

## ***EXPERT OPINION***

**by Assoc. Prof. Denitsa Rumenova Teofanova, PhD,**

SU "St. Kliment Ohridski", Faculty of Biology, Department of Biochemistry, member of the Scientific Jury, appointed by Order No. RD-38-264 from 31.05.2024. of the Rector of SU "St. Kliment Ohridski", Prof. Georgi Valchev, for awarding the Educational and Scientific Degree "Doctor" in

Field of higher education: 5. Technical sciences,

Professional field: 5.11 Biotechnologies

PhD program: Technology of biologically active substances

**REGARDING:**

**Dissertation topic:** "Properties and characteristics of newly isolated lactic acid bacteria strains and application in model probiotic products for oral health"

**Author of the dissertation:** Nikola Nikolov Atanasov, full-time PhD student at the Biotechnology Department of the Faculty of Biology, SU "St. Kliment Ohridski"

**Scientific supervisor:** Assoc. prof. Dilyana Nikolova, PhD

### **1. General presentation of the procedure and the PhD student**

#### **1.1. Documents**

The materials submitted by Nikola Nikolov Atanasov include the following required documents according to the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Terms and Conditions for Acquiring Scientific Degrees and Holding Academic Positions at SU "St. Kliment Ohridski": 1) Dissertation - 50 points according to the minimum national requirements for the ESD "Doctor"; 2) Abstract in Bulgarian and English; 3) An extract from the "Authors" information system with the PhD student's contributions, which also contains a list of scientific publications, including those related to the dissertation, which are also provided in full text. They bring him 36.66 points out of the required 30 according to the minimum national requirements for the ESD "Doctor"; 4) CV; 5) Copy of the Master's degree qualification diploma; 6) Certificate of passed exams from the individual plan; 7) Plagiarism report with annexes; 8) Order for enrollment and dismissal from the PhD program, with the right of defense; 9) Declaration of originality and authenticity; 10) Order for the appointment of a scientific jury and 11) Certificate of compliance with the minimum national requirements for ESD "Doctor" - available 86.66 points out of 80 required according to the minimum national requirements for the ESD "Doctor".

The dissertation corresponds to the criteria for acquiring scientific degrees and holding academic positions in SU for professional field 5.11 Biotechnologies.

#### **1.2. Introduction of the PhD student**

Nikola Atanasov obtained his first "Professional Bachelor" degree with the specialty Dental Technician in 2014. in the Medical University - College of Medicine and second Bachelor's degree in Biotechnologies in 2018. in the Faculty of Biology of the SU "St. Kliment Ohridski". He graduated with a Master's degree in Industrial Biotechnologies in 2020, then in the period 2020-2023. was a full-time PhD student in the Biotechnology Department of the Faculty of Biology. He acquired additional professional work experience as a Biologist, Researcher R1 and subsequently Assistant in the Biotechnology Department, a position he holds up to date. He participated in a number of scientific projects and scientific forums. All this shows a

scientific interest, combined with the upgrading of knowledge, experience and skills, thanks to additional courses, internships and summer schools.

### **1.3. Personal impressions of the PhD student**

I have known Nikola Atanasov since 2015. as his teacher within the framework of his studies in the Bachelor's degree, and consequently also through collaborative professional contacts, which continue to this day, between the colleagues from the Biotechnology Department, where he was a PhD student, and the laboratory of the Department of Biochemistry, in which I work. He has always shown curiosity, a desire to upgrade his knowledge and skills in various areas of the scientific sphere, hard work, dedication and persistence in every endeavor. His responsible attitude in the implementation of various activities related to his dissertation work and in his participation in scientific research projects is indisputable. It should be noted the serious progress that this colleague has made in recent years in terms of increasing his qualification skills, which is evidenced not only by the successfully passed exams during the PhD studies, but also by the above-mentioned courses, participation in scientific forums in the promotion of the achieved results and the training of undergraduate students within the framework of the compulsory pedagogical activity.

### **1.4. Plagiarism**

Based on the plagiarism check annexes, it is clear that the detected similarity rates for the three criteria do not affect the original results obtained, are not the result of illegal borrowing and copying of foreign text, and do not bear signs of plagiarism.

