Описано позитивний вплив пробіотиків на організм людини. Описана здатність пробіотичних мікроорганізмів накопичувати неорганічні форми селену, утворюючи органічні. Охарактеризовано вплив концентрацій селеніту натрію на приріст біомаси лакто- та біфідобактерій. Визначено умови максимального накопичення селену в культурах мікроорганізмів. Досліджено мікробіологічні та органолептичні показники створеної селенвмісної дієтичної добавки

Ключові слова: пробіотики, дієтичні добавки, селеніт натрію, лактобактерії, біфідобактерії, селенопротеїни, оптична густина

Описано положительное воздействие пробиотиков на организм человека. Описана способность пробиотических микроорганизмов накапливать неорганические формы селена, образовывая органические. Охарактеризовано влияние концентраций селенита натрия на прирост биомассы лакто- и бифидобактерий. Определены условия максимального накопления селена в культурах микроорганизмов. Описаны микробиологические и органолептические показатели созданной селенсодержащей добавки

Ключевые слова: функциональные продукты, пробиотики, диетические добавки, селенит натрия, лактобактерии, селенопротеины, оптическая плотность

UDC [579.864+579.873]:546.33'234-021.434:613.292
DOI: 10.15587/1729-4061.2018.120785

DEVELOPMENT OF THE BIOTECHNOLOGY FOR OBTAINING A DIETARY SUPPLEMENT FROM THE SELENIUM-CONTAINING PROBIOTIC CULTURES LACTOBACILLUS ACIDOPHILUS 412/307 AND BIFIDOBACTERIUM BIFIDUM 1

N. Zykova
Postgraduate student
E-mail: zav380@yahoo.com
L. Kaprelyants
Doctor of Technical Sciences, Professor, Head of Department
E-mail: leonid@onaft.edu.ua
A. Petrosyants
PhD, Associate Professor
E-mail: arsenico78@mail.ru
A. Zykov
PhD
Department of processes, equipment, energy management**
E-mail: zav380@yahoo.com
*Department of Biochemistry,
Microbiology and Physiology of Nutrition**
**Odessa National Academy of Food Technologies
Kanatna str., 112, Odessa, Ukraine, 65039

1. Introduction

At present, stresses, unsatisfactory conditions in the environment, disruption of nutrition lead to a decrease in the level of immune protection of people. This is the cause of chronic illnesses as it results in the deterioration of health and decreased labor performance [1].

Recent years have seen a wide popularity of the concept of functional food products, which implies development of theoretical provisions, as well as production, implementation, and consumption of functional foods. The concept of functional food first originated in Japan in the 80s of the twentieth century. The main components of functional products were determined to be nutritional value, pleasant taste, positive physiological effect.

Functional food products are intended for systematic use in the composition of food diet by all age groups of healthy people, which results in the improved state of health [2].

The products of functional purpose include those, which are obtained by increasing or decreasing the percentage of separate components of food: proteins, amino acids, lipids, vitamins, food fibers, micro- and macroelements, oligosaccharides, polyunsaturated fatty acids, alkaloids, isoprenes, cholines, probiotics, antioxidants [1, 2].

One of the components, which can improve useful properties of functional foods is the microelement selenium. The priority pathways in which selenium is received by the human organism are alimentary – 90 % with food, and 10 % – with water [3, 4].

Selenium enters human body in organic form, with the products of plant and animal origin. However, an important problem, in Ukraine and other countries of the world, is the insufficient amount of the microelement selenium that the human body received from food products. The reason may be the use of synthetic fertilizers, unsatisfactory environmental situation, which leads to the acidification of soils and their contamination with industrial waste. As a result, the process of inorganic selenium accumulation by plants becomes more difficult. In the products of plant and animal origin selenium is in the form of selenomethionine and selenocysteine.

When penetrating the human body, the organic forms of selenium are built into proteins replacing methionine and cysteine and performing their functions. Selenium ions activate the redox enzymes glutathione peroxidase, glutathione reductase, cytochromes, participate in moving the electrons from hemoglobin to oxygen, and support the exchange of cysteine [4]. More than half of the total content of selenium in the body is in the form of selenoproteins. Antiviral, anti-apoptotic, antioxidant functions of selenium are associated with the expression of selenium-dependent enzymes. Selenomethionine, selenocysteine are non-toxic and can be consumed at higher-than-daily dosage of the microelement.

Insufficient selenium intake by the human body may cause reduced immune protection level, weakness, skin diseases, ischemic heart disease, hypertension, arrested mental and physical development [5].

Creating food products enriched with organic forms of selenium, as well as dietary supplements based on it, is an important task. The use of such products makes up for the insufficient intake of selenium into the body.

2. Literature review and problem statement

At present, we can distinguish the following groups of functional products in Ukraine: breakfast cereals, dairy products, margarines and soft drinks, specialized foods. One of the important groups of functional foods are products with probiotic properties [2]. Probiotics are important components of the new products of functional purpose and dietary supplements [5].

Among the microorganisms with high probiotic properties most widely used in the food industry are representatives of the genera *Lactobacillus*, *Bifidobacterium*, *Propionibacterium, Streptococcus*. Specifically, the consortium of the genera *Lactobacillus acidophillus DSM 20079 and DSM 20242, Bifidobacterium bifidum DSM 20082, DSM 20215, DSM 20239 and DSM 20456* with the addition of prebiotics serves as a component of dietary supplements [6].

Selenium-containing preparations, being developed now, can be used as dietary supplements, or as basic components to create products of functional purpose. One of such products is the preparation "Selenopropioniks" based on the selenium-containing culture of propionic acid bacteria *Propionibacterium freudenreichii subsp. shermanii KM 186*. There are also preparations based on selenium-enriched yeast, in particular, *Saccharomyces cerevisiae NCYC 1026*, or the beer yeast of strain 8 AM (S.S. Chervona). Yeast have proven to be active accumulators of selenium, due to their high content of protein [7, 8]. The dietary supplements were developed based on SeMet – selenium-containing amino acid. A positive effect of SeMet on the health of laboratory animals, including mice, was reported [9]. Adding selenomethionine to the diet of mice enabled the activation of the immune system by increasing the activity of natural killers and phagocytic activity of macrophages [9].

Creation of selenium-containing dietary supplements with improved functional properties is an important task. In our work, it implies the enrichment of symbiotic culture of probiotics with selenium.

The mechanism of probiotic action involves a probiotic dose of microorganisms finding its way to colon, after passing through the stomach and duodenum. In the parts of the colon the microorganisms-probiotics are attached to epithelial cells – the cells that line the inner surface of the intestine [10–12]. Probiotics feed on nutrients that are found in the large intestine and reproduce there. In the course of their life activity, they produce organic acids that stimulate the work of intestines and antagonistic effect relative to the pathogenic and conditionally pathogenic microorganisms [13]. Microorganisms synthesize vitamins of groups B and K, antibiotic-like substances [14]. They participating in the development of immunoglobulins of groups A1 and A2 and stimulate the phagocytic activity [15–17].

It is known that probiotics are capable of accumulating and biotransforming the inorganic forms of selenium (selenites, selenates) into organic (selenomethionine, selenocysteine) [18]. Characteristic of the organic forms of selenium obtained with the participation of different types of microorganisms is given in Table 1.

