AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

The Characteristics of Lactic Acid Bacteria Isolated in North Ossetia-Alania

Boris Georgievich Tsugkiev, Ruslan Gelbertovich Kabisov, Andrey Georgievich Petrukovich, Ella Viktorovna Ramonova, Valentina Batyrbekovna Tsugkieva, Eteri Illarionovna Rekhviashvilli

Gorsky State Agrarian University. 37 Kirov Street, Vladikavkaz, 362040, The Republic of North Osetia-Alania,

ARTICLE INFO

Article history:
Received 25 May 2014
Received in revised form
12 June 2014
Accepted 25 June 2014
Available online 15 July 2014

Keywords:
Lactic acid microorganisms, strains, antagonism, technological properties, the isolation of pure cultures.

ABSTRACT

The lactic acid bacteria are widely spread in the environment, in the human and animal bodies. Generally, they play very important role in their habitats. So, the following issues are discussed in this article: the isolation of pure cultures of lactic acid microorganisms from various natural substrates, the establishing of their species, the selection of probiotic cultures with high and stable potential, and the study of their biological characteristics and technological properties. The antagonism of 13 strains of lactic acid microorganisms isolated in the Republic of North Ossetia-Alania in comparison with 9 members of pathogenic and conditionally pathogenic microflora was determined. The new bacterial sourdough, as well as the probiotic and functional food products based on the studied strains of lactic acid bacteria were obtained. The research provides a significant contribution to the use of direct application sourdough in the food, agricultural, and biotechnological industries.

To Cite This Article: Boris Georgievich Tsugkiev, Ruslan Gelbertovich Kabisov, Andrey Georgievich Petrukovich, Ella Viktorovna Ramonova, Valentina Batyrbekovna Tsugkieva, Eteri Illarionovna Rekhviashvilli., The Characteristics of Lactic Acid Bacteria Isolated in North Ossetia-Alania. ***Adv. Environ. Biol., 8(13),*** *335-340, 2014*

INTRODUCTION

One of the most promising and demanded direction of microbiological research is the discovery of the new strains of lactic acid bacteria. They can be used to create probiotic products and functional foods [1]. Nowadays, the production of new dairy products based on the probiotics is one of the fastest growing segments of the food industry [2].

Some lactic acid bacteria have been detected on the surface of various plants in a small amount: up to 10^2 - 10^4 cells per 1 g of the plant [3]. While the other ones have been detected in greater amounts on the surface of vegetables, cereals, legumes plants: up to 10^4-10^6 cells per 1 g of plant material [4, 5].

The ability of lactic acid bacteria to form the aromatic components out of the amino acid is closely related to their glutamate dehydrogenase activity. This is the main criterion of aroma-forming microorganism's selection. These bacteria can be used as a starter culture or co-culture for the intensification of the product aroma-forming [6].

The antagonism of lactobacilli towards the numerous saprophytic and pathogenic bacteria can be explained not only by their acids production, but also by the emissions of specific antibiotic substances [7]. The antibiotic activity of the combined sourdoughs is significantly higher than the one of the individual cultures [8]. V.I. Ganina determined that the strains detected in the regions of their practical use are better adapted to local raw materials [9].

The particular significance of lactobacilli antagonistic properties study is explained by the implementation of the co-culture method into the technological cycles. This method is promising for the creation of drugs and products based on some lactobacilli strains [10].

Some authors working in North Ossetia-Alania have isolated 148 lactobacillus strains out of 25 different natural sources (plants, raw milk, vegetables, kefir grains, the faeces of farm animals) [11].

The goals of the research were: the isolation of pure cultures of lactic acid bacteria out of various natural substrates, the determination of their species, the study of their biological features and the most important production valuable properties, the creation of fermented functional foods on their basis, as well as production testing of the most active strains.

Corresponding Author: Boris Georgievich Tsugkiev, Gorsky State Agrarian University. 37 Kirov Street, Vladikavkaz, 362040, The Republic of North Osetia-Alania.