## **2. Characteristics of the thesis**

### **2.1. Relevance and significance of the dissertation topic**

The relevance and significance of the topic of submitted dissertation work are indisputable and can be considered in several aspects. One of them is the fact that in recent years probiotics have been the subject of increased interest and are an extremely attractive topic in terms of their potential and benefits for good physical condition and increasing the health status of people. Moreover, expanding and detailing the knowledge of the natural and pathogenic oral microflora will not only be of fundamental importance, but will also contribute significantly to the maintenance of good oral health and hygiene. On the other hand, linking the above-mentioned two factors will provide a scientifically sound basis for strategies in the development of probiotic products for the prevention and therapy of pathological conditions. A large part of the dissertation work concerns this extremely modern, contemporary alternative to the classically used medications – probiotic microorganisms included in products that are also with better biological tolerance. The effects of their metabolism or of products they can synthesize, as well as their action as natural antagonists of various pathogens can contribute positively above all to the prevention of oral diseases, as well as to their therapy, and from there to the overall state of health of people. And last but not least, the importance and relevance of the topic is confirmed by the publications on the topic, which are not only in high-impact and reputable journals, but already have citations.

## **2.2. Structure of the dissertation, persuasiveness of the obtained results, interpretations, conclusions, and scientific contributions**

The dissertation is prepared according to the generally accepted scheme. It is written on 149 standard printed pages and comprises the following sections, including sub-sections: Title Page (1 page), Acknowledgments and information about the dissertation (2 pages), Table of Contents (1 page), Description of figures and tables (2 pages), List of Abbreviations Used (1 page), Introduction (1 page), Literature Review (39 pages), Aim and tasks (2 pages), Materials and methods (12 pages), Results and discussion (47 pages), Final Conclusions (2 pages), Statement of contributions (1 page), List of scientific publications related to the dissertation (1 page), List of references (35 pages) and Appendices (2 pages). The information presented in it is illustrated with 26 figures (19 of which in the "Results and Discussion" section and 2 in the Appendices) and 18 tables (12 of which in the "Results and Discussion" section), which are of sufficiently good quality.

The dissertation is written in an appropriate scientific language, all rules are observed, there are a small number of semantic, technical and stylistic errors and it is of sufficient volume to acquire the PhD degree. There is a good balance in terms of the optimal ratio between sections, with the "Results and Discussion" section dominating the "Literature Review". A more positive impression would have been made by separating the "Results" and "Discussion" sections, which would show the PhD student's ability to interpret the obtained data and distinguish the obtained results from their discussion, which is an important skill acquired at this level of education.

The literature used includes 467 sources (1 in Cyrillic, 465 in Latin and 1 link to Internet sources), of which over 60% are from the last 10 years. I consider the number of sources in the bibliographic reference to be too large, but they are correctly selected according to the need of the subject.

*The introduction* is well structured, provides information and demonstrates the importance of the subject matter by outlining the foundations on which the work rests.

*The literature review* is extremely detailed and covers all aspects of the subject matter on which the work rests. It includes detailed data on the natural human oral microbiome, starting from its localization in the oral cavity, its composition and diversity, as well as its effects on oral and, accordingly, systemic health and factors inducing pathological conditions. The latter are considered in the light of causal relationships between microorganisms in the oral cavity, often related to the imbalance of natural and pathogenic ones. This first part could have been described more systematized and concentrated. Probiotic microorganisms and, in particular, oral ones are described in great detail, giving the absolutely necessary characteristics for them to be defined as such and their potential mechanisms of action. Special attention is paid to the representatives of lactic acid bacteria, their probiotic properties and the ways in which they can manifest them. The options for the application of lactic acid bacteria in various products for the therapy and prevention of oral diseases are also considered.

It becomes clear that the PhD student is well acquainted in the subject matter and in addition to good theoretical background, his ability to work with scientific literature and to systematize the available information is proven.

*The aim* of the dissertation is clearly formulated and hindered by the relevance of the topic. *The tasks* related to the planned scientific research activities to achieve the stated aim are also adequately set.

