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RESEARCH OF TECHNOLOGICAL AND BIOTECHNOLOGICAL PROCESSES OF CULTIVATION OF LACTIC ACID SYMBIONTS

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Abstract. The main technological and biotechnological features of symbiont cultivation based on microbial cultures *Lactobacillus plantarum* and *Lactococcus diacetylactis* and various prebiotics have been studied in this work. Cultures of probiotic microorganisms were chosen. The main technological characteristics of several types of dietary fibers from pumpkin seeds produced by WTF "Farmakom", "Richoil" and "Agroselprom" were studied, namely their fractional composition. In all studied samples, three fractions with the screenings of sieves 059, 044, and 037 were separated. In "Richoil" fibers, the content of screening fraction of 044 sieve was the highest and accounted for up to 80%. The yield of the fine fraction with the throughs of sieve 037 in all studied fiber samples was 3–10%. The adsorption capacity of pumpkin seed dietary fibers has been studied. The throughs fraction of the sieve 044 was found to actively adsorb the cells of the producer microorganism. For pumpkin seed dietary fibers, the water-retaining capacity was proven to be 2.5 g of water per gram of sample. The influence of mass fraction of pumpkin seed dietary fibers on cultivation of *Lactobacillus plantarum* and *Lactococcus diacetylactis* was studied. The mass fraction of 0.5 g of dietary fibers per 10 ml of culture medium was shown to be optimal. The dynamics of acid accumulation in the process of cultivation of symbiotic microorganisms *Lactobacillus plantarum* and *Lactococcus diacetylactis* in milk with different prebiotics (pumpkin seed dietary fibers, lactulose, and amaranth oil) was studied. The dietary fibers from pumpkin seeds was shown to have a significant effect on the β-galactosidase activity of symbiont microorganisms and to accelerate their acid production. A clot formed in the presence of fibers in 4.0–4.5 hours. *Lactococcus diacetylactis* was found to be the predominant culture in the symbiotic interactions in the presence of pumpkin seed dietary fibers. As a result of culturing the selected symbionts with pumpkin seed dietary fibers in the finished product, we obtain a predictable complex of metabolites, the majority of which would be produced by the predominant culture *Lactococcus diacetylactis*.

Key words: microbiota, symbiosis, probiotic, metabolic probiotic, dietary fibers, lactic acid bacteria, cultivation.

Introduction. Formulation of the problem

According to economic predictions, the global biotechnology market by 2025 will be 2–3 trillion US dollars. Even today, the growth rate of this area in some sectors ranges from 5–9 to 30% annually [1]. Biotechnological products on the Ukrainian market are usually represented by foreign companies. For example, Ukraine annually imports 100% of forage amino acids for agriculture, 100% of enzymes for household chemicals, 100% of lactic acid, 93% of biologically active food additives, 75% of forage enzyme preparations, and 80% of forage and veterinary antibiotics [1].

With the sustainable development of industrial biotechnology, when controlled biosynthesis produces many new products with specified properties, this situation can be changed. The priority sub-directions of industrial biotechnologies for Ukraine can be:

– production of enzymes, which can significantly reduce their imports;

– deeper processing of food raw materials involving biotechnological processes;

– production of food additives and biologically active additives to improve the beneficial properties of food, prebiotics, probiotics and synbiotics [1].

The development of the latter sub-direction is an important part of Ukraine's industrial development. With its help, important social problems can be solved in the state, which will significantly reduce the socio-economic damage from morbidity and reduce the burden on the medical sector, which is especially true in the today's pandemic. However, probiotics are not always highly effective. When the probiotic drug enters the gastrointestinal tract, only 5% of lyophilized bacteria are activated [2]. The high cost of probiotics based on living microorganisms also limits their use. After all, to preserve the valuable properties of probiotics, specialized protection of cells from aggressive environments in the stomach are used, like capsules of acid-resistant materials, immobilization of cells on various matrices, or lyophilization. However, living cells, when leaving the capsule or matrix, come to a state of stress and poorly colonize the intestine [2]. The latest generation of probiotics, which contain metabolic products of normal human intestinal microbiota, are immediately included in the intestinal metabolic processes via the regulation of the functioning of biofilms on the macroorganism's mucous membranes. The technology of their manufacture is much simpler. That is why the solution to the problem of treating dysbiosis or other diseases is to develop and implement in clinical practice probiotics based on microbial metabolites, which, according to the new classification, are called metabolic type probiotics, metabiotics, or postbiotics [3]. Considering the above, the study of important technological and biotechnological processes for further development of biotechnology of metabolic probiotics based on lactic acid symbionts is relevant.

Analysis of recent research and publications

Symbiosis is a form of close coexistence of different species of microorganisms, in which the partners together participate in the settlement of their relationships under the influence of external factors. The relationship of the macroorganism with its own, endogenous microbiota has also a complex multi-vector nature, and is realized at the metabolic, regulatory, intracellular and genetic levels. Under natural conditions, there is no biochemical process, no function of living macroorganisms, which would be carried out without the direct or indirect participation of symbiotic microorganisms [4, 5].

