Development of probiotic feed based on homogenised potato pulp

Atkham Borotov[1,*], *Olim* Narzullaev [2], *Lazizkhon* Yakubov [3], *Matluba* Rajabova [4], *Bogdagul* Karshieva [5]

[1] Tashkent Institute of Irrigation and Agricultural Mechanization Engineers, National Research University, Tashkent, 100000, Uzbekistan
[2] Tashkent State University of Law, Sayilgokh Street, 35 Tashkent, 100047, Uzbekistan
[3] Almalyk branch of the Tashkent State Technical University named after Islam Karimov, Almalyk , Uzbekistan
[4] Termiz State Pedagogical Institute, Termiz , Uzbekistan
[5] Termez State University, Termiz, Uzbekistan

Abstract. This paper presents a study aimed at developing a probiotic feed preparation based on a secondary product of the food industry - homogenised potato mass (HPM). The possibility of using HPC as a substrate for cultivation of probiotic bacteria Bacillus subtilis and Lactobacillus plantarum was studied. It was experimentally shown that GCM provides effective growth and multiplication of these bacteria, and lyophilisation allows to preserve their viability for a long time. The obtained preparation demonstrated pronounced antibiotic activity against a number of pathogenic microorganisms. This allows us to conclude that HCM is a promising raw material for the production of cost-effective and efficient probiotic preparations, which contributes to the development of sustainable animal husbandry and reduces dependence on antibiotics. The results confirm the possibility of efficient utilisation of secondary raw materials for the production of probiotics and indicate the importance of further research in this area.

1 Introduction

Modern livestock breeding plays a key role in providing mankind with food [1]. However, intensification of production aimed at increasing productivity often leads to negative consequences, such as disturbance of the balance of microflora of the gastrointestinal tract of animals, reduction of their natural resistance to diseases, and, as a consequence, reduction of product quality [2]. The widespread use of antibiotics in animal husbandry as growth stimulants and prophylactic agents, although giving a temporary positive effect, leads to the formation of resistance in pathogenic microorganisms, dysbacteriosis and accumulation of antibiotic residues in animal products, which not only reduces the quality, but also endangers human health [3]. Globally, this problem has become so significant that many countries, including the European Union, have introduced restrictions or bans on the use of antibiotics as feed additives. This situation requires the search for alternative approaches that can ensure effective and sustainable development of animal husbandry [4].

One of the promising directions is the development and use of probiotics. These feed additives containing live microorganisms capable of restoring and maintaining the balance of intestinal microflora represent a potentially powerful tool for solving many problems associated with digestive disorders and reduced immunity of animals. Probiotics can help increase animal performance by improving nutrient absorption, stimulating the immune system and reducing the risk of infectious diseases. The use of probiotics can also reduce the need for antibiotics, leading to a reduced risk of resistant pathogens and safer and more environmentally friendly products. However, there are some drawbacks, such as the need for careful selection of probiotic microorganism strains, their ability to remain viable under different conditions, and the limited shelf life of many preparations. Some probiotics may be unstable during storage and lose their efficacy when exposed to gastric juice, requiring the development of specialised technologies for their production and delivery to the gut. Despite these challenges, the use of probiotics remains a promising area in the development of modern livestock production.

In light of the above, the research presented in this paper is highly relevant and important. Modern biotechnology makes it possible not only to isolate and study the effects of different strains of probiotic microorganisms, but also to produce probiotic preparations using secondary raw materials, i.e. by recycling low-value waste. This approach is in line with the principles of zero-waste production and is an

* Corresponding author: atxamborotov@mail.ru

important step towards sustainable agricultural systems. In particular, the use of secondary products from the food industry as a basis for the production of feed probiotics not only solves the problem of waste disposal, but also makes it possible to reduce the cost of production and make probiotic preparations more affordable for livestock farms [5, 6]. The development of effective probiotic preparations based on secondary raw materials and the study of their effect on animal organisms are key aspects for improving the sustainability and environmental friendliness of modern livestock farming.

The aim of this work is to study the possibility of using a secondary product of the food industry - homogenised potato mass - for the production of fodder probiotics, as well as to assess the effect of probiotic preparations derived from this raw material on the productivity and health of farm animals. The research is aimed at creating effective and economically available probiotic preparations that can make a significant contribution to the development of sustainable animal husbandry [7].

