

REVIEW

by **Prof. Dr. Velizar Kostadinov Gochev,**
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dissertation for the award of the educational and scientific degree "**PhD**"

by: field of higher education 5. Technical sciences, professional field 5.11 Biotechnology
Doctoral Program Technology of biologically active substances

Author Nikola Nikolov Atanasov

Topic: *Properties and characterization of newly isolated lactic acid bacteria strains and their application in model probiotic oral health products.*

Scientific supervisor: *Assoc. Prof. Dilyana Nikolova, PhD, Department of Biotechnology, Faculty of Biology, Sofia University "St. Kliment Ohridski*

1. General description of the submitted materials

By Order No. RD-38-264 of 31.05.2024 of the Rector of Sofia University "St. Kliment Ohridski" (Sofia University), I have been appointed as a member of the scientific jury for providing the procedure for the defence of the dissertation on "*Properties and characterization of newly isolated strains of lactic acid bacteria and their application in model probiotic products for oral health*" for the acquisition of the educational and scientific degree "Doctor" in the field of higher education 5. Technical sciences., professional field 5.11 Biotechnology, doctoral program Technology of biologically active substances. The author of the dissertation is Nikola Nikolov Atanasov - PhD student in full-time study at the Department of Biotechnology with scientific supervisor Assoc. Prof. Dr. Dilyana Nikolova from SU. The set of materials submitted by Nikola Nikolov Atanasov on electronic media is in compliance with the requirements of the Academic Staff Development Act in the Republic of Bulgaria (ASDA) and the Regulations for Academic Staff Development of Sofia University (RASDSU), and includes all required documents.

2. Brief biographical data about the PhD student

Nikola Atanasov graduated from Sofia University and holds a bachelor's degree in professional field 5.11 Biotechnology, specialty Biotechnology, and a master's degree in professional field 5.11 Biotechnology, specialty Industrial Biotechnology. In the period from 2018 to 2020, she worked as a biologist at SU, from 2021 to 2023 as an R1 researcher under

Project BG05M2OP001-1.002-0012, and from 2024 as an assistant professor at the Department of Biotechnology of SU. In the period from 2017 to the present, he has participated in 6 research projects and passed a number of courses to improve his qualifications.

All this shows that the PhD student is purposefully developing professionally in the field of biotechnology.

3. Relevance of the subject matter and appropriateness of the set goals and objectives

For decades, probiotic microorganisms with a positive effect on human health and the possibility of their incorporation into various products, mainly food and cosmetics, have been the subject of sustained research interest, demonstrating the magnitude of this scientific problem. Attempts by a number of research teams to isolate new strains of probiotic lactic acid bacteria (LAB) with a specific metabolic profile and a wide range of different forms of biological activity (antimicrobial, antioxidant, anti-inflammatory, etc.), mainly from specific national fermentation products, are ongoing. All this aims to satisfy the high demands of modern consumers for the consumption of natural products that not only satisfy the plastic and energy metabolism of the body, but also exhibit a health-protective effect.

In this line of thought, I believe that the dissertation's topic is definitely topical, albeit in the field of a long and detailed problem developed in science.

4. Knowledge of the problem

I judge the level of knowledge of the research problem by the literature review, which is based on over 450 relevant and appropriately selected literature sources. The review discusses the composition of the oral microbiome in normal and imbalanced states and describes the factors influencing the dynamics of the oral microbiota composition. A characterization of the family Lactobacillaceae is presented with emphasis on the probiotic properties of its representatives. Much of the information is synthetically presented in tabular form. Different groups of products (tablets, pastilles, candies, etc.) including probiotic IBCs in their composition are briefly described. The review ends with a brief conclusion, which motivates the necessity of developing the thesis. The scope of the literature review and the manner in which the review is formatted give me reason to believe that the PhD student is familiar with the problem under development to a degree that allows him/her to correctly and clearly formulate the research objective, properly plan the experimental tasks and implement the experimental design of the study.