For example, the beneficial intestinal microbiota shows its gratitude for the warm habitat and high amount of nutrients to the macroorganism by production of essential regulatory metabolites, synthesis of vitamins, antibiotics and thus providing protection against "intruding microbes" that constantly try to penetrate our "technological zone".

The complex symbiotic relationships of eukaryotic and prokaryotic cells of the human microbiome are regulated by certain mechanisms that allow to control the number and composition of respective consortia, to prevent competition between them for nutrient substrates and to exchange metabolites for mutual benefit [6].

The development of dysbiosis and the emergence of many diseases or syndromes can occur under the influence of various physicochemical and biological factors, in fact due to dysfunction of the symbiotic relationship between the host organism and its microbiota. Many scientists have no doubt that symbiotic microorganisms present in the host organism significantly affect such processes [5, 6]. Not only live active microbial cells carried by biological fluids throughout the body can affect these, but also the metabolites of symbiotic microorganisms and their enzymes. Metabolites and fragments of cells, having entered the bloodstream and lymph and various organs and tissues from them, show their regulatory effect there [7]. Despite the active work of scientists in this direction, the system of symbiosis regulation is currently poorly studied.

From the physiology of biological fluids it is known that the metabolic exchange of 70% of blood plasma fluid on a limited surface occurs in 1 min. Therefore, metabolites and small fragments of microbial biopolymers enter the bloodstream almost immediately after their formation. The markers of various microorganisms have been found in the blood. Various microorganisms were found to produce a total of about 200 fatty acids, which differ from those synthesized by the human body. Comparative chromato-mass-spectrometric study of the composition of these chemical markers in the blood and sites of intestinal mucosa revealed a correlation of markers in the blood and habitats of the jejunum, ileum, colon and human feces [8].

In recent decades, scientists from different countries have been actively working to create probiotics and functional foods based on lactic acid bacteria, the normal inhabitants of the intestine, which would treat the human microbiocenosis from the outside. The following cultures have gained worldwide recognition as probiotics: *Bacillus subtilis, Bifidobacterium adolescentis, B. bifidum, B. infantis, B. longum, Enterococcus faecalis, E. faecium, Escherichia coli, Lactobacillus acidophilus. L. casei, L. delbruecki subsp, L. bulgaricus, L. helveticus, L. fermentum, L. lactis, L. rhamnosus, L. salivarius, L. plantarum, Lactococcus, Leuconostoc, Pediococcus, Propionibacterium acnes, Saccharomyces boulardii, Streptococcus cremoris, S. lactis, S. salivarius subsp. thermophilus, Clostridium butyricum*. All these microorganisms are found in a healthy person's body in ratios specific for each individual [4, 6].

The study of the plasmid composition of lactic acid bacteria revealed plasmids encoding for the

transport and metabolism of lactose, sucrose, galactose, mannose, xylose, citrate, proteins, for bacteriocin synthesis and exopolysaccharide production, nisin, antibiotic, ultraviolet and viral resistance [9-11].

Based on these cultures, technologies were developed and a large number of therapeutic and prophylactic treatments and functional food products were created, these were classified according to their composition into monoprobiotics, associated probiotics, multiprobiotics, and synbiotics. It is predicted that in the next few decades, probiotic treatments and products will reduce human consumption of medical chemotherapeutics by more than a third, especially of those used for preventive purposes [4–7].

Unfortunately, the positive effect of probiotics, even with long-term use, is often transient, and moreover, despite the safety of probiotics, supplements and functional foods, people who have long used live probiotic microorganisms have been reported to develop various complications (laccidemia in babies, autoimmune diseases, allergic manifestations, opportunistic infections, dysbiotic conditions etc.). One of the main reasons for the ineffectiveness of probiotics is the alien nature of microorganisms that are part of them, and insufficient consideration of the high species, individual and anatomical specificity of the own (indigenous) microbiota of the patients who are treated with these remedies for microecological disorders. As a result, the strains of microorganisms that exhibit in vitro probiotic activity are not always active in the human body [2].

However, this does not mean we should not help our own microbiota. It is better to use prebiotics and metabiotics instead of probiotics. Since they are not living microorganisms, they do not have the side effects typical of probiotics. They do not compete with our own microbiota for nutrients, but only help it by their regulatory and stimulating effects.

Metabolite probiotics are the probiotics that contain metabolic products of the normal human gut microbiota. They affect the physiological functions and biological reactions of the body both directly by interventing in the metabolic activity of tissue cells of corresponding organs and indirectly by regulating the biofilms on the mucous membranes of the macroorganism. All types of endogenous digestion in the human body (cavity, parietal and membrane) are carried out by their own enzymes and occur in the stomach and upper gastrointestinal tract (GIT). Symbiotic digestion is carried out with the assistance of anaerobic intestinal microbiota and mainly in the ascending parts of the colon [12-14]. This decomposes not only previously undigested food residues (mainly plant fibers), but also other organic compounds. Low molecular weight metabolites are formed as a result of their incomplete oxidation under anaerobic conditions (fermentation). Under normal physiological conditions, proteolytic and saccharolytic bacteria jointly participate in this process.

