

R E V I E W

From: Prof. Svetla Danova, PhD,

Institute of Microbiology "Stefan Angelov" (IMikB), BAS

on PhD thesis titled:

"Functional and Technological Characteristics of Newly Isolated Strains of Lactic Acid Bacteria from Traditional Foods",

for the educational and scientific degree “**Doctor**” in the field of higher education 4. *Natural Sciences, Mathematics and Informatics*, professional field 5.11. *Biotechnology*, doctoral programme *Technology of biologically active substances*

Presented by **Ramize Hoxha**, PhD student

Scientific supervisor: **Assoc. Prof. Dilyana Nikolova**

ON THE PROCEDURE:

The current procedure for the acquisition of ONS "Doctor" is conducted on the basis of Art. 4 of ZRASRB, in connection with §7 of the Transitional and final provisions of the Regulations on the terms and conditions for acquiring scientific degrees and occupying academic positions at SU "St. Kl. Ohridski", Decision of the Faculty Council of the Faculty of Biology (Protocol No. 6) dated 26.03.2024. Pursuant to Order (No. RD-38-163 dated 01.04.2024.) of the Rector of Sofia University "St. Kliment Ohridski" I was elected as a member of the Scientific Jury for the above-mentioned competition and I was designated as a reviewer at its first meeting. In my capacity as such, I declare that there is no conflict of interest within the meaning of § 1, item 2a of the additional provisions of the ZRSARB between me and the candidate under the procedure for the ONS "Doctor" and I am not subject to the restrictions under Art. 33 of ZRASRB.

The absence of plagiarism in the candidate's scientific works has been proven according to the law.

RELEVANCE AND IMPORTANCE OF THE PROBLEM

The thesis submitted to me for review is devoted to a topical scientific problem in the field of modern biotechnology - the search for new bacterial strains with potential for the development of functional foods and probiotics. Nowadays, consumers are well informed and aware of the importance of food, of safety in the food chain and the link to health. This leads to higher demands on food additives, their usefulness and safety. In this context, there is a growing interest in lactic acid bacteria (LAB) and new opportunities are being sought for their inclusion in functional foods and diets. They have been shown to be a factor in food safety and are associated with the health of the human gastrointestinal tract and the prevention of dysbiosis and associated disease-causing abnormalities. The PhD student and her supervisor focused on

this particular scientific challenge - characterising different aspects of biological activity in original newly isolated strains of lactic acid bacteria from different origins, assessing their applicability in the formulation of a new functional product. Above presented gives me a reason to emphasize the innovativeness and relevance of the developed thesis.

SCOPE AND STRUCTURE OF THE DISSERTATION

The PhD thesis is written in 143 standard computer pages (1 pt), which include 116 pages of text and 27 pages of references and appendixes. The requirements for the form, content and proportion of the individual sections have been met as follows: *Introduction* - 1 p., *Literature review* - 51 pages; *Aim and objectives* - 2 pages.; *Materials and methods* - 12 pages, *Results and discussion* - 45 p., *Conclusions* - 2 pages, *Contributions* - 1 page, *References* - 22 p.; *Appendices* - 2 pages. The abbreviations used are duly indicated. The introduction is very focused and presents both the unresolved issues and the need for the development in a concentrated form. Excellent impression is made by the precise scientific style and correct grammatical Bulgarian language, which are also important for the presentation of the work. The PhD thesis is richly illustrated with 27 figures and 21 tables. They are numbered separately in each section of the thesis. I would recommend that the doctoral student numbers the figures and tables consecutively or prepare a list of the pages on which they are presented with their designation.

