test strains were studied: *Escherichia coli* B-6954, *Bacillus fastidiosus* B-5651, *Pseudomonas fluorescens* B-3502, *Pseudomonas aeruginosa* ATCC 9027, *Leuconostoc mesenteroides* B-8404, *Candida albicans* ATCC 885-653 and *Staphylococcus aureus* ATCC 25923. Table 5 provides the results of the studies of antagonistic properties of the strains of microorganisms isolated from national Kazakh sour-milk drinks.

Table 3. Distinctive physiological and biochemical features of the strains isolated from sour-milk Kazakh products

Name of the substratum	Name of the microbial strain									
	Lactobacillus delbrueckii subsp. bulgaricus	*Lactobacillus paracasei*	*Lactobacillus acidophilus*	*Lactobacillus fermentum*	*Lactococcus lactis subsp. lactis*	*Lactococcus lactis subsp. lactis biovar diacetilactis*	*Pediococcus damnosus*	*Streptococcus thermophilu*	*Pediococcus acidilactici*	*Lactobacillus gallinarum*
Control	–	–	–	–	–	–	–	–	–	–
Glycerin	+	+	+	+	+	+	+	–	–	–
Erythritol	–	–	–	+	–	–	–	–	–	–
D-arabinose	–	–	–	–	–	–	+	+	+	–
L-arabinose	–	+	+	+	+	–	–	+	+	–
D-ribose	+	+	+	+	–	+	–	+	+	+
D-xylose	–	+	–	–	–	–	+	–	–	+
L-xylose	–	–	–	–	–	–	–	–	–	–
D-adonitol	+	–	–	–	–	+	+	–	–	–
Methyl-βD-xylopyranoside	–	–	+	–	–	–	+	–	–	+
D-galactose	+	–	–	–	–	+	–	+	+	+
D-glucose	+	+	–	+	+	+	+	+	+	+
D-fructose	+	+	+	+	–	+	–	–	–	+
D-mannose	+	+	+	+	+	+	+	+	+	+
L-sorbose	–	–	–	–	–	–	–	–	–	–
L-rhamnose	–	–	–	–	–	–	–	–	–	–
Dulcitol	–	–	–	–	–	–	–	–	–	–
Inosite	+	–	–	–	–	+	+	–	–	–
D-mannite	+	+	–	+	–	+	+	+	+	–
D-sorbite	–	+	+	+	–	–	–	–	–	–
Methyl-αD-mannopyranoside	+	–	–	–	–	+	+	–	–	–
Methyl-αD-glucopyranoside	+	+	+	–	+	+	+	+	–	–
N-acetylglucosamin	+	+	+	+	–	+	+	+	+	+
Amygdalin	+	+	+	+	+	+	+	+	–	+
Arbutin	+	+	+	+	+	+	–	+	+	–
Aesculin	+	+	+	+	+	+	+	+	–	+
Salicin	+	+	+	+	+	+	+	+	+	+
D-cellobiose	+	+	+	+	+	+	+	+	–	+
D-maltose	+	+	–	+	+	+	+	+	+	+
D-lactose	+	–	–	–	–	+	–	+	+	+
D-melibiose	+	–	–	–	–	+		–	–	–
D-sucrose	+	+	+	+	+	+	+	+	+	+
D-trehalose	+	+	+	+	+	+	–	–	–	–
Inulin	–	–	+	–	–	–	–	–	–	–
D-melezitose	+	–	–	–	–	+	+	–	–	–
D-raffinose	+	+	+	+	+	+	+	+	–	+
Starch	+	+	+	+	–	+	+	–	–	+
Glycogen	+	+	+	+	–	+	–	–	–	–
Xylitol	–	–	–	–	–	–	–	–	–	+
Gentiobiose	+	+	+	+	+	+	–	+	+	+
D-turanose	+	–	+	–	–	+	+	–	–	–
D-lyxose	–	–	–	–	–	–	+	–	–	–
D-tagatose	–	–	–	–	+	–	–	–	–	–
D-fucose	–	–	–	–	–	–	–	+	+	–
L-fucose	+	+	–	–	–	+	+	–	–	+
D-arabitol	–	–	–	–	–	–	+	–	–	–
L-arabitol	–	–	–	–	–	–	–	–	–	–

Designations: "+" is a positive result; "–" is a negative result.