Table 1

Biotransformation of selenium by microorganisms

Microorganisms	Form of biotransformed selenium
Lb. casei	SeCys, Se(0)
Lb. plantarum	SeCys
Lb. delbrueckii subsp. bulgaricus	SeCys
Lb. rhamnosus LB3	Se(0)
Lb. fermentum LB7	Se(0)
Lb. bulgaricus	Se(0)
B. animalis 01	SeMet
E. faecium LAB 1	Se(0)
B. animalis 01	SeMet
Lb. acidophilus LA 5	SeCys, Se(0)
Bifidobacterium BB 12	SeCys
Streptococcus thermophilus	Se(0)

Selenium, which is present as SeCys, SeMet in selenoproteins, is the protector against a number of diseases, including cancer, tiroidyn-dependent diseases, inhibits the development of pathogenic microorganisms, exhibits high antioxidant property. In medicine, organic selenium demonstrates high biological activity and low toxicity [19]. The daily need for organic selenium is, on average, 80–200 μg, in contrast to its inorganic forms, the daily need in which is 70–90 μg [20–22].

The process of transformation of inorganic forms of selenium by microorganisms is shown in Fig. 1.

Synthesis of selenocysteine is performed on specialized tRNA, which also include it in the growing peptide chain. The primary and secondary structure of selenocysteine-specific tRNA, tRNASec, differ from those in the standard tRNA in several aspects. Thus, the acceptor region contains 8 pairs of bases in bacteria, and 10 – in eukaryotic cells, and a longer T-loop [23, 24]; in addition, tRNASec is characterized by the replacement of several rather conservative base pairs. First, tRNASec binds to serine using the enzyme of seril-tRNA ligase, however, the formed complex of Ser-tRNASec does not come into translation because it is not recognized by normal translational factors (EF-Tu in bacteria; and eEF1A in eukaryotic cells) [25, 26]. The residue of serine, bound by tRNA, converts into the residue of selenocysteine by the enzyme selenocysteine synthetase.

In this case, there forms a complex of Sec- tRNASec, which specifically binds to the alternative translational factor (SelB or mSelB (or eEFSec), which delivers it directly to the ribosome, translates mRNA for selenoprotein. The specificity of this transport is predetermined by the presence of an additional protein domain (in bacteria, SelB), or an additional sub-unit (SBP2 for eukaryotic mSelB/eEFSec), which binds with the appropriate element of the secondary structure of mRNA, formed by the element SECIS emerges (Kurek E. et al.) [27].

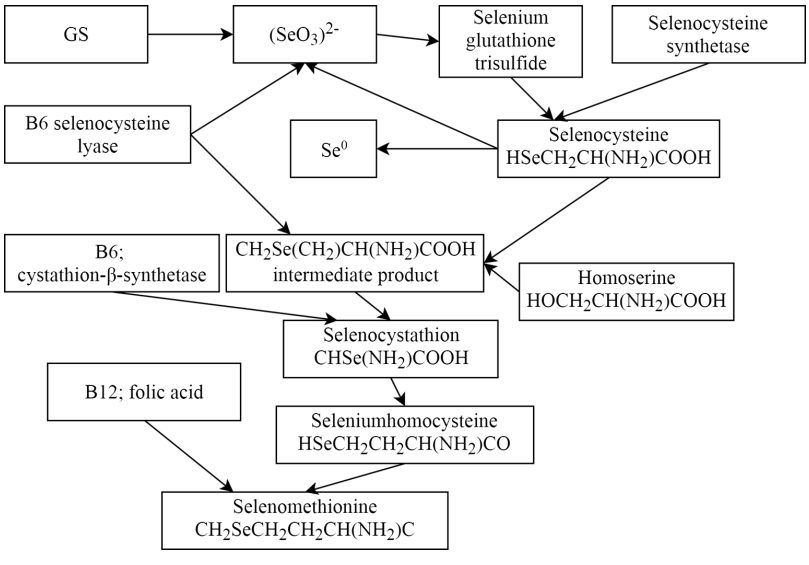

Fig. 1. Generalized scheme of biosynthesis of organic forms of selenium in bacterial cells

Following the biotransformation in the bacterial cell, about 32 % of selenium are found in the membranes, 22 % are part of the cell wall, 52 % are included in the composition of amino acids and soluble proteins of protoplasm. 72 % of them are in the fraction of proteins and amino acids, 1 % is bound with lipids, and 27 % of selenium are in non-organic form [28].

Owing to their properties, probiotics are capable of improving the state of human health and serve as a cheap source of the organic forms of selenium. Microorganisms are the original biomatrices for organic selenium [25–27]. The method of obtaining organic forms of selenium from inorganic sources, using microorganisms, is energy-efficient and environmentally safe.

Therefore, we consider research aimed at the creation of the biotechnology for obtaining the selenium-containing dietary supplement based on the symbiotic culture of probiotic microorganisms to be promising. In this case, it is advisable to choose, as the microorganisms to be studied, the cultures of lacto- and bifidobacteria with high probiotic properties, specifically *Lactobacillus acidophilus 412/307* and *Bifidobacterium bifidum I.* This can be justified by the fact that the lacto- and bifidobacteria are normal inhabitants of the gastrointestinal tract. Bifidobacteria are one of the first microorganisms that penetrate the body of the newborn with mother's milk. They are the first to colonize the gastrointestinal tract, preventing the development of pathogenic and conditionally pathogenic microorganisms. Lactobacilli are characterized by acid resistance that helps them withstand conditions of passing the gastrointestinal tract. Data from the scientific literature indicate bacterial synergy between lacto- and bifidobacteria, which manifests itself in the improved capability for adhesion to GIT. In addition, the joint cultivation of lacto- and bifidobacteria creates favorable conditions for the accumulation of bifidobacteria, by bringing down the redox potential to the values required for their development.

Malnutrition, reduced level of immune protection cause disruption of the microbial balance of the intestine. This may be the reason for lowering the quantitative content of lacto- and bifidobacteria in it. That is why one of the tasks of present research was the development of a dietary supplement from selenium-containing probiotics [5]. Useful action of the developed dietary supplement (DS) is based on a double positive effect exerted on the human body by the metabolites of microorganisms-probiotics and the organic form of selenium. Consuming the developed DS makes it possible to improve the levels of the body's immune protection, as well as the antioxidant protection of body cells, to remove the ions of heavy metals, all of which, taken together, provides a better state of health.

3. The aim and objectives of the study

The aim of present study was to develop a biotechnology of selenium-containing probiotics and a dietary supplement. Adding to the diet of the dietary supplement with a strictly controlled quantitative content of selenium in it would make it possible to compensate for the daily need of the microelement.

To accomplish the aim, the following tasks have been set:

– to select the preparations of inorganic selenium;

– to explore kinetic parameters of the growth of microorganisms and the accumulation of organic form of selenium by the microorganisms-probiotics;

– to devise a technological scheme for obtaining selenium-containing probiotics;

– to examine basic microbiological and physical-chemical indicators of the resulting product.