LITERATURE AWARENESS AND GOAL AND OBJECTIVE SETTING

The literature review is closely logically linked to the subsequent sections of the thesis. It has been prepared in a focused and specific manner and includes all aspects of the research. It clearly highlights the reasoned and literature-supported rationale for the need for the development to address the demands of the modern consumer. Functional foods and lactic acid bacteria are discussed in a logical sequence, together with their applicability and the role of probiotic lactic acid bacteria. It clearly demonstrates the PhD student's understanding of the role of the polyphasic-taxonomic approach and molecular genetic methods in modern taxonomy and in the study of the lactic acid bacteria (LAB). She is familiar with and has summarized very well the changes in the taxonomy of one of the most important representatives of the LAB, the former genus *Lactobacillus*, presenting recent data on 23 newly formed genera. Ramise Hodja makes an original transition from presenting interesting studies on LABs over the last 10 years to traditional foods, as a source of new LAB strains with functional and probiotic properties. The detailed review of the Bulgarian experience in this aspect correctly reflecting the achievements so far is very impressive. This enables the reader to assess the new directions in

the development. the complexity of the scientific challenge that the PhD student and supervisor have to solve. On the basis of the analysis in the literature review, a goal is derived: *“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 novel food products with improved functional characteristics and health effects”*. Four specific, interrelated and logically following tasks are formulated with 15 detailed sub-tasks. I would like to emphasize the ambition (in a good sense) of the planned work in relation to the time frame of a full-time PhD. Ramize Hoxha skillfully combined classical and modern microbiological, biochemical and molecular genetic methods, experimental model systems in solving not only the scientific challenge but also obtaining an applied result with a prototype of a functional dairy product.

The work cites 321 publications, of which 4 in Cyrillic and 317 in Latin, corresponding to each of the set tasks. The review presents the state of the problem historically and currently, citing articles from the last 5-10 years.

EVALUATION OF THE MATERIALS AND METHODS USED

The Materials and Methods section demonstrates a wide range of routine and advanced molecular methods tailored to the specific requirements of the tasks. They are skillfully selected and enable the experiments to be performed correctly and reliable results to be obtained. They are presented in the same accurate and concise writing format. The PhD student selected traditional fermented foods from the region of Gora, Albania for the purpose of the thesis. This is a poorly explored mountainous region, with extensive agriculture and well-preserved traditions for preparing a variety of fermented foods. Together with her supervisor, they cleverly combined the latest experimental protocols published in the research of leading international teams with original solutions in characterizing the functional and technological characteristics of newly isolated LABs from the selected 7 traditional fermentation products, of which one plant-based - fermented fruits according to an old recipe; one meat-based - Shischejeki sudzhuk and 5 dairy products (yoghurt, cheese, cheese, from goat or cow fresh milk according to authentic recipes). Microbiological and physiological and biochemical were the main criteria and a control group of 12 strains was established - subject of a comprehensive evaluation according to EFSA criteria for in vitro selection of candidate probiotics. Taxonomically relevant assays- catalase, oxidase, peroxidase and Gram-staining and biochemical profile based on 49 carbon sources, as well as enzymatic activities were correctly selected as well as enzymatic activities - protease, peptidase, coagulating ability, API Zym -19

enzymes profile, etc. A wide range of molecular genetic PCR and sequence analyses support the identification of newly isolated IBCs and in the assessment of their functionality as microorganisms with probiotic potential and technological relevance. The inclusion of these various superimposing microbiological and physiological assays, molecular methods and the variety of functional and antimicrobial activity tests (anti-viral, anti-bacterial and anti-fungal) as well as molecular-biological techniques to demonstrate the genetic basis of this biological activity are highly impressive.

Excellent theoretical background is evident in the choice of methodological approaches. Ramise Hoxha has chosen algorithms that lead to unambiguous and accurate results. She skillfully used statistical programs for processing the results (t-test, ANOVA).

EVALUATION OF THE RESULTS OBTAINED

The scientific work of Ramize Hoxha, as a whole and in essence, is a large-scale study, of a scientifically fundamental nature, in which the new data obtained were used and innovatively developed to prototypes of new probiotic products. The development was based on the aspiration to establish a scientific basis for the determination of probiotic potential and the selection of effective scientifically proven strains -probiotics, with relevance for application in food technology for health. The scientific and applied character of the development in the professional field, 5.11. Biotechnology, gives me a reason for high evaluation and a testimonial for successful preparation of a well-rounded biotechnologist. I am justified both by the technical design of the thesis and the thorough literature awareness demonstrated in the literature review and discussion of the results. In my view, its merits lie in several points:

□ The PhD student develops in detail, with scientific methods, a problem arising from the needs of practice (health care, food and the demand for useful supplements) and proposes it as a scientific product with a clear application, evident from the stated goal.

