

OPINION

by Prof. Dr. Penka Angelova Moncheva, Faculty of Biology, SU "St. Kl. Ohrid"

of a dissertation for awarding the educational and scientific degree "doctor"

by: area of higher education 4. Natural sciences, mathematics and informatics

Professional field 4.3. Biological Sciences

doctoral program Microbiology

Author: Gloria Biserova Georgieva

Topic: „Physiological and biochemical characteristics of the plant-microbial symbiosis of representatives of the genus *Pseudomonas*“

Supervisor: Assoc. prof. Dr. Trayana Nedeva

Scientific consultant: Prof. Dr. Petya Hristova

1. General presentation of the materials under the procedure

By order No. RD-38-473 of 24.07.2024 of the Rector of Sofia university "St. Kliment Ohridski" I have been appointed as a member of the scientific jury in connection with the defense of a dissertation work for the acquisition of the educational and scientific degree "doctor" in professional field 4.3. Biological Sciences, PhD program "Microbiology". The topic of the dissertation is "Physiological and biochemical characterization of the plant-microbial symbiosis of representatives of the genus *Pseudomonas*", and its author is Gloria Biserova Georgieva - full-time doctoral student at the Department of "General and Industrial Microbiology" with supervisor Assoc. prof. Dr. Trayana Nedeva and scientific consultant Prof. Dr. Petya Hristova from SU.

The set of electronic materials presented by Gloria Georgieva is in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations on the Development of the Academic Staff of the Sofia University and includes all the documents required for the procedure.

2. Brief biographical data for the doctoral student

Gloria Georgieva completed her higher education at Sofia University "St. Kliment Ohridski", Faculty of Biology and holds a bachelor's degree in "Biotechnologies" (2019) and a master's degree in "Industrial biotechnologies" (2021). In the period 2021-2024, she is a full-time doctoral student in the Doctoral Program "Microbiology" at the Department of "General and Industrial Microbiology" at the Faculty of Biology. From 2021 until now, she has been working in the position of "Technologist-Biotechnological Processes" at "Terra Power". During the academic year 2023-2024, she was a part-time assistant in Microbiology at the department where she is a full-time doctoral student. Gloria Georgieva has excellent language skills in English.

3. Characteristics of the dissertation work

3.1. Relevance of the dissertation topic, appropriateness of the tasks

The increasing population of the earth and the related intensification of cultivation of agricultural crops leads to a shortage of nutritional components in the soil, which affects yields. On the other hand, increasing industrialization has a negative effect on the environment, including the soil. For the past 20 years, specialists working on this problem, which is of great importance to all of humanity, have been conducting scientific research aimed at finding solutions that will have the least possible adverse effect on the environment. Of particular interest are bacteria/microorganisms that stimulate plant growth and development through various mechanisms. Bacteria of the genus *Pseudomonas* are a focus of research related to the improvement of plant development and biocontrol of plant diseases. The aim of the present thesis is related to the selection and study of bacterial strains of the genus *Pseudomonas* for the study of plant-microbial symbiosis and proving their stimulating effect on plants. In view of the above, I believe that the topic of the dissertation is actual and significant in scientific and applied terms. There are three main tasks to be completed,

each with several sub-tasks, which I consider to be purposeful and their implementation will lead to the achievement of the goal.

3.2. Knowing the problem

Reading the literature review of the dissertation gives me reason to conclude that the doctoral student knows in depth the scientific problem underlying the presented work. An in-depth literature review of the main questions on the topic was carried out: the role of soil microorganisms, and in particular rhizosphere ones, which stimulate plant growth and the mechanisms for this; the importance of microorganisms in the phyllosphere; the role of PGPR from the genus *Pseudomonas*, which are the subject of research in the dissertation; plant-microbial symbiosis; the application of PGPR as biological control agents in conventional agriculture, etc. The literature review is based on 347 up-to-date sources corresponding to the subject of the dissertation. About 92% of the cited literature was published after 2000, with the majority of it after 2010. This shows that the doctoral student has excellent knowledge of the current state of the scientific problem being developed.

3.3 Research methodology

For the implementation of the tasks, a combination of various methods - classical microbiological, enzymological, analytical, molecular-genetic, etc. - has been selected and used, which correspond to the tasks, as a result of which the goal will be realized.