Table 4. Antibiotic resistance of the strains isolated from sour-milk Kazakh products

Antibiotic	Name of the microbial strain									
	Lactobacillus delbrueckii subsp. bulgaricus	*Lactobacillus paracasei*	*Lactobacillus acidophilus*	*Lactobacillus fermentum*	*Lactococcus lactis subsp. lactis*	*Lactococcus lactis subsp. lactis biovar diacetilactis*	*Pediococcus damnosus*	*Streptococcus thermophilu*	*Pediococcus acidilactici*	*Lactobacillus gallinarum*
Ampicillin	32.0 ± ± 1.9	33.2 ± ± 1.9	19.5 ± ± 1.2	26.5 ± ± 1.6	35.6 ± ± 1.8	29.9 ± ± 1.5	32.2 ± ± 1.6	21.6 ± ± 1.3	0	19.8 ± ± 1.2
Benzylpeni cillin	20.7 ± ± 1.2	18.7 ± ± 1.1	22.5 ± ± 1.3	19.0 ± ± 1.1	17.4 ± ± 1.0	18.5 ± ± 1.1	17.9 ± ± 1.0	22.6 ± ± 1.3	0	24.3 ± ± 1.4
Doxycycline	18.4 ± ± 1.2	22.3 ± ± 1.3	34.0 ± ± 2.0	31.5 ± ± 1.9	0	0	17.6 ± ± 1.0	31.9 ± ± 1.9	35.7 ± ± 2.1	0
Kanamycin	5.0 ± ± 0.3	3.0 ± ± 0.2	0	0	15.6 ± ± 0.8	0	0	0	0	0
Carbeni cillin	14.5 ± ± 0.8	12.4 ± ± 0.7	16.7 ± ± 0.9	13.0 ± ± 0.7	8.6 ± ± 0.5	11.2 ± ± 0.6	18.9 ± ± 1.1	12.3 ± ± 0.7	15.8 ± ± 0.9	9.3 ± ± 0.5
Chloromy-cetin	32.0 ± ± 1.9	28.7 ± ± 1.7	26.4 ± ± 1.5	30.6 ± ± 1.8	37.0 ± ± 1.9	32.0 ± ± 1.6	28.4 ± ± 1.7	31.6 ± ± 1.8	27.8 ± ± 1.4	28.9 ± ± 1.7
Methicillin	15.6 ± ± 0.9	13.5 ± ± 0.8	22.3 ± ± 1.3	21.0 ± ± 1.2	14.5 ± ± 0.8	28.9 ± ± 1.7	12.6 ± ± 0.7	0	0	14.9 ± ± 0.1
Neomycin	7.0 ± ± 0.4	6.0 ± ± 0.3	0	5.0 ± ± 0.3	2.0 ± ± 0.1	0	6.3 ± ± 0.3	0	0	3.4 ± ± 0.2
Oxacilline	9.5 ± ± 0.5	10.7 ± ± 0.6	16.0 ± ± 0.9	14.0 ± ± 0.8	28.5 ± ± 1.4	25.6 ± ± 1.3	8.3 ± ± 0.5	13.2 ± ± 0.8	0	26.8 ± ± 1.6
Oleandomy cin	27.5 ± ± 1.6	22.6 ± ± 1.3	15.4 ± 0.9	24.0 ± ± 1.4	26.9 ± ± 1.6	23.6 ± ± 1.4	22.7 ± ± 1.3	15.8 ± ± 0.5	19.7 ± ± 0.5	24.8 ± 1.6
Ristomycin	11.7 ± ± 0.7	8.5 ± ± 0.5	7.0 ± ± 0.4	9.0 ± ± 0.5	12. 1± ± 0.7	6.8 ± ± 0.4	7.2 ± ± 0.4	7.0 ± ± 0.2	9.2 ± ± 0.2	7.3 ± ± 0.4
Sizomicin	0	0	0	3.0 ± ± 0.1	0	0	0	0	0	2.0 ± ± 0.1
Streptomy cin	2.0 ± ± 0.1	4.5 ± ± 0.2	10.5 ± ± 0.6	6.0 ± ± 0.2	0	9.8 ± ± 0.6	3.6 ± ± 0.2	0	0	2.8 ± ± 0.1
Tetracycline	24.0 ± ± 1.4	26.7 ± ± 1.6	32.0 ± ± 1.9	27.6 ± ± 1.6	0	24.5 ± ± 1.2	19.8 ± ± 1.1	0	34.2 ± ± 1.7	22.7 ± ± 1.3
Cefalexin	32.0 ± ± 1.9	25.0 ± ± 1.5	19.6 ± ± 1.1	21.4 ± ± 1.3	0	0	18.2 ± ± 1.1	21.5 ± ± 1.4	32.9 ± ± 1.6	33.8 ± ± 1.7