4. Materials and equipment used in the experiment

We studied the following objects: the probiotic culture *Lactobacillus acdophilus* strain *412/307* borrowed from the Museum of the Department of Biochemistry, Microbiology and Physiology of Nutrition at ONAKhT (Ukraine), and *Bifidobacterium bifidum I* – the preparation "Bifidumbacterin", manufactured by company Biopharma. We used sodium selenite Na_2SeO_3 (TOV NVP Hemel, hch) as a source of selenium. The environment for the cultivation of lactobacilli was chosen to be MRS broth (proteosopepton; meat extract; glucose; yeast extract; Twin-80; sodium acetate; ammonium citrate) and the environment based on cheese whey (cheese whey, milk, sodium acetate; magnesium sulfate; corn extract). For the cultivation of bifidobacteria we have chosen corn-lactose medium (lactose; peptone; sodium citrate; potassium; sodium phosphate; ascorbic acid; corn extract; water).

The examined materials and equipment used in the experiment, the procedure for determining the indicators of the properties of samples are described in detail in paper [29].

5. Results of research into development of a selenium-containing dietary supplement

The initial stage of research implied finding a source of selenium adding which to the cultivation medium of microorganisms would determine its maximum biological availability for the microorganisms. The examples of existing sources of the inorganic forms of selenium, which can be used as a source for their accumulation by microorganisms, are: sodium selenite – Na_2SeO_3, selenic acid – H_2SeO_4, sodium selenite – Na_2SeO_4. We added to the cultivation media of microorganisms the sources of inorganic selenium in the amount of 5 μg/cm^3. The dynamics of accumulation of the organic forms of selenium is shown in Fig. 2.

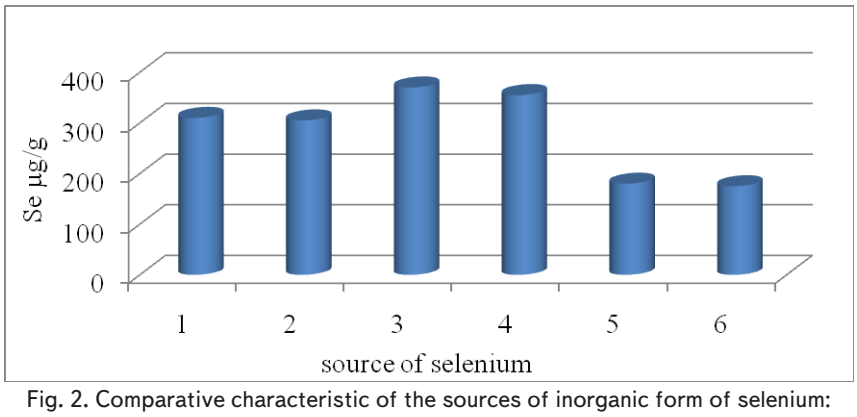

Fig. 2. Comparative characteristic of the sources of inorganic form of selenium: 1 – H_2SeO_4 biotransformed by lactobacilli; 2 – H_2SeO_4 biotransformed by bifidobacteria; 3 – Na_2SeO_3 biotransformed by lactobacilli; 4 – Na_2SeO_3 biotransformed by bifidobacteria; 5 – Na_2SeO_4 biotransformed by lactobacilli; 6 – Na_2SeO_4 biotransformed by bifidobacteria

It was established that sodium selenite is better accumulated both by the culture *Lactobacillus acdophilus* strain *412/307* and the culture *Bifidobacterium bifidum I*.

Sodium selenite was subsequently used in the research and introduced it in the amount from 0.5 μg/cm^3 to 20 μg/cm^3. The environment without added sodium selenite served as control.

Changes in the indicators of optical density in the process of cultivation of lactobacilli were determined at all stages of cultivation, Fig. 3.

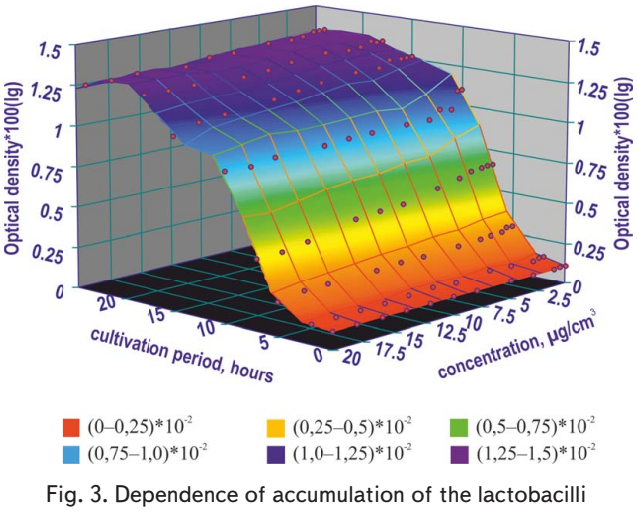

Fig. 3. Dependence of accumulation of the lactobacilli biomass on the concentration of sodium selenite when cultured in MRS medium

We found the relationship between the concentration of Na_2SeO_3 and the accumulation intensity of microorganism biomass. Thus, at the concentrations of Na_2SeO_3 equal to 0.5–5 μg/cm^3 the indicators of OD were not essentially different from control. An increase in the concentration of Na_2SeO_3 to 8–20 μg/cm^3 led to a slowdown in the dynamics of accumulation of lactobacilli biomass compared with control.

The content of selenium in the cells of microorganisms directly depends on its concentration in the environment of cultivation. This is due to the lack of a mechanism that would limit its intake. Constant delivery of selenium to cells leads to its maximal accumulation in the organic form and in the form of nanostructures Se^0. Part of the zero-valent selenium enters the cultivation environment providing for its pink coloration.

Increasing the concentration of selenium causes the acidification of pH of cytoplasm, reduces the protein content in the biomass and increases the concentration of carbohydrates and lipids, which, taken together, causes the stressed state of the cell. The consequences are the disruption of biochemical processes in the cell, which can lead to apoptosis.

Indicators of specific growth rate and duration of generation in the process of cultivation of lactobacilli in MRS medium were determined based on the indicators of optical density, Table 2.

It was established that an increase in the concentration of sodium selenite in the environment of cultivation to 8–20 μg/cm^3 leads to a gradual delay in the indicators of specific growth rate and to the increase in the indicators of generation period.

Table 2

Change in the indicators of specific growth rate and duration of generation during cultivation of lactobacilli in MRS medium (n=3, P≥0.95)

Amount of sodium selenite, μg/cm^3	Cultivation duration, hours							
	0–5 hours	0–10 hours	0–15	0–24	0–5 hours	0–10 hours	0–15 hours	0–24 hours
	Specific growth rate, hour^{-1}				Duration of generation, hours			
0	0.35	0.20	0.132	0.079	1.98	3.50	5.20	8.7
0.5	0.35	0.20	0.132	0.079	1.98	3.50	5.20	8.7
1	0.35	0.20	0.132	0.079	1.98	3.50	5.20	8.70
2	0.36	0.20	0.130	0.079	1.92	3.50	5.30	8.70
3	0.36	0.21	0.139	0.084	1.92	3.30	4.90	8.20
5	0.35	0.18	0.119	0.072	1.98	3.85	5.70	9.50
8	0.34	0.18	0.116	0.070	2.03	3.85	5.90	9.80
10	0.33	0.17	0.110	0.067	2.10	4.0	6.20	10.2
12	0.32	0.17	0.10	0.066	2.15	4.0	6.90	10.4
14	0.32	0.16	0.10	0.066	2.15	4.30	6.90	10.4
16	0.31	0.16	0.10	0.065	2.20	4.30	6.90	10.6
18	0.31	0.16	0.10	0.064	2.20	4.30	6.90	10.7
20	0.31	0.16	0.10	0.064	2.20	4.30	6.90	10.7

Indicators of optical density obtained in the process of cultivation of bifidobacteria in corn-lactose environment with sodium selenite are shown in Fig. 4.

