SCIENTIFIC OPINION

by **Prof. Maria Bogomilova Angelova-Dyankova**, DSc, Stephan Angeloff Institute of Microbiology at the Bulgarian Academy of Sciences
on a dissertation submitted for the educational and scientific degree "doctor" in the professional field: 4.3. Biological Sciences, doctoral program Biochemistry
Author: **Teodora Mincheva Panayotova**
Topic: **Study of the properties of biologically active peptides obtained by enzymatic hydrolysis with proteolytic enzymes from lactic acid bacteria**
Scientific supervisor: **Prof. Dr. Iliya Nikolov Iliev**

1. General presentation of the procedure and the doctoral student

By order No. RD-22-742 of 07.04.2026 of the Rector of Plovdiv University "Paisiy Hilendarski" (PU), I have been appointed as a member of the scientific jury for ensuring a procedure for the defense of a dissertation on the topic "Research on the properties of biologically active peptides obtained by enzymatic hydrolysis with proteolytic enzymes from lactic acid bacteria" for the acquisition of the educational and scientific degree "doctor" in the field of higher education 4. Natural sciences, mathematics, and informatics, professional direction 4.3. Biological sciences, doctoral program in Biochemistry. The author of the dissertation is Teodora Mincheva Panayotova, a part-time doctoral student at the Department of Biochemistry and Microbiology, with scientific supervisor Prof. Dr. Iliya Iliev from PU.

The electronic set of materials presented by Teodora Panayotova is in accordance with Art. 36 (1) of the Regulations for the Development of Academic Staff.

Teodora Panayotova graduated from secondary education in 2006 at the Vocational High School of Tourism "Prof. Dr. Asen Zlatarov", Plovdiv. In 2010, she graduated as a bachelor at the University of Food Technologies, Plovdiv with a specialty "Meat and Milk Technology". In 2011, she received a master's degree in Control and Safety of Foods of Animal Origin at the same university. Since 2012, she has been working as a researcher at "LB Bulgarikum" EAD, Sofia.

2. Relevance of the topic

Natural bioactive peptides (BPs) have emerged as key candidates in modern science, thanks to their versatile biological activity and diverse applications in biomedicine, biotechnology, and the food industry. These molecules exhibit a wide pharmacological spectrum, including antimicrobial, antiplatelet, antioxidant, antihypertensive, and antitumor properties, which positions them as potent therapeutic agents and essential functional food ingredients. According to the WHO, diseases such as cancer, diabetes, and hypertension cause 36 million deaths per year. One of the most significant peptide-based drugs, licensed and commercialized as early as the 1920s, is insulin. Nowadays, functional foods, supplements, or drugs containing bioactive peptides can improve the health of millions of patients. Their importance is confirmed by the fact that the global market for bioactive

peptides in 2025 amounted to $ 2.76 billion, with a forecast for 2035 of $4.63 billion. Due to their inherent structural diversity, biocompatibility, and biodegradability, biopeptides offer a desirable safety profile, minimizing the systemic toxicity and undesirable effects of synthetic alternatives. The most widely used method for obtaining bioactive peptides is hydrolysis with enzymes obtained from plants, animals, or microorganisms. This method adds important advantages to the development of environmentally friendly technologies. At the same time, new strategies are proposed to obtain higher yields, as well as to reduce the time and cost of protein processing.

However, various challenges hinder the production of peptides on an industrial scale, such as low yield, unconfirmed mechanisms of action, and the selection of appropriate resources for mass production, which reveals a serious niche for scientific research and business requirements. The efforts of research groups worldwide are aimed at finding appropriate sources of proteolytic enzymes and protein resources. It is these aspects of the problem that are in the focus of attention of doctoral student Teodora Panayotova and her scientific supervisor, Prof. Iliya Iliev, which emphasizes the relevance and perspective of the development.

