Metabolic activity of B. subtilis and B. bifidum when cultivated on grain nutrients

Gayane Svazlyan[1,*], *Viktor* Popov[2]

[1]Federal State Budgetary Scientific Institution " Federal Agricultural Kursk Research Center ", Karla Marksa str. 70B, Kursk, Russian Federation
[2]Federal State Budgetary Scientific Institution " Federal Agricultural Kursk Research Center ", 305021, Karla Marksa str., 70B, Kursk, Russian Federation

Abstract. This article presents the results of research aimed at evaluating the biological effectiveness of probiotics, the mechanisms of their effect on the macroorganism and the prospects for the development of a new generation of biologics based on metabolites of probiotic cultures. In this studies, special attention is paid to the production of active metabolites by cultivating probiotic microorganisms Bacillus subtilis strain DSM-32424 and Bifidobacterium bifidum No. 1 on grain nutrient medias which was created on the basis of sprouted and non-sprouted grains of oats of the Nemchinovsky variety and grains of wheat Bezostnaya-100 variety. The obtained results make it possible to form scientifically based approaches to the use of probiotics and their metabolites, in order to increase the productivity of farm animals by optimizing digestive processes and strengthening their immune system.

1 Introduction

Within the framework of modern livestock production conditions, the productive and reproductive indicators of animals increasingly depend not only on their genetic potential, but also on a properly organized diet that supports the healthy functioning of metabolic processes. Studies report that the genetic potential of animal productivity is closely related to feeding as the main element of technology which ensures high levels of physiological state of animals [1-4]. The introduction of biologically active additives based on probiotics into the diet allows to mobilize the genetic potential and strengthen key metabolic processes, maintaining them at an optimal level. In this regard, the development of scientifically based and highly effective probiotic and metabiotic supplements aimed at maintaining a healthy intestinal microbiota and enhancing nonspecific resistance of the body is becoming increasingly important [5-7].

Probiotics are biological additives consisting of living microorganisms that have a positive effect on animal health, primarily by maintaining microbial balance in the intestine. They stimulate metabolic and immune processes, which contributes to an increase in overall productivity and stability of physiological parameters [8-12]. Probiotics play a role not only as productivity stimulants, but also as critical tools for optimizing energy

[*] Corresponding author: manukyang@yandex.ru

resources, directing them to increase the efficiency of metabolism and, consequently, to increase productivity. It is important to note that such drugs, despite the fact that they are not indispensable for life, significantly contribute to the stabilization and increase of production indicators [13-16]. However, the resistance of probiotics in the gastrointestinal tract varies, and often a significant part of them (about 70%) is excreted from the organism, which makes it necessary to search for more stable and effective solutions to prolong their effects.

Due to the fact that probiotic microorganisms entering the gastrointestinal tract, only about 5% retain their viability, despite their widespread use, they become ineffective. In modern science, a new generation of drugs based on probiotic microorganisms, metabiotics, are more widely used. These are drugs containing active metabolites of probiotic microorganisms [17-20].

Metabiotics, representing the products of the metabolic activity of probiotic cultures, become a logical continuation of the probiotic concept. They contain a whole complex of active substances, including enzymes, peptides, amino acids, bacteriocins and lysozyme, which have a beneficial effect on the body, maintaining a healthy microflora and preventing the development of pathogenic microorganisms. Unlike traditional probiotics, metabiotics have increased biological availability and a more stable effect on the intestinal microflora. This makes them a promising direction for the creation of new feed additives aimed at correcting the microbiota, stimulating immune reactions and maintaining stable metabolic processes in animals. The introduction of metabiotics into the diet helps to improve digestive functions, increase the absorption of nutrients and maintain a healthy state of intestinal microbiocenosis [21].

The development and application of metabiotics is a promising approach in the formation of microbiocenosis of the intestinal microflora. In this regard, the creation of biologically active feed additives based on them is an important task for correcting the intestinal microbiota and stimulating nonspecific resistance of the body [22-25].

The development of new symbiotic drugs and the study of their interaction with the macroorganism to optimize and maintain the microecology of animals is an important area in the scientific strategy for the development of a probiotic concept based on the extraction of metabolites of probiotic cultures from various nutrient

The authors have identified the main scientific provisions of the probiotic concept for the development of symbiotic biologically active additives of a new generation (metabiotics) for the correction of metabolism. In this regard, we consider it expedient to provide a brief justification of the main provisions aimed at correcting metabolism.