Among such metabolites, short-chain volatile fatty acids (SVFA) deserve special attention: acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic and isocaproic acids. In the human colon, SVFA are represented mainly by acetic, propionic and butyric acids [6].

Recently, the range of enriched fermented dairy products and their analogues containing functional ingredients (plant fiber, minerals, polyunsaturated fatty acids, some oligosaccharides, etc.) has significantly expanded.These components affect not only the process of growing producer microorganisms, but also the accumulation of metabolites that depend on this effect in the cells themselves or in the culture medium [5,8,15].

Pumpkin seed dietary fiber (PSDF) from is a complicated biopolymer plant complex that Ukrainian producers have begun to actively market, often named “fiber”. Pumpkin is grown at a large scale and its meal (after squeezing the oil), which contains other components besides the fiber, can be a potential source of the prebiotic component in the cultivation of microorganisms and may have a positive effect on this process. Dietary fiber from pumpkin seeds is a matrix consisting of high molecular weight polysaccharides and lignin, typical of plant cell walls, resistant to human digestive enzymes. As a functional ingredient of food, fiber has a beneficial effect both on individual body systems and the human body as a whole. At the same time, dietary fibers is used as a technological additive that changes the structure and properties of the product. Soluble dietary fibers have the properties of fillers and structurants. During the process of cultivation with lactic acid bacteria individual components may be released from the dietary fibers (e. g., cucurbitin – an anthelmintic substance) and enter the complex of metabolites in the nutrient medium [16,17].

A lot of researchers’ attention is attracted by vegetable oils, which have a combined effect on the cytoplasmic membranes of cells, the composition and redox potential of media. Among them, a special place is occupied by amaranth oil. The chemical composition of amaranth oil contains riboflavin (vitamin B_2), tocopherol (vitamin E), thiamine (vitamin B_1), vitamins D, provitamin A, chlorophyll, choline, fatty acids, steroids, phytosterols, and polyunsaturated fatty acids, which are extremely unique because they contain a balanced complex of omega-3 and omega-6. This allows the use of amaranth oil in the treatment of many diseases. [18-20]. That is why it attracted attention as a source of a complex of biologically active substances used to compare the effects of different types of prebiotics on the process of cultivation of the studied microorganisms.

Management of technological processes of microbial synthesis is based on the regulation of

biochemical reactions carried out by the producer in specific cultivation conditions and characteristic of the whole process. These include pathways of assimilation of the main carbon and nitrogen-containing components from the environment, synthesis of low molecular weight metabolites, reactions of energy metabolism. Knowledge of the mechanisms of synthesis regulation is necessary not only for the production of enzymes as target products, but also for the synthesis of low molecular weight compounds, such as secondary metabolites. Some of them may be precursors for synthesis of substances required by human organism and nutrients for its own microbiota.

After full completion of the biotechnological cycle, such a preparation may contain not only short-chain fatty acids, antibiotic substances, cucurbitin, fragments of bio-organic molecules that are part of the cell membrane and organelles of lactic acid microorganisms, but also other substances that promote the development of beneficial human microbiota, as they are close to it by their nature.

The **purpose** of this work was to study the technological and biotechnological parameters of lactic acid microorganisms growth important for the development of metabolic probiotic technology and to optimize the nutrient medium involving various prebiotic substances.

To achieve this target, the following **tasks** were planned:

- to select lactic acid symbiotic microorganisms and to optimize the composition of the nutrient medium by adding various prebiotic substances;
- to study the fractional composition of dietary fiber from pumpkin seeds of different manufactures;
- to investigate the adsorption capacity of different fractions of the studied samples of PSDF;
- to study the water retaining capacity (WRC) of PSDF;
- to determine the optimal mass content of PSDF in the cultivation of *Lactobacillus plantarum* and *Lactococcus diacetylactis*;
- to study the dynamics of acidity changes in the culture medium with the optimal mass content of dietary fiber;
- to study the relationship and development of symbiotic microorganisms in the culture medium with dietary fiber.

Research materials and methods

The cultures of *Lactobacillus plantarum* and *Lactococcus diacetylactis* provided by the Museum of Cultures of the Department of Biochemistry, Microbiology and Physiology of Nutrition ONAFT were used in this work..

As dietary fiber, we used the PSDF from three manufacturers: TOV VTF "Farmacom" – with a content of 26.5 g of protein, 9.5 g of fat, 18.2 g of carbohydrates; private enterprise "Richoil" – with a content of 43.7 g of protein, 9.8 g of fat, 35.3 g of carbohydrates, and Agroselprom (Ukraine, Dnipro) – with a content of 35.3 g of protein, 9.1 g of fat, 34.3 g of carbohydrates.

As a source of lactulose, we used the preparation "Normaze", manufactured by L. Molteni & C. dei F. lli Alitti Societa di Esercizio S. P. A., S. S. 67 (Tosca Romagnola) Localita Granatieri-50018 Scandicci, Italy (lactulose content 10 g in 15 ml of syrup, excipients: citric acid, monohydrate, cream flavor, water).