3.4. Characterization and evaluation of the dissertation work

The structure of the dissertation corresponds to the generally accepted for this type of work and contains the following sections: Introduction (1 page), Literature review (45 pages), Working hypothesis (2 pages), Aim and tasks (2 pages), Materials and methods (21 pages), Results and discussion (86 pages), Conclusions (2 pages), Contributions (2 pages), Appendices and References. The dissertation includes a list of the doctoral student's publications, participation in scientific forums and citations of her scientific works. The purpose of the dissertation is clearly formulated, and for its achievement 3 main tasks are set, which correspond to the purpose of the work. Experimental work was carried out on each task, the results of which are presented, very well illustrated and discussed in the light of what is known about the relevant issues in the scientific literature. Illustrative material includes very well-designed 69 figures and 10 tables. Results of the screening of isolates of the genus *Pseudomonas* for the ability to exert a PGP effect on plants have been reported, with a rather extensive phenotypic characterization of the same by a number of characters (morphological, assimilation, enzyme profile, production of iron-binding proteins, biosynthesis of lytic enzymes, catalase activity, solubilization of phosphorus-containing inorganic compounds, ability to nitrogen fixation, biosynthesis of phytohormones, identification by 16S rRNA sequencing). As a result of the first task, two strains (1S4 and 1046) belonging to the species *P. chlororaphis* and *P. yamanorum* were selected for further research. The next task is to prove the PGP potential of the selected strains mentioned above, which includes results from optimization of strain cultivation conditions, optimization of phytohormone biosynthesis, study of phenazine-1-carboxylic acid biosynthesis, siderophore production, antifungal activity, optimization the preservation and stability of biologically active fermentation products from the both strains. The third task is related to the PGR potential of the two strains, and the plant-microbial symbiosis between them and technical crops and ornamental plants was investigated. The effect of these strains on the germination of corn and soybean seeds was monitored (4 indicators each); on the germination of wheat seeds; the plant-microbial symbiosis between the two strains and potted ornamental plants (primrose, chrysanthemum, cyclamen) was evaluated based on several plant biometric indicators. Results of a vessel experiment with primrose, in which solutions of dry forms of the investigated microorganisms were used, are also presented. Based on the obtained results and their discussion, I can conclude that all the tasks set have been fulfilled. Eight conclusions were drawn, based on the results. I would only note that the formulation of some of the conclusions gives them the character of a reported result rather than of conclusions derived from the specific results obtained.

The dissertation contains contributions that are well formulated by the doctoral student, some of which are defined as original and confirmatory. I will mark as more important only the original contributions.

1. After extensive screening, two strains were selected - *P. chlororaphis* 1S4 and *P. yamanorum* 1046, which were proven to have a PGP effect on technical and potted ornamental plants, which defines them as potential agents for application in agricultural practice.

2. The proven antifungal activity of the two strains against phytopathogens of the genus *Fusarium* reveals the possibility of their inclusion as components of biopreparations for biological control.

3. An economically viable variant of the culture medium for stimulating the synthesis of IAA by the strains was developed, in which the inducer synthetic L-tryptophan was replaced by L-tryptophan of microbial origin, in which the synthesis of IAA increased by about 34%.

4. A prototype series of fresh and dry cultures of the two *Pseudomonas* strains was constructed with the potential for application as an ecologically appropriate alternative to conventional fertilizers.

4. Evaluation of publications on the dissertation work

Doctoral student Gloria Georgieva has presented three scientific publications in journals indexed in Scopus databases, one of which is with quartile Q3 and two with quartile Q4, i.e. the total number of points from indicator G is 39 (out of a required 30 points), according to the minimum national requirements for "Doctor" degree. Two of the publications have been accepted for printing, for which the necessary certificates from the journal's editors have been presented. Gloria Georgieva is the first author of all three publications, demonstrating her leading role in both the experimental work and their preparation for publication. One of her articles has also received two citations. These publications contain the results of her dissertation work. Four posters were presented, three of them in international scientific forums.

5. Abstract

The abstract is formatted according to the requirements and faithfully reflects the main results presented in the dissertation.

6. Critical remarks and recommendations

I have no significant critical remarks about the dissertation submitted for review, except for the technical errors noticed in some places.

7. Personal impressions

I do not know the doctoral student and I have no personal impressions of her, but the dissertation work with the obtained results and its design give me reason to conclude that Gloria Georgieva is a well-prepared young researcher in the given field, who has acquired the skills to conduct scientific research, analyze, summarize and design the results in scientific papers.

8. Questions to the doctoral student

1. What research and steps are necessary to realize the practical application of the two investigated strains as PGP agents, as well as for their inclusion in preparations for biocontrol of phytopathogenic fungi of the genus *Fusarium*?

Conclusion

The dissertation presented by Gloria Georgieva contains results of a scientific-applied contribution nature. Some of the contributions are original and others confirmatory. The work meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations on the Development of the Academic Staff of the Sofia University. The doctoral student has in-depth theoretical and methodological knowledge in the field of microbiology and on the specific topic, which is confirmed by the presentation of the results, their analysis, summary and interpretation and the very good design of the illustrative material of the dissertation work and the publications related to it.

On the basis of the above, I confidently give my positive assessment of the conducted research, the results of which are reflected in the dissertation work, and allow myself to recommend to the respected scientific jury to award the educational and scientific degree "doctor" to Gloria Biserova Georgieva in the area of higher education: 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, PhD program Microbiology.

20.09.2024.

Prepared the opinion:
(Prof. Dr. Penka Moncheva)