Journal of Agricultural Science and Technology A 16 (2026) 126-133
doi: 10.17265/2161-6256/2026.03.002

Innovative Probiotic Preparations Based on Bacteria of the Genus *Bacillus*: A Contribution to the Development of the Feed Base for Livestock Farming and Veterinary Medicine

Natalia Sverchkova, and Emiliya Kalamiyets

The State Scientific and Production Association "Chemical Synthesis and Biotechnology", Belarus National Academy of Sciences, 220084 Minsk, Belarus

Abstract: The studies on the isolation, selection and study of strains of spore-forming bacteria with high antimicrobial and hydrolytic (protease, cellulolytic, xylanase, amylolytic) activity were conducted. Six most active cultures were selected, which were identified and deposited in the Belarusian collection of non-pathogenic microorganisms under the numbers: *Bacillus velezensis* BIM B-454, *B. velezensis* BIM B-497, *B. velezensis* BIM B-713, *B. velezensis* BIM B-844, *B. velezensis* BIM B-845, *B. velezensis* BIM B-1125. As a result of molecular genetic studies, the complete nucleotide sequence of the genome of *B. velezensis* BIM B-454 bacteria was obtained for the first time. The features of the chromosome organization of the strain were determined. The whole genome sequences have been annotated and deposited in the GenBank database under accession numbers CP082262.1-CP082265.1. Based on the studied bacterial strains, technologies have been developed for producing the probiotic feed additives Sporobact, Sporobact-K (*B. velezensis* BIM B-497 and *B. velezensis* BIM B-713) and the veterinary drugs Bacinil-K (*B. velezensis* BIM B-454), Emilin (*B. velezensis* BIM B-845), and Bacto-Health (*B. velezensis* BIM B-1125). These developments are characterized by a high degree of novelty, correspond to the global level of development of biological science, and make a significant contribution to the development of the "green economy".

Key words: Microbiology, biotechnology, high-tech types of products, probiotic preparations.

1. Introduction

Producing livestock products with veterinary and sanitary safety is a crucial element in addressing the issue of healthy human nutrition. Due to the intensification of livestock farming, primarily based on the use of feed that ensures effective growth and development in the shortest possible time, as well as the declining effectiveness of antibiotic therapy, there has been a significant increase in the incidence of diseases in farm animals. The use of probiotics is considered a key element in the transition to environmentally friendly livestock products.

Corresponding author: Natalia Sverchkova, Ph.D., As. Prof., research fields: biotechnology, physiology and biochemistry of microorganisms.

Probiotics are live microbial supplements that have a beneficial effect on the animal body by improving intestinal microbial balance, metabolic and immune processes. They are safe and non-toxic to animals and poultry, do not accumulate in the body, and do not induce resistance in pathogenic microbiota.

The effectiveness of probiotics is largely determined by the production technologies used. The most well-known probiotics are those based on representatives of the normal intestinal biocenosis, in particular bifidobacteria and lactobacilli. However, preparations based on these bacteria are inferior in stability to those produced using spore-forming bacteria of the genus *Bacillus*, which have several advantages over other representatives of exogenous microflora: the vast majority of representatives of the

genus *Bacillus* are safe for the host even at high concentrations, are capable of increasing the non-specific resistance of the host, exhibit antagonistic activity against a wide range of pathogenic and opportunistic microorganisms, possess high enzymatic activity, are characterized by resistance to lytic enzymes and high viability in the gastrointestinal tract, are distinguished by technologically advanced production, shelf stability, and environmental safety.

The global market for probiotic preparations for livestock and feed production offers a wide range of products, represented by biotechnology companies in the European Union, the United States, and Russia. In Belarus, due to the ban on the use of antibiotics for prophylactic purposes and to stimulate the growth and productivity of farm animals, considerable attention has been paid in recent years to the development of domestic production of probiotic preparations. This requires the development of a wide range of preparations with high antimicrobial, immunostimulating, and antioxidant activity. Their use in livestock, poultry, and industrial fish farming will improve the epizootic situation on farms at all stages of animal, poultry, and fish production and produce high-quality, environmentally friendly commercial products competitive in foreign markets [1-6].