Metabiotics are metabolic products or structural components of probiotic microorganisms [16].

In the complex of active metabolites, an important factor is the presence of amino acids, vitamins, organic acids and enzymes, due to the activity of which digestion improves; the immune defense of the body is enhanced.

2 Microecology

It should be noted that the normal microflora of the gastrointestinal tract competes for many pathogens and the mechanisms of suppressing their growth are quite diverse. The main one is the selective binding of cell surface receptors, especially epithelial ones. Most representatives of the resident microflora show pronounced antagonism, directed, among other things, against pathogenic species.

2.1 Stimulation of the immune system

The role of normal microflora as an antigenic stimulator of the immune system is equally important. The absence of a normal microbial biocenosis causes numerous violations of it. During normal colonization, numerous microbes induce the formation of low titers of antibodies (AT), the target of which is unlikely to be specific species. These antibodies (AT) are predominantly Ig class A released onto the surface of the mucous membranes. On the one hand, IgA forms the basis of local immunity to penetrating pathogens and prevents commensals from penetrating deep tissues, on the other hand, they contribute to maintaining homeostasis of the mucous membranes themselves.

2.2 Metabolism

It is difficult to overestimate the role of normal intestinal microflora in various metabolic processes and maintaining their balance. For example, the metabolism of some substances includes their excretion from the liver into the intestinal lumen, followed by a return to it; a similar hepatic-intestinal cycle is characteristic of some sexual hormones and bile salts. In the vast majority of these products are excreted in the form of glucuronides or sulfates, which are not capable of reabsorption in such a form. Their absorption is caused by intestinal bacteria that produce glucuronidases and sulfatases, so the physiological significance of such properties is quite obvious. It should be noted that the same sulfatases can also have an adverse effect, which was established by the example of the artificial sweetener cyclamate. The enzyme converted cyclamate into the carcinogenic product cyclohexamine, which causes malignant transformations of the bladder epithelium. A generally accepted fact is the leading role of normal microflora in providing the human body with Fe2+, Ca2+ ions, vitamins K, D and many B vitamins (especially Bp riboflavin, nicotinic, folic and pantothenic acids). The advantages of metabiotics are justified by the fact that they have high bioavailability, unlike probiotic microorganisms [25].

It should be noted that the developed provisions (control and optimization of fermentation factors of components, qualitative and quantitative composition of synbiotic biologically active additive (laboratory studies)) They include specific tasks to achieve the set research goal.

The purpose of the research is the scientific and practical justification of metabiotics as a new generation of biologically active additives in the development of the probiotic concept.

2.3 Objects and methods of research

The research was conducted in the Laboratory of Veterinary Medicine and Biotechnology of the Federal State Budgetary Scientific Institution "Federal Agricultural Kursk Research Center " (FGBNU "Kursk FARC "), Kursk, Russian Federation (RF), using high-precision methods to study the properties of probiotic metabolites. For the cultivation of probiotic cultures, strains of B. subtilis (DSM-32424) and B. bifidum (No.1) were selected. These strains were grown on nutrient media based on wheat and oats. Sprouted and non-sprouted grain of naked oats of the Nemchinovsky variety was used as a substrate for the B. subtilis strain, and wheat grain of the Bezostnaya—100 variety was used for the B. bifidum strain. The preparation of the cultural material included the preliminary sterilization of the substrate and its preparation by soaking and germination, which made it possible to create favorable conditions for the growth of probiotic cultures and their metabolic activity.

In order to maintain a stable temperature regime and pH level of the medium, cultivation was carried out in a thermostat at a temperature of 37 ± 1 ° C for two weeks, which provided optimal conditions for the growth and activity of microorganisms. The cultivation process and the number of probiotic cultures were monitored in the Goryaev chamber with a Levenhuk 740T microscope. The amino acid composition was analyzed by capillary electrophoresis in accordance with GOST R 55569-2013, and the protein content was determined according to GOST 32044.1-2012. For statistical data processing, methods of variational analysis and the Student's t-test were used statistically significant at P≤0.05, which allowed to obtain reliable results on the quantitative and qualitative characteristics of biological products.

