R E V I E W

by: assoc. prof. Mihail Iliev (Department of General and Industrial microbiology, Faculty of Biology, SU), a member of the Scientific Jury according to Order No. **RD 38-473/24.07.2024** of the Rector of Sofia University "St. Kliment Ohridski".

Concerning dissertation entitled “Physiological and biochemical characteristics of the plant - microbial symbiosis of representatives of the genus *Pseudomonas*”.

For the award of the educational and scientific degree "PhD" in field of higher education 4. 3 Biological sciences (Microbiology). PhD candidate Gloria Biserova Georgieva, scientific supervisor assoc. prof. Trayana Nedeva, Faculty of Biology, SU.

I declare that I have no conflict of interest as per Article 4(5) of the Law on the Development of Academic Staff in the Republic of Bulgaria (LDASRB). This review was prepared in accordance with the statutory documents - LDASRB and its Implementing Rules, and the recommendations of the Faculty Council of the Biological Faculty regarding the criteria for acquiring scientific degrees, which are consistent with them.

I. Evaluation of the Structure and Layout of the Dissertation

The reviewed dissertation is structured according to the requirements for such types of scientific developments. It consists of 217 A4 pages and contains the following main sections: 1. Introduction (1 page); 2. Literature Review (41 pages); 3. Research Hypothesis (3 pages); 4. Objectives and Tasks (2 pages); 5. Materials and Methods (21 pages); 6. Results and Discussion (74 pages); 7. Conclusions (4 pages); 8. Contributions (2 pages). The cited literature includes 343 titles. Information about the doctoral candidate's publications and presentations at scientific forums, as well as observed citations, is also provided. Good impression is made by the excellent technical layout of the work.

II. Relevance and Significance of the Dissertation Topic

The topic "Physiological and Biochemical Characteristics of Plant-Microbial Symbiosis of Representatives from the Genus *Pseudomonas*" is highly relevant and timely, as it focuses scientifically on the complex interactions between plants and specific microorganisms, particularly bacteria from the genus Pseudomonas. These interactions are crucial for

maintaining ecological balance in nature and have significant implications in various fields, including agriculture, environmental sustainability, and sustainable farming. Investigating the physiological and biochemical aspects of these relationships can lead to a better understanding of the mechanisms underlying plant-microbe interactions and potentially identify new targets for improving crop productivity and resilience to environmental stressors. Additionally, understanding the role of Pseudomonas microorganisms in biological control can help develop effective and environmentally friendly alternatives to chemical pesticides. The dissertation that explores this topic will undoubtedly contribute to our understanding of these complex interactions and pave the way for innovative solutions to global challenges related to population growth and rapid industrialization.

III. Literature Review

The literature review is structured appropriately. At the outset, Section 1. "*The Role of Microorganisms in Soil - Rhizosphere Microorganisms Promoting Plant Growth*" is placed adequately. This section serves as a basis for the subsequent divisions of the literature review, which delve into the specific roles of plant Growth-Promoting bacteria and their potential applications in sustainable agricultural practices. Historically, the focus on microbiology has completely changed humanity's perception of soil itself. From the archaic definition of Kraut (1853) and Raman (1912) - "*soil is the uppermost, covering layer of the Earth's c*rust" to the revolutionary idea of Docuchayev - "*Soil is not a dead body (just a geological product), but a specific living organism that germinates and develops according to its own la*ws." The changing concept is indeed the soil microbial component. The emphasis is placed on plant Growth-Promoting microorganisms (PGPM). The undeniable benefit of studying them is that PGPM can help reduce dependence on chemical fertilizers and pesticides, leading to more sustainable agricultural practices. Correctly defined and presented concepts such as "Rhizosphere" and "Rhizobiome" are commendable. I consider it appropriate to include numerous examples highlighting the relationship between plants and associated microorganisms. In this part, I recommend the inclusion and discussion of the following publications: 1. Jones, C. S., & Wilson, D. R. (2012). Rhizosphere Bacteria: Their Role in Plant Growth and Development and Potential Benefits for Sustainable Agriculture. In Plant Growth Promotion and Biocontrol Agents (pp. 1-26). CRC Press. 2. Jones, C. S., & Wilson, D. R. (2012). Rhizosphere bacteria: Their contribution to nitrogen cycling and potential benefits for sustainable agriculture. In Plant Growth Promotion and Biocontrol Agents (pp. 27-46). CRC Press. New concepts such as "*Natural Intelligence Farming*", "*The Nitrogen Solution*", and "*Fungal Energy Channel*" can

be introduced, which will contribute to the evaluation of the extremely complex character of the processes occurring in the rhizosphere, which are microbially mediated.

In the subsequent parts of the section, the correct and detailed focus is placed on the target taxon of the study, namely representatives of the genus *Pseudomonas*, and the knowledge of their involvement in the complex character of symbiotic relationships in the soil.