Amaranth oil used for research was "Amarant korolivsky", cold-pressed, SFG "Olena", 2019 (squalene content 7.5%), TUU 10.4-36553354-001-2012.

The typical probiotic microorganisms *Lactobacillus plantarum* and *Lactococcus diacetylactis*, were selected as probiotics because they are normal microbiota of the human intestine, have a high colonizing capacity, form mainly lactic acid, have been tested for symbiotic effect at many manufactures of probiotic preparations, and are facultative anaerobes [3,8-14].

PSDF of various manufacturers, lactulose (a classic prebiotic) and amaranth oil were chosen as prebiotics.

As a nutrient medium, we used milk of 0.5% fat "Na zdorovya" manufactured by TOV "Lustdorf" as the most acceptable, organic nutrient medium for selected symbionts.

To obtain starting cultures for laboratory investigations, we used sterile media for lactic acid microorganisms: MRS (agar of de Man J., Rogosa M. and Sharpe M.) and hydrolyzed milk.

To study the WRC of PSDF and compare this trait with others, we used wheat bran of 2020 harvest, obtained from DP Kulindor KHP, and wheat fiber (wheat bran biomodified by hydrolase enzymes), provided by the Department of Biochemistry, Microbiology and Nutrition Physiology.

Determination of the fractional composition of PSDF. Studies of the fractional composition were performed on the device "Rozsivok laboratorny RLU-1". A weight portion of PSDF was sifted through a set of sieves: 164, 109, 059, 041, 025, 020, and 016, then the percentage of fraction on each sieve was determined according to the weight of the original sample.

Determination of adsorption capacity of PSDF. Determination of the adsorption capacity of different fractions of PSDF was carried out using a laboratory centrifuge, hydrolyzed milk and a freshly grown culture of symbionts. To do this, 1 ml of starting culture of *Lactobacillus plantarum* was added to 1 ml of hydrolyzed milk. The number of cells was counted using a Goryaev chamber. Then, in three test tubes, 10 ml of hydrolyzed milk, 1 ml of *Lactobacillus plantarum* starting culture and 0.5 g of each fraction of dietary fiber of sample 1 (manufactured by "Richoil") were similarly mixed. After thorough mixing and

centrifugation, cells from the supernatant not adsorbed by the fibers were counted again in the chamber. The adsorption capacity of each fraction was determined by the difference.

Determination of water retaining capacity (WRC) of PSDF. A 1 g portion of the test samples was soaked for 24 hours. The solid phase was separated from the liquid by centrifugation at 6000 rpm for 15 min. The supernatant was drained, the tube was placed in a sloped position on filter paper to remove excess moisture and weighed after 10 minutes. WHC was expressed as the amount of water rentained in 1 g of the test sample, and was calculated by the formula 1:

$$WRC = \frac{c-b}{a}, \quad (1)$$

where *a* is the weight of tested sample, g;
b is the weight of the test-tube with dry portion, g;
c is the weight of the test-tube with moist portion.

Determination of milk acidity. The acidity of milk is due to the content of lactic acid and phosphoric and lactic salts, proteins etc. Acidity was determined in Turner degrees (a conventional unit corresponding to a mass fraction of 0.1 N NaOH in ml spent to neutralize 100 ml of milk). 10 ml of milk were poured into a 50-100 ml conical flask, 20 ml of distilled water added, and this was titrated with 0.1 N NaOH solution in the presence of phenolphthalein indicator, until it turned pink. Acidity is an important indicator, which for fresh milk should not be higher than 22°T.

Determination of numbers of lactobacilli and lactococci. The number of cells of lactic acid bacteria was counted according to the method described in DSTU 7999:2015 "Food. Methods for determining lactic acid bacteria". The method is based on the ability of thermophilic lactic acid bacteria to grow in skim milk at a temperature of 37±1°C and to form a clot there within 72 hours.

The choice of dilutions for inoculation was made based on the most probable content of these microorganisms in the product. In this work, dilutions were prepared in the conventional way up to 10^{15}. From each of the last three or four dilutions, 1 ml was added to 2 parallel tubes with sterile skim milk and incubated at 37±1°C for 72 hours. During this time, milk, under the action of lactic acid microorganisms, ferments, forming a clot. A microscopic mount was prepared from the clot, the morphology of microorganisms was studied and the numbers counted in a Goryaev chamber followed by calculation into lg CFU/ml. The fraction of lactobacilli in the test samples was calculated taking into account the number of test tubes with fermented milk and their dilution, according to the table of results depending on the number of clots in test tubes.

The cells were also counted during the growth process in the Goryaev chamber, taking into account the degree of dilution of the selected sample. The number of cells in 1 ml:

$$x = (\alpha \cdot 4000 \cdot \frac{b}{c}) \cdot 1000, \quad (2)$$

where α is the number of cells in 5 (or 10) large squares of the grid;
b is the dilution of the initial substrate;
c is the number of small squares involved in counting.

To count the cells after clot formation, to study the cultural and morphological characteristics of lactic acid bacteria and to establish their probiotic dose, all samples, after a series of dilutions, were also inoculated by pour-plating on cabbage agar and incubated for two days at 37°C.