Table 5. Antagonistic properties of the strains of microorganisms isolated from national Kazakh sour-milk drinks

Name of the strain	Name of the strain									
	Lactobacillus delbrueckii subsp. bulgaricus	*Lactococcus lactis subsp. lactis*	*Lactobacillus paracasei*	*Pediococcus acidilactici*	*Lactobacillus acidophilus*	*Lactobacillus fermentum*	*Lactococcus lactis subsp. lactis biovar*	*Pediococcus damnosus*	*Streptococcus thermophilus*	*Lactobacillus gallinarum*
Escherichia coli B-6954	0	0	0	0	0	0	0	9	0	9
Bacillus fastidiosus B-5651	7	9	10	7	0	0	0	3	0	8
Pseudomonas fluorescens B-3502	0	6	0	0	0	0	0	0	0	8
Pseudomonas aeruginosa ATCC 9027	8	0	0	0	0	0	0	0	0	0
Leuconostoc mesenteroides B-8404	0	3	1	0	0	0	0	6	0	3
Candida albicans ATCC 885-653	0	3	0	0	0	0	0	3	0	0
Staphylococcus aureus ATCC 25923	0	8	0	7	0	0	0	2	0	6

The analysis of empirical data on the study of antagonistic properties of the strains of microorganisms isolated from national Kazakh sour-milk drinks presented in Table 5 testifies that such natural strains as *Escherichia coli* B-6954, *Bacillus fastidiosus* B-5651, *Pseudomonas fluorescens* B-3502, *Pseudomonas aeruginosa* ATCC 9027, *Leuconostoc mesenteroides* B-8404, *Candida albicans* ATCC 885-653 and *Staphylococcus aureus* ATCC 25923 turned out to be the most sensitive to metabolites of lactic bacteria. Such strains as *Lactobacillus delbrueckii subsp. bulgaricus, Lactococcuslactis subsp. lactis, Pediococcus acidilactici, Lactobacillus paracasei, Pediococcus damnosus and Lactobacillus gallinarum* show the highest antagonistic activity. Thus, for example, the microorganism strain *Lactobacillus delbrueckii subsp. bulgaricus* has an adverse effect on the strains *Bacillus fastidiosus* B-5651 and *Pseudomonas aeruginosa* ATCC 9027; the strain *Lactococcus lactis subsp. lactis* has an adverse effect on the strains *Bacillus fastidiosus* B-5651, *Pseudomonas fluorescens* B-3502, *Leuconostoc mesenteroides* B-8404, *Candida albicans* ATCC 885-653 and *Staphylococcus aureus* ATCC 25923; the strain *Lactobacillus paracasei* shows antimicrobic properties against *Bacillus fastidiosus* B-5651 and *Leuconostoc mesenteroides* B-8404; the strain *Pediococcus acidilactici* has an adverse effect on *Bacillus fastidiosus* B-5651 and *Staphylococcus aureus* ATCC 25923; the microorganism strain *Pediococcus damnosus* shows antimicrobic properties against *Escherichia coli* B-6954, *Bacillus fastidiosus* B-5651, *Leuconostoc mesenteroides* B-8404, *Candida albicans* ATCC 885-653 and *Staphylococcus aureus* ATCC 25923; the microorganism strain *Lactobacillus gallinarum* shows antimicrobic properties against *Escherichia coli* B-6954, *Bacillus fastidiosus* B-5651, *Pseudomonas fluorescens* B-3502, *Leuconostoc mesenteroides* B-8404 and *Staphylococcus aureus* ATCC 25923.