The above defined the goal of research aimed at isolating new highly active strains of bacteria of the genus *Bacillus* and developing, on their basis, competitive import-substituting biotechnologies for the production and use of probiotic preparations.

2. Materials and Methods

The main objects of the study were selected and studied: bacterial strains *Bacillus velezensis* BIM B-454, *Bacillus velezensis* BIM B-497, *Bacillus velezensis* BIM B-713 with high enzymatic and antagonistic activity towards bacterial pathogens of animals and birds, isolated by us from the rumen of ruminants, soil, wastewater of a livestock complex, as well as strains *Bacillus velezensis* BIM B-844, *Bacillus velezensis* BIM B-845, *Bacillus velezensis* BIM B-1125 — antagonists of bacterial pathogens of fish, isolated from soil and river silt. These strains formed the basis of probiotic veterinary drugs (Bacinil-K, Emilin, Bacto-Health) and feed additives (Sporobact, Sporobact-K).

Opportunistic bacteria of the genera *Escherichia, Staphylococcus, Streptococcus, Klebsiella, Proteus, Pasterella,* and *Salmonella* — causative agents of infectious diseases of the gastrointestinal tract and respiratory system of animals and poultry — as well as bacteria of the genera *Aeromonas, Pseudomonas*, and *Sphingobacterium* — causative agents of bacterial diseases of fish — were used as test objects to determine the antagonistic activity of the studied cultures.

Selection and study of the physiological, biochemical and molecular genetic properties of antagonist bacteria were carried out using the methods described in [7, 8]. Bacterial species identification was performed using MALDI-TOF mass spectrometry. Antagonistic enzymatic activity was studied according to [8-12].

Submerged cultivation of antagonist bacteria was carried out on Meynell's nutrient medium in modifications [19] in Erlenmeyer flasks on a shaker (200 rpm) at a temperature of 28-30 °C for 48 hours; in 10-liter laboratory fermenters ANKUM (RF) (stirring mode 200 ± 10 rpm, temperature 30 ± 2 °C, air supply mode 0.8-1.2 l/l min), pilot-industrial fermenters LiFlus (Republic of Korea) with a volume of 100 l (fill factor 0.6, stirring mode 200 ± 20 rpm, temperature 30 ± 2 °C, air supply mode 0.8-1.2 l/l min).

Mass spectrometric analysis of the lipopeptide content in the extract was performed by high-performance liquid chromatography (HPLC) on an Agilent 1260 Infinity chromatograph (Agilent Technologies, USA) with an Accurate Mass Q-TOF 6530 hybrid high-resolution quadrupole-time-of-flight

mass spectrometer (Agilent Technologies, USA).

The effectiveness of stress factors (temperature shock) was assessed spectrophotometrically by the formation of heat shock proteins in liquid cultures of antagonist bacteria. Protein fractionation by molecular weight was performed by vertical SDS-PAGE in a polyacrylamide gel (10%) in a vertical electrophoresis chamber (Laemmli system) [13, 14].

The preparation of the DNA library for whole genome sequencing was carried out using commercial NEBNext Ultra II kits (E7805S and E7335S, New England Biolabs, United Kingdom) for subsequent Illumina sequencing and Ligation Sequencing Kit (SQK-LSK109, Oxford Nanopore Technologies, country) for subsequent nanopore sequencing. Whole genome DNA sequencing. High-throughput genome sequencing was performed on MiSeq device (Illumina, Russia) using MiSeq Reagent Kit v3 (2 × 301 nucleotides) and MinIon MK 1B (Oxford Nanopore Technologies) using an R9.4.1 flow cell. Genome assembly and bioinformatic analysis. Reads obtained on the MySec device were collected to contigs using the SPAdes program (https://cab.spbu.ru/software/spades/), version 3.14.1. Reads obtained on the MinIon device were collected to contigs using the Fly program (https://github.com/fenderglass/Flye), version 2.8.2. To analyze the frequency of occurrence of contigs and search for matching ends, the "combinator-FQ.py" script from the "TsAGII-Raznoe" software package was used (https://github.com/masikol/cager-misc). Pylon v. 1.23 was used to correct errors in draft genome sequences using high-quality MySec reads. (https://github.com/broadinstitute/pilon/). Functional annotation of nucleotide sequences was performed using the Rapid Annotations Using Subsystems Technology (RAST) service (https://rast.nmpdr.org/). Genetic maps were visualized using the CGView server (https://cgview.ca/) or the SnapGene Viewer 5.2 program. The search for prophage sequences in the analyzed genome was performed using the Faster web server (https://phaster.ca/). The antiSMASH 6.0 web server (https://antismash.secondarymetabolites.org) was used to identify, annotate, and analyze gene clusters of secondary metabolite biosynthesis. The search for mobile genetic elements in the bacterial genome was performed using the ISfinder web server (https://www-is.biotoul.fr/index.php).