The information is presented correctly and logically. My only comment here is the categorical statement "With the greatest diversity on Earth is characterized by microorganisms, represented by 10^{12} species (Locey & Lennon, 2016)" (p. 13). In the cited article, this value is based on scaling and mathematical modeling assumptions. The known and described diversity is significantly lower.

IV. Research Hypothesis

I find the formulation of the Research Hypothesis to be extremely appropriate and illustrative in terms of the experimental scheme and expected results. As a whole, the research hypothesis offers a systematic approach to investigating the PGP potential of *Pseudomonas* bacteria, which combines laboratory and field studies. If successfully realized, this could lead to the discovery of new beneficial bacterial strains and improve our understanding of the role of microorganisms in promoting plant growth and maintaining ecological stability.

V. Aim and Tasks

Overall, the objectives and tasks set forth in the dissertation are well-structured and sequential, providing a comprehensive approach to examining the PGP potential of Pseudomonas bacteria and their application in agriculture and horticulture.

VI. Materials and Methodology

Detailed and accurate representation of the entire methodology engaged in such a comprehensive study is presented. The volume corresponds to the tasks set and serves as an attestation to their credibility.

VII. Results and Discussion

The research logically begins with a detailed description of the used microbial strains, including analysis of their assimilation characteristics.

1. The finding that none of the strains reduce nitrates to nitrites is a positive discovery, as nitrites can be toxic to plants at high concentrations. The presence of nitrite reductase activity in three out of five strains is desirable, as it helps prevent nitrate toxicity and improves nitrogen uptake by plants. However, it is important to note that while the presence of nitrite reductase is a desirable characteristic for PGP bacteria, it is not the sole factor determining the effectiveness of these bacteria in promoting plant growth.

2. The lack of production of indole and glucose fermentation in the studied strains is not necessarily a negative trait, as these characteristics are not universally present in all PGP bacteria. The positive test for L-arginine dehydrogenase in strains G-52 and 1046 indicates that these strains can degrade L-arginine, which is an important nitrogen source for plants. This ability is a desirable characteristic for PGP bacteria, as it promotes plant growth by providing access to additional sources of nitrogen.

3. The lack of urease activity in the studied strains is a positive discovery, as urease activity can lead to loss of nitrogen under the form of ammonia, which can be toxic to plants. Urease is an enzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Although this process can be beneficial for some plants by increasing the availability of nitrogen, it can also be harmful if it occurs excessively or if it releases toxic levels of ammonia. In the context of PGP bacteria, the ability to degrade urea can be both beneficial and detrimental. On one hand, the degradation of urea can provide an additional source of nitrogen for plants. On the other hand, excessive degradation of urea can lead to the release of toxic levels of ammonia, which can inhibit plant growth and disrupt the balance of nutrients in the soil.

4. The hydrolysis of esculin by β-glucosidase demonstrated by strain 1S4 is an interesting characteristic.

5. The established enzyme profile shows that all five investigated strains possess the potential to degrade a wide spectrum of organic compounds, which may be beneficial for the host plants and overall soil health.

6. The CAS analysis reveals that all strains produce siderophores, with strain 1S4 showing the highest capacity for this.

7. The presence of proteases in four of the investigated strains may be beneficial for the host plants through the breakdown of proteins in plant material and improvement of their availability for absorption.

8. The four strains that demonstrate clear catalase activity (R6, Or5, 1046, and G-52) could potentially contribute to plant growth by protecting against oxidative stress.

9. The chromatographic analysis reveals that all five strains produce most of the investigated plant growth regulators, with the main difference being quantitative. This finding suggests that these strains can contribute to plant growth and defense through the production of PRRs, although the specific effect may vary depending on the level of production by each strain. This characteristic, along with other potential PGP characteristics, may make these strains effective agents for enhancing plant growth.

Considering the above, I fully endorse the choice of two strains, namely ***Pseudomonas chlororaphis* 1S4** and ***Pseudomonas yamanor*um 1046**, as appropriate for the next stages of the experimental design.

The subsequent optimization of the cultivation process has been carried out adequately and logically. The stepwise approach to the process of periodic cultivation with optimization with respect to carbon, nitrogen, and phosphorus sources demonstrates the effectiveness of this method in identifying optimal working concentrations of these nutrient sources for the selected strains. The parameter optical density observed in this experiment provides additional information on the growth and metabolism of bacterial strains under different cultivation conditions. The trend recorded for extracellular protein indicates that the lowest values of optical density were observed in the variants with ½C and ½P, suggesting that reducing the concentrations of carbon and phosphorus may slow down the growth and metabolism of bacterial cells. The results of the experiment show that the best performance for all parameters was achieved in the variants with double concentrations of the three nutrient sources (carbon, nitrogen, and phosphorus). This implies that increasing the concentrations of these nutrient sources may significantly enhance the growth and metabolism of bacterial cells, leading to improved production of desired products. However, the economic feasibility of implementing these variations on an industrial scale is a major concern. The so-called base concentration (B) variant shows good performance, indicating that increasing the concentrations of nutrient sources may not be necessary for achieving the desired performance. The data from this experiment show that the 168-hour cultivation period results in viable cultures with strong potential for biosynthesis of desired products during the stationary phase of growth. The selected strains are adaptable to cultivation conditions and can maintain stable production of desired products throughout the cultivation period. These results can be used for further