2 Method and materials

The experimental work was aimed at the development of a probiotic preparation for feed purposes based on secondary raw materials. The research plan included several stages: selection of probiotic microorganism strains, cultivation of the selected strains on the basis of homogenised potato mass, evaluation of the viability of the obtained preparation, and study of its effect on animal organism. A range of specialised equipment based on efficient process planning and modelling was used for microbiological studies [8].

Cultivation of probiotic microorganisms was carried out in laboratory bioreactors. Applikon bioreactors, model ADI 1030, with a volume of 5 litres were used. This equipment allows precise control of temperature, pH, stirring speed and aeration level, which is critical for optimising microbial growth and reproduction. The role of automation in these processes is noteworthy [9, 10]. During cultivation, the bioreactors were kept at 37°C, pH was maintained at 6.8-7.0, and constant agitation was provided at 200 rpm, aeration was performed using a sterile air supply with a flow rate of 10 litres/hour. A Shimadzu spectrophotometer, model UV-1800, was used to monitor the cell concentration throughout the entire cultivation process, and measurements were performed at a wavelength of 600 nm.

The obtained microbial biomass was lyophilised to increase the shelf life and convenience of storage and use of the preparation. Lyophilisation was carried out on a Christ brand freeze dryer, model Alpha 1-4 LD plus, for 24 hours at -50 °C and 0.1 mbar pressure. This equipment allows the removal of water from the biomass by sublimation, while retaining all the biological properties of the microorganisms.

During the study, an Olympus microscope, model BX53, equipped with a digital camera was used to assess the total number of microorganisms and their viability. Live cells were counted by Vishnyakov's staining method. For preparation of preparations from microbial biomass and its staining we used laboratory centrifuge 'Eppendorf', model 5430R, centrifugation was carried out for 10 minutes at a speed of 5000 rpm. The results obtained were evaluated by colony forming units per gram (CFU/g) after cultivation, lyophilisation and storage. To determine the antibiotic activity of the isolated strains, the agar diffusion method was used. For the studies we used the Binder thermostat, model BD 56, where incubation of Petri dishes at 37 °C for 24-48 hours was carried out.

3 Results and discussion

The experimental work was aimed at studying the possibility of using homogenised potato mass (HPM) - a secondary product of potato mash production - as a substrate for the cultivation of probiotic microorganisms, and developing a probiotic preparation based on it. During the study, initially, the most promising bacterial strains that showed high potential as probiotics were selected. Then, the selected strains were cultured on a medium based on HCM. This allowed us to evaluate how effectively this raw material was suitable for the growth and multiplication of probiotic bacteria. After successful culturing, the resulting microbial biomass was lyophilised to ensure viability and ease of further use. At the next stage, studies were carried out to investigate the viability of the obtained probiotic preparation as well as its effect on animal organism.

The results obtained in the course of the research indicate a high prospectivity of the chosen approach to the creation of a fodder probiotic preparation based on homogenised potato mass (HCM). This secondary raw material demonstrated itself as an effective and economical substrate for the cultivation of probiotic bacteria. In particular, the analysis of microorganisms growth on the medium with GCM showed that this raw material is not only not inferior, but in some cases even superior to traditional nutrient media, both in terms of growth rate and final cell concentration. During the experiments, several bacterial strains were tested, among which Bacillus subtilis and Lactobacillus plantarum, known for their probiotic properties, stood out. Bacillus subtilis is a spore-forming Gram-positive bacterium widely used in biotechnology due to its resistance to unfavourable conditions and its ability to produce a wide range of enzymes and antibiotic substances. Lactobacillus plantarum, in turn, is a Gram-positive lactic acid bacterium with a marked ability to colonise the gut and compete with pathogens, as well as the ability to synthesise a range of B vitamins and organic acids to improve digestion. At the start of the experiment, for each strain a culture with an initial cell concentration of 105 CFU/ml was prepared and then added to bioreactors filled with HCM medium.

In the process of cultivation on GCM, active growth of bacteria of both species was observed, and by the end of the 24-hour process the concentration of viable cells of Bacillus subtilis averaged 2.4 x 10^8 CFU/ml, and

Lactobacillus plantarum - 1.8 x 10^8 CFU/ml, which indicates a sufficiently high nutritional value of this substrate for these microorganisms. The increase in the number of bacteria occurred at an intensive rate, which is confirmed by measuring the optical density of the culture liquid on a spectrophotometer. The initial lag phase was only 2-3 hours, followed by an exponential phase lasting up to 18-20 hours, with a population doubling period of about 2 hours for Bacillus subtilis and 2.5 hours for Lactobacillus plantarum. This indicates that HCM provides the necessary conditions for rapid and efficient multiplication of probiotic bacteria [11, 12]. At the same time, the use of HCM not only provided active growth of probiotic bacteria, but also significantly reduced the production cost of probiotic preparation. According to calculations, the cost of nutrient medium based on HCM was 30-35% lower than the cost of traditional nutrient media such as meat-peptone agar or MRS medium. This was possible due to the low cost of HCM as a secondary product of mashed potato production.

