

STATEMENT

by Assoc.Prof. Zdravka Yancheva Velkova, PhD
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Member of the academic jury set to render a decision on a procedure for the acquisition of educational and scientific degree “Doctor” (PhD) according to the Classifier of the areas of higher education 5. “Technical sciences”, professional field 5.11. “Biotechnologies”, Doctoral program “Technology of biologically active substances”

Author: Nikola Nikolov Atanasov

Form of doctoral studies: Full-time study

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

Scientific Supervisor: Assoc. Prof. Dilyana Nikolova, PhD, Department of Biotechnology, Faculty of Biology, Sofia University “St. Kliment Ohridski”

1. General presentation of procedure and the doctoral student

This statement is prepared in response to order No RD-38-264/31.05.2024 г. issued by the Rector of the University of Sofia “St. Kliment Ohridski” (SU). The package of materials presented to me in electronic form, for acquiring the educational and academic degree “Doctor” is in accordance with the Act on Development of Academic Staff of the Republic of Bulgaria (DASRB), the Regulations for its implantation and the Regulations for Academic Development of SU and includes all necessary documents.

Nikola Atanasov graduated from Sofia University “St. Kliment Ohridski” in 2018 where he received a bachelor’s degree in Biotechnologies, and in 2020 – a master’s degree in Industrial Biotechnologies.

Nikola Atanasov was enrolled as a PhD student at the Department of Biotechnology, SU, on July 20, 2020, and on July 20, 2023, was deducted with the right of defense.

From 2018 to 2020, he worked as a biologist at the Faculty of Biology of the SU, and since the beginning of this year he has held the position of assistant.

2. Relevance of the topic and expediency of the set goals and objectives

Nowadays, probiotics are defined as live microorganisms that, when consumed in suitable quantities, confer a health benefit to the host. Probiotic products have demonstrated promising results as anti-inflammatory, anti-carcinogenic, antimicrobial, antioxidant and immunomodulatory agents. Probiotic microorganisms are mainly lactic acid bacteria (LAB).

There is considerable interest in the search of new probiotic strains. The process is complex and involves isolation, characterization, identification, evaluation of their probiotic potential, formulation of probiotic products, and demonstration of positive effects on human health.

Oral diseases are common and can have a significant impact on the quality of human life. In recent years, intensive *in vitro* and *in vivo* studies have been conducted related to the role of probiotics in the prevention of various oral diseases - caries, fungal infections, periodontal disease, halitosis, etc. It has been found that probiotic strains interact with the oral microbiota, exhibit antipathogenic effects and maintain a healthy microbial balance.

The goal of the dissertation is actual and clearly stated – “Isolation, identification and characterisation of new LAB strains from the oral microbiome, evaluation of their probiotic potential, antimicrobial interactions, and investigating their potential application in a model probiotic product for oral health”.

To achieve the goal, nine tasks with corresponding subtasks, which are 20 in total, have been set.

3. Knowledge of the problem

The literature review is presented in 39 standard pages and is illustrated with three tables and five figures.

The design of the review and the systematic presentation of the information in it show that the doctoral student is familiar with the topic being developed.

4. Research methodology

All used materials and research methods are correctly described. The experimental researches are correctly structured, the separate stages are in logical sequence, which is a prerequisite for successful realization of the set goal. The PhD student has mastered and applied a number of classic and modern microbiological, biochemical and molecular methods. The obtained results were processed statistically.

5. Characteristics and evaluation of the dissertation, conclusions, and contributions

The dissertation is 149 pages long and is illustrated with 26 figures and 18 tables. The bibliography includes 467 literary sources (1 is in Cyrillic, and the remaining 466 - in Latin). The dissertation is structured as follows: Introduction (1 page); Literature review (39 pages); Purpose and tasks (2 pages); Results and discussion (47 pages); Conclusions (2 pages); Contributions (1 page); List of scientific publications on the dissertation (1 page); Bibliography (35 pages); Applications (2 pages). Detailed lists of all figures and tables in the dissertation (2 pages) and abbreviations used (1 page) are presented.

The introduction is focused on the increased interest in bacterial probiotics for oral health.

The literature review is well structured, providing detailed information on the human oral microbiome, microbial balance and imbalance in the oral cavity, and the relationship between oral

and systemic health. Information on probiotic microorganisms, probiotic properties of *Lactobacillaceae* genus, their specific mechanisms of action in the oral cavity and application in probiotic oral health products is presented. At the end of the review, a brief conclusion is presented, which motivates the purpose of the dissertation and the necessity of the planned research.

The Results and Discussion section is presented in accordance with the formulated tasks.

Twelve new LAB strains isolated from the human oral microbiome are the subject of the study. They have been characterized and identified to species by applying morphological, physiological-biochemical and molecular biological approaches. The probiotic potential of the newly isolated LAB strains has been investigated according to the following indicators: survival ability and growth dynamics under simulated conditions of the different departments of the gastrointestinal tract (GIT), adhesive abilities, antibiotic resistance, total antioxidant capacity, antagonistic activity against microbial test pathogens, including oral test pathogens, and survival ability during lyophilization and storage. A scoring system is proposed to evaluate the probiotic potential of the studied LAB strains. Two of them are selected, which have clearly demonstrated functional and probiotic characteristics. The selected strains *L. fermentum* N 2 and *L. delbrueckii subsp. lactis* VG 2, are included in the composition of two model probiotic products for oral health.

All obtained results have been analyzed in detail and compared with those obtained by other researchers. The experimental data are very well illustrated with 21 figures and 15 tables.

Based on the conducted research, 13 conclusions are formulated, which correctly reflect the obtained results. The presented contributions are five and can be classified as scientific and scientific applied in nature.

6. Evaluation of the thesis publications and the personal contribution of the doctoral student

Nikola Atanasov has presented 3 scientific publications on the thesis, in all of them he is the first author. All articles are published in journals, referenced and indexed in the global databases of Scopus and Web of Science, of quartile Q2 and Q3, according to SCImago Journal & Country Rank. Three citations have been noticed. The doctoral student has participated in 6 national and international scientific forums. He participated in 2 scientific projects and one national program.

7. Assessment of the extended abstract of the doctoral thesis

The extended abstract is prepared according to the requirements and reflects the content of the dissertation.

8. Critical notes and recommendations

I have no critical notes and recommendations.

9. Conclusion

Nikola Atanasov has acquired competence in terms of knowledge in a specific scientific field, demonstrated qualities and skills for independent conduct of scientific research, presentation and interpretation of the obtained results.

The presented dissertation contains scientific and scientific-applied results that represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Application and the Regulations for Academic Development of Sofia University "St. Kliment Ohridski".

Based on the analysis of the conducted research and the presented scientific works, I give my positive assessment of the presented dissertation work and recommend to the respected scientific jury to award Nikola Nikolov Atanasov the educational and scientific degree "Doctor" in area of higher education 5. Technical sciences, professional field 5.11 Biotechnologies (Technology of biologically active substances).

Reviewer:

/Assoc. Prof. Zdravka Velkova, PhD/

July 18, 2024