REVIEW

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on a dissertation submitted to a Scientific Jury, formed by order № RD-38-163/01.04.2024 of the Rector of Sofia University "St. Kliment Ohridski” for obtaining the educational and scientific degree "Doctor" in Professional field: 5 Technical sciences, professional direction 5.11 Biotechnologies (Technology of biologically active substances).

Author of the dissertation: **Ramize Hoxha**

Topic of the dissertation: **Functional and technological characteristics of newly isolated lactic acid bacteria strains from traditional foods**

Supervisor: **Assoc. Prof. Dilyana Nikolova**, **PhD**

This review has been prepared in accordance with the normative documents - Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for its implementation and the Recommendations of the Faculty Council of the Faculty of Biology on the criteria for acquiring scientific degrees and occupying academic positions in the SU for professional direction 5.11. Biotechnologies.

1. Data on the doctoral student

Doctoral candidate Ramize Hoxha obtained a bachelor's degree in "Biotechnology" in 2015 at the University of Tirana, Albania, and in 2017 she received a master's degree in "Molecular and Industrial Biotechnology" at the same university. After her graduation, she was enrolled as a full-time doctoral student in professional direction 5.11. Biotechnology, doctoral program "Technology of biologically active substances" at the "Biotechnology" department of the Faculty of Biology, according to Order No. RD-20-1097/11.07.2018 of the Rector of SU "St. Cl. Ohridski", and by Order No. RD-20-919/09.05.2023 she was charged with the right of defense on the basis of Art. 74, paragraph 1 of the Law on Higher Education, art. 24, paragraph 2 and 6 of the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria, Art. 63, paragraph 2 and 6 of the Regulations on the

terms and conditions for acquiring scientific degrees and occupying academic positions at SU "St. Cl. Ohridski" and decision of the Faculty Council of the Faculty of Biology dated 04/25/2023. During the period of regular doctoral studies, Ramize Hoxha successfully passed all the necessary exams.

2. Relevance and significance of the dissertation topic

The dissertation work presented by Ramize Hoxha is dedicated to the biotechnologically important lactic acid bacteria, which have a proven role in human health as probiotics. The beneficial effects associated with probiotics are numerous and most of them are well researched and documented in the literature. The selection of lactic acid bacteria to be used as probiotics, nutritional supplements or biotherapeutic agents is a complex and multi-step process. Probiotic bacteria have the potential to influence the immune response in a desired direction as well. In recent years, science and industry have been working hard to meet the growing demand for new probiotic products. The search for new strains with proven beneficial effects on health, different functional characteristics, probiotic and biotechnologically relevant indicators is important to meet the growing needs of the market and obtain a variety of functional foods. The intake of food supplements containing probiotics has a proven positive effect on the health of the consumer, and in recent years a lot of data has been accumulated proving the successful prevention and treatment of intestinal infections by probiotics, antitumor effect, positive influence in heart diseases. Probiotics provide a natural and body-friendly alternative for dealing with local dysfunctions of the gastrointestinal tract, as well as the associated conditions of single, acute and/or chronic inflammatory processes. At present, probiotic products have become a promising opportunity for the ever-open market of innovations aimed at improving people's quality of life. Therefore, the development of probiotic products is of interest, both from a purely scientific and economic point of view.

The doctoral student's research is aimed at isolating new strains of lactic acid bacteria from the microbiota of traditionally prepared dairy, meat and spontaneously fermented products and studying their functional and technological properties for application in new food products with improved functional characteristics and health effects. That is why I consider that the dissertation work presented to me for review by

Ramize Hoxha is up-to-date and definitely of practical importance. The content of the dissertation fully covers the announced nomenclature specialty in professional direction 5.11 Biotechnologies, scientific specialty "Technology of biologically active substances" for the award of the ONS "Doctor".

3. Evaluation of the structure of the dissertation work

The dissertation is written on 143 standard pages of text, A4 format, following the generally accepted scheme as follows: Introduction - 1 page, Literature review - 50 pages, Aim and tasks - 2 pages, Materials and methods - 12 pages, Results and discussion - 45 pages, Conclusions - 2 pages, Contributions - 1 page; References - 20 pages (323 sources), Appendices - 2 pages. The recommended ratios between the individual parts of the work are respected. The concise scientific style and technical layout of the dissertation make a very good impression. The work is very well illustrated as the results are summarized and presented in 18 tables and 22 figures, as well as rich photographic material and 1 appendix reflecting the obtained results.