Industrial trials of probiotic feed additives and veterinary drugs were conducted at livestock, poultry, and fish farms in the Republic of Belarus in collaboration with staff from relevant organizations. The effectiveness of the probiotic feed additives was assessed based on live weight, average daily gain, and relative growth rate, as well as the health status of the test animals through daily visual observation and morphological and biochemical blood analysis. Conversion efficiency and feed consumption per 1 kg of live weight gain were also assessed. The effectiveness of the probiotic veterinary drugs was assessed based on the duration of the disease, the number of recovered animals, therapeutic efficacy, and live weight gain in both test and control animals.

Statistical data processing was performed using Microsoft Excel 2016 and Statistica for Windows, v. 10.0 [15]. Calculations were performed in triplicate, taking into account the standard deviation. The t-test was used to establish the reliability of differences in mean values with a statistical significance level of $p < 0.05$.

3. Results and Discussion

It is well known that one of the most important stages in the development of probiotic products for veterinary medicine and feed production, effective against a wide range of pathogens affecting farm animals, poultry, and aquaculture fish, is the search for an active producer. This producer must meet a number of requirements when included in a probiotic product. Of particular importance are probiotics based on bacteria of the genus *Bacillus*, which are resistant to elevated temperatures, ensuring the preservation of

their properties during feed preparation using various methods (extrusion, expansion, pelleting, etc.) [3-6].

The advantages of our developed technologies for the production of microbial products for veterinary medicine and feed production lie in the fact that they are produced using strains of spore-forming bacteria with a range of economically beneficial properties, ensuring effective control of pathogenic microbiota and increased feed digestibility.

Based on their morphological, cultural, physiological, biochemical, and molecular genetic properties, the cultures we selected were identified as *Bacillus velezensis* and deposited in the Belarusian Collection of Non-Pathogenic Microorganisms under the numbers: *B. velezensis* BIM B-454, *B. velezensis* BIM B-497, *B. velezensis* BIM B-713, *B. velezensis* BIM B-844, *B. velezensis* BIM B-845, and *B. velezensis* BIM B-1125. The cultures produce a wide range of antimicrobial metabolites (surfactin, fengycin, bacillomycin, iturin, etc.) and exhibit high enzymatic activity. Quantitative determination of hydrolytic enzymes showed that protease activity of the studied cultures on a nutrient medium with molasses varied in the range from 18.6 to 19.3 U/ml with a maximum in *B. velezensis* BIM B-713 (19.3 U/ml). The best results for endo-1, 4-β-glucanase activity were noted for *B. velezensis* BIM B-713 (1.2 U/ml), *B. velezensis* BIM B-1125 (1.1 U/ml), *B. velezensis* BIM B-845 (1.041 U/ml). Xylanase activity of bacteria varied in the range from 4.78 U/ml (*B. velezensis* BIM B-713) to 5.14 U/ml (*B. velezensis* BIM B-845). Amylolytic activity ranges from 34.6 U/ml in *B. velezensis* BIM B-454 to 69.77 U/ml in *B. velezensis* BIM B-713 [16].

The results of veterinary toxicological tests showed that the studied bacterial strains are not pathogenic, toxigenic, or allergenic, do not inhibit the growth of normal intestinal microbiota, belong to exogenous transient (self-eliminating) microflora, and are resistant to acid stress (pH from 2 to 10), bile (from 0.3 to 10%), and the antibiotics ampicillin (300 μg/ml), streptomycin (20 μg/ml), and chloramphenicol (10 μg/ml); they do not contain plasmids carrying antibiotic resistance genes. The high antagonistic activity of the cultures against pathogenic and opportunistic microflora of farm animals, birds, and fish, the presence of a spectrum of hydrolytic enzymes, and the absence of cross-antagonistic activity in the selected cultures made it possible to use them together to create consortia with complementary properties (in terms of antagonistic and enzymatic activity) [17-19].