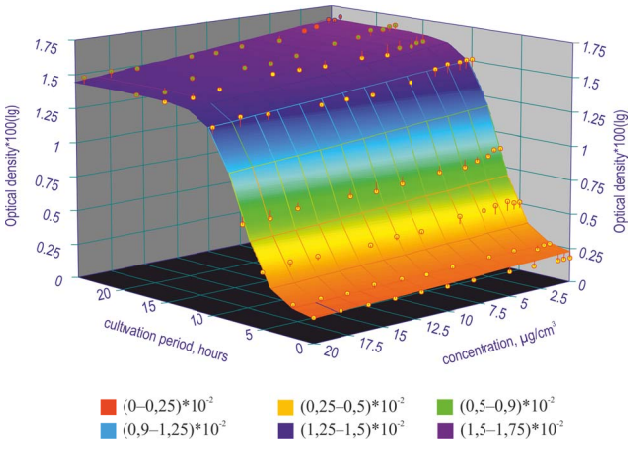

Fig. 4. Dependence of bifidobacteria biomass accumulation on the concentration of sodium selenite when cultured in corn-lactose environment

We determined that over first 2 hours of culturing, OD indicators in all samples remained close to the initial values. From hour 5 to hour 15, we registered a sharp increase in the OD indicators, which in control were equal to 1.58 units, and in the sample containing sodium selenite in the amount of 20 μg/cm^3 – 1.43 units. Dynamic accumulation of bifidobacteria biomass occurred over the indicated period of time. However, we observed strong inhibition of the growth of microorganism biomass under the influence of growing concentrations of sodium selenite, specifically 14–20 μg/cm^3. A less intensive increase in the biomass was registered from hour 15 to hour 24 of cultivation. This is explained by a gradual cultivation environment exhaustion over time and the accumulation of a growing amount of Se^0 and H_2Se^- inside bacterial cells.

Indicators of specific growth rate and duration of bifidobacteria generation are given in Table 3.

We established the relationship between indicators of SGR and DG and the concentration of sodium selenite in the environment of cultivation. Thus, growing concentrations of Na_2SeO_3 cause a delay in SGR and an increase in the DG values, which, accordingly, affects the yield of selenium-containing biomass.

During cultivation process of the biomass of lacto- and bifidobacteria we determined a change in the indicators of colony-forming units (CFU/cm^3) over time. A change in the indicators of CFU/cm^3 of the culture *Lactobacillus acdophilus* over time is shown in Fig. 5.

It was established that an increase in the concentrations of sodium selenite in the environment of cultivation to 8–20 μg/cm^3 leads to a decrease in the dynamics of accumulation of the selenium-containing culture of lactobacilli.

A change in the indicators of CFU/cm^3 of the culture *Bifidobacterium bifidum* over time is shown in Fig. 6.

It was established that the concentrations of sodium selenite in the range of 5–20 μg/cm^3 lead to the gradual suppression of the accumulation of biomass of the examined culture of lactobacilli.

Quantitative content of the selenium accumulated by the examined probiotic microorganisms is given in Table 4.

Table 3

Indicators of specific growth rate and duration of generation (n=3, P≥0.95)

Amount of sodium selenite, μg/cm^3	Cultivation duration, hours							
	0–5 hours	0–10 hours	0–15 hours	0–24 hours	0–5 hours	0–10 hours	0–15 hours	0–24 hours
	Specific growth rate, hour^{-1}				Duration of generation, hours			
0	0.26	0.149	0.10	0.06	2.65	4.63	6.90	11.1
0.5	0.26	0.15	0.09	0.05	2.65	4.60	7.04	11.8
1	0.26	0.15	0.09	0.05	2.65	4.60	7.04	11.8
2	0.26	0.149	0.09	0.05	2.65	4.63	7.04	11.6
3	0.24	0.142	0.09	0.05	2.87	4.85	7.20	12.1
5	0.24	0.142	0.09	0.05	2.87	4.85	7.30	12.3
8	0.23	0.138	0.09	0.05	3.0	5.0	7.60	12.7
10	0.23	0.13	0.08	0.05	3.0	5.10	7.70	12.7
12	0.23	0.135	0.08	0.05	3.0	5.10	7.70	13.0
14	0.23	0.13	0.08	0.05	3.0	5.10	7.90	13.2
16	0.23	0.13	0.08	0.05	3.0	5.10	7.90	13.2
18	0.22	0.125	0.08	0.04	3.10	5.50	8.30	14.0
20	0.22	0.125	0.08	0.04	3.10	5.50	8.40	14.0

The initial factor that characterizes the intensity of biomass accumulation by selenium was the indicators of intensity of luminescence of the examined samples.

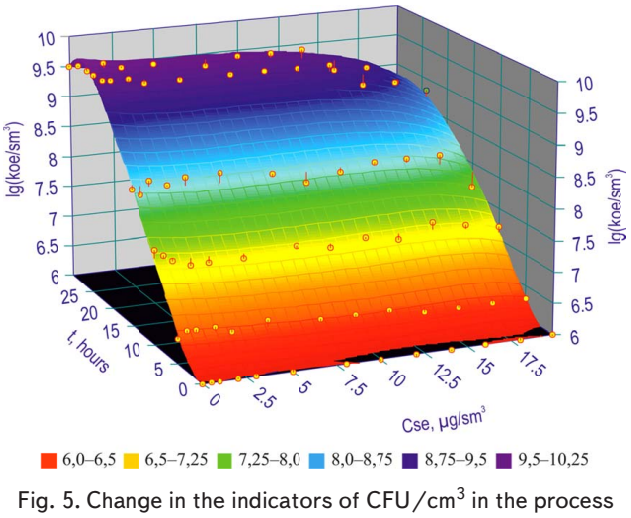

Fig. 5. Change in the indicators of CFU/cm^3 in the process of cultivation of lactobacilli in MRS environment: lg(koe/cm^3) – decimal logarithm of CFU/cm^3, t – period of cultivation, Cse – concentration of the inorganic form of selenium in the environment

Maximum values of the intensity of luminescence for lacto- and bifidobacteria were registered at 20 μg/cm^3. For

lactobacilli – 98, for bifidobacteria – 52. This corresponds to the content of selenium of 4,698 μg/g for lactobacilli, and 3,149 μg/g of selenium for bifidobacteria.