3. Understanding the problem

The dissertation is written in a traditional style, containing the relevant sections. It comprises 182 standard computer pages, including 144 pages of text, 30 pages of literature, and eight pages of appendices. The material presented is balanced in terms of the volume and ratio of the individual parts. The literature review is purposeful and specific, covering all aspects of the study. It reflects 324 publications, one for each of the tasks set. The review presents the current state of the problem, correctly taking into account historical developments and achievements to date. The available literature on the characteristics of lactic acid bacteria (LAB) and their potential to synthesize proteolytic enzymes is discussed in detail. Attention is paid to the types of enzymes and transporters involved. Achievements in the field of bioactive peptides of different origins are analyzed in detail, with a focus on those obtained from milk and dairy products. The literature review also includes a section discussing methods of obtaining bioactive peptides, with an analysis of the challenges associated with each method. The main emphasis is on the physiological properties of biologically active peptides and the mechanism of their action. The wide scope of knowledge and analytical style of presentation of the literature data are noteworthy, as are the 15 figures and 5 tables included in the review, which facilitate readers' understanding of the scientific data. This leads me to conclude that doctoral student T. Panayotova is well acquainted with the subject of her dissertation. Based on the analysis in the literature review, the present dissertation aims to obtain new data on the synthesis of biologically active peptides with ACE-inhibiting activity when cultivating lactic acid bacteria in nutrient media containing vegetable proteins. Five specific, interrelated, and consecutive tasks were formulated to achieve this goal, covering all the mandatory stages of such a study.

4. Research methodology

The "Materials and Methods" section demonstrates an impressive set of methods, tailored to the specific requirements of the experiment. They are both routine and modern, microbiological, biochemical, molecular biological, etc. In addition, they are understandable and allow for the correct conduct of experiments and obtaining reliable results. I would like to note the impressive number of collections of lactobacilli of four species, identified by the nucleotide sequence of their 16S-rRNA gene (16S-ribosomal RNA). Modern methods were used to demonstrate enzyme hydrolysis, ACE inhibitory activity, electrophoretic analysis with tris-tricine SDS-polyacrylamide gel electrophoresis, ultra-high-performance liquid chromatography with mass spectrometry (UHPLC-MS) for the demonstration of low-molecular peptides, etc.

5. Characteristics and evaluation of the dissertation work and contributions

The section "Results and discussion" presents data on the implementation of an extremely significant task in theoretical and applied aspects - obtaining and characterizing a bioactive peptide with ACE-inhibiting activity. This is a large-scale study, illustrated with 28 figures and 4 tables from its own results. It is distinguished by a clearly expressed logical sequence of the experimental stages, which gives the development a characteristic of integrity.

Starting with screening of the LAB strains for secretion of proteolytic enzymes with subsequent comparison of the degree of proteolysis in lactobacilli of different species and under different conditions, the doctoral candidate selected 30 strains of *Lactobacillus delbrueckii* subsp. *Bulgaricus*, 11 strains of *Lactobacillus helveticus*, and 3 strains of *Lacticaseibacillus casei*, which she studied regarding the indicators of cell growth and acid formation in relation to the degree of proteolysis. In the second stage of the dissertation, data were obtained on the degree of proteolysis as a function of strain type and plant protein type in the culture medium. Based on the generalized analysis, the most suitable strains for the following experiments were identified. Much attention was paid to the effect of plant proteins on the level of specific proteolytic activity. The results determined the effective producers *L. helveticus* h70 and *L. helveticus* b244 and added new information to the regulation of enzyme synthesis through the control mechanism, nitrogen catabolite repression. In addition, the doctoral student also suggests regulation of gene expression in response to environmental conditions.

The potential of 4 strains of *L. helveticus* and 1 strain of *L. casei* to degrade peptides to free amino acids by 4 types of peptidases was studied in detail. The results obtained complement the knowledge regarding the characteristics of peptidases synthesized by lactobacilli.