Cultivation of microorganisms. Lactobacilli, in order to determine the adsorption capacity of the fibers, were cultured by pour-plating on sterile MRS medium at 37±1°C for 72 hours. To study the behavior of symbiotic cultures of *Lactobacillus plantarum* and *Lactococcus diacetylactis*, cultivation was performed on milk in heterophase conditions in Erlenmeyer flasks of 250 ml volume at 37±1°C without stirring or aeration. The fibers introduced into each flask at the rate of 0.5 g per 10 ml of milk were pre-sterilized. Cultures of *Lactobacillus plantarum* and *Lactococcus diacetylactis* were applied in a volume of 10% of the total culture medium in equal mass fractions. To determine the mass fraction of fibers, the growth was performed on milk in heterophase conditions in tubes with sterile milk at 37±1°C without stirring. Additionally, microscopy of isolated colonies was performed to establish morphological features.

Determination of the optimal mass fraction of PSDF. A row of test tubes with different mass fractions of introduced fibers from 0.1 to 1.0 g were prepared for the study. 10 ml of milk were added to each of these test tubes. Tubes with milk without the fibers served as a control. All tubes were sterilized in an autoclave. After cooling, a fresh 72-hour culture of *Lactobacillus plantarum* and *Lactococcus diacetylactis* was added to each tube in a volume of 10% of the total volume of the culture medium in equal mass fractions. Cultivation was performed at 37±1°C. Tubes with different fiber mass fractions and the control were collected every hour and titrated with 0.1 N NaOH solution in the presence of phenolphthalein until turned pink. Simultaneously, the formation of a clot was surveyed. The main part of the research was conducted in the laboratories of the Biochemistry, Microbiology and Physiology of Nutrition Department, some research was performed in the laboratories of Grain Processing technology Department of ONAFT.

Results of the research and their discussion

An important way to create products that provide healthy nutrition (functional products) is to enrich basic products with missing functional ingredients and develop new technologies for obtaining these products. Dietary fiber is today one of the most sought after and most widely used dietary ingredients.

The efficiency of interaction of PSDF in joint cultivation with lactic acid bacteria depends not only on the catalytic activity of lactic acid bacteria and fermentation conditions, but also on the specifics of processed raw materials and technological conditions for target products. The chemical composition of pumpkin fibers we selected from different manufacturers did not vary significantly. The content of proteins in them ranged from 33.8 to 43.7%, carbohydrates from 32.8 to 36.3%, lipids from 9.1 to 9.8% per 100 g of product.

In addition to the main biochemical indicators of raw materials, technological indicators are essential for the further conduct of the biotechnological process. These include bulk density, fractional composition, the possibility of transportation, wettability, etc. Since the particle size of PSDF can significantly affect the choice of technological parameters of the cultivation process and on mass transfer and adsorption processes, the fractional composition of dietary fiber of different manufacturers was studied. The research results are shown in Fig.1.

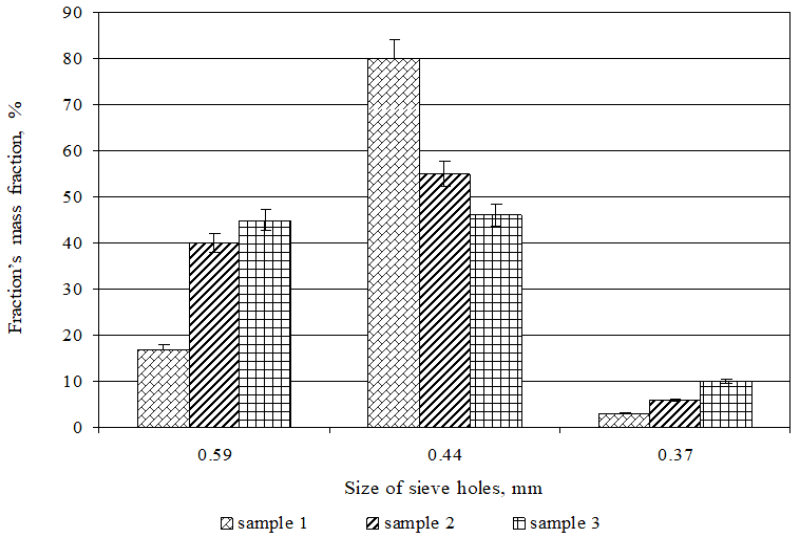

Fig. 1. Fractional composition of PSDF from three manufacturers: *sample 1* – private manufacture "Richoil"; *sample 2* – TOV "Agroselprom"; *sample 3* – TOV "Farmakom".

As a result of research, the fractional composition of PSDF from different manufacturers was found to vary insignificantly. Although in all studied samples there were clearly three fractions with the screening of sieves 059, 044, and 037 of sample 1 (manufacturer – private enterprise "Richoil"), the content of the fraction with the sieve 044 screening was the highest. The yield of the fine fraction with the screening of the sieve 037 was extremely small for all producers, accounting for 3-10%. All three samples by their particle size can be used for cultivation of bacteria with subsequent use for drying the finished product in a spray dryer. That is why the dietary fiber of sample 1 (private company "Richoil") was chosen for further research, as it had the highest yield of medium-sized fraction.

