REVIEW

of a dissertation on the topic:

"NEW PRODUCERS OF PREBIOTIC CARBOHYDRATES FROM BULGARIAN YOGURT" for the acquisition of the ESD "Doctor" in the field of higher education: 4.3. Natural Sciences, scientific specialty Microbiology

Author: Ivan Ivanov, full-time doctoral student, dismissed with the right to defend on 19.12.2023

Scientific supervisors: Prof. Penka Petrova, DSc and Prof. Kaloyan Petrov, DSc

Reviewer: Prof. Dr. Lyudmila Kabaivanova

Ivan Dimitrov Ivanov was born on September 22, 1978. He graduated from Thrace University - Stara Zagora, where he obtained a Master's degree in Veterinary Medicine (2004). He additionally specialized in pedagogy at the Faculty of Agriculture of Thrace University (2002), as well as practical training in animal husbandry technologies in China (2007). He also holds a "veterinary technician" qualification. Since 2004, he has been gaining diverse professional experience as a veterinary inspector, assistant at the Institute of Animal Sciences - Kostinbrod, consultant in the animal husbandry sector, and since 2020 he has been an assistant at the Laboratory of Genetics, Department of General Microbiology of IMikB.

The present dissertation work of Assistant Professor Ivan Ivanov is submitted for evaluation for the acquisition of the educational and scientific degree "Doctor" in the professional field 4.3. Natural Sciences, scientific specialty Microbiology.

The topic of the dissertation is relevant both from a scientific and an applied point of view. Prebiotics, and in particular galactooligosaccharides (GOS), occupy a key place in the development of functional foods. According to international market analyses, over the past decade the global prebiotics market has been growing by over 8% annually and already exceeds 6 billion US dollars. The demand is increasing due to the proven positive impact of prebiotics on the intestinal microbiota, the immune system and the prevention of metabolic diseases. In this context, the study of new bacterial producers of GOS from Bulgarian yogurt is timely and economically promising.

The dissertation is 120 pages long, structured as is customary, in the following sections: introduction, literature review (44 pages), aim and objectives, materials and methods (12

pages), results and discussion (30 pages), conclusions and contributions. The work contains 13 tables and 24 figures.

The introduction presents in a synthesized form the importance of fermented foods in human nutrition. They are healthy and have their role in improving the quality of life of people. Used since ancient times, fermented foods remain the subject of intensive scientific research in modern nutrigenomics, microbiology and biotechnology. The relevance and significance of the research to which the presented scientific work is dedicated is clearly justified. An emblematic dairy product stands out, which is Bulgarian yogurt, whose qualities and functional properties are undeniable, as well as the role of the specific microflora, mainly including *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

The literature review provides a historical overview of the emergence of fermented foods, as well as scientific achievements in the field in our time, with emphasis on the importance of the participating microorganisms and the health-beneficial metabolites contained in traditional Bulgarian fermented dairy products. Based on the existing scientific literature, the role of microbial metabolites generated during the fermentation process is highlighted. The beneficial effects of the consumption of dairy foods and the strains of lactic acid bacteria contained in them, as preventive and therapeutic agents, are indicated. The mechanisms of biological detoxification by lactic acid bacteria, the potential of galactooligosaccharides as prebiotics, the structural and functional role of oligosaccharides in human and animal milk, and the possibilities for biotechnological production of prebiotic ingredients with high added value are reviewed.

The list of cited literature contains 218 sources covering the period 1990 - 2025. This emphasizes the long-term efforts of scientists around the world to clarify the key functions of microorganisms and the metabolites they produce with proven potential for improving health, for their use in modern food science and industry, as well as the high degree of relevance of the topic being developed.

The purpose of the dissertation is clearly stated.

The six tasks form a specific plan for conducting research in the correct sequence and logical connection between them.

The **Materials and Methods** section is presented in 4 parts, which describe classical and mainly modern analysis approaches used in the course of the development. Molecular genetic methods are described in detail.

In the chapter **Results and Discussion**, significant data are presented, following the set goal of the dissertation work. The biodiversity of Bulgarian yogurts has been determined using metagenomics. New starter and companion strains of lactic acid bacteria have been isolated and identified using modern genetic approaches, and the resulting bioactive metabolites have been studied using precise analytical methods. This dissertation provides new data on the composition, enzyme profile and metabolic capabilities of strains originating from authentic Bulgarian products. New aspects of the genetic and functional diversity of *Lactobacillus bulgaricus* are shown and specific strain differences in the enzymatic profile of *L. bulgaricus* are described, which determine different potential for synthesis of functional oligosaccharides. The presence of the accompanying microflora in home-made yogurts from different regions of Bulgaria has been proven, an indicator of the great biodiversity, which leads to the production of unique metabolites with probiotic value such as organic acids, esters, exopolysaccharides, and galactooligosaccharides, which contribute to the health characteristics of the product. New perspectives for using the isolated microorganisms in the development of functional foods with proven positive effects on the overall health of people have been revealed.

The central part of the results is the description of the experiments for cloning and heterologous expression of the lacZ gene, which encodes the enzyme beta-galactosidase in *L. bulgaricus* strain 43, isolated from the Rhodope Mountains. After overexpression in *E. coli*, the enzyme was purified and the temperature and pH optima were determined, the influence of divalent metal ions on its activity was noted. In vitro processes of enzymatic synthesis of galactooligosaccharides from the substrate lactose with different initial concentrations were carried out and a high yield of GOS was achieved, as can be seen from the comparison with similar results obtained by other researchers.

The Results and Discussion section ends with a **Conclusion**, which emphasizes the most important achievements, which logically leads to the formulated conclusions and contributions. They are clearly stated and correspond to the results obtained in the course of the scientific work, and I cannot fail to note: the identification of rare species of lactic acid bacteria, including *L. equicursoris*, reported for the first time in yogurt; the evidence obtained that the lacZ gene from *L. bulgaricus* strain 43 encodes a new enzyme β-galactosidase with 21 unique amino acid substitutions, providing its higher activity compared to other similar enzymes in the species; the tetrameric structure of the β-galactosidase of L. bulgaricus strain 43, determined by the amino acids Glycine 191, Glycine 215 and Serine 214; The successful heterologous expression of the enzyme in *E. coli* strain BL21 (DE3) resulted in the production of a recombinant enzyme

with enormous activity (over 3000 U/mL), as well as the unusually high transgalactosidase activity of the Bulgarian isolate *L. bulgaricus* 43.

I accept author contributions of a scientific-fundamental and scientific-applied nature.

The abstract has been prepared according to the requirements and corresponds to the data presented in the dissertation.

Conclusion

The dissertation submitted to me on the topic: "NEW PRODUCERS OF PREBIOTIC CARBOHYDRATES FROM BULGARIAN YOGURT" contains scientific and scientific-practical results, representing significant scientific contributions. The author has managed to publish the obtained results in 3 scientific publications with impact factor and open access, which speaks of their significance. This is also an important reason for me to strongly recommend to the members of the Scientific Council of the Institute of Microbiology "Stephan Angeloff" - BAS to vote positively for the acquisition of the ESD "Doctor" by Asst. Ivan Dimitrov Ivanov.

07.10.2025 **Reviewed by:**

(Prof. Dr. Lyudmila Kabaivanova)