The majority of the experimental work focuses on proving the ACE-inhibitory activity of biologically active peptides in media containing plant proteins. Combined nutrient media schemes for synthesising peptides with high ACE-inhibitory activity were proposed, and strain L. helveticus b244 was selected as the most effective producer as a result. By the end of the study, data had been obtained on the physicochemical characteristics of the formed peptides. Modern methods proved

that the ACE-inhibitory activity of strains L. helveticus h70 and L. helveticus b244 is due to low-molecular peptides present when the strains are cultivated on combined nutrient media containing milk and plant proteins. The results obtained add important information to this new research area.

It is a good idea to formulate conclusions and final comments after each subsection, summarising the obtained data and their significance in an informative way. This makes it easier for the reader to orient themselves in the work and perceive its achievements. I would like to emphasise the important role played by the scientific supervisor, Prof. Iliya Iliev, a leading scientist in the field of the doctoral thesis. In my opinion, he has contributed to the excellent presentation of the material.

The evidence in the 'Results and Discussion' section, especially the excellently designed tables and figures, enables the reader to correctly understand it. The presentation of the results is skilfully combined with a discussion of all stages of the work. A thorough and professional discussion of the data has been carried out using appropriate literary sources. This creates the impression of comparability with what has been published by other authors. The discussion also demonstrates the doctoral student's serious experimental work and in-depth knowledge of the field.

In my opinion, the conclusions are a logical consequence of the experimental data, providing the necessary information about the value of the research conducted. I would recommend that they be more specifically defined, albeit more in number. Furthermore, I agree with the wording of the contributions and would like to emphasise their importance in terms of both theory and scientific application.

6. Assessment of the doctoral student's publications and personal contribution

The data from the dissertation are included in four scientific articles, three of which have been published in specialised journals: Bulgarian Chemical Communications and Acta Microbiologica Bulgarica (referenced in Scopus with a quartile of Q4), and Food Science and Applied Biotechnology (with a quartile of Q2). The doctoral student has presented her results at three scientific forums. This leads me to believe that Teodora Panayotova's results have become known to the national and international scientific communities. She is the first author of all the scientific publications and contributions, which proves her significant contribution to the development of the dissertation.

7. Abstract

The abstract is properly structured and adequately reflects the essence and results of the dissertation.

8. Critical notes, recommendations, and questions

I have no serious criticisms of the dissertation.

To doctoral student Teodora Panayotova, I have the following questions:

1. In what way are the bioactive peptides with ACE inhibitory activity obtained in this dissertation superior to some commercial preparations?

2. Which of the results obtained are promising for further research, and what experiments should be conducted?

Recommendation:

Given that the review is highly informative, with excellent illustrative material, and will be useful to many specialists, I recommend that it be published in a specialised journal.

CONCLUSION

In conclusion, I would like to emphasize that the doctoral student has fulfilled the requirements of the Law on the Scientific and Technological Development of the Republic of Bulgaria, as well as those in the Regulations to it of the University "Paisii Hilendarski" for acquiring the educational and scientific degree "doctor". The material presented by Teodora Panayotova is suitable for a dissertation, and the topic offers a modern perspective on an important issue in theory and practice. The experiments were correctly set up and conducted, and the results obtained are reliable and provide a solid basis for further scientific and applied developments. A significant amount of experimental work has been carried out, and the problem has been studied in depth from a modern perspective. The dissertation makes significant contributions of practical importance. In addition to the above, I would like to add that Teodora Panayotova leaves the doctoral programme as a well-prepared specialist in biochemistry who has mastered a large number of modern methods and gained experience in interpreting scientific data.

Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation, abstract, achieved results, and contributions, and I propose to the esteemed scientific jury to award the educational and scientific degree "doctor" to **TEODORA MINCHEVA PANAYOTOVA** in the field of higher education: 4. Natural Sciences, Mathematics and Informatics; professional direction 4.3. Biological Sciences, doctoral program in Biochemistry.

16/05/2026

Sofia

Prepared by: .....

/Prof. Maria Angelova-Dyankova, DSc/