When determining biocompatibility the isolated strains are subdivided into four categories: biocompatible strains, bioincompatible strains, strains with weak antagonism and strains that have a high antagonistic effect. Table 6 provides the results of the study.

Interpreting the results of the study presented in Table 6, we draw a conclusion that full compatibility is only characteristic of four strains – *Lactobacillus gallinarum (kurunga), Streptococcus thermophilus (koumiss), Lactococcus lactis subsp. lactis (ayran) and Pediococcus damnosus (chegen).*

Thus, it was established during the study that of ten strains of microorganisms isolated from national Kazakh sour-milk drinks the most suitable for further studying for the purpose of development of technology of a direct application starter are the microorganisms isolated from kurunga – *Lactobacillus gallinarum*, koumiss – *Streptococcus thermophilus*, ayran – *Lactococcus lactis subsp. lactis* and chegen – *Pediococcus damnosus*.

Table 7 provides the compositions of culture media.

Table 6. Biocompatibility of the strains of microorganisms isolated from national Kazakh sour-milk drinks

Name of the strain	Name of the strain									
	Lactobacillus delbrueckii subsp. bulgaricus	*Lactococcus lactis subsp. lactis*	*Lactobacillus paracasei*	*Pediococcus acidilactici*	*Lactobacillus acidophilus*	*Lactobacillus fermentum*	*Lactococcus lactis subsp. lactis biovar*	*Pediococcus damnosus*	*Streptococcus thermophilus*	*Lactobacillus gallinarum*
Lactobacillus delbrueckii subsp. bulgaricus		+	+	–	+	±	–	+	+	+
Lactococcus lactis subsp. lactis	+		+	–	±	±	–	+	±	+
Lactobacillus paracasei	+	+		–	±	+	–	±	+	+
Pediococcus acidilactici	±	+	–			+	+	+	+	±
Lactobacillus acidophilus	+	±	+	±		+	+	+	+	+
Lactobacillus fermentum	±	+	+	+			+	+	±	+
Lactococcus lactis subsp. lactis biovar	+	+	+	–	±	–		±	+	+
Pediococcus damnosus	–	+	–	±	–	–	+		+	+
Streptococcus thermophilus	–	+	–	–	±	–	+	±		+
Lactobacillus gallinarum	–	+	±	–	±	±	+	+	±	

Notes. "+" stands for biocompatible strains; "–" stands for incompatible strains; "±" stands for partially compatible strains

Table 7. Variants of culture media for the cultivation of consortium of microorganisms

Name of the component	Number of the culture medium depending on the content of components, g/l			
	1	2	3	4
proteose-peptone	10	5	15	–
meat extract	15	10	8	–
yeast extract	10	5	8	5
glucose	15	20	5	20
Tween-80	1	1	1	1
ammonium citrate	2	1	2	1
sodium acetate	5	–	2.5	5
magnesium sulfate	0.1	–	–	0.1
manganic sulfate	0.05	0.05	–	0.05
sodium hydrophosphate	0.5	1.0	1.5	2.0
agar-agar	12.0	15.0	15.0	14.0
methyl rosaniline	0.0001	0.0002	0.0001	–
casein hydrolysate	15.0	12.5	20.0	8.0
papain digest of soy flour	5.0	2.5	–	5.0
sodium chloride	4.0	3.0	1.0	1.0
sodium citrate	0.5	0.5	–	1.0
sodium sulphite	0.1	0.2	0.1	0.2
L-cystine	0.1	0.05	–	0.2
potassium phosphate dibasic	1.5	0.5	–	2.0
sodium azide	0.05	0.1	0.1	0.2
ammonium citrate dibasic	1.2	0.8	1.0	2.0
sodium acetate	5.0	–	1.0	2.5