□ Its solution has been achieved through a diverse panel of correctly selected scientific methods, the results obtained have been objectively interpreted and used for a stepwise and building-up study to reach a prototype product.

□ The logical sequence of the development is noteworthy. Each stage includes conclusively proven material that is a starting point for further direction of the research. This gives the dissertation a characteristic of comprehensiveness. The selection of methodological approaches and the inclusion of state-of-the-art genetic and molecular methods (MALDI TOF, Nuclear Magnetic Resonance, etc.) complementing established and classical ones prove that the PhD student is a well-rounded young professional with a wealth of practical and theoretical readiness

and knowledge. She successfully solved specific experimental problems, choosing the most accurate and discriminative approach.

□ To the merits of the dissertation are added both the large volume and reliability of the scientific data obtained and the premises on which the contributions are built. Here, I would like to mention the rich experimental material, formed in 4 subsections, corresponding to the 4 experimental tasks, with which the stated goal was achieved. They are analyzed objectively and are systematized in 18 tables and 22 figures. The figures are in colour and very well designed. Statistical processing of the data proves their reliability and enables correct selection of the newly isolated 12 strains of LAB promising for practice.

The PhD student is familiar with recent changes in taxonomy and systematics of the ICB and skillfully selects the protocols for species identification. I appreciate the polyphasic-taxonomic approach and the inclusion of MALDI -TOF as an upgrade to classical biochemical and sequence analyses.

Ramize Hoxha not only ascertained the useful functional characteristics of these original strains of ICB , newly isolated from traditional and unstudied fermentation dairy products from Albania, but also searched for the genetic basis of their probiotic and technological potential. Correctly selected primers were used to characterize the proteolytic enzymes, which are also the basis of their applicability for probiotic yogurts.

The risk for transfer of antibiotic resistance genes in fulfilling the mandatory controls, according to EFSA's Qualified Presumption of Safety (QPS), was assessed.

An original element is the summary protocol for quantitative assessment and comparison of the potential of individual strains. Although a conclusion would give more clarity on the multiple results obtained. This note does not diminish the value of the work only would facilitate the reading and objective evaluation of the volume of new data obtained. I find convincing evidence of their value in the 4 published papers. 3 of them in scientific journals with IF and impact rank. Two of them have even already been cited. Therefore, the results of the work have also received international recognition,. In each of the 4 papers, the PhD student is ranked first, which gives me reason to believe that she had a crucial involvement in the experiments performed.

The conclusions are a logical consequence of the experimental data. They detail the performance of the set tasks. Some of them may have a shorter statement, but it is evident that the PhD student sought to summarise all the results obtained. Therefore, their number corresponds to the number of subtasks formulated. It is clear from these that the objective has been achieved.

I also accept the formulated contributions, which although not separated have an original and confirmatory character. In this regard, and because Ramise Hoxha has modestly noted the importance of the new strains characterized, I would like to ask her :

Which one of the strains and in what form would you choose and implement in practice?

In conclusion, I would like to emphasize that the material is dissertable, the topic is topical, the PhD student has mastered a wide range of modern methods, the experiments are methodologically set correctly, the results obtained are reliable and are a solid basis for further scientific and applied developments, original scientific and applied contributions stand out.

On the basis of the analysis made, and taking into account the relevance and level of the work, I confidently propose to the esteemed members of the Scientific Jury to award Ramize Hoxha the degree of Doctor of Education and Research" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field, 5.11. Biotechnology, doctoral programme.

Sofia, 17.05.2024

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(Prof. Dr. Svetla Danova,DS.c.