5. Research methodology

The experimental scheme of the thesis follows the mandatory steps for any biotechnological process, including isolation of pure culture from a target group of microorganisms, morphological and physiological-biological characterization of the isolates, and the application of species discriminative molecular biological and bioinformatic approaches for correct and definitive species identification of the isolates. All species-identified isolates of the IBC were subjected to an evaluation of their probiotic potential by a set of indicators, including survival in simulated human gastrointestinal tract conditions, antibiotic resistance, antimicrobial activity, adhesiveness, etc. The PhD student successfully combined classical microbiological, modern molecular biological, and bioinformatics approaches, achieving the stated goal and providing adequate answers to the problems addressed in the thesis.

6. Characteristics and evaluation of the thesis

The dissertation is structured in the accepted manner, including the sections Introduction (2 p.), Literature Review (39 p.), Aim and Objectives (2 p.), Materials and Methods (12 p.), Results and Discussion (47 p.), Conclusions (2 p.), Contributions (p.), List of References, and 2 p. Appendices. The thesis maintains optimal proportions between its various sections. The aim of the dissertation is clearly formulated, and 9 research tasks with their corresponding sub-tasks are set to achieve it. The experimental work started with the processing of 74 baseline samples from 16 healthy volunteers, and out of 64 oral microbial isolates, 12 isolates were identified as presumptive MCD. After 16S rRNA gene sequencing, protein profiling by MALDI-TOF, combined with classical microbiological approaches, the isolates were species-identified with a high percentage of correctness and species discriminability and assigned to the species *Limosilactobacillus fermentum*, *Latilactobacillus curvatus*, *Lactobacillus delbrueckii subsp. sunkii*, *L. delbrueckii subsp. lactis*, *Lactocaseibacillus rhamnosus*, *L. paracasei* and *Weissella confusa*. The survival of all species-identified isolates was monitored in simulated conditions of different compartments of the digestive system, i.e., the oral cavity, stomach, and small intestine. The adhesive, autoaggregation, antioxidant, biofilm-forming and antimicrobial activities of the isolates were determined, and the MAP index and survival of the strains under lyophilization conditions were also determined. The probiotic potential of the strains was evaluated on the basis of a complex scoring system, and it was reported that most of them possessed probiotic potential above 60 %, with the most pronounced one being strain *L. fermentum* TC 3-11 - 74.3 %. Two strains of *L. fermentum* N2 and *L. delbrueckii subsp. lactis* VG2 were selected for incorporation into model probiotic oral health products, and the survival

of the strains in the products was monitored and their microbiological stability was determined with respect to the indices of both number of microorganisms and E. coli. These studies give a certain completeness to the study. The dissertation's experimental design is well-structured, both in terms of the study's sequence and scope. The results obtained are correctly interpreted. Thirteen conclusions are formulated, which follow directly from the results obtained. On the basis of the obtained results 5 contributions have been formulated, which I define as scientifically applied with original character.

7. Assessment of the publications on the dissertation

The PhD student has submitted three scientific publications in journals indexed in the Scopus and Web of Science databases with quartiles Q2 and Q3. In all three publications, the PhD student is the lead author, which is evidence of his active role in the implementation of the publications, and all publications contain significant results of the dissertation.

8. Abstract of the thesis

The abstract is formatted according to the requirements of the relevant regulations and reflects the main results achieved in the thesis.

9. Critical comments and recommendations

I have no critical remarks about the dissertation submitted for review.

10. Personal impressions

I do not know the PhD student and have no personal impressions.

CONCLUSION

The dissertation contains scientific and applied results which represent an original contribution to science and meet all the requirements of the PRASRB and PRACSU. The dissertation shows that the PhD student possesses in-depth theoretical knowledge and professional skills in the scientific specialty of Technology of biologically active substances, demonstrating qualities and skills for independent scientific research. Because of the above, I confidently give my positive assessment of the research conducted, presented by the above-reviewed dissertation, abstract, results, and contributions, and propose that the honorable scientific jury award the degree of Doctor of Education and Science to Nikola Nikolov

Atanasov in the field of higher education. 5. 5 Technical Sciences, professional field 5.11
Biotechnology, doctoral program Technology of biologically active substances.

18.07.2024 г.

Reviewer:

(Prof. Dr. Velizar Gotchev)