

STATEMENT

for the educational and scientific degree "PhD" in the professional field 5.11 Biotechnology, doctoral program Technology of biologically active substances

Dissertant: Ramize Hoxha

Dissertation Topic: FUNCTIONAL AND TECHNOLOGICAL CHARACTERISTICS OF NEWLY ISOLATED STRAINS OF LACTIC ACID BACTERIA FROM TRADITIONAL FOODS

Statement by: Assoc. Prof. Dr. Sci. Lyuben Ivanov Zagorchev, Department of Biochemistry, Faculty of Biology, Sofia University "St. Kliment Ohridski", member of the jury according to the order RD-38-163 of 01.04.2024 of the Rector of Sofia University "St. Kliment Ohridski".

I. General data on the applicant and chronology of the defense procedure

Ramise Hoxha was born on 26.02.1994 in Kocus, Albania as Ramise Ibrahimaj. She graduated consecutively with a BSc in Biotechnology (in 2015) and an MSc in Molecular and Industrial Biotechnology (in 2017) from the University of Tirana, Albania. The Master's degree is officially recognized by the National Center for Information and Documentation. In 2018 Ramise Hoxha was enrolled in a full-time PhD program at the Department of Biotechnology, PhD program "Technology of Biologically Active Substances" with scientific supervisor Assoc. Prof. Dr. Dilyana Nikolova and PhD term 15.07.2021 according to order RD 20-1097 dated 11.07.2018. After completion of the individual plan, she was discharged with the right of defense according to order RD-20-919 dated 09.05.2023.

The preliminary defense of the dissertation was held before an expanded departmental council of the Department of Biotechnology on 19.03.2024, when a positive decision on the readiness for formal defense was made and the procedure for such defense was initiated. The scientific jury was appointed by order RD-38-163 of 01.04.2024 of the Rector of the University of Sofia "St. Kliment Ohridski" and at its first regular meeting, on 17.04.2024, made a positive decision to admit the candidate to the defense and selected reviewers and deadlines for the submission of reviews and opinions, as well as a date for the official defense.

II. Completion of the minimum state requirements and the individual plan

According to the documents submitted, Ramise Hoxha has passed the minimum grade in the specialty and two curriculum examinations with an excellent grade and the minimum grade in the Western language (English) with a very good grade. According to the Regulation for the application of the Law for the Development of Academic Staff in the Republic of Bulgaria in the professional field 5.11 Biotechnology, a total of 90 points are required, of which 50 points from a submitted Dissertation (Indicator Group A, indicator 1) and 30 points from scientific publications (Indicator Group D, indicators 1 to 7). The dissertation and the abstract have been submitted in Bulgarian and English, thus satisfying the requirements for Indicator Group A. The dissertant has submitted three publications in refereed journals (Indicator D.3) and one scientific publication in a non-refereed journal (Indicator D.4), which carry a total of 40 points. The analysis shows that the necessary minimum state requirements have been met, which was also reported during the first meeting of the Scientific Jury.

III. Dissertation Analysis

1. *Relevance of the issues and correspondence with the professional field and the doctoral program*

The dissertation work is dedicated to the isolation and characterization of new strains of lactic acid bacteria (LAB) from traditional fermented foods from the Republic of Albania, the study of their characteristics, and their application to obtain functional products with useful properties. LABs represent a group of microorganisms known for millennia (through the food products obtained as a consequence of their activity), and of increasing relevance (with the advances of biotechnological sciences) due to their beneficial properties and beneficial effects on human health.

Particularly commendable is the choice of source for the isolation of LABs in the present work - traditional, poorly studied food products from the Republic of Albania. Current thinking in this area of science identifies such products as a rich source of indigenous strains of LABs that possess unique and/or superior qualities to commercialized strains. The importance of such developments for the assessment and conservation of biological and genetic diversity cannot be underestimated, due to the fact that the unique microbiota in these foods has formed and evolved under specific climatic conditions and under selective human pressure.