The introduction purposefully introduces the actuality and essence of the developed topic - isolation of new strains of lactic acid bacteria from the microbiota of traditionally prepared dairy, meat and spontaneously fermented products and the study of their functional and technological properties for application in new food products with improved functional characteristics and health effects.

4. Literature review

The literature review is concise and at the same time a large amount of scientific information has been analyzed. It contains several sections reflecting the current trends in the scientific problem. Of the cited literary sources, 2/3 are from the last 10 years. This is proof of excellent theoretical preparation of the doctoral student and is a prerequisite for successful work. The literature review is written in a high scientific style and shows a very good awareness in the field of the dissertation topic, ability to handle literary sources, to summarize and analyze, ability to present modern information for a wide range of specialists. Several main themes are distinguished in the literature review, closely related to each other. The doctoral student presented a description of functional foods and their health benefits, focusing in detail on lactic acid bacteria (LAB), their application and the biologically active substances synthesized by them. In the next

section of the literature review, the doctoral student focuses on classical and modern methods in LAB research - whole-genome sequencing, quantitative polymerase chain reaction, PCR, etc. The doctoral student also draws attention to the studies conducted so far on the LAB by foreign authors worldwide, as well as the contribution of Bulgarian scientists in this field. Traditional foods as sources of new LAB strains with functional and probiotic properties are discussed in detail in the literature review, and the PhD student has described in detail these foods specific to the Gora region of Albania.

5. Purpose and tasks

Based on the information presented, the doctoral student clearly and precisely formulates the goal and the 4 experimental main tasks and 15 subtasks related to its achievement, which is evidence of a well-thought-out and thorough approach to the implementation of scientific research. The main tasks are: isolation of new strains of LAB from the microbiota of traditional fermented products, study of functional and probiotic properties of the newly isolated strains of LAB, determination of basic technological characteristics of the studied strains, inclusion of selected strains of the new isolates in a model product and determination of basic product characteristics.

6. Materials and methods

In the Materials and methods section, the newly isolated microorganisms, reagents and consumables used in the study, as well as the necessary equipment, are precisely described. The research was carried out with the help of classic and modern methods suitable for achieving the set goals and objectives - microbiological, biochemical, genetic and microscopic methods. All of them are described in great detail, which would allow them to be successfully reproduced by anyone interested. The individual stages of the experimental work are well presented in this section and provide an opportunity to conduct the experiments correctly and obtain reliable results.

7. Results, discussion and conclusions

In the "Results and discussion" section, a large volume of experimental work is included, following the course of the set tasks and methodical approaches. The results are presented in detail, well analyzed and discussed, compared with the literature data. The dissertation research begins with the collection of samples of fermented products prepared by traditional technology and the isolation of new strains of LAB. Experiments

were carried out to identify the newly isolated 12 LAB strains using a polyphasic taxonomic approach and sequence analysis of the 16S rDNA gene. With a high percentage of confidence - 98.8%, two strains were identified as belonging to *L. delbrueckii ssp. bulgaricus*, 7 strains are from the species *Lactiplantibacillus plantarum*, 1 strain from the species *Loigolactobacillus coryniformis*, 1 strain from the species *Lactilactobacillus sakei*, and 1 strain from the species *Pediococcus pentosaceus*. The next stage of the research is devoted to the study of the functional and probiotic properties of the newly isolated strains, applying a complex approach. Good antimicrobial activity of the newly isolated strains was found against Gram (+) and Gram (-) test-pathogenic bacteria, as well as against mold food-associated contaminants. Antiviral activity has also been demonstrated in some of the strains. In the newly isolated strains, a well-expressed aminopeptidase enzyme profile was also observed, as well as antibiotic multiresistance. For good probiotic indicators, well-expressed auto- and co-aggregation abilities are also important, and very good indicators were recorded in some of the studied strains. The survival of the strains under direct exposure to stress factors characteristic of the upper parts of the GIT, presence of pancreatin and bile salts was also monitored. Strain KS 5-12 (*L. lantarum*) was determined to have the best probiotic potential. Model yogurt products were obtained with starter cultures of the newly isolated strains, and it was proved that they retain their vitality and activity, as well as their functional and probiotic potential until the end of the storage period. The sensory characteristics of yogurt inoculated with strain KZM 2-11-3 (*L. delbruekii ssp. bulgaricus*) were also evaluated, being the closest to the control, corresponding to the metabolic profile, including aroma-determining components, defining the strain as very good candidate for inclusion in model functional products. The results are illustrated with appropriate and well-formed figures, tables and photographic material.

