OPINION

By Assoc. prof. Galina Radeva, PhD

Roumen