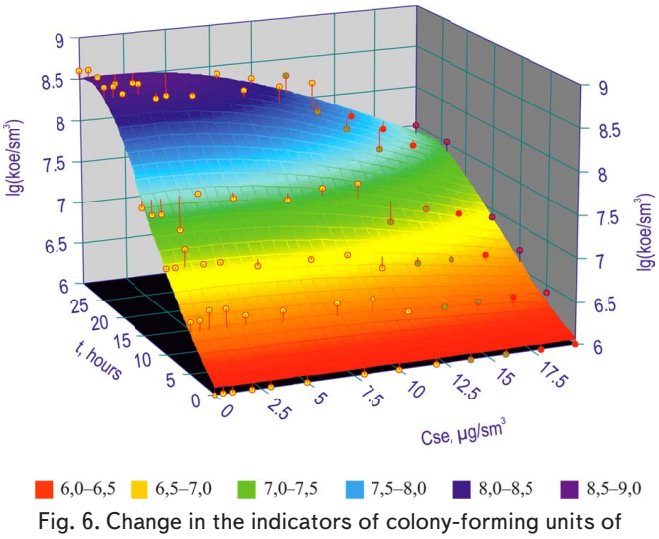

Fig. 6. Change in the indicators of colony-forming units of biomass of bifidobacteria during cultivation in the corn-lactose environment: lg(koe/cm³) – decimal logarithm of CFU/cm³, *t* – duration of cultivation, Cse – concentration of the inorganic form of selenium in the environment

It was noted that an increase in the concentration of selenium led to a decrease in the level of accumulation of biomass of lactobacilli and bifidobacteria. The lowest indicators of CFU/cm³ and, at the same time, the highest indicators of the amount of accumulated selenium were observed in samples containing sodium selenite in the amount of 14–20 μg/cm³.

This phenomenon can be explained by the lack of a mechanism for regulating the level of intake of selenium into the bacterial cell. In this case, the amount of accumulated selenium is directly proportional to the amount of selenium introduced to the environment for the cultivation of microorganisms.

The data obtained allowed us to characterize the relationship between the quantitative content of organic selenium in the examined cultures of lacto- and bifidobacteria, the duration of cultivation, and the content of inorganic selenium in the cultivation environments. The obtained dependences for the cultivation of lacto- and bifidobacteria are shown in Fig. 7, 8.

Table 4

Dynamics of selenium accumulation by the cultures of bacteria (n=3, P≥0.95)

Culture of microorganisms	Concentration of sodium selenite prior to cultivation, μg/cm³	Intensity of luminescence	Content, μg per 1 g of dry biomass
Lactobacillus acidophilus 412/307	Control	3	31.5
	0.5	10	105.0
	1	13	195.0
	2	18	450.0
	5	25	975.0
	10	48	2,116.0
	14	70	3,252.0
	20	98	4,698.0
Bifidobacterium bifidum I	Control	3	31.5
	0.5	8	97.5
	1	10	200.0
	2	14	435.0
	5	20	787.5
	10	38	1,599.7
	14	50	2,322.5
	20	52	3,149.0

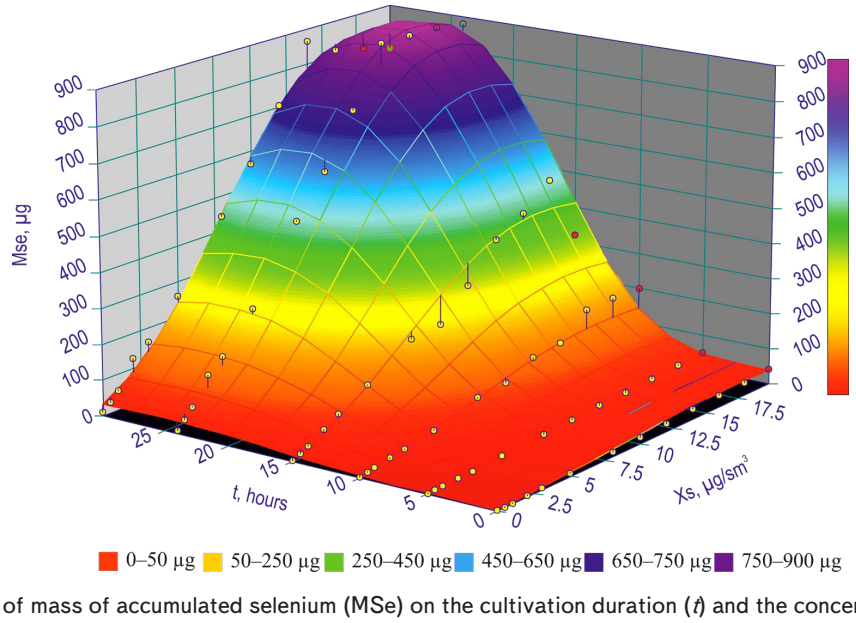

Fig. 7. Dependence of mass of accumulated selenium (MSe) on the cultivation duration (*t*) and the concentration of inorganic selenium (Xse) for the culture of lactobacilli

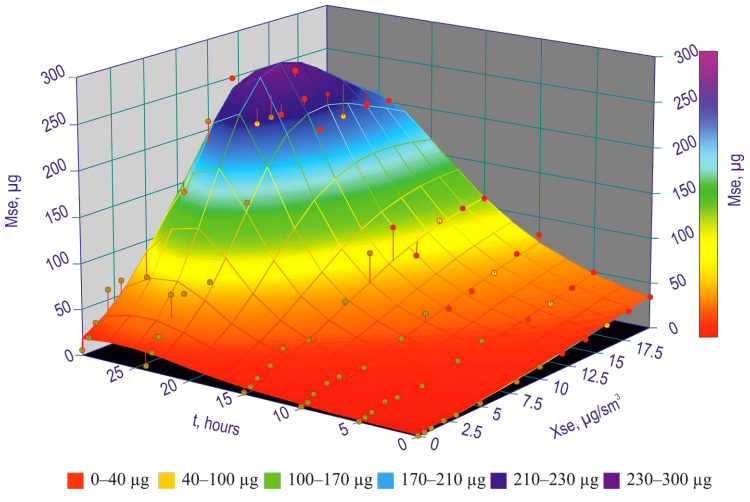

Fig. 8. Dependence of mass of accumulated selenium (*MSe*) on the cultivation duration (*t*) and the concentration of inorganic selenium (*Xse*) for the culture of bifidobacteria

These dependences can be described mathematically using equation 1 – for lactobacilli, and equation 2 – for bifidobacteria.

$$MSe=a+b\cdot Xse+c\cdot Xse^2+d\cdot Xse^3+e\cdot t+f\cdot t^2+g\cdot t^3, \quad (1)$$

$$MSe=\frac{a+b\cdot Xse+c\cdot t+d\cdot t^2}{1+e\cdot Xse+f\cdot Xse^2+g\cdot t+h\cdot t^2}, \quad (2)$$

where *a, b, c, d, e, f, g, h* are the numerical constants.

These equations are continuous and differentiated over the entire period of change in parameters. That is why it is possible to find the values for parameters of the concentration and duration of cultivation of microorganisms that would provide for the largest production of organic selenium employing classic methods of optimization.

For lactobacilli, maximum accumulation of organic selenium in the biomass occurs in 25 hours at the concentration of sodium selenite in the environment of cultivation of 16 µg/cm^3. For bifidobacteria, respectively, in 26 hours at the concentration of sodium selenite of 12 µg/cm^3.

When creating a selenium-containing dietary supplement, it is important to take into consideration the quantitative content of organic selenium in the resulting product. The daily need for a given microelement is 70–90 µg for women and men, respectively. It is known that the organic forms of selenium are much safer for the human body compared to the inorganic forms. That is why it is allowed to consume this form of selenium in the amount of up to 200 µg per day.