In the "*Materials and methods*" section, the techniques used are described in detail. It is evident that they are adequately selected and meet the set tasks. They are presented in a way suitable for reproducing the experiments and accompanied by data analysis methods. Some additional clarifications could be given such as the PCR program, processing of the volunteer samples, the number of which could be reported here rather than in the "Results and Discussion" section, the "optimal temperatures for the test pathogens" and the matrices for the molded chewing candies. The large range and variety of learned techniques is clearly evident, which indicates a good methodological background of the PhD student.

The results obtained from the conducted research are described consistently and in accordance with the tasks set in the "*Results and Discussion*" section and are well illustrated. The newly isolated strains of lactic acid bacteria are characterized in detail by using a multidisciplinary approach and covering a number of morphological, physiological, biochemical and genetic features. Species are taxonomically identified unambiguously and their survival in the conditions of the oral cavity (showing good survival) and the gastrointestinal tract (part of the strains showed sensitivity and growth inhibition) is evaluated. The adhesive abilities, antibiotic resistance and antioxidant capacity of the studied lactic acid bacteria strains were defined again by multiplex analysis based on different indicators and a good probiotic potential was determined. Antagonistic activity of the studied lactic acid bacteria strains with microbial test pathogens confirms the previous studies for some of them and provides an opportunity to determine the main mechanisms of antagonistic activity. Appropriate lyoprotective media were formulated to maintain the stability of the respective lactic acid bacteria strains during long-term storage. Based on expressed complex probiotic potential, two strains are selected, and included in two types of model products, with cocoa containing one determined as a more suitable matrix.

The interpretation of the obtained data is extremely well placed in the light of the available data in the literature of recent years, but the discussion could be more convincing and definitive in terms of a generalized relationship between the results and their explanations. Some of the legends of the figures and tables could be more detailed for the purpose of autonomous informativeness (e.g. Tables 5 and 8), and I believe it is appropriate to show the correlation analysis for antagonistic properties of the lactic acid bacteria.

Based on the obtained results, **13 conclusions**, which logically follow the results of the conducted research and **5 contributions** were formulated. I fully accept the contributions presented in the dissertation.

### **2.3. Correspondence between the Abstract and the dissertation**

The abstract is prepared in accordance with the requirements and presents in an abridged version the study, the experimental work, the results obtained, and the conclusions and contributions made on their basis. The English and Bulgarian versions are identical. I consider it correct to include the cited sources in the form of a bibliographic reference list at the end of the abstracts.

### **3. Questions for the PhD student**

I have the following questions for the PhD student:

1. Why did you perform the experiments against only two pathogens in most of the analyzes when determining the antagonistic activity of the studied lactic acid bacteria strains with microbial test pathogens?
2. Does it make sense to study combined probiotic activity between several lactic acid bacteria strains at the same time and what do you think the effect would be in a comparative aspect?
4. Based on what has been achieved so far, in what direction do you think future research should be directed?

### **5. Conclusion**

In conclusion, I believe that the presented dissertation work represents a complete and in-depth study on an undeniably relevant and extremely interesting, including for me, topic, incorporating modern and innovative techniques and bringing with it corresponding contributions to the scientific field. It demonstrates the PhD student's sound theoretical background and the acquired wide range of methodological skills in the analysis and solving of problems. Regardless of the remarks made, I believe that the dissertation fully complies with the Law on the Development of the Academic Staff in the Republic of Bulgaria, as well as with the Regulations for the Terms and Conditions for Acquiring Scientific Degrees and Holding Academic Positions at SU "St. Kliment Ohridski", for awarding the educational and scientific degree "Doctor".

All the above gives me grounds to positively evaluate the presented dissertation work, convinced to vote "YES" and to recommend to the other members of the esteemed Scientific Jury to support the awarding of the educational and scientific degree "Doctor" in professional field 5.11. Biotechnologies to Nikola Nikolov Atanasov.

25. 07. 2024.

Prepared the expert opinion: .....

Sofia

/Assoc. Prof. Denitsa Teofanova, PhD/