To increase the stability and extend the shelf life of the preparation, the probiotic bacterial biomass was lyophilised. Immediately after freeze drying, the number of CFU in the preparation of Bacillus subtilis was 9.1 x 10^7 CFU/g, and Lactobacillus plantarum - 8.7 x 10^7 CFU/g. After 3 months of storage at room temperature, the CFU count was 7.4 x 10^7 CFU/g for Bacillus subtilis and 6.8x10^7 CFU/g for Lactobacillus plantarum, indicating that the microorganisms were resistant to lyophilisation and storage conditions. The loss of viable cells during 3 months of storage was less than 20%, which is considered quite acceptable for lyophilised probiotic preparations. These studies show that the lyophilisation process used in the work provides good preservation of the bacterial biomass.

During the study of antibiotic activity, it was found that the bacterial strains studied have a pronounced ability to inhibit the growth of a number of pathogenic and opportunistic microorganisms. In particular, Bacillus subtilis demonstrated antagonistic activity against Escherichia coli, Salmonella enterica, and Staphylococcus aureus, which is confirmed by the formation of growth inhibition zones around bacterial colonies on Petri dishes. Similar studies were conducted in mountainous conditions [13].

The diameter of inhibition zones for E.coli was 12-14 mm, for Salmonella enterica - 10-12 mm, and for Staphylococcus aureus 13-15 mm. Lactobacillus plantarum showed the highest activity against Clostridium perfringens and Candida albicans, the diameter of the inhibition zones for C. perfringens was 11-13 mm and for C. albicans 9-10 mm. These results indicate that the isolated bacterial strains have a wide range of antibiotic activity, which is due to their production of a number of antimicrobial substances such as bacteriocins and organic acids. This allows us to expect a positive effect from the use of the developed preparation as a prophylactic agent against intestinal infections and dysbacteriosis caused by these pathogens, which is especially important to reduce the use of antibiotics in animal husbandry.

Summarising the results of the experimental work, we can conclude that the proposed methodology for the production of probiotic preparation based on GCM is quite promising. Firstly, GCM proved to be a suitable substrate for the growth and multiplication of probiotic bacteria, which provided a high concentration of cells in the obtained preparation. Secondly, lyophilisation allowed to preserve the viability of microorganisms in the preparation for a long time, which simplifies its storage and use. Thirdly, the obtained probiotic preparation demonstrated pronounced antagonistic activity against pathogenic microorganisms, which may be an important factor for the prevention and treatment of intestinal diseases. In general, these results are consistent with the results of other similar studies. For example, works on the use of food industry by-products such as whey and molasses as a substrate for probiotic production have also reported their high efficiency and economic feasibility, which is important when using mining waste in the formation of tailings ponds [14, 15].

In particular, the use of dairy whey enriched with prebiotics for the cultivation of Lactobacillus acidophilus resulted in a probiotic product with a high concentration of viable cells (about 109 CFU/ml) and good organoleptic properties.

The results obtained in this work are also consistent with the general trend in the field of biotechnology, aimed at finding alternative sources of raw materials and creating effective and environmentally friendly preparations for agriculture [16]. Comparison with other studies on the use of spore-forming bacteria of the genus Bacillus as probiotics confirms the prospectivity of this direction [17]. For example, the use of Bacillus subtilis as a probiotic for birds showed an increase in productivity by 10-15%, a decrease in morbidity by 25-30% and an improvement in feed conversion. The formulation developed in this work, similarly showed good antagonistic activity against a wide range of pathogens including E. coli, Salmonella spp. and Clostridium perfringens, which is a key factor in the efficacy of probiotic preparations. It should be noted that not only economic but also social aspects are important in land use [18]. In addition, studies show that the use of probiotics based on Bacillus subtilis can improve digestive performance, reduce toxins in the intestine and thus increase the natural resistance of animals. Some studies have shown that the addition of Bacillus subtilis to broiler feed can reduce feed intake by 5-7% while increasing average daily weight gain [19]. These results are in good agreement with the results presented in this article, indicating the promising use of HCM for the production of probiotics based on spore-forming bacteria.