The analysis of results of the choice of optimum composition of culture medium for the cultivation of symbiotic consortium of the microorganisms provided in Fig. 1 testifies that the maximum viability and activity of the microorganisms which are part of consortium is observed when it is cultivated on culture medium No. 2. The concentration of microorganisms amounts to 28.3 CFU/g after 24 h of cultivation on culture medium No. 2 $\cdot 10^6$ which is 15% higher than the concentration of the microorganisms grown up on culture medium No. 3 and is 32% higher than the concentration of the microorganisms grown up on culture medium No. 4 under equal conditions.

Fig. 2 provides the results of selection of composition of culture medium for the cultivation of consortium of microorganisms.

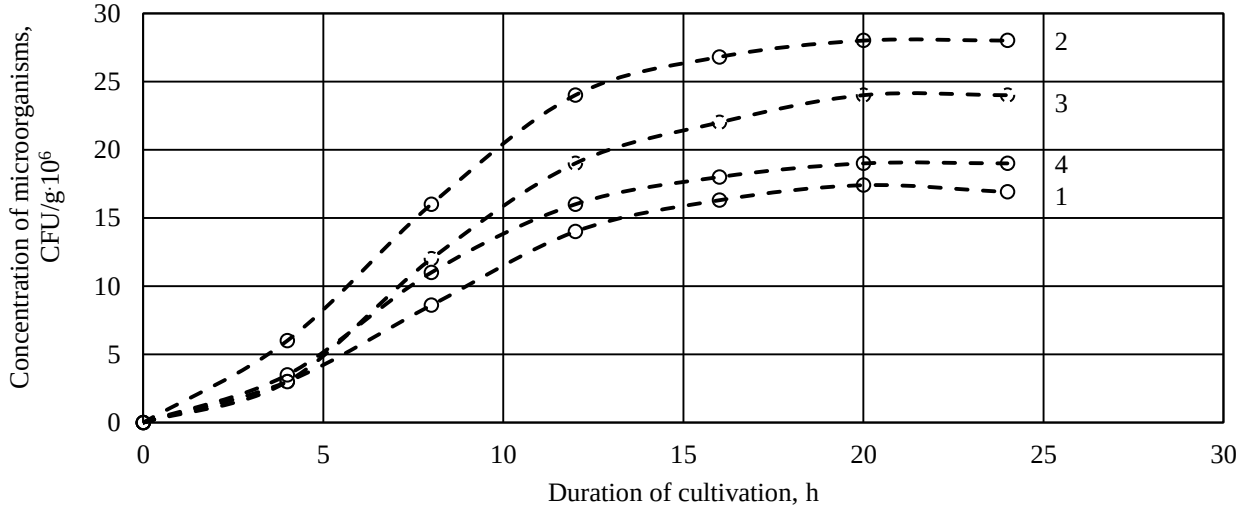

Fig. 2. Influence of composition of culture medium on the viability and activity of symbiotic consortium: (1) culture medium No. 1; (2) culture medium No. 2; (3) culture medium No. 3; (4) culture medium No. 4.

CONCLUSIONS

(1) Such strains of lactobacilli as *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus thermophiles*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. lactis biovar diacetilactis*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Pediococcus damnosus*, *Pediococcus acidilactici* and *Lactobacillus gallinarum* have been isolated from national sour-milk food products: chegen, ayran, koumiss, kurunga.