Advances in Environmental Biology, 8(13) August 2014, Pages: 335-340

Methodology:

The research materials were 13 strains of lactic acid microorganisms isolated in North Ossetia-Alania and deposited in the Russian National Collection of Industrial Microorganisms, Unitary Enterprise State Research Institute of Genetics (Russian National Collection of Industrial Microorganisms (VKPM)): Enterococcus durans VKPM B-8731, Enterococcus durans VKPM-B 10093, Enterococcus hirae VKPM B-9069, Enterococcus hirae VKPM B-10088, Enterococcus hirae VKPM B-10090, Enterococcus hirae VKPM -B 10091, Streptococcus thermophilus VKPM B-10089, Lactococcus casei VKPM B-8730, Lactococcus paracasei VKPM B-10092, Lactobacterium gallinarum VKPM B-10131, Lactobacterium gallinarum VKPM B-10132, Lactobacterium gallinarum VKPM B-10133, Lactobacterium gallinarum VKPM B-10134.

The species determination of local Lactobacillus strains was done according to L.A. Bannikova's method (1975). The studied parameters of the research are: morphological, cultural, physiological, biochemical, and tinctorial properties: Gram staining; growth in milk at various temperatures; resistance to sodium chloride, methylene blue, phenol and bile; growth in meat-peptone broth; NH3 culture formation from arginine; thermal resistance; the rate of milk coagulation; limit of acid; ability to ferment carbohydrates and alcohols.

The lactic acid bacteria were isolated out of fermented milk and derived products of functional food. Their following properties were determined and studied: the nutritional value – calculation method; organoleptic characteristics – according to GOST R 51331-99; acidity – in accordance with GOST 3624-92; the mass fraction of fat – in accordance with GOST 5867-90; the mass fraction of protein – in accordance with GOST 23327-98; the mass fraction of dry matter – according to GOST 3626-73; microbial count – the serial dilution method and according to GOST 10444.11; antibiotic activity – the agar diffusion method.

RESULTS AND DISCUSSIONS

In order to isolate the pure cultures of lactic acid bacteria, the samples from various natural sources were selected: plants, fermented and fresh vegetables, kefir fungus, faeces piglets, calves and lambs, raw milk, etc.

The milk of 0.5% fat was chosen as a nutrient medium of natural sources for the primary selection of lactic acid bacteria. The isolation of pure cultures of lactic acid bacteria was done according to the conventional method. The pieces of various natural substrate samples were ground in a sterile porcelain mortar, and then they were used for the inoculation in sterile skimmed milk done with a bacteriological loop in test tubes. The purity of cultures was achieved by daily passaging.

In order to isolate the pure cultures of lactic acid bacteria, the plants pieces of leaves, stems, berries, and slices of vegetables were used. The samples out of raw milk and feces of animals were also processed in sterile milk using a bacteriological loop. The cell cultures were incubated at temperatures of 30-37 and 45^{o}C until the clot formation. Transplantation of enriched culture was carried out in sterilized 0.5% fat milk to a smooth dense bunch without breaks or gas bubbles. After each transplantation, the contents of the test tubes were tested for culture purity.

To obtain the isolated colonies, in order to isolate pure cultures of lactobacillus strains, MRS agar was used.

As a result of the physiological and biochemical research, the species affiliation of 13 local lactobacillus strains was established. These physiological and biochemical studies of lactic acid microorganisms' identification are confirmed by certificates of the national patent in the Russian National Collection of Industrial Microorganisms State Research Institute of Genetics, by the 16S RNA analyses, and the conferment of their collection numbers. The strains patents of the Russian Federation were obtained.

The quality, nutritional value, therapeutic and prophylactic properties of fermented milk products directly depend on the used microorganisms' strains. In order to obtain these high quality bacterial products, it is necessary to provide a constant breeding research: isolation and selection of new lactic acid microorganisms' strains with the necessary properties for each type of fermenting product.

Table 1: Characteristics of Lactobacillus strains.