3 Research results

1. Analysis of biotechnological aspects of probiotic cultivation B. *subtillis* in a grain nutrient medium based on naked oats and B. *bifidum* in a grain nutrient medium based on wheat.

When cultivating probiotic microorganisms, the choice of a nutrient medium is an important factor. Wheat of the "Bezostnaya-100" variety contains protein from 15%, gluten — from 27%. Naked oats of the Nemchinovsky-61 variety contain 11.6-15.3% protein in grain, 5.7-7.1% vegetable oil, 57.0-70.1% starch. It is known that the germination of grain is characterized by the activation of a complex of enzymes that enhances the metabolic processes in the grain, while nutrients are hydrolyzed into compounds easily digestible for the body [26, 27].

The preparation of grain nutrient media was carried out in the following sequence: cleaning and disinfection of grain; soaking the grains; germination of grains.

After washing, the grains were disinfected with an aqueous solution of iodine, soaking was carried out twice with an interval of 6 hours. For germination, the grain was placed in a uniform layer (2-3 cm) in a dish (enameled tray). The grain was germinated at T 15oC until the sprouts reached 1.5-2.0 times the grain size. To prepare the nutrient medium, a thermostat was placed in glass containers, gradually bringing the temperature to 90 ° C (10 ° C/hour).

The cultivation of microorganisms was carried out in a filler liquid at 37 ° C, pH 7.5.

The seed material for cultivation was probiotic microorganisms of daily cultures (15 * 10^6 CFU/cm^3) of bifidobacteria and subtilis.

As a result of the research, a high activity of CFU B. bifidum was revealed when cultivated on nutrient media on day 6. At the same time, CFU activity on the medium from sprouted grain (SW) is 21% higher compared to the medium from non-sprouted grain (W) on the third day (Figure 1). On the 14th day of cultivation, the indicators of the number of microorganisms based on SW were 15 * 10^6 KG / cm3, which was 20% higher than in the medium of W. The maximum differences in the number of B.bifidum in experimental samples were observed on the sixth and ninth days and amounted to 28% and 31%.

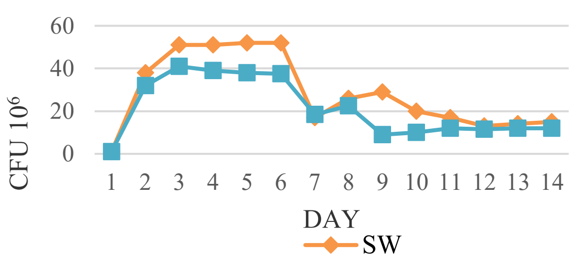

Fig. 1. Dynamics of the amount of CFU B.bifidum when cultivated on grain nutrient media (SW-sprouted wheat, W-non-sprouted wheat)

During cultivation, the dynamics of changes in the pH of the medium were studied (Figure 2). At the beginning of cultivation, for the first three days, there was a decrease in the indicator in both samples from 7.5 to 5. On the 14th day of the experiment, the pH level in the medium based on sprouted grain (SW) compared with the medium (W) was 3% lower and amounted to 4.74.

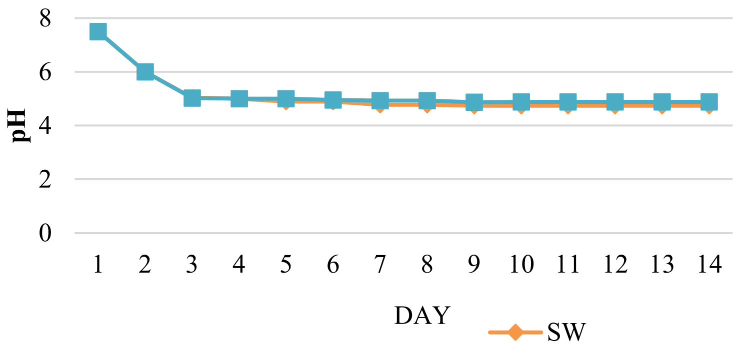

Fig. 2. pH dynamics during cultivation of B. bifidum

It is important to note that when cultivating CFU B. subtilis, certain features in the growth of the number of microorganisms are observed. The largest amount was recorded on the sixth day, amounting to 58*10^6 CFU/cm3 in the medium of sprouted oats (Figure 3).