The next stage of the research involved creation of the dietary supplement "Selenobifilact", based on the symbiotic selenium-containing culture of microorganisms with probiotic properties. Characteristic of the resulting product is given in Table 5. Using a fluorometric method for studying the quantitative content of organic selenium in the examined biomasses of microorganisms, it was established that adding even 1 µg/cm^3 of the inorganic form of selenium provides the resulting product with the content of organic selenium equal to 200 µg/g of dry biomass.

The finished product contained bifidobacteria in the amount of 1.2×10^8 CFU/cm^3, lactobacilli – 1.0×10^9 CFU/cm^3. Quantitative content of selenium in the finished product was 202.5± ±1 µg/g.

Technological scheme for obtaining the dietary supplement "Selenobifilact" is shown in Fig. 9.

Presented scheme describes the technology of obtaining the biologically active additive "Selenobifilact" based on the symbiotic selenium-containing culture *Lactobacillus acidophillus 412/307* and *Bifidobacterium bifidum I.*

Table 5

Physical-chemical parameters of the preparation "Selenobifilact"

Indicator	Characteristic
Physical appearance	Powder with crystalline or porous mass
Taste and flavor	Specific
Color	Beige
Quantitative content of selenium, µg/g	202.5±1

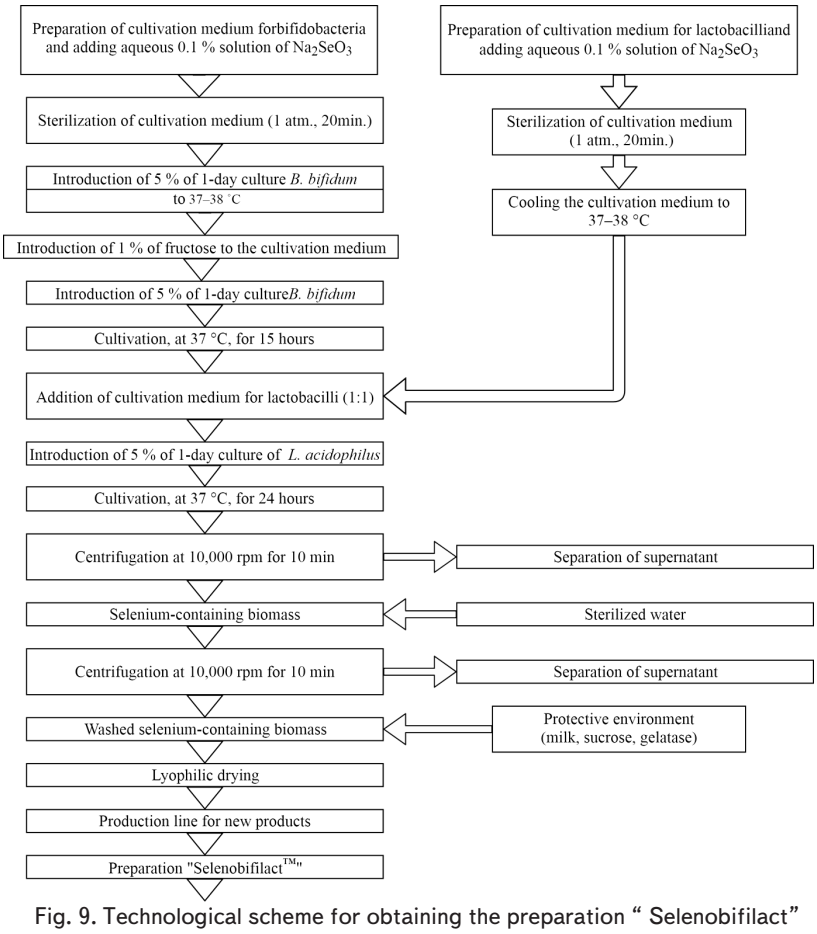

Fig. 9. Technological scheme for obtaining the preparation " Selenobifilact"

6. Discussion of results of obtaining a selenium-containing dietary supplement

The first stage of research involved choosing the optimal source of inorganic selenium, adding which to the environment of cultivation of microorganisms would enable its active biotransformation by microorganisms into organic form. The examples of such inorganic forms of selenium were Na_2SeO_3 – sodium selenite, H_2SeO_4 – selenic acid, Na_2SeO_4 – sodium selenate.

Inorganic forms of selenium were introduced to the environment of cultivation of microorganisms in the amount of 5 μg/cm^3. It was established that the culture of lacto- and bifidobacteria are most active when biotransforming sodium selenite into organic form. By using the fluorometric method, it was established that the quantitative content of organic selenium in the culture *Lactobacillus acidophilus* was 370 μg/g, *Bifidobacterium bifidum* – 355 μg/g.

To test the impact of increasing concentrations of sodium selenite on the dynamics of biomass accumulation of lacto- and bifidobacteria, it was added to the cultivation medium of microorganisms in the amount from 0.5 to 20 μg/cm^3.

Indicators of optical density (OD) make it possible to register a change in the amount of biomass of the examined microorganisms over the duration of cultivation. Samples in the amount of 5 cm^3 were taken to cuvettes at a distance of 1 cm^3, every two hours. It was registered that the sodium selenite concentrations in the amount of 0.5–5 μg/cm^3 had not a significant impact on the process of biomass accumulation of microorganisms (the indicators derived were close to control). Increasing concentrations of Na_2SeO_3 (8–20 μg/cm^3) caused a gradual slowdown in the process of accumulation of biomass of lactobacilli, at all stages of cultivation. The concentrations of Na_2SeO_3 at the level of 0.5–3 μg/cm^3 did not exert any inhibiting effect on the dynamics of accumulation of bifidobacteria biomass. However, the concentrations of sodium selenite at the level of 5–20 μg/cm^3 did lead to inhibiting the process of accumulation of bifidobacteria biomass. The lowest indicators for OD were recorded in the sample containing sodium selenite in the amount of 20 μg/cm^3 (both in the cultures of lactobacilli and bifidobacteria).

Value of the indicators of specific growth rate (SGR) and duration of generation (DG) characterized the rate of accumulation of the examined biomass of microorganisms. It was established that at all stages of cultivation the concentrations of Na_2SeO_3 in the amount of 8–20 μg/cm^3 led to a delay in SGR and increased DG of lactobacilli culture, while the concentrations of Na_2SeO_3 from 5 to 20 μg/cm^3 resulted in a delay of SGR and an increase in DG of bifidobacteria culture.

In parallel with the indicators of OD, DG, and SGR we determined the number of colony-forming units of lacto- and bifidobacteria per 1 cm^3 of the cultivation medium. Indicators for CFU/cm^3 make it possible to register the number of live microorganisms in a culture. It was established that over the first 4 hours there occurred the adaptation of lacto- and bifidobacteria to the cultivation medium. From hour 5 to hour 15 hour, we observed a phase of exponential growth of microorganisms, which was characterized by the rapid accumulation of biomass of selenium-containing microorganisms. The phase of exponential growth was gradually replaced with the stationary phase. The indicators of CFU/cm^3 made it possible to register the process of suppressing the growth of biomass of lactobacilli in the samples with the concentration of sodium selenite of 8–20 μg/cm^3 and 5–20 μg/cm^3.