The present thesis represents a complete study from the isolation of the strains, through their taxonomic identification by phenotypic and genetic methods, cultivation, analytical methods to investigate functional and probiotic properties, and finally the production and characterization of a model product, yogurt.

2. *Structure*

The dissertation is written in 143 pages and follows a standard outline. The results are illustrated with 18 tables and 22 figures, and the data are not duplicated in the tables and figures. I believe a good balance has been achieved between the different sections of the thesis, but the number of sources cited is too large and could be reduced. The dissertation is written literately, with relatively few spelling and grammatical errors. The figures and photographs are clear and legible, but more work could be done on the quality of presentation.

3. *Contents*

This literature review sequentially reviews the current literature on functional (fermented) foods and their microbiota, LABs in particular, their taxonomy and basic characteristics, their health effects, and their application in biotechnology. The literature review concludes with an overview of current methods in the study of LABs and a review of traditional foods in the region under study. The information is presented in an accessible manner, and in sufficient detail to gain a clear understanding of the current state of the problem. The volume could be condensed and some references to relatively outdated literature sources omitted. For example, the phylogenetic tree of the LABs that is attached as illustrative material is from 1998, and in recent years molecular methods have contributed significantly to changing the taxonomy of virtually all groups of organisms. An up-to-date taxonomic classification from the year 2020 is cited below, which text provides sufficient information.

The aim of the thesis follows naturally from the literature review and is achievable within the period of full-time doctoral study. The tasks set satisfy the aim and are adequately divided into several (four) main groups. The methods are described in detail, perhaps too descriptively, and logically follow the set tasks. The spectrum of methods applied is impressive and reinforces the general impression of a very systematic and ambitious work, eventually brought to completion. In this section, there is a greater saturation of technical errors and perhaps a lack of citations in certain places.

The results are presented systematically and are richly illustrated. The discussion is integrated into the presentation of the results and places them in the light of recent research in the field. 13 Conclusions and 6 Contributions are formulated, which I accept, but some of them could be reworked so that they do not sound like results. Overall, the dissertation leaves an impression of comprehensiveness and completeness that is relatively rare.

4. Abstract

The abstract is a well-structured self-contained body of text and gives a good idea of the qualities of the thesis. Some of the results could be abbreviated to reduce the length, while it is appropriate that the sources cited in the discussion appear as a References Cited list. The English version coincides with the Bulgarian version.

IV. Analysis of the scientific publications and the contribution of the dissertation

There are four scientific publications attached to the dissertation, three of which are in journals with impact factor or impact rank published in 2023 and one in a non-refereed journal published in 2022. I would like to note that at the date of abstract submission, the publications had generated 7 citations, and these were fairly evenly distributed. At the present time, according to Scopus alone, the number of citations has doubled, which is a testimonial to the significance of the data obtained. The results presented in the articles are present in the dissertation in their entirety.

The contribution of the thesis, beyond the formulated contributions, consists in the focused approach to the study of the microbiota of traditional local fermented products, with which the Balkan Peninsula abounds. This scientific field is not new, and I would even say that it is well established in the Faculty of Biology of Sofia University, but the presented dissertation is a rare example of a multidisciplinary and complete research in this direction.

V. Conclusion

I believe that the presented dissertation fully meets all the state and institutional requirements for the award of the educational and scientific degree "Doctor". In spite of several critical remarks, the scientific value and the technical layout are beyond doubt. I have the following questions for the dissertation:

- 1. Does she consider the NMR spectroscopic method to be the most appropriate for the metabolic analyses performed and what other alternative would she use?**
- 2. Why was OPLS-DA analysis chosen instead of say PCA or PCoA analysis?**
- 3. Concerning the results for antibiotic resistance, which is observed to a significant extent in the isolates, what is it due to and how good is it that it is present?**

In conclusion, I vote convincingly FOR the award of the degree of Doctor of Education and Research in the professional field 5.11. Biotechnology to Ramise Hoxha.

21.05.2024

Sofia

/Assoc. Prof. Dr. Sci. Lyuben Zagorchev/