When analyzing the dynamics of cultivation of probiotic microorganisms, a certain variability is noted (Figure 3). The CFU dynamics of B. subtilis in the sample of sprouted oats (OP) increased by 15.5% compared to the sample of non-sprouted oats (O) and reached 58*106 CFU/cm3 on the sixth day. After active CFU growth on the nutrient medium from the OP, on the eighth day, a decrease in the growth of microorganisms to 5.5*106 CFU/cm3 is observed. in the process of further cultivation, there is an increase in the number of CFU. On the tenth day, the number of BEDS increased to 30*106 CFU/cm3 and the growth rate remained at 28*106 CFU/cm3 until the end of the cultivation experiment period.

In the O-based nutrient medium, up to the ninth day, there is a decrease in dynamics to 9.5*106 CFU/cm3, and from the tenth day, an increase to 20*106 CFU/cm3 is observed.

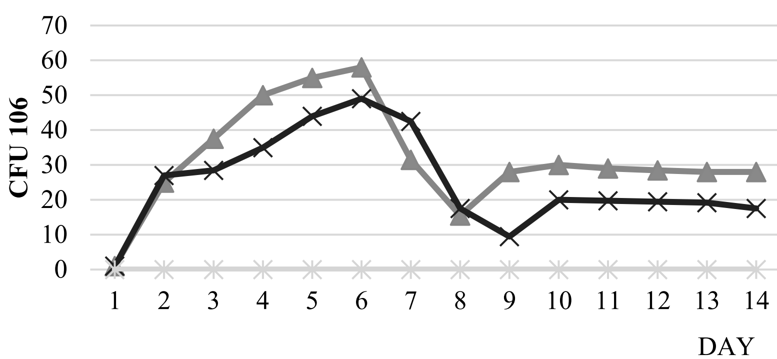

Fig. 3. Dynamics of CFU quantity during cultivation of B. subtilis on grain nutrient media SO-sprouted oats, O-not sprouted oats

2. Scientific and practical substantiation of the qualitative and quantitative composition of the experimental probiotic composition. When analyzing the content of proteinogenic amino acids of nutrient media during the cultivation of the probiotic microorganism B.

bifidumo, certain variability in their ratio is noted. In a comparative assessment, the content of these indicators in nutrient media was higher than 28.3% in a medium based on non-sprouted grain, the content of proline was by18.9%, leucine and isoleucine by 9.4%, methionine by 30.96%. At the same time, the indicators in the sample based on sprouted wheat in the ratio of amino acids are dominated by proline (34%), leucine and isoleucine (9.9% each), as well as alanine (9.5%).

Thus, as a result of the conducted studies, it was found that the probiotic microorganism B. bifidum was 21.16% higher during cultivation CFU, and the pH level was 3% lower in a medium based on sprouted wheat. Quantitative and qualitative differences in the content of proteinogenic amino acids and organic acids were revealed in experimental samples of B. bifidum suspension.

Thus, the resulting composition contains probiotic microorganisms, their active metabolites and cellular components of the grain nutrient medium, which makes it possible to determine the resulting composition as a liquid biologically active additive.

Probiotic suspension is a biologically active additive containing at least 28*106 CFU/ml of probiotic microorganisms and 0.7-0.8 crude protein, as well as proteinogenic amino acids.

During the experiment, when analyzing the qualitative and quantitative composition of amino acids in nutrient media based on sprouted and ungrouted grain of naked oats during cultivation of the probiotic microorganism B. subtilis, the crude protein content was 0.7-0.8 g/l and the following variability was noted (Figure 4).