In addition, the PSDF is a potent sorbent. Since the cultivation of lactic acid microorganisms was carried out in the presence of a strong sorbent, its adsorption capacity was previously studied, because a strong sorbent can adsorb the introduced producer culture and inhibit the cultivation process from the beginning. The research results are shown in Fig. 2.

The results showed that the screening of sieve 037 has the greatest adsorption capacity of cells. The smallest was in the screening of sieve 059. Since the yield of these fractions is a total of 20%, they will not significantly affect the adsorption of cells during cultivation.

That is, the fibers can be used without fractionation. However, the fraction of the highest yield (screening of sieve 044) quite actively adsorbs the producer cells (35%). This must be taken into account when cultivating lactic acid bacteria, so the bioreactor for growing lactic acid bacteria in the presence of PSDF must be equipped with a stirring device with a speed of no more than 30 rpm.

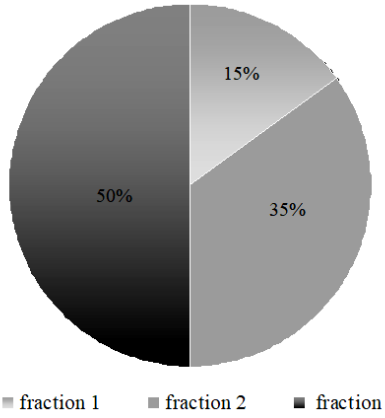

Fig. 2. Adsorption capacity of various fractions of PSDF: *fraction 1* – sieve 059 screening*; fraction 2* – sieve 044 screening*; fraction 3* – sieve 037 screening.

Optimization of mass fraction of PSDF in the process of cultivation of *Lactobacillus plantarum* and *Lactococcus diacetylactis*. One of the most important characteristics of biopolymer complexes of plants represented by dietary fibers is their WRC. The WRC is due to the affinity of components of plant biopolymer complexes to water and also to their ability to influence the processes of delayed absorption of carbohydrates in the small intestine and to affect the bacterial decomposition of bile salts in the large intestine, which normalizes its peristalsis. On the basis of experimental data the WRC of plant biopolymers was compared (fig. 3).

Moisture absorption occurs first due to adsorption on the surface layer, and then via the distribution throughout the polymer by active diffusion. Biopolymer complexes of plant nature are known to be capable of adsorbing not only water, but also acids that are metabolites. Therefore, it can be stated that the PSDF does not have a high WRC which will not affect both the adsorption of acids (and other biologically active substances) in the intestine and moisture retention during drying of the preparation.

Determination of mass fraction of PSDF in the process of cultivation of *Lactobacillus plantarum* and *Lactococcus diacetylactis*. According to the tasks set in this paper, the next step was to optimize the introduction of a mass fraction of prebiotic substance into the nutrient medium and to study the behavior and development of selected symbiotic microorganisms in culture medium in order to obtain a predictable complex of metabolites that can serve as a basis for creation of a metabiotic preparation (Table 1).

According to the results, the process of lactic acid formation in the culture medium is significantly accelerated in the presence of PSDF. After as much as 4.5 hours a uniform non-porous clot was observed in tubes with a mass fraction of fibers of 0.3, 0.5 and 0.7. At the same time, in control tubes the clot was observed after 7 hours. Titration of all tubes after one day showed a significant increase in acidity in samples with a mass fraction of 0.7, and 1.0 g of fibers, reaching 160°T.

Taking into account all the established features of the studied biopolymer complex – the PSDF – and the features of cultivation and active accumulation of acidity, the optimal introduction was the mass fraction of 0.5 g of PSDF per 10 ml of culture medium. The further research was conducted with this mass fraction.

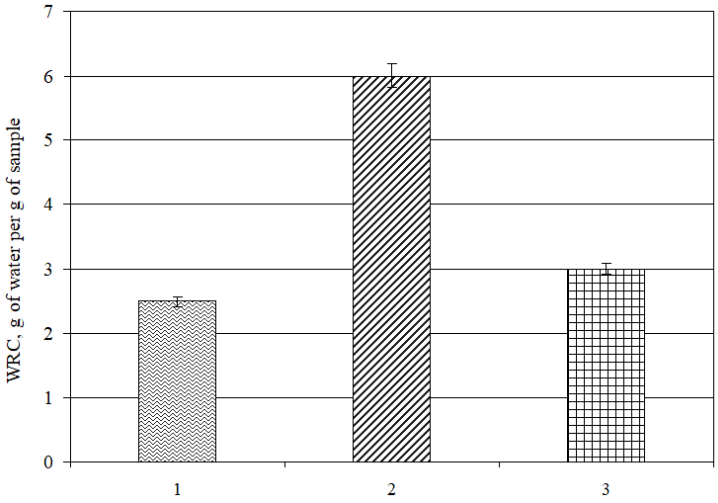

Fig. 3. Water-retaining capacity of plant raw material: *1* – PSDF, *2* – wheat fibers, *3* – wheat bran.