As a result of molecular genetic studies, the complete nucleotide sequence of the *B. velezensis* BIM B-454 bacterial genome was obtained for the first time. It was shown that the genome of this strain is represented by a circular chromosome, 4,204,088 bp in size, and three plasmids of 6,990 bp, 6,587 bp, and 6,117 bp in size, respectively. The bacterial chromosome contains 4,209 predicted genes. The features of the strain's chromosome organization were determined, including the presence of unique nucleotide sequences (the BgIII type II restriction enzyme gene) and mobile genetic elements with a unique localization (ProPhageX phage), which can be used in typing the studied strain. The full-genome sequences were annotated and deposited in the GenBank database under the accession numbers CP082262.1-CP082265.1. The *B. velezensis* strain BIM B-454 D has a large number of genetic determinants responsible for economically valuable traits (antibacterial, antifungal, etc.). Fourteen loci have been identified in the bacterial chromosome that encode genes for the synthesis of substances with antagonistic activity of various chemical natures: lipopeptides (surfactin, fengycin), polyketides (bacilene, difficidin, macrolactin), the siderophore bacillibactin, the dipeptide bacilizin, the azole-containing peptide plantazolin, etc. Molecular genetic analysis of the *B. velezensis* BIM B-454 genome in comparison with other *B. velezensis* genomes is described in detail in the article [20]. The complete nucleotide sequence can serve as the basis for a detailed functional analysis of practically significant properties of microorganisms of

the genus Bacillus. The antagonistic activity of the *B. velezensis* strain BIM B-454 D is due to the production of a complex of antimicrobial compounds, including cyclic lipopeptides of nonribosomal synthesis: surfactin A, fengycin A, and bacillomycin LA. Key factors stimulating the synthesis of these metabolites have been identified; in particular, the effect of heat shock on the intensification of the synthesis of active lipopeptides (surfactin A) has been established [20].

Antimicrobial metabolites of *B. velezensis* BIM B-1125, an antagonist of pathogens in valuable fish species, were isolated and characterized. Extracellular localization of the antimicrobial compounds was established, and their stability over a wide temperature range of 50-100 °C and pH 2-10 was demonstrated. Thin-layer chromatography and liquid chromatography-mass spectrometry experimentally demonstrated the production of lipopeptide metabolites by the strain, belonging to the iturin and surfactin families (iturin A, iturin A4, iturin isomers A6-A7, surfactin A, surfactin C, and surfactin isomers B) [21].

In studies aimed at increasing the productivity of selected cultures, it was established that targeted exposure to stress factors (heat exposure at 60 °C for 20 min) contributed to improved growth characteristics of bacteria and increased their resistance to adverse environmental factors [22]. When adding 5-10% of the heat-treated inoculum, activation of growth and synthesis of target metabolites by the liquid culture was observed: the CFU titer increased from 2.1×10^9/ml to 4.7×10^9/ml, and antagonistic activity against *E. coli* and *Staphylococcus* sp. was 29.3 ± 0.9 mm and 31.0 ± 0.5, respectively. The selected conditions of temperature treatment of *B. velezensis* BIM B-454 D seed material (60 ºC, 20 min) and the optimized mass transfer mode in the fermenter (aeration intensity of 1 liter of air/l min, stirring speed of 200 rpm) make it possible to increase the productivity of the culture, reduce the fermentation duration from 48 h to 36 h and obtain a liquid preparation with higher CFU and spore titers (by 3.0-3.5 times), antagonistic activity (by 15-20%), and enzymatic activity (protease - 10.7-19.5 U/ml, cellulase - 0.9-1.2 U/ml, xylanase - 4.2-5.3 U/ml) [22].