By using the fluorometric method, we established the relationship between the amount of sodium selenite in the cultivation medium and the number of biotransformed organic selenium in microorganisms. The resulting selenium-containing culture of lacto- and bifidobacteria was separated from cultivation environments (which contained part of undiluted selenium) using the centrifugation method (10 minutes at 10,000 rpm). The resulting selenium-containing biomass was washed with sterilized water from the remnants of undiluted selenium. Next, the biomass was added with a cryoprotective environment (milk, gelatin, sucrose) in the ratio of 1:1, and then it was lyophilically dried. Next, we selected 0.1 g of dry selenium-containing preparation and determined the amount of accumulated selenium. Using indicators of the intensity of luminescence, we determined the content of organic selenium in the dry biomass of lacto- and bifidobacteria. It was established that increasing concentrations of sodium selenite in environments cultivation led to an increase in the amount of organic selenium in microorganisms. Thus, maximum indicators of the quantitative content of organic selenium in the culture of lactobacilli at the level of 4,698 μg/g were registered in the sample containing sodium selenite in the amount of 20 μg/cm^3. The maximum amount of organic selenium for bifidobacteria reached 3,149 μg/g in the sample containing Na_2SeO_3 in the amount of 20 μg/cm^3.

We have created a selenite-containing dietary supplement based on the symbiotic selenium-containing culture of lacto- and bifidobacteria "Selenobifilact". For this purpose, we introduced to the sterile corn-lactose environment a 0.1-% solution of sodium selenite in the amount of 1 μg/cm^3. As a growth stimulant for the culture of bifidobacteria we used fructose (which was introduced in the amount of 1 %). The inoculum of 1-day culture of bifidobacteria was introduced in the amount of 5 %. The cultivation lasted for 15 hours, at 37 °C. Next, we added an environment for the cultivation of lactobacilli in the ratio of 1:1, and introduced inoculum of the 1-day culture of lactobacilli (in the amount of 5 %). The cultivation was carried out at 37 °C. Obtained selenium-containing symbiotic biomass of microorganisms was separated from the cultivation environment using the centrifugation method (10 minutes at 10,000 rpm). The biomass was washed with sterilized water in order to remove the undiluted selenium. Next, we added a cryoprotective environment and lyophilically dried it.

Selenium-containing preparations based on live lacto- and bifidobacteria enriched with selenium could be used in liquid forms and capsules, liquid preparations, yogurts enriched with selenium-containing preparations, suspensions, tablets.

The amount of organic selenium in received DS was 202,5±1 μg/g. The quantitative content of lactobacilli in it was 1.0×10^9 CFU/cm^3; and the number of bifidobacteria was 1.2×10^8 CFU/cm^3.

We have developed a new technology for producing organic bioselenium in the composition of probiotic cultures, which makes it possible to obtain dry and liquid forms of the finished product.

The developed preparation "Selenobifilact" could be used as a dietary food supplement. "Selenobifilact" contains a probiotic dose of the viable cells *Lactobacillus acidophillus 412/307* and *Bifidobacterium bifidum I* that can colonize the intestine and restore its normal microbiota. Probiotic microorganisms in the process of their metabolisms synthesize lactic, acetic, propionic, butyric, capric acids providing for the antagonistic activity against pathogenic and conditionally pathogenic micro-

organisms. Probiotics synthesize lysozyme, hydrogen peroxide, enabling the antimicrobial activity, as well as produce vitamins of groups B and K. DS contains the organic form of selenium, which makes up for the lack of selenium in the human body and ensures antioxidant, antimutagenic, anticarcinogenic effect.

We have conducted pilot medical-biological testing on outbred white mice. The selenium-containing preparation was introduced to the daily diet in the amount equal to 5 μg per day per one mouse. We found in the process of examination that the experimental group of mice, similar to control group, the wool cover of animals was dry, glossy, visible mucous is pale pink. We did not reveal in the course of the experiment any statistically significant changes in the body mass of mice from the examined group compared with control. Every 10 days, we took the samples of faeces from the examined animals. The quantitative content of lacto- and bifidobacteria was then determined. In the course of the experiment we noted a growth of the content of lacto- and bifidobacteria. We observed a growth of the content of lactobacilli by 1.5 orders of magnitude at the end of the experiments, in comparison with control group. The number of bifidobacteria increased by 2 orders of magnitude.

The result of present research is the developed biotechnology for obtaining the selenium-containing functional ingredient – the dietary supplement, which is a new generation of probiotics with enhanced antioxidant, antimutagenic, anti-cancer effect due to selenomethionine and selenocysteine biotransformed by microorganisms. In other words, the preparation "Selenobifilact" has a dual biological action, as a probiotic and a source of organic selenium.

We consider the shortcoming of present study the insufficient medical-biological research, planned to be undertaken during further experiments.

In the future, the technology for obtaining a dietary supplement will require additional production-technical research into designing and development of the technological scheme of the process in order to improve parameters of the technology.

7. Conclusions

1. We have chosen preparations of inorganic selenium. In the process of adding the sources of inorganic selenium to the cultivation media of the examined microorganisms, it was determined that sodium selenite (Na_2SeO_3) performs best in terms of accumulation and biotransformation. Adding Na_2SeO_3 to the cultivation environments of lacto- and bifidobacteria ensured the resulting content of organic selenium in the biomass of lactobacilli in the amount of 370 μg, and in the biomass of bifidobacteria – 355 μg. In the subsequent work, we used sodium selenite as a source of inorganic selenium.

2. We have studied kinetic indicators of the growth of microorganisms and the accumulation of organic forms of selenium by microorganisms-probiotics. The indicators that characterize a growth of the biomass are the indicators of optical density, specific growth rate, duration of generation, colony-creating units. It was established that the concentrations of Na_2SeO_3 in the amount of 0.5–5 μg/cm^3 do not inhibit the accumulation process of lactobacilli biomass. Such concentrations for bifidobacteria are 0.5–3 μg/cm^3. It was established that an increase in the concentrations of Na_2SeO_3 in the cultivation environment causes an increase in the content of organic selenium in the derived biomasses of microorganisms. Thus, at a concentration of Na_2SeO_3 equal to 0.5 μg/cm^3, the amount of organic selenium in the biomass of lactobacilli was 105 μg/g of dry biomass, for bifidobacteria – 97.5 μg/g. At a concentration of Na_2SeO_3 equal to 20 μg/cm^3, the quantitative content of organic selenium in the biomass of lactobacilli was 4,698 μg per 1 gram of dry biomass; for bifidobacteria – 3,149 μg/g.

3. Based on the results obtained, we devised a technological scheme for obtaining the dietary supplement "Selenobifilact", based on the selenium-containing symbiotic culture Lactobacillus acidophilus and Bifidobacterium bifidum whose special feature is obtaining a comprehensive dietary supplement, which combines the probiotic and antioxidant properties.

4. We have investigated the microbiological and physical-chemical indicators of the obtained preparation. The quantitative content of lactobacilli in the preparation "Selenobifilact" was 1.0×10^9 CFU/cm^3, that of bifidobacteria – 1.2×10^8 CFU/cm^3. The content of organic selenium was equal to 202.5±1 μg/g.