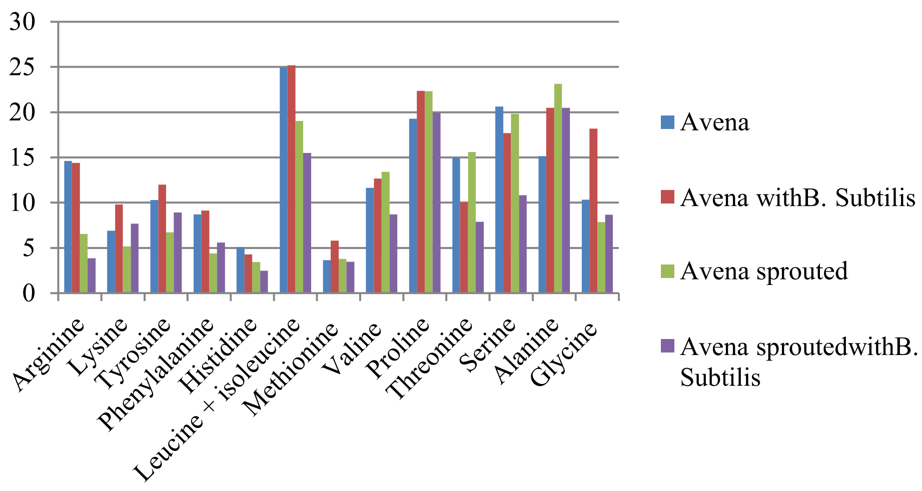

Fig. 4. Indicators of the amino acid composition of the probiotic suspension

The concentrations of glycine, lysine, and alanine in the OP-based medium were higher by 76.4%, 41.8%, and 35.5%, while arginine, leucine + isoleucine, valine, threonine, and serine significantly decreased (by an average of 37.8%)

The obtained experimental samples can be used as a biologically active additive containing probiotic microorganisms and their metabolites.

4 Conclusion

As a result of the research, high activity of metabolites of B. subtilis and B. bifidum strains was revealed when cultivated on nutrient media from sprouted grain. The qualitative and quantitative composition of the probiotic suspension confirms its high protein value.

The use of sprouted oat and wheat grains as a substrate has significantly improved the quantitative and qualitative parameters of the biological product, enriching its composition with essential amino acids and organic acids, which makes this approach promising for the development of new probiotic and metabiotic additives.

References

1. N. Belousov. Compound feed and genetics — key factors of productivity increase, Pig breeding, **2**, 82-85 (2018) (in Russ).
2. S.I. Kononenko, Actual problems of feeding organization in modern conditions, Polythematic network electronic scientific journal of Kuban State Agrarian University, **115**, 951-80 (2016) (in Russ).
3. D. Abadjieva, M. Chervenkov, K. Shumkov, A. Shimkus, A. Shimkiene and E. Kistanova, Improving of productive and reproductive performances in rabbits by biologically active feed additives, Modern Trends in Livestock Production: Proceedings of the 10th International Symposium, October 2-4. (Institute for Animal Husbandry, Belgrade-Zemun, Serbia, 2013) 288-298.
4. E.V. Karpenko, M.V. Postnova and V.S. Grishin, Biotechnological techniques for increasing the productive effect of feed for farm animals, Vestnik VolGU Natural sciences, **Series 11, 7 (1),** 19-22 (2017) (in Russ).
5. L.I. Podobed N.P. Buryakov, and G.Yu. Laptev, *Raising a calf from birth to a highly productive cow technological, feed and veterinary aspects* (St. Petersburg, 2017) p. 580 (in Russ).
6. L. Gamko, I.I. Sidorov, A.G. Menyakina, T.L. Talyzina and N.P. Bazutko, Probiotic additives as part of a feed mixture; influence on the productivity of fattening young animals, Pig breeding, **6,** 29-31 (2020) (in Russ).
7. R.K. Miluev, D.V. Engovatov and V.F. Engovatov, The effect of a new pre-starter feed on the metabolism and immune status of piglets, Feeding of farm animals and feed production, **8(18),** 42-51 (2020) (in Russ).
8. L.N. Gamko, I.I. Sidorov, T.L. Talyzina and Yu.N. Chernenok, *Probiotics to replace antibiotics*: monograph (Bryansk, 2015) 136 p (in Russ).
9. A.N. Panin and N.I. Malik, Probiotics are an integral component of rational animal feeding, Veterinary medicine, **7**, 19-22 (2006) (in Russ).
10. R. Patel and H.L. Dupont, New approaches to bacteriotherapy: Prebiotics, probiotics of the new generation and synbiotics Klin, *Infect. Dis* V 60 EXT, **2**, 108–21 (2015) DOI: 10.1093/cid/civ177
11. N.A. Ushakova, R.V. Nekrasov, I.V. Pravdin, N.V. Sverchkova, E.I. Kolomeits and D.S. Pavlov, Mechanisms of the influence of probiotics on symbiotic digestion, *Proceedings of the Russian Academy of Sciences* The series is biological, **5,** 468-76 (2015) (in Russ).
12. Aarti Singh, VishakhaVishwakarma, Barkha Singhal, Metabiotics: The Functional Metabolic Signatures of Probiotics: Current State-of-Art and Future Research Priorities – Metabiotics: Probiotics Effector Molecules, Advances in Bioscience and Biotechnology, **9,** 147-189 (2018) DOI: 10.4236/abb.2018.94012.
13. V.S. Popov, N.M. Naumov and G.A. Svazlyan, Scientific and practical justification and aspects of the use of metabolic probiotics in the correction of microbiocenosis, Bulletin of the UlGAU, **4**, 198-205 (2020) DOI 10.18286/1816-4501-2020-4-198-205 (in Russ).