Scaling up the fermentation process for obtaining probiotics in industrial-type devices (LiFlus-300) using the developed approaches (fractional feeding of the substrate, thermal activation of the seed material (60 ºC, 20 min)), ensures an increase in the titer and antagonistic activity of the final product (by 15-20%), a reduction in the duration of fermentation (up to 42-44 hours) and, as a consequence, a reduction in energy and labor costs for the production of the drug, as well as the cost of the final product (by 15-17%).

The developed dry formulation of probiotic preparations (Emilin, Bacto-Health) and feed additives (Sporobact, Sporobact-K) based on spore-forming bacteria using tripoli and wheat flour as carriers helps maintain bacterial viability over a long period (at least 24 months) and is characterized by prolonged action. According to veterinary toxicological testing, the preparations are non-pathogenic, non-toxic, and non-toxigenic, and do not affect the quality of livestock, poultry, or fish products.

Microbial Preparations for Veterinary Medicine and Feed Production

Between 2011 and 2024, we developed and commercialized 12 original technologies for producing probiotics for veterinary and feed use with antimicrobial, enzymatic, immunostimulating, and antioxidant activity based on spore-forming cultures. As examples, we will give the following.

A waste-free, competitive technology for producing the probiotic product Bacinil-K, based on the spore-forming bacteria *B. velezesis* BIM B-454 D, has been developed. This product is intended to correct the gastrointestinal microbiota and stimulate the immune system in cattle, pigs, and poultry. The product exhibits high antagonistic activity against pathogenic and opportunistic bacteria that cause animal infectious diseases, including *E. coli*,

Staphylococcus aureus, *Streptococcus* sp., *Salmonella* et al. According to production tests, the product has a prophylactic efficacy of 93.3% and a therapeutic efficacy of 96.0%. The duration of gastrointestinal diseases in animals is reduced by 1.9-2.4 times, and the average daily live weight gain reaches 15-20%. Bacinil-K is used in various agricultural enterprises and livestock complexes specializing in the raising and fattening of young cattle and pigs, as well as in the production of broiler chicken meat.

An effective, environmentally friendly technology for producing and using the probiotic preparation Emilin has been developed. It is based on the bacteria *B. velezesis* BIM B-844 and *B. velezesis* BIM B-845 D and exhibits high antimicrobial activity. This preparation is used for the prevention and treatment of bacterial diseases in fish of the carp family (pond carp, wild carp, their hybrids, etc.). The preparation is administered with feed. It affects representatives of opportunistic and pathogenic microflora, activates the body's non-specific defense systems, causing an increase in such parameters as the bactericidal activity of blood serum (BAS) by 17.7-24.4%, phagocytic activity (PA) by 17.0-28.8%, phagocytic index (PI) by 1.8-2.6%, and the phagocytic number (PC) by almost 2 times. The probiotic has a beneficial effect on the vitality of fish and their ability to withstand stress during wintering. Fish fed the product survive winter better, are less susceptible to bacterial infections, and begin feeding earlier and more actively. The survival rate from winter is 8% higher, and weight gain is 10% higher than in fish not fed the probiotic.

Bacto-Health, a probiotic preparation based on *B. velezesis* bacteria, BIM B-1125 D, for the prevention and treatment of bacterial diseases in valuable fish species, has both a direct effect on opportunistic microflora and an indirect effect by activating the body's non-specific defense systems. Application of the probiotic at a concentration of 400 g/t of feed when feeding according to fish farming standards (3% of fish weight), as well as its use in baths at a concentration of 10 g/m³ with a 20-minute exposure, has a pronounced positive effect on the level of natural (non-specific) resistance in sturgeon and salmon, including indicators of cellular and humoral immunity, causing an increase in such indicators as BASK, FA, FI, and FC. After a 5-day course of probiotic feeding, significant differences were observed between the experimental and control fish. In sterlet fed with the probiotic Bacto-Health, BASK was 32.2% higher, FA was 30.9% higher, FI was 109% higher (more than 2 times), and FC, which characterizes leukocyte aggressiveness, was 175% higher (2.53 versus 0.92). In trout from the experimental group, BASK was 69.4% higher, FA was 48.9% higher, FI was 83.8% higher, and FC was 172% higher (4.55 versus 1.67).