References

1. Kapreliants L. V., Yorhachova K. I. Funktsionalni produkty. Odessa: Druk, 2003. 312 p.
2. Spirichev V. B. Obogashchenie pishchevyh produktov mikronutrientami: nauchnye podhody i prakticheskie resheniya // Pishchevaya promyshlennost'. 2003. Issue 3. P. 10–16.
3. Kapreliants L. V., Trehub N. S. Kultyvuvannia bifido- i laktobakteriyi na seredovyshchakh iz selenitom natriyu // Kharchova nauka i tekhnolohiya. 2016. Issue 10. P. 26–30.
4. Reilly C. Selenium: A new entrant into the functional food arena // Trends in Food Science & Technology. 1998. Vol. 9, Issue 3. P. 114–118. doi: 10.1016/s0924-2244(98)00027-2
5. Gut microbiota limits heavy metals burden caused by chronic oral exposure / Breton J., Daniel C., Dewulf J., Pothion S., Froux N., Sauty M. et. al. // Toxicology Letters. 2013. Vol. 222, Issue 2. P. 132–138. doi: 10.1016/j.toxlet.2013.07.021
6. Goderska K., Nowak J., Czarnecki Z. Comparison of Lactobacillus acidophilus and Bifidobacterium bifidum species in media supplemented with selected saccharides including prebiotics // Technologia Alimentaria. 2008. Vol. 7, Issue 2. P. 5–20.
7. Rajashree K., Muthukumar T. Selection of culture medium and conditions for the production of selenium enriched Saccharomyces cerevisiae // African journal of Biotechnology. 2013. Vol. 12, Issue 20. P. 2972–2977.
8. Chervonaya S. S., Usacheva O. A., Mirhaev M. N. Vliyanie selenita natriya na rost drozhzhevyh kletok v proizvodtve piva // Mater. Mezhdunarodnogo s'ezda terapevtov i diagnostov. 2005. P. 194–195.
9. L-selenomethionine as a source of selenium added for nutritional purposes to food supplements // EFSA Journal. 2009. Vol. 7, Issue 7. P. 1082. doi: 10.2903/j.efsa.2009.1082

10. Baranovs'kiy A. Yu., Kondrashina E. A. Disbakterioz i disbioz kishechnika. Moscow: Grant, 2002. 224 p.
11. Moyseenok A. G., Kudryacheva L. A., Rusina E. D. Bifidobakterii i ih ispol'zovanie v klinike, meditsinskoy promyshlennosti i sel'skom hozyaystve. Moscow: Agropromizdat, 1996. 120 p.
12. Review article: bifidobacteria as probiotic agents – physiological effects and clinical benefits / Picard C., Fioramonti J., Francois A., Robinson T., Neant F., Matuchansky C. // Alimentary Pharmacology and Therapeutics. 2005. Vol. 22, Issue 6. P. 495–512. doi: 10.1111/j.1365-2036.2005.02615.x
13. Hadadji M., Bensoltane A. Growth and lactic acid production by Bifidobacterium longum and Lactobacillus acidophilus in goat's milk // African Journal of Biotechnology. 2006. Vol. 5, Issue 6. P. 505–509.
14. Mannitol production by lactic acid bacteria: a review / Wisselink H. W., Weusthuis R. A., Eggink G., Hugenholtz J., Grobben G. J. // International Dairy Journal. 2002. Vol. 12, Issue 2-3. P. 151–161. doi: 10.1016/s0958-6946(01)00153-4
15. Ruas-Madiedo P., Hugenholtz J., Zoon P. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria // International Dairy Journal. 2002. Vol. 12, Issue 2-3. P. 163–171. doi: 10.1016/s0958-6946(01)00160-1
16. Fávaro-Trindade C. S., Grosso C. R. F. Microencapsulation of L. acidophilus (La-05) and B. lactis (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile // Journal of Microencapsulation. 2002. Vol. 19, Issue 4. P. 485–494. doi: 10.1080/02652040210140715
17. Hassan A. N., Frank J. F., Shalabi S. I. Factors affecting capsule size and production by lactic acid bacteria used as dairy starter cultures // International Journal of Food Microbiology. 2001. Vol. 64, Issue 1-2. P. 199–203. doi: 10.1016/s0168-1605(00)00427-x
18. Skal'naya M. G., Notova S. V. Makro- i mikroelementy v pitanii sovremennogo cheloveka: ekologo-fiziologicheskie i sotsial'nye aspekty. Moscow: ROSMEM, 2004. 300 p.
19. Tapiero, H., Townsend, D. M., Tew, K. D. The antioxidant role of selenium and seleno-compounds // Biomedicine & Pharmacotherapy // Biomedicine & Pharmacotherapy. 2003. Vol. 57, Issue 3-4. P. 134–144. doi: 10.1016/s0753-3322(03)00035-0
20. Gorelikova G. A. Teoreticheskie i prakticheskie aspekty razrabotki pishchevyh produktov, obogashchennyh selenom: monografiya. Moscow: Grant, 2008. 235 p.
21. Zaretskaya E. S., Gmoshinskiy I. V., Mazo V. K. Novye pishchevye istochniki mikroelementov: kontrol' kachestva // Rossiyskiy zhurnal gastroenterologii, gepatologii, koloproktologii. 2002. Issue 5.
22. Sneddon A. Selenium nutrition and its impact on health // Food & Health Innovation Service. 2012.
23. Mikulasova M., Dusinsky R. Probiotic supplementation: What nurse practitioners need to know to recommend safe and effective formulations // J. Biomed Res. Int. 2013. Issue 13. P. 78–94.
24. Selenium nanoparticle-enriched Lactobacillus brevis causes more efficient immune responses in vivo and reduces the liver metastasis in metastatic form of mouse breast cancer / Yazdi M., Mahdavi M., Setayesh N., Esfandyar M., Shahverdi A. // DARU Journal of Pharmaceutical Sciences. 2013. Vol. 21, Issue 1. P. 33. doi: 10.1186/2008-2231-21-33
25. Selective enumeration and identification of mixed cultures of Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, L. acidophilus, L. paracasei subsp. paracasei and Bifidobacterium lactis in fermented milk / Tabasco R., Paarup T., Janer C., Peláez C., Requena T. // International Dairy Journal. 2007. Vol. 17, Issue 9. P. 1107–1114. doi: 10.1016/j.idairyj.2007.01.010
26. Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications / Burrell A. L., Dozier W. A., Davis A. J., Compton M. M., Freeman M. E., Vendrell P. F., Ward T. L. // British Poultry Science. 2004. Vol. 45, Issue 2. P. 225–263. doi: 10.1080/00071660410001715867
27. Kurek E., Rusrczynska A. Bio-transformation of selenium in Se-enriched bacterial strains of Lactobacillus casei // Raczniki Panstuowego zakladu hihieny. 2016. Vol. 67, Issue 3. P. 253–262.
28. Elemental, Nano-Sized (100-500 nm) Selenium Production by Probiotic Lactic Acid Bacteria / Eszenyi P., Sztrik A., Babka B., Prokisch J. // International Journal of Bioscience, Biochemistry and Bioinformatics. 2011. P. 148–152. doi: 10.7763/ijbbb.2011.v1.27
29. Development of biotechnology of getting selenium nanostructures with Lactobacillus acidophilus culture / Kaprellyanz L., Zykova N., Petrosyants A., Zykov A. // EUREKA: Life Sciences. 2018. Issue 1. P. 54–60. doi: 10.21303/2504-5695.2018.00560