(2) Cultural, morphological, physiological and biochemical properties have been studied, antibiotic resistance, antagonistic activity and biocompatibility of the microorganisms isolated from national Kazakh drinks have been determined. It has been established that the isolated microorganisms have a stick-like shape of 0.6 x 3.5 to 6.0 x 0.7 microns in size characteristic of bacteria of the sort *Lactobacillus* and are Gram-positive and nonspore-forming. It has been shown that strains considerably differ from each other in the consumption of other carbohydrates. It has been established that all the studied strains are sensitive to the effect of β-lactam antibiotics which inhibit the synthesis of cellular wall (ampicillin), except *Streptococcus thermophilus*. The analysis of biocompatibility has shown that full compatibility is only characteristic of four strains – *Lactobacillus gallinarum (kurunga), Streptococcus thermophilus (koumiss), Lactococcus lactis subsp. lactis (ayran) and Pediococcus damnosus (chegen).*

(3) Such strains of microorganisms as *Lactobacillus gallinarum, Streptococcus thermophilus, Lactococcus lactis subsp. lactis and Pediococcus damnosus* have been chosen to form a symbiotic consortium.

(4) Optimum conditions of cultivation of symbiotic consortium of microorganisms have been selected. It has been established that the growth of microorganisms is the maximum when the composition of culture medium is the following: proteose-peptone – 5.00; meat extract – 10.00; yeast extract – 5.00; glucose – 20.00; Tween-80 – 1.00; ammonium citrate – 1.00; manganese sulfate – 0.05; sodium hydrophosphate – 1.00; agar-agar – 15.00; methyl rosaniline – 0.0002; casein hydrolyzate – 12.50; papain digest of soy flour 2.50; sodium chloride – 3.00; sodium citrate – 0.50; sodium sulfite – 0.20; L-cystine – 0.05; sodium azide – 0.10; potassium phosphate dibasic – 0.50; ammonium citrate dibasic – 0.80.

REFERENCES

1. Özer B.H. and Kirmaci H.A. Functional milks and dairy beverages. *International Journal of Dairy Technology*, 2010, vol. 63, no. 1, pp. 1–15. DOI: 10.1111/j.1471-0307.2009.00547.x.
2. Astakhova L., Babich O., Prosekov A., et al. Short chain fatty acids (SCFA) reprogram gene expression in human malignant epithelial and lymphoid cells. *PLoS ONE*, 2016, vol. 11, no. 7, e0154102. DOI: 10.1371/journal.pone.0154102.
3. Zimina M.I., Sukhih S.A., Babich O.O., Noskova S.Yu., Abrashina A.A., and Prosekov A.Yu. Investigating antibiotic activity of the genus Bacillus strains and properties of their bacteriocins in order to develop next-generation pharmaceuticals. *Foods and Raw Materials*, 2016, vol. 4, no.2, pp. 95–100. DOI: 10.21179/2308-4057-2016-2-92-100.
4. Michaelidou A. and Steijns J. Nutritional and technological aspects of minor bioactive components in milk and whey: Growth factors, vitamins and nucleotides. *International Dairy Journal*, 2006, vol. 16, no. 11, pp. 1421–1426. DOI: 10.1016/j.idairyj.2006.06.018.
5. Side C. *Food Product Development: Based on Experience*. Iowa, Blackwell publishing company, 2008. 199 p.
6. Surkov I.V., Prosekov A.Yu., Ermolaeva E.O., et al. Evaluation and preventing measures of technological risk of food technology. *Modern Applied Science*, 2015, no. 4, pp. 45–52. DOI: http://dx.doi.org/10.5539/mas.v9n4p45.
7. Novoselova M.V. and Prosekov A.Yu. Technological options for the production of lactoferrin. *Food and Raw Materials*, 2016, vol. 4, no. 1, pp. 90–101. DOI: 10.21179/2308-4057-2016-1-90-101.
8. Kinsella J.E. and Morr C.V. Milk proteins: Physicochemical and functional properties. *CRC Critical Reviews in Food Science and Nutrition*, 1984, vol. 21, no. 3, pp. 197–220. DOI: http://dx.doi.org/10.1080/10408398409527401.
9. Dyshlyuk L., Babich O., Belova D., and Prosekov A. Comparative analysis of physical and chemical properties of biodegradable edible films of various compositions. *Journal of food process engineering*, 2017, vol. 40, n/a, e12331. DOI: 10.1111/jfpe.12331.
10. Jakubczak A. and Stachetska M. Health benefits resulting from probiotic bacteria consumption. *Adv. Agrycultyral Science*, 2011, no. 14, pp. 53–63.
11. Juszczyk S. The rational managing rule and the way of improving the effectiveness of milk production. *Zagadn. Ekon. roln.*, 2007, no. 3, pp. 56–71.
12. Korhonen H. and Pihlanto A. Bioactive peptides: Production and functionality. *International Dairy Journal*, 2006, vol. 16, no. 9, pp. 946–956. DOI: https://doi.org/10.1016/j.idairyj.2005.10.012.
13. Prosekov A.Yu. and Ivanova S.A. Providing food security in the existing tendencies of population growth and political and economic instability in the world. *Foods and Raw Materials*, 2016, vol. 4, no. 2, pp. 201–211. DOI: 10.21179/2308-4057-2016-2-201-211.
14. Zamberlin S., Dolencic S.I., Kelava N., and Samarzija D. Probiotic bacterium Lactobacillus rhamnosus: Benefical and a dverse effects on human health. *Milchwissenschaft*, 2012, no. 1, pp. 30–33.
15. Gorska S., Grycko P., Rybka J., et al. Exopolysaccharides of lactic acid bacteria: structure and biosynthesis. *PostepyHig Med Dosw*, 2007, vol. 61, pp. 805–818.