Probiotic feed additives Sporobact, Sporobact-K (based on *B. velezensis* 497 and *B. velezensis* 713 with complementary properties) are intended to increase the bioavailability of feed and correct the microbiocenosis of the gastrointestinal tract of pigs, poultry, and young cattle. Probiotics have complex enzymatic (proteolytic, cellulolytic, xylanase) and antagonistic activities, improve the quality and digestibility of feed, reduce its contamination with pathogenic and opportunistic microflora, and have therapeutic and prophylactic properties. Experiments have shown that the use of probiotics in compound feed rations for young pigs promotes the activation of protein, carbohydrate, and mineral metabolism, improved absorption of feed nutrients, and a decrease in feed costs per 1 kg of live weight gain by 4.7-5.8%. For broiler chickens, there was an increase in live weight by 3.2-6.2%, an increase in the growing efficiency index by 25.4-61.2%, a decrease in feed costs per 1 kg of live weight gain by 3.2-10.3%; for young cattle — an increase in the average daily gain of animals by 11.2%, a decrease in feed costs per 1 kg of live weight gain by 11.1%; in the diets of lactating cows — intensification of digestion processes and an increase in milk productivity of animals, an increase

in the palatability of roughage, digestibility of feed nutrients (a decrease in feed costs to obtain 1 kg of milk by 7.4%), an increase in milk yield of natural (by 6.1%) and basic (by 6.5%) fat milk, as well as an improvement in its qualitative composition (an increase in protein and fat content by 0.02%).

Based on the results of industrial trials, the use of our developed probiotic biopreparations contributes to: a 45-50% reduction in the treatment time for animal diseases; an increase in weight gain by 6-10%; a reduction in feed costs per 1 kg of weight gain by 12-18%; and an improvement in the quality of agricultural products. In terms of technological and microbiological characteristics, Belarusian probiotic products are comparable to the best foreign analogues.

4. Conclusion

Our results provide compelling evidence that the use of probiotics is a promising approach to preventing and treating diseases in farm animals, poultry, and fish.

Probiotics are becoming an integral part of modern animal husbandry, ensuring environmental friendliness and improving product quality. Regular use promotes significant weight gain in young animals, strengthens the body's defenses, and restores microbial balance in the gastrointestinal tract, thereby ensuring high effectiveness in preventing gastrointestinal infections.

Research focuses on using specific bacterial strains isolated from the microbiome of farm animals as the basis for probiotic feed additives to normalize microflora, accelerate weight gain, and improve feed quality, ensuring environmentally friendly production.

The results of this research have formed the basis for the development of competitive technologies for the production and use of biotechnological products for various purposes. The development of these technologies facilitates the solution of complex agricultural challenges and the introduction of new types of competitive, export-oriented biotechnological products to the domestic market, aligning with the 5th and 6th technological paradigms. These developments are highly innovative, correspond to the global level of development of biological science and make a significant contribution to the development of the "green economy".

References

[1] Donkova, N. V., and Donkov S. A. 2021. "Enzymatic activity of bacteria of the genus *bacillus* during hydrolysis of statch-containing raw materials." *Bulletin of Krasnoyarsk State Agrarian University* 5: 174-179.

[2] Fomichov, N. V., Bogolubova, N. V., and Romanov, V. N. 2020. "Comparative evaluation of natural feed additives for their functional effects on digestion processes and rumen microbiota in sheep." *Agricultural biology* 55 (4): 770-783.

[3] Afanaseva, U. G., Korbmacher, E. R., and Kolodina E. V. 2023. "Probiotics as an alternative to feed antibiotics." *Bulletin of the Altai State Agrarian University* 2: 65-72.

[4] "Probiotics in animal feed market by livestock, source, form and region – global forecast to 2025." *Markets and Markets*. https://www.marketsandmarketsblog.com

[5] Pohilenko, V. D., and Perelygin, V. V. 2007. "Probiotics based on spore-forming bacteria and their safety." *Chemical and biological safety* 2/3: 20-41.

[6] Iliashenko, A. N. 2024. "Naturpro bacillary probiotic in pig farming technology" *Our agriculture: veterinary and animal husbandry* 6: 2-7.