optimization of the cultivation process and development of efficient strategies for producing desired products. The results related to phenazine-1-carboxylic acid production and siderophore production from the target strains, as well as their antifungal capacity, have been correctly conducted and logically interpreted.

Summary of this part of the experimental scheme - the optimization of the cultivation process, the biosynthesis of the desired products, and the maintenance of product stability of Pseudomonas chlororaphis 1S4 and Pseudomonas yamanorum 1046 demonstrate the potential The established profile of the selected strains allows for the implementation of the next major part of the experimental scheme, i.e., determining the PGP potential of *Pseudomonas chlororaphis* 1S4 and *Pseudomonas yamanorum* 1046: plant-microbial symbiosis with technical crops and ornamental plants.

The study of plant-microbial symbiosis of *Ps. chlorora*phis 1S4 and *Ps. yama*norum 1046 with model plant systems from technical crops and ornamental plants in different phases of the vegetation period demonstrates the broad range of potential benefits from these strains for plants as producers of beneficial compounds and potential agents for plant protection.

It has been found that *Ps. chlororaphis* 1S4 and *Ps. yamanorum* 1046 enhance the germination efficiency of seeds from technical crops such as corn, soybean, and wheat, as assessed by the biometric parameters of the root system of the model plants. This suggests that these strains can be used as biofertilizers to promote the growth and development of cultural plants.

I highly appreciate the clear photographic evidence and graphical/tabular presentation of the results from this part.

The conducted experiments and obtained results emphasize the potential of *Pseudomonas chlororaphis* 1S4 and *Pseudomonas yamanorum* 1046 as biopesticides, biocontrol agents, and biofertilizers. The optimization of cultivation conditions, biosynthesis of desired products, and retention of stability of biologically active fragmentation products from these strains demonstrates their versatility and potential for practical application in sustainable agriculture. Understanding the regulatory mechanisms controlling the production of these compounds can help develop strategies to improve the efficacy of these compounds as biopesticides or plant protection agents.

VIII. Conclusions/Contributions

Based on the conducted experiments, 9 conclusions have been drawn. They are logical consequences of the experimental work, accurately reflect the obtained results, and provide a clear, correct, and precise answer to the stated goal and tasks of the scientific research. Additionally, 5 contributions have been identified, whose formulation I consider correct and fully reflecting the practical potential of the scientific development.

IX. Participation of the PhD student in the preparation of the thesis

The PhD student developed the thesis in the Department of General and Industrial Microbiology at SU and the research department of an industry-affiliated entity focused on the subject matter. Her participation in various scientific forums and reporting of results from the thesis leads me to believe that the completion of the thesis work was entirely her own endeavor.

- **Publications**
  The PhD student submitted a list of 3 publications related to the topic (2 publications in Q4 journals; 1 publication in a Q3 journal), where she is the first author. There were 4 participations in scientific conferences.
- **Abstract of the thesis**
  The abstract contains 58 pages and accurately reflects the most essential results obtained during the development of the doctoral thesis. It is well structured, contains summarized data and scientific interpretation of the obtained results. The conclusions and contributions remain unchanged. I believe that the abstract meets all the necessary requirements.

X. Administrative Documents

The administrative document, provided by the candidate, meet the requirements.

XI. Questions

What are the potential challenges and limitations associated with the practical application of these strains as biopesticides or biocontrol agents, and how might they be overcome?

CONCLUSION

The presented doctoral thesis in terms of volume, format, and content fully meets the requirements of the LDASRB, the Rules for its Application, and the Rules of Sofia University "St. Kliment Ohridski". It is a complete study that provides valuable scientific information. The

topic is current, the research was conducted methodically correctly, a wide range of modern methods was mastered, a serious amount of research activity was performed, demonstrated was the ability to interpret the received results and draw conclusions and contributions. The doctoral thesis is original, with scientific and applied contributions and is the work of the author.

Based on the information provided above, I am convinced that the doctoral thesis is of high scientific quality, the required competencies have been acquired, and I will confidently vote in favor of awarding the educational and scientific degree "Doctor" to Gloria Biserova Georgieva.

Reviewer:

/assoc. prof. Mihail Iliev/

Date: 21.09. 2024