16. *GOST 31702-2013. Ayran. Tekhnicheskie usloviya* [State Standard 31702-2013. Ayran. Technical conditions]. Moscow: Standards Publ., 2014. 17 p.
17. *GOST R 52974-2008. Kumys. Tekhnicheskie usloviya* [State Standard R 52974-2008. Kumis. Technical conditions]. Moscow: Standards Publ., 2010. 15 p.
18. *GOST 10382-85. Konservy molochnye. Produkty kislomolochnye sukhie. Tekhnicheskie usloviya* (State Standard 10382-85. Canned milk. Sour-milk products dry. Technical conditions]. Moscow: Publishing House of Standards, 1987. 25 p.
19. Prosekov A.Yu. and Ostroumov L.A. Innovation management biotechnology of starter cultures. *Food Processing: Techniques and Technology*, 2016, vol. 43, no. 4, pp. 64–69. (In Russian).
20. Robinson R.K. (ed.) *Dairy Microbiology Handbook. The Microbiology of Milk and Milk Products*. USA: John Wiley & Sons, 2002. 765 p.
21. Shishin M.V. and Prosekov A.Yu. Investigation of morphological and antimicrobial properties of intestinal tract microorganisms. *Food Processing: Techniques and Technology*, 2015, vol. 39, no. 4, pp. 131–137. (In Russian).
22. Lewisi M.J. Microbiological issues associated with heat treated milks. *J. Soc. Dairy Technol*., 2002, no. 3, pp. 121–125.
23. Ludikhuyze L., Claeys W., and Hendrickx M. Combined Pressure—temperature Inactivation of Alkaline Phosphatase in Bovine Milk: A Kinetic Study. *Journal of Food Science*, 2000, vol. 65, no. l, pp. 155–160. DOI: 10.1111/j.1365-2621.2000.tb15972.x.
24. Morris G. and Harding S. Polysaccharides, Microbial. *Applied Microbiology: Industrial*, 2009, pp. 482–494.
25. Akalin A.S. and Una G. The influence of milk supplementarion on the microbiological stability and textural characteristics of fermented milk. *Milchwissenschaft*, 2010, no. 3, pp. 291–294.

Please cite this article in press as: Sukhikh S.A., Krumlikov V.Yu., Evsukova A.O., and Asyakina L.K. Formation and study of symbiotic consortium of lactobacilli to receive a direct application starter. *Foods and Raw Materials*, 2017, vol. 5, no. 1, pp. 51–62. DOI: 10.21179/2308-4057-2017-1-51-62.