14. R. Parker, Probiotics, the other half of the antibiotic story, Animal Nutrition and Health, **29**, 4-8 (2014)
15. A.V. Andreeva and O.N. Nikolaeva, Probiotics, their influence on the intestinal microbiota, Scientific notes of the educational institution "Vitebsk order" Badge of Honor "state Academy of veterinary medicine, **54 (1),** 86-89 (2018).
16. M.D. Ardatskaya, Probiotics, prebiotics and metabiotics in the correction of microecological intestinal disorders, Medical Council*,* **13**, 94-99 (2015) (in Russ).
17. M.D. Ardatskaya, L.G. Stolyarova and E.V. Arkhipova, Metabiotics as a natural development of the probiotic concept, Difficult Patient*,* **6-7**, 35-39 (2017) (in Russ).
18. R.V. Magomedaliev, M.G. Nekrasov, V.V. Chabaev, I.M. Javakhia, E.V. Glagoleva and M.I. Kartashov, The influence of the probiotic complex on productive qualities and metabolic processes in growing fattened young pigs, Agrarian Science, **1**, 22-26 (2020) (in Russ).
19. A.V. Strekalovskikh and A.I. Belousov, Scientific justification of the use of probiotics and metabiotics for the prevention of gastrointestinal diseases (literature review), Youth and science, **3**, 30 (2018) (in Russ).
20. B. Sánchez, S. Delgado, S. Blanco, A. Míquez, A. Lourenço, Gueimonde, and A Margolles, Probiocitcs, gut microbiota, and their influence on host health and disease, Mol. Nutr. Food Res, **61 (1)** (2017) doi: 10.1002/mnfr.201600240.
21. V.A. Neschislyaev, P.A. Mokin and T.V. Fedorova, On the issue of developing highly effective metabolic probiotics, Actual directions of scientific research: from theory to practice, **2-1 (8),** 15-17 (2016) (in Russ).
22. B.A. Shenderov, Metabiotics: novel idea or natural development of probiotic conception, MicrobEcol Health Dis, **24**, 203–99 (2013)
23. M Roselli, R Pieper, C Rogel-Gaillard, H Vries, M Bailey, H Smidt and C Lauridsen, Immunomodulating effects of probiotics for microbiota modulation, gut health and disease resistance in pigs, Animal Feed Science and Technology, **233,** 104-119 (2017) doi:10.1016/j.anifeedsci.2017.07.011
24. S. Lebeer, P.A. Bron, M.L. Marco, J.-P. Van Pijkeren, M. O'Conell Motherway, C. Hill, B. Pot, S. Roos and T. Klaenhammer, Identification of probiotic effector molecules: present state and future perspectives, Current Opinion in Biotechnology, **49**, 217 -23 (2018)
25. V.S. Popov, N.V. Vorobyeva, G.A. Svazlyan, N.M. Naumov and O.A. Gryaznova, Overview of Metabiotics and Probiotic Cultures During Fermentation of Molasses, Systematic Reviewsin Pharmacy, **11(9)**, 813-17 (2020) DOI:10.18411/srp.2020.9.115.1
26. A.Yu. Veselova, Intensification of the preliminary preparation of cereal crops in the conditions of the development of a new technology, Bulletin of the NGIEI*,* **2, 6 (7),** 27-37 (2011) (in Russ).
27. H.-T. Nguyen, D.-H. Truong, S.Ly. Kouhonde, H. Razafindralambo and F. Delvigne, Biochemical engineering approaches for increasing viability and functionality of probiotic bacteria, IntJMolSci, **17(6),** 867 (2016) doi: 10.3390/ijms17060867.