The results of the obtained experimental data in this section are particularly valuable, as they are of a scientific-applied nature and form to a large extent the contributions of this dissertation work. The good scientific style, the logical sequence of the individual research stages, as well as the objective and in-depth discussion reflecting the published data of recent years are impressive. The results are correctly presented and discussed by the doctoral student. On the basis of the experimental work, 13 conclusions

were formulated, arising logically from the obtained results and providing accurate information about the value of the conducted experiments. In my opinion, the conclusions of the work well done are many and could be combined - conclusion 7 and 8, as well as conclusion 9 with 13 for the purpose of a tighter presentation.

8. Scientific and applied contributions

In the presented dissertation, 6 original scientific and scientific-applied contributions stand out, which proves the importance of the scientific development. The contributions are presented concisely without unnecessary comments and as more important stand out the proven applicability of the KZM 2-11-3 and KC 5-12 strains with bioprotective and probiotic potential for inclusion in starter cultures for the production of new functional foods with certain health benefits. For the first time, antiviral activity against human herpes virus was found in a strain of *L. delbruekii ssp. bulgaricus*. The applicability of NMR spectroscopy as a high-tech method for differentiating different types of sour milk according to their specific metabolic profile determined by the strains used was also confirmed.

9. Publications and participations related to the dissertation

In connection with the dissertation, Ramizeh Hoxha has presented 4 publications published in refereed and indexed journals, which have already received citations. In all publications, the doctoral student is the first author, which clearly reflects her leading role in the conducted research, and I consider that the implementation of the dissertation work is entirely her work. The results of the dissertation work have been reported at 11 national and international scientific conferences with reports and posters. The publication activity fully complies with the normative documents - PRASRB and the Recommendations of the FS of BF on the criteria for acquiring scientific degrees. In addition to her dissertation work, Ramize Hoxha increased her scientific capacity by being a participant in 3 scientific projects related to the field of her dissertation topic, funded by the Scientific Research Окха of Sofia University "St. Kliment Ohridski".

10. Abstract

The presented abstract fully reflects all the important highlights of the scientific research and the obtained results, and the most important elements of all sections are presented in abbreviated form, without, of course, the literature review. It is very well

designed according to the requirements of the Regulations for the Application of the PRASRB.

11. Recommendations, comments and questions

I have no critical notes on the work and its presentation. There are some minor technical errors which in no way detract from the quality of the dissertation.

I have the following question for the doctoral student:

i) How do you explain the fact that in your studies strain KZM 2-11-3 (*L. bulgaricus*) did not report the ability to survive direct exposure to pepsin and pH 2 within 3 hours, and at the same time prove probiotic potential and applicability of this strain for inclusion in starter cultures for the production of novel functional foods with defined health benefits?

ii) Would you continue your research in the field of functional foods and what would you emphasize in your work so that these foods are used by consumers on a daily basis?

12. Acquired competence and compliance with the requirements of the educational and scientific degree "Doctor"

During the implementation of the experimental work on the dissertation by Ramize Hoxha, it is clearly evident that she has acquired competence in terms of knowledge in the specific scientific field, skills in applying a complex methodological approach to solving the tasks set, skills in working with scientific literature, as well as analyzing and summarizes scientific information. She has mastered a variety of methods - microbiological, biochemical, molecular genetic, widely used in biological research, as well as competence for analysis and evaluation of the obtained results.

13. Conclusion

The presented dissertation work is a proof of precisely performed experiments and thoroughly interpreted results, which shows a high professional culture of Ramize Hoxha. I believe that in terms of topicality, volume of research, achieved scientific contributions, as well as publication activity, the dissertation work fully meets the requirements of PRASRB and the Regulations of SU "St. Cl. Ohridski" for the acquisition of the educational and scientific degree "Doctor". With conviction, I will vote

positively, and I recommend the members of the Scientific Jury to vote positively for awarding the educational and scientific degree "Doctor" in professional direction 5.11. Biotechnologies (Technology of biologically active substances) by Ramize Hoxha.

Sofia, 16.05.2024 **Reviewer:**

/prof. Nelly Georgieva, PhD/