Table 1 – Determination of mass fraction of PSDF during cultivation of *Lactobacillus plantarum* and *Lactococcus diacetylactis* ($n = 3$, $p \geq 0,95$)

№	Intervals of taking samples, hours	Acidity of dairy, °T					
		Mass fraction of PSDF, g per 10 ml					Control (no fibers)
		0.1	0.3	0.5	0.7	1	–
1	0	22	22	22	22	22	22
2	1	27	27	28	28	30	25
3	2	40	41	41	41	58	32
4	3	50	58	63	68	70	38
5	4	55	72	100	100	110	48
6	5	60	82	102	110	112	58
7	6	65	84	110	120	130	60
8	24	131	136	150	160	160	118

Study of dynamics of acidity in the process of cultivation of *Lactobacillus plantarum* and *Lactococcus diacetylactis* in presence of various prebiotics. PSDF was added to each flask at the rate of 0.5 g per 10 ml of milk, and then pre-sterilized. The prebiotic lactulose and the amaranth oil were added aseptically at the rate of 1 ml per 10 ml of sterile milk. The starting cultures, *Lactobacillus plantarum* and *Lactococcus diacetylactis*, grown in laboratory conditions on MRS medium, were added in the amount of 10% of the total culture medium volume in equal mass fractions. Cultivation of the same lactic acid symbionts without the prebiotics served as the control [20].

In the process of cultivation, the acidity was determined hourly, and every two hours all cells were counted using Goryaev chambers and the cell ratio of cultured microorganisms was determined, because the relationships of major symbiotic cultures in their cultivation are important for biotechnological processes and for obtaining a complex of predicted metabolites. The results of these studies are shown in tables 2 and 3.

As shown by the results represented in table 2, dietary fiber from pumpkin seeds significantly accelerates the accumulation of acids in the culture medium. After 4.5–5.0 hours of cultivation, the appearance of a uniform, non-porous clot was observed, while in other samples it was formed 1.5–2.5 hours later.

Table 2 – Dynamics in acidity during cultivation of lactic acid symbionts (n = 3, p ≥ 0,95)

Samples	Acidity (°T) and duration of cultivation (hours)									
	0	1	2	3	4	5	6	7	8	9
Amaranth oil	21	25	36	36	40	40	45	50	55	60
PSDF	21	25	38	40	45	50	58	60	65	70
Lactulose	21	25	36	38	44	46	47	50	57	62
Control	21	23	24	26	30	35	38	40	25	50

Table 3 – Changes in ratios of *Lactobacillus plantarum* and *Lactococcus diacetylactis* cells during cultivation with various prebiotics (n = 3, p ≥ 0,95)

Samples	Number of cells and duration of cultivation (hours)							
	2		4		6		8	
	L. plantarum	*Lc. diacetilactis*	*L. plantarum*	*Lc. diacetilactis*	*L. plantarum*	*Lc. diacetilactis*	*L. plantarum*	*Lc. diacetilactis*
Amaranth oil	$3 \cdot 10^2$	$1 \cdot 10^2$	$4 \cdot 10^3$	$2 \cdot 10^2$	$5 \cdot 10^5$	$2 \cdot 10^2$	$14 \cdot 10^6$	$3 \cdot 10^2$
PSDF	$1 \cdot 10^2$	$5 \cdot 10^2$	$2 \cdot 10^2$	$9 \cdot 10^4$	$2 \cdot 10^2$	$7 \cdot 10^6$	$2 \cdot 10^2$	$7 \cdot 10^6$
Lactulose	$4 \cdot 10^2$	$2 \cdot 10^2$	$4 \cdot 10^3$	$2 \cdot 10^2$	$5 \cdot 10^5$	$11 \cdot 10^2$	$7 \cdot 10^6$	$12 \cdot 10^2$
Control	$2 \cdot 10^2$	$1 \cdot 10^2$	$3 \cdot 10^2$	$2 \cdot 10^2$	$4 \cdot 10^3$	$2 \cdot 10^2$	$6 \cdot 10^3$	$3 \cdot 10^2$

According to these results, a conclusion can be made that the relationship of symbionts depends significantly on the prebiotic component of the medium. For example, in the presence of pumpkin seed fibers, the predominant culture was *Lactococcus diacetylactis*. At the eighth hour of cultivation of symbionts, the number of cells of this microorganism in the culture medium was 3.5 times higher than of others. The environment with amaranth oil was dominated by the culture of *Lactobacillus plantarum*. *Lactobacillus plantarum* dominated also in the environment without the prebiotics. Microscopy of the studied samples showed a normal physiological state of lactobacilli and lactococci.

Conclusions

1. The fractional composition of dietary fiber from pumpkin seeds was studied for products of of TOV VTF "Farmacom", private enterprise "Richoil" and Agroselprom (Ukraine, Dnipro). In all studied samples, there were three distinct fractions with sieve 059, 044, and 037 screenings. In sample 1 (private enterprise "Richoil") the content of the fraction with the screening of sieve 044 was the highest and amounted to 80%. The yield of the fine fraction with the screening of sieve 037 was extremely small for all manfacturers at an amount of 3-10%. All three samples by their particle size can be used for the cultivation of bacteria followed by the use of a spray dryer.