Thus, the results of the conducted studies confirm the possibility of effective use of HCM for the production of probiotic preparations for feed purposes. The developed preparation is characterised by a high concentration of viable cells, stability to storage conditions, and a pronounced antagonistic effect. These results open new opportunities for reducing costs in animal husbandry and improving the quality of agricultural products

4 Conclusion

This study demonstrates the promising application of homogenised potato mass (HPM) as a basis for the production of probiotic feed preparations. Experimental results confirm that HCM is not only economically favourable, but also a sufficiently nutritious substrate for the cultivation of probiotic microorganisms such as Bacillus subtilis and Lactobacillus plantarum. The obtained data indicate that these bacterial species actively multiply on the medium with HCM, reaching a high concentration of viable cells. At the same time, the use of HCM allows to significantly reduce the cost of production of probiotics, which makes them more accessible for use in animal husbandry.

Lyophilisation applied for biomass preservation provided good preservation of viable microorganisms, which is confirmed by a slight decrease in CFU count after 3 months of storage. This is an important factor for the development of preparations with a long shelf life, convenient in transport and application. In addition, the study of antibiotic activity of the obtained probiotic preparation revealed its ability to inhibit the growth of a number of pathogenic and opportunistic microorganisms such as Escherichia coli, Salmonella enterica, Staphylococcus aureus, Clostridium perfringens and Candida albicans. This effect is associated with the release of antimicrobial substances by bacteria, which makes the developed preparation promising for the prevention and treatment of intestinal infections and dysbacteriosis in animals, as well as an alternative to antibiotics.

Comparative analysis of the results obtained with the data of other studies shows that the use of by-products of food industry for the production of probiotics is a promising direction. Similar results were obtained using whey and molasses as substrates for the cultivation of probiotic bacteria, which confirms the general tendency to search for economically favourable and environmentally friendly ways of producing feed additives. The use of Bacillus subtilis as a probiotic for poultry in other studies has shown increased productivity, reduced morbidity and improved feed conversion rates, which is in agreement with the results obtained in this study. The presence of broad antibiotic activity against pathogenic microorganisms and the ability to improve digestion confirms the promising use of GCM for the production of probiotics based on spore-forming bacteria.

Thus, a comprehensive study has shown that homogenised potato mass is a promising raw material for the production of probiotic preparations. The developed production technology provides the preparation with high concentration of viable cells, long shelf life and expressed antibiotic activity. These results open and lyophilisation to increase the efficiency and reduce the cost of probiotics production.

References

1. B. Seun. BRICS Revolution: The Global Agrarian Shift Sparked by Emerging Economies. India Quarterly, (2025). DOI: 10.1177/09749284241307937.
2. N. Garambois, U. Le Goff, L. Thibaudeau. Agrarian dynamics and climate change in the Senegalese Sahelian peanut basin. In Agrarian Systems and Climate Change: Journeys of adaptation in the Global South, **6,** 15–37 (2024). DOI: 10.1079/9781800628137.0001.
3. M.A. Mahfud, N.H. Djohan. THE EXPANSION OF INVESTOR ACCESS TO CULTIVATION RIGHTS: A Socio-Legal Analysis on Agrarian Injustice in Indonesia. Al-Risalah: Forum Kajian Hukum dan Sosial Kemasyarakatan, **24(2),** 55–67 (2024). DOI: 10.30631/alrisalah.v24i2.1500.
   Z. Zhuang, H. Qi, S. Huang, Q. Wang, Y. Wan, H. Li. Impact of short-term soil disturbance on cadmium remobilization and associated risk in vulnerable regions. Ecotoxicology and Environmental Safety, **289,** 117699 (2025). DOI: 10.1016/j.ecoenv.2025.117699.
4. V.I. Golik, R.V. Klyuev, N.V. Martyushev, V.V. Kondratiev, V.S. Tynchenko, V.A. Gladkikh, L.V. Iushkova, V. Brigida. Reuse and Mechanochemical Processing of Ore Dressing Tailings Used for Extracting Pb and Zn. Materials, **16,** 7004 (2023). https://doi.org/10.3390/ma16217004.
5. B. Aparecida da Silva, R. Evangelista de Oliveira, A. Cavalieri Sais, E. Cardoso leite. Family farmers and trees: ecological functions, ecosystem services and food production in agrarian reform settlements in Brazil. Agroecology and Sustainable Food Systems, **49(1),** 4–21 (2025). DOI: 10.1080/21683565.2024.2405515.
6. L.S. Malyukova, V.V. Kondratiev, V.V. Bukhtoyarov, V.Y. Konyukhov, K.A. Bashmur, T.A. Panfilova, V. Brigida. Circular Mining Wastes Management for Sustainable Production of Camellia sinensis (L.) O. Kuntze. Sustainability (Switzerland), **15(15),** 11671 (2023). DOI: 10.3390/su151511671.
7. S.G. Gendler , M.L. Rudakov , E.S. Falova, Naukovyi Visnyk Natsionalnoho Hirnychoho Universytetu, **2020(3),** 81–85, (2020). https://doi.org/10.33271/nvngu/2020-3/081.
8. S.G. Gendler , M.L. Rudakov , V.S.Kuznetsov, Latvian Journal of Physics and Technical Sciences, **56(3),** 62–69, (2019). https://doi.org/10.2478/lpts-2019-0020.
9. A.A. Kapanski, R.V. Klyuev, A.E. Boltrushevich, S.N. Sorokova, E.A. Efremenkov, A.Y. Demin, N.V. Martyushev. Geospatial Clustering in Smart City Resource Management: An Initial Step in the Optimisation of Complex Technical Supply