Stains	Isolation source	Morphology	Milk coagulation speed, h	Acidity reserve, ^{o}T
Ent. durans VKPM B-8731	cucumber	cocci	5	112
Ent. durans VKPM B-10093	kefir grains	cocci	9	92
Ent. hirae VKPM B-9069	leaves of yacón	cocci	5	142
Ent. hirae VKPM B-10088	kefir grains	cocci	9	100
Ent. hirae VKPM B-10090	flowers of burclover	cocci	9	201
Ent. hirae VKPM B-10091	kefir grains	cocci	9	95
Str. thermophilus VKPM B-10089	dianthus arenarius	cocci	6	120
L. casei VKPM B-8730	faeces of piglets	bacilli	9	235
L. paracasei VKPM B-10092	sauerkraut	bacilli	9	343
Lbm. gallinarum VKPM B-10131	kefir grains	bacilli	6	300
Lbm. gallinarum VKPM B-10132	dianthus arenarius	bacilli	6	337
Lbm. gallinarum VKPM B-10133	malva pusilla	bacilli	6	357
Lbm. gallinarum VKPM B-10134	kefir grains	bacilli	7	288

As it is shown in the Table 1, the strains of the bacteria being examined have different sources of isolation, morphology, and acid-forming ability.

The speed of a clot formation is very important practical characteristics applied in the production of dairy products. The high activity of acid formation can increase the productivity per time unit. The acid-forming ability of lactic acid microorganisms' strains of the Gorsky State Agrarian University breeding was 5 to 9 hours.

The acidity limit level of fermented milk is important technological characteristics as well, because this factor determines the quality, the conditions, and the storage period of the final product.

The highest acidity limit level was observed for the rod strains – 235-357 ° T, and the lowest one for the cocci (92-142 ° T).

The content of living cells of lactic acid bacteria in dairy products is one of the key indicators of their quality. It was proved that the microbial count of the milk samples fermented by studied bacteria strains is high: 10^9 - 10^{10} CFU / cm^3.

The rate of antagonistic activity and the ability to synthesize antibiotic substance are various for each individual strain; this fact confirms the necessity of further research (provided in different conditions) of probiotic cultures isolated from various substrates.

So we have established the antagonistic activity of lactic acid bacteria strains isolated in North Ossetia-Alania, towards to the pathogenic and conditionally pathogenic microflora.

The conditionally pathogenic and pathogenic bacteria were used as a test cultures: Escherichia coli, Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Proteus vulgaris, Moraxella catarrhalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus mesentericus obtained from bacteriological laboratory of the Federal State Institution of public health "The center of Hygiene and Epidemiology in North Ossetia-Alania."

The antagonist activity of local breeding lactic acid microorganisms' strains towards pathogenic and conditionally pathogenic bacteria was determined by the method of agar diffusion, using a solid nutrient medium – IPA - and a sterile paper cylinder of 1 cm^3. The test microbe sensitivity of the synthesized lactobacilli to the antibiotic substances was determined by its growth delay or death caused by the minimum concentration of the product within 16-18 hours.

The test culture was inoculated into Petri dishes in the form of the continuous lawn on the dried IPA medium using Drygalski spatulas. The paper cylinders were placed tightly (at the same distance from the center of the cup and from one another) on agar with the help of sterile forceps. The cylinders were filled with pure cultures of lactobacillus, and being kept in thermostat at 37^{o}C.

The zone of growth inhibition and the diameter (including the diameter of the cylinder) were measured with a ruler. The presence of the growth of the test organisms indicates their resistance to the particular culture of lactic acid bacteria, meanwhile the lack of growth is an indicator of the high sensitivity of microbes towards antibiotic substances synthesized by certain strains of bacteria.

Thus, the indicator of the low sensitivity is the sterility zone of up to 15 mm; the area of 15-25 mm indicates a sufficient sensitivity of the test microbes to the effects of lactic acid bacteria; while the indicator of a high sensitivity is considered to be a zone of growth inhibition inferior 25 mm. Therefore, the larger th zone of test microbes' growth inhibition is, the higher the sensitivity of lactic acid bacteria against those particular strains is.