2. The adsorption capacity of food biopolymer complexes from pumpkin seeds has been studied. It was established that the fibers can be used without fractionation. However, the most important fraction (screening of the sieve 044) fraction quite actively sorbs the cells of the producer microorganism – by 35%. This must be taken into account when culturing

lactic acid bacteria in industrial conditions, so the bioreactor must be equipped with a low-speed mixing device.

3. The water retaining capacity of biopolymers of plant nature has been studied. For dietary fiber from pumpkin seeds, the WRC was found to be 2.5 g of water per g of sample. The dietary fibers from pumpkin seeds were proven to not have a high water absorption capacity, which will not affect the absorption of short-chain acids and other soluble biologically active substances in the intestine and will positively affect the drying process during the dry preparation of the metabiotic.

4. The influence of mass fraction of dietary fiber from pumpkin seeds on the traits of cultivation and on active accumulation of acidity has been studied. Mass fractions of dietary fiber 0.7 g and 1.0 g dramatically increase the acidity in the culture medium, and after a day of storage cause the phenomenon of syneresis. The mass fraction of 0.5 g of dietary fiber from pumpkin seeds per 10 ml of culture medium was shown to be optimal.

5. The dynamics of acid accumulation in the process of cultivation with different prebiotics has been studied. As a result of research, all substances – prebiotics used by us – were found to have accelerated the fermentation of milk by selected cultures of symbionts. The fastest clot formation was observed in the sample with dietary fiber from pumpkin seeds – it took 4.0–4.5 h, which indicated a significant effect of PSDF on β-galactosidase activity of symbiotic microorganisms and on their accelerated production of acids.

6. In the presence of dietary fiber from pumpkin seeds, the dominant culture was found to be *Lactococcus diacetylactis*. When cultivated in the presence of lactulose and amaranth oil, *Lactobacillus plantarum* dominated. As a result of cultivation of selected symbionts in the presence of pumpkin seed fibers, in the finished product we obtain a predictable complex of metabolites, most of which will be produced by the predominant culture of *Lactococcus diacetylactis* (lactic, acetic, and propionic acids, aromatic substances, bacteriocins).

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ДОСЛІДЖЕННЯ ТЕХНОЛОГІЧНИХ ТА БІОТЕХНОЛОГІЧНИХ ПРОЦЕСІВ КУЛЬТИВУВАННЯ МОЛОЧНОКИСЛИХ СИМБІОНТІВ

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Анотація. У роботі вивчено основні технологічні та біотехнологічні особливості культивування симбіонтів на основі культур мікроорганізмів *Lactobacillus plantarum* та *Lactococcus diacetylactis* та пребіотиків різної природи. Вивчено основні технологічні характеристики кількох видів харчових волокон з насіння гарбуза виробництва ВТФ «Фармаком», «Річойл» та «Агросельпром», А САМЕ, досліджено їхній фракційний склад. У всіх зразках виділЕНО три фракції зі сходом сит: 059; 044; 037. У волокнах «Річойл» вміст фракції зі сходом сита 044 був найбільшим і складав 80%. Вихід дрібної фракції зі сходом сита 037 у всіх досліджених зразках волокон складав 3–10%. Вивчено сорбційну здатність харчових волокон з насіння гарбуза. Встановлено, що фракція, отримана через сито 044 активно сорбує клітини мікроорганізма-продуцента. Доведено, що для харчових волокон з насіння гарбуза водоутримувальна здатність становить 2,5 г води/г зразка. Досліджено вплив масової частки харчових волокон з насіння гарбуза на культивування *Lactobacillus plantarum* та *Lactococcus diacetylactis*. Встановлено, що оптимальною є масова частка 0,5 г харчових волокон з насіння гарбуза до 10 $см^3$ культурального середовища. Вивчено динаміку накопичення кислот у процесі культивування симбіотичних мікроорганізмів *Lactobacillus plantarum* та *Lactococcus diacetylactis* на молоці з різними пребіотиками – харчовими волокнами з насіння гарбуза, лактулозою та олією амаранту. Встановлено, що харчові волокна з насіння гарбуза мають суттєвий вплив на β-галактозидазну активність мікроорганізмів – симбіонтів та прискорюють продукування ними кислот. Згусток у присутності волокон утворився – за 4,0 – 4,5 год. Встановлено, що у присутності харчових волокон з насіння гарбуза домінуючою культурою у симбіотичних взаємовідносинах була *Lactococcus diacetylactis*. У результаті культивування обраних симбіонтів з волокнами насіння гарбуза у готовому продукті отримаємо, прогнозований комплекс метаболітів, переважну більшість з яких продукуватиме домінуюча культура *Lactococcus diacetylactis*.

Ключові слова: мікробіота, симбіоз, пробіотик, метаболітний пробіотик, харчові волокна, молочнокислі бактерії, культивування.