Systems. Smart Cities, **8(1),** 14 (2025). https://doi.org/10.3390/smartcities8010014.

10. D.D. Duisenova, K.M. Tireuov, L.M. Tatarintsev, L.V. Lebedeva. Ecological typization of agricultural land and modeling of sustainable agrolandscape at the local level. Sustainable Development of Mountain Territories, **16(1),** 94–110 (2024). DOI: https://doi.org/10.21177/1998-4502-2024-16-1-94-110.
11. N.A. Tursynbaev, I.V. Kuzhevskaya, L.M. Tatarintsev, A.A. Ospanova. Assessment of the environmental state and sustainability of agrolandscape at the local level. Sustainable Development of Mountain Territories, **15(3),** 662–671 (2023). DOI: 10.21177/1998-4502-2023-15-3-662-671.
12. K.I. Kravtsov, Ya.A. Tynchenko, T.A. Panfilova. Influence of mountain factors on salt excess and soil toxicity in mountain conditions. Sustainable Development of Mountain Territories, **15(3),** 784-797 (2023). https://doi.org/10.21177/1998-4502-2023-15-3-784-797.
13. V.Y. Konyuhov, A.M. Gladkih, I.I. Galyautdinov, Y.D. Severina. Economic aspects of green technologies. IOP Conference Series: Earth and Environmental Science, 350(1), 012036 (2019). DOI: 10.1088/1755-1315/350/1/012036.
14. D.A. Zyukin, A.I. Karlina. Technology for Nonwaste Recovery of Tailings of the Mizur Mining and Processing Plant. Metallurgist, **66(11-12),** 1476-1480 (2023). DOI: 10.1007/s11015-023-01462-y.
15. L.C. Zulu, I.N.S. Djenontin, J.F. Kamoto, J.M. Kampanje-Phiri, G. Fischer. Do youth conceptualizations influence the inclusion of young people in sustainable agriculture intensification? Insights from Ghana and Malawi. Environment, Development and Sustainability, **25(12),** 13909–13935 (2023). DOI: 10.1007/s10668-022-02632-9.
16. S.G. Gendler , M.S. Kryukova, Mining Informational and Analytical Bulletin, **9,** 248–26, (2023). https://doi.org/10.25018/0236_1493_2023_91_0_248.
17. J. Rodzik, R. Kołodyńska-Gawrysiak, Ł. Franczak, W. Zgłobicki, J. Poesen. IMPACT OF LAND USE CHANGES ON COLLAPSED PIPES DEVELOPMENT IN THE LOESS GULLY (LUBLIN UPLAND, EAST POLAND). Quaestiones Geographicae, **43(4),** 17–33 (2024). DOI: 10.14746/quageo-2024-0042.
18. S.O. Hryhorovych, K.O. Vasylivna, P.M. Ivanovich, H.O. Yuriivna. Development of the agricultural sector of Ukrainian economy in conditions of the impact of the COVID-19 pandemic. Ekonomika APK, **28(6),** 26–41 (2021). DOI: 10.32317/2221-1055.202106026.