The results of antagonistic activity of 13 local lactic acid bacteria strains are presented in Table 2.

The results of Table 2 data analysis point out that the antagonistic activity of pure lactic acid of the local breeding bacteria cultures against the pathogenic and conditionally pathogenic bacteria is quite high. But it varies for different strains.

It was proved that the sterility zone towards Escherichia coli was from 17 to 34 mm; Staphylococcus aureus - from 21 to 35 mm; Staphylococcus saprophyticus - from 12 to 30 mm; Staphylococcus epidermidis - from 12 to 28 mm; Proteus vulgaris - 13 to 29 mm; Moraxella catarrhalis - from 13 to 32 mm; Pseudomonas aeruginosa - from 12 to 30 mm; Klebsiella pneumoniae - from 13 to 18 mm, and Bacillus mesentericus - from 14 to 31 mm.

The research has shown that most antagonistic activity against the majority of pathogenic and conditionally pathogenic strains of lactic acid bacteria is observed for Lbm.gallinarum VKPM B-10131, Lbm.gallinarum VKPM B-10132, Lbm.gallinarum VKPM B-10134, and L.casei VKPM B -8730 (Table 3 and Figure 1).

It should be noted that the studied strains of lactic acid microorganisms have unequal ability to inhibit the growth of pathogenic and conditionally pathogenic microflora. This fact should be taken into consideration while selecting the pure cultures in order to obtain the probiotic products which could be used for the prevention and treatment of certain diseases.

Table 2: Antagonistic activity of lactic acid bacteria to the test microbes (the sterility zone, mm) n = 10.

Lactobacillus strains	Test microbes								
	Escherichia coli	Staphylococcus aureus	Staphylococcus saprophyticus	Staphylococcus epidermidis	Proteus vulgaris	Moraxella catarrhalis	Pseudomonas aeruginosa	Klebsiella pneumoniae	Bacillus mesentericus
Ent. durans VKPM B-8731	24	26	15	20	19	22	23	14	21
Ent. durans VKPM B-10093	26	21	14	14	20	14	21	13	15
Ent. hirae VKPM B-9069	26	24	14	16	18	15	23	14	19
Ent. hirae VKPM B-10088	26	24	15	15	16	15	17	13	18
Ent. hirae VKPM B-10090	20	23	12	15	20	18	16	13	14
Ent. hirae VKPM B-10091	25	25	13	16	13	17	13	13	15
Str. thermophilus VKPM B-10089	17	23	16	18	17	15	25	13	25
L. casei VKPM B-8730	30	29	22	26	23	24	19	18	28
L. paracasei VKPM B-10092	26	22	18	12	15	13	12	13	14
Lbm. gallinarum VKPM B-10131	25	35	24	24	29	23	22	18	31
Lbm. gallinarum VKPM B-10132	28	23	25	28	29	32	26	14	29
Lbm. gallinarum VKPM B-10133	25	21	16	24	17	28	21	15	28
Lbm. gallinarum VKPM B-10134	34	26	30	21	28	24	30	16	30

Table 3: The antagonists of pathogenic and conditionally pathogenic microflora.

Test microbes	The best antagonists strains
Escherichia coli	Lbm.gallinarum VKPM B-10134, L.casei VKPM B-8730
Staphylococcus aureus	Lbm.gallinarum VKPM B-10131, L.casei VKPM B-8730
Staphylococcus saprophyticus	Lbm.gallinarum VKPM B-10134, Lbm.gallinarum VKPM B-10132
Staphylococcus epidermidis	Lbm.gallinarum VKPM B-10132, L.casei VKPM B-8730
Proteus vulgaris	Lbm.gallinarum VKPM B-10131, Lbm.gallinarum VKPM B-10132
Moraxella catarrhalis	Lbm.gallinarum VKPM B-10132, Lbm.gallinarum VKPM B-10133
Pseudomonas aeruginosa	Lbm.gallinarum VKPM B-10132, Lbm.gallinarum VKPM B-10134
Klebsiella pneumoniae	L.casei VKPM B-8730, Lbm.gallinarum VKPM B-10131
Bacillus mesentericus	Lbm.gallinarum VKPM B-10131, Lbm.gallinarum VKPM B-10134

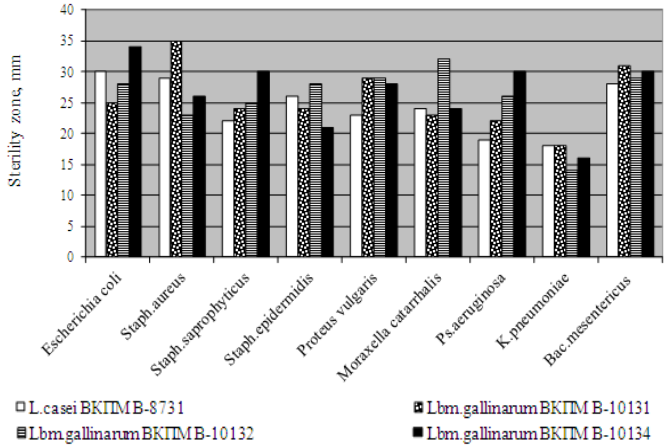

Fig. 1: The antagonists of pathogenic and conditionally pathogenic microflora.

The new probiotics and functional food products with dietary and curative properties based on isolated strains of lactic acid bacteria were obtained at the Research Institute of Biotechnology, Gorsky State Agrarian University. 8 patents for the invention and one positive decision to the further grant patent of the Russian Federation were obtained:

- No 2441910 "The strain of Streptococcus thermophilus VKPM B-10089 is used for the preparation of dairy products";
- No 2449011 "The strain of Lactobacillus gallinarum VKPM B-10131 is used for the preparation of dairy products";
- No 2461617 "The strain of Lactobacillus paracasei is used for the production of fermented beverages";
- No 2461618 "The strain of Lactobacillus gallinarum is used for the production of dairy products";

- No 2461619 "The strain of Lactobacillus gallinarum is used for the production of dairy products";
- No 2505600 "The bacterial sourdough of the pure lactic acid microorganisms cultures is used for the preparation of dairy products";
- No 2505601 "The bacterial sourdough of the pure lactic acid microorganisms cultures is used for the preparation of dairy products";
- No 2480017 "the method of sour cream "Lakomka" preparation;
- Application No 2012140624/10 dated 21.09.2012, the "The method of soured milk production out of buttermilk" (decision to grant a patent dated 02.06.2014).

The environmental pollution, the widespread use of antibiotics and chemicals, stress and other adverse factors lead to disturbances in the composition of the normal intestinal microflora, digestion and metabolism processes, which reduces immune activity of the human body and facilitates the growth of diseases. That is why, the research related to the expansion of the functional products range based on new types of micro-organisms become more and more up to date.

In the laboratories of the Research Institute of Biotechnology, Gorsky State Agrarian University, we have developed the technology of obtaining the fermented dairy products based on the local breeding pure cultures of lactic acid microorganisms' strains and their combinations.

The sour cream "Lakomka" (Russian Federation patent number 2480017). The strains of lactic acid microorganisms Ent.durans, Ent.hirae VKPM, and Str.thermophilus were used. This sour cream has a pronounced flavor with a pleasant taste, dense texture, it is homogeneous and white. The fat content is 20%, the protein mass fraction is 2.5%. and titratable acidity is 70ºT. The sour cream "Lakomka" can be recommended for the prevention and treatment of the gastrointestinal tract diseases.

Buttermilk soured milk. To produce soured milk out of buttermilk, lactic acid bacteria strains Ent.hirae VKPM, Ent.durans VKPM, and Str.thermophilus were used. It is identified that in average the mass fraction of solids in the final product is 9.5%, fat – 0.5%, protein – 3.2%, carbohydrates – 4.7%. The number of microorganisms per 1 ml of product is 10^{10} cells. Energy value is 37 kcal. The main peculiarities of this product are: high biological value and low caloric content. Due to the presence of live probiotic lactobacilli, choline, lecithin, the unsaturated fatty acids, this soured milk can be used for the treatment of acute and chronic gastritis, hepatitis, cholecystitis, pancreatitis, diseases of the nervous system, kidney, gastrointestinal tract; it might be also used while fighting against obesity.

The functional food fermented milk product "Selen +". In order to obtain the fermented milk product "Selen +" the strains of Ent.durans, Ent. hirae, and Str. Thermophilus were used. This product is a white homogeneous mass of creamy consistency with pure, dairy taste and aroma. The average acidity of the product is 80°T, the fat mass fraction is 0.5%, protein – 2.8%, selecsen – 0.025%; the energy value is 35 kcal. Selecsen contained in the product helps to improve the functional state of the cardiovascular system, to normalize cholesterol metabolism, and to increase functional activity of the antioxidant system.

The functional food fermented milk product enriched with iodine. In order to obtain the fermented milk product enriched with iodine, the sourdough of lactobacillus strains Ent.hirae and Ent.durans was used. The obtained product has milky-white color; it is homogeneous; its taste and smell are pure and dairy. The mass fraction of solids in the final product is about 9%, fat – 0.5%, protein - 2.9%, the additives "Iodine-active" – 0.05%; and the acidity is 85 °T. The energy value of the final product is 35 kcal. Due to the high titer of live lactic acid bacteria and the presence of biologically active additives (organic iodine compound incorporated in the milk protein molecule) the product may be used for the prophylaxis and treatment of dysbacteriosis and other diseases of the gastrointestinal tract, as well as iodine deficiency disorders.

The functional food fermented milk product enriched with Spirulina Plantex. In order to obtain the fermented milk product enriched with Spirulina plantex, the sourdough of lactobacillus strains Str.thermophilus, Enterococus hirae, and Ent.durans was used. The mass fraction of solids in the final product, on the average, is 11-12%, fat – 0.7%, protein – 4.5%, Spirulina plantex – 0.3%; the acidity is 80 °T. Due to the presence of high titers of "live" cells of probiotic lactobacilli and spirulina, the product has a wide range of positive effects on the human body: it suppresses conditionally pathogenic and pathogenic microorganisms; it normalizes the metabolism; it improves the resistance against bacterial infections; it increases nonspecific resistance of the body and stimulates the immune system; and it compensates vitamin and mineral deficiencies.

The functional food symbiotic fermented milk product enriched with fiber. In order to produce the fermented milk product enriched with fiber, the lactobacilli strains Str.thermophilus VKMP and Ent.durans were used. The research has proved that the average mass fraction of fat in the product is 0.5% and the protein is 2.9%; the acidity is 70ºT. The energy value of the final product is 35 kcal. The microcrystalline cellulose (MCC), when being in the gastrointestinal tract, forms a stable colloidal dispersion or gel. Its particles adsorb the components of the decay products and toxins. The use of MCC inhibits the absorption of cholesterol into the bloodstream; it activates secret and motor function of small and large intestines, causing the feeling of satiety and suppresses appetite. Intestinal microflora while processing fiber, synthesizes PP, C, and B group vitamins.

The functional food symbiotic fermented milk product enriched with lactulose. This dairy product is based on a pure culture of Lbm.gallinarum. The average acidity of the product is up to 80 ° T; the mass fraction of fat is 0.5%, protein – 2.6%, lactulose – 3%. The energy value of the final product is 35 kcal. This product stimulates proliferation of lactic acid bacteria as well as the intestinal peristalsis, it provides laxative and hypoazotemic effects.

It is important to emphasize that all the developed products are natural ones. They are characterized by pronounced prophylactic properties. The products contain a high number of "live" cells: up to 10^{10} CFU/cm^3. These products can be used for the prevention of the gastrointestinal tract diseases. They can also provide the positive impacts while fighting against the obesity, having heart, liver, and kidneys diseases.