[7] *Methods of General Bacteriology*. 1984. Gerhard, F. (Ed.). M.: Mir. V. 3. p. 264.

[8] *Microbiology Workshop*. 2005. Netrusov, A. I. (Ed.). M.: Akademia. p. 608.

[9] Petrova, I. S., and Vintunaite, M. M. 1980. "Determination of proteolytic activity of enzyme preparations of microbiological origin." *Applied biochemistry and microbiology* 2 (2): 322-327.

[10] Sinitsyn, A. P., Gusakov, A. V., and Chernoglazov, V. M. 1995. "Bioconversion of lignocellulosic materials." M.: MGU: 146-147.

[11] *Enzyme Preparations. Methods for Determination of Xylanase Enzymatic Activity: GOST R 53047-2008*. 2009. p. 15.

[12] *Enzyme Preparations. Methods for Determination of Amylolytic Activity: GOST 20264.4-89*. 1989. p. 27.

[13] Struchkova, I. V. 2012. *Theoretical and Practical Principles of Protein Electrophoresis in Polyacrylamide Gel. N. Novgorod*, p. 60.

[14] Berezhnaia, A. V., Romanovskaya, T. V., and Kolomiets, E. I., 2019. "Thermal shock as a factor in increasing the

productivity of the periodic process for obtaining the biopesticide Betaprotectin." In *Microbial Biotechnology: Fundamental and Applied Aspects*, Proceedings of the *XI International Scientific Conf.*: 131-132.

[15] Kozlov, A. U., Mhitoran, V. S., and Shishov, V. F. 2021. Statistical data analysis in MS Excel. M.: INFRA-M. p. 320.

[16] Sverchkova, N. 2025. "Enzymatic and antagonistic activities of Probiotic Bacterial Strains *Bacillus velezensis* – The Basis of Feed Additives and Veterinary Drugs." In *Microbial Biotechnology: Fundamental and Applied Aspects*, Collected Papers of Institute of Microbiology, NAS Belarus, Vol. 17: 249-262.

[17] Ushakova, N. A., Nekrasov, R. V., Pravdin, V. G., Kravtsova, L. Z., Bobrovskaya, O. I., and Pavlov, D. S. 2012. "A new generation of probiotic preparations for feed use." *Fundamental Research* 1: 184-192.

[18] Sverchkova, N., Kolosovskaya, M., Romanovskaya, T., Zhuk, G., Kolomiets, E., Krasochko, P., and Lomako, Y. 2012. "New probiotic product for prevention and treatment of enteritis and respiratory diseases of farm stock." In: Varfolomeev, S. D., Zaikov, G. E, Krylova., L. P. (Eds.), *Biochemistry and Biotechnology: Research and Development.* New York: Nova Science Publishers: 106-112.

[19] Sverchkova, N. Romanovskaya, T., Zhuk, G., Kolomiets E., Degtyarik, S., Asadchaya, R., and Ageets, V. 2016. "Probiotic preparation for commercial pisciculture." In: Berhardt, L. V. (Ed.), *Advances in Medicine and Biology* New-York: Nova Science Publishers: 149-163.

[20] Sverchkova, N., Akhremchuk, A., Valentovich, L., and Kolomiets, E. 2022. "A molekular genetic analysis of the bacterial genome of *Bacillus velezensis* BIM B-454 D: the basis of a probiotic preparation for veterinary practice." *Applied Biochemistry and Microbiology* 58 (1): S75-S85.

[21] Kantor, K. V., Proskurnina, I. A., Sverchkova, N. V., and Kolomiets, E. I. 2022. "Analysis of antimicrobial metabolites of bacteria *bacillus amyloliquefaciens* BIM B-1125." *Doclady NAS Belarusi* 66 (3): 321-329.

[22] Kolomiets, E. I., Romanovskaya, T. V., Sverchkova, N. V., Berezhnaia, A. V., Zhuk, G. V., Girilovich, N. I., Stempitsky, V. R., Kuleschov, A. A., and Michaluk, A. V. 2015. "Improving the technology for producting the probiotic Bacinil K for feed production." In *Microbial biotechnology: Fundamental and Applied Aspects*, Collected Papers of Institute of Microbiology, NAS Belarus, Vol. 7: 144-159.