The search of new industry-valuable microorganisms' cultures, their multiple, symbiotic combinations, improvement of the technological processes, the introduction of new sources of raw materials being a dresser, contributes to a considerable expansion of the food and dairy products range, as well as to a fuller utilization of the dairy industry wastes.

Practical application of probiotics has been provided at: the Open Joint-Stock Company Poultry Farm "Mikhaylovskaya", the Republic of North Ossetia-Alania; Poultry Farm "Ingushetia", the Republic of Ingushetia; the Limited Liability Company Pig Farm "Kirovskiy", the Republic of North Ossetia-Alania.

The inclusion of the milk fermented by strains of lactic acid bacteria of the Gorsky State Agrarian University breeding into the diet of young pigs allows (by the end of the experiment) to increase: the live weight by 12.4 kg, or 12.3%; the absolute increase in body weight by 12.2 kg or 14.7%; and the average daily gain by 68 g or 14.7%.

The inclusion of the milk fermented by the sourdough of local lactic acid bacteria strains into the diet of broiler chickens allowed, by the end of the experiment, to increase: the live weight by 18.2%; the absolute increase in the body weight by 18.5%; the average daily gain by 18.7%; and the livestock safety by 5%.

The use of lactobacillus strains isolated in North Ossetia-Alania as a probiotic for the animals and birds feeding is economically beneficial. The increase in pork and poultry profitability due to reduction of feed costs and increase in live weight gain is observed.

The practical application of the developed products provides a significant extension of functional food range. It also contributes to the realization of bioresource potential of young pigs and broiler chickens. It increases the economic efficiency of rearing livestock and poultry due to an increase in live weight gain and reduction of deaths caused by intestinal infections.

To summarize, the obtained results prove the feasibility of using the pure lactic acid microorganisms' cultures of local selection and their associations. Having a high antagonistic activity, they can be used for the preparation of bacterial starter cultures, probiotics, and functional food products.

REFERENCES

[1] Soloveva, I.V., A.G. Tochilina and N.A. Novikov, 2010. Study of the biological properties of new strains of the genus Lactobacillus. General Biology. Bulletin of N.I. Lobachevskiy Nizhny Novgorod University, 2(2): 462-468.
[2] Karycheva, O.V., 2007. New cultures for fermented milk products in the range of "Hansen" company. Dairy industry, 11: 28.
[3] Deger, H., 1932. Milchwirtsch. Zeit, 37(37a – 38a): 39.
[4] Allen, L.A., J. Harrison, S.J. Watson and W.S. Ferguson, 1937, J. Agric. Sci., 27. –P.271.
[5] Stark, P. and J.M. Sherman, 1935. J. Bacteriol., 30. –P.639.
[6] Tonous, C. Glutamat dehydrogenase activity: a major criterion for the selection of flavour-producing lactic acid bacteria strains / C. Tonous et al. // Ant. Van Leeuw, 2002. – V. 82. – № 1-4. – P. 271-278.
[7] Rozhkova, T.V., 2006. Russian market of starter cultures. Dairy industry, 3: 23.
[8] Hamagaeva, I.S. and N.V. Mitypova, 2006. Combination starter for fermented milk products. Dairy industry, 9: 35.
[9] Ganina, V.I., N.V. Ananyeva and T.V. Rozhkov, 2005. Integrated approach of domestic starter cultures development for direct inoculation. Dairy industry, 11: 23.
[10] Golovach, T.N., 2004. Experience of co-cultivation of lactobacilli. Microbiological magazine, 6: 23-25.
[11] Tsugkiev, B.G., R.G. Kabisov, A.G. Petrukovich, E.V. Ramonova, A.M. Khoziev and I.B. Tsugkieva, 2012. Practical use of lactic acid bacteria of Gorsky State Agrarian University breeding as a decision of urgent agribusiness issues. News of Gorsky State Agrarian University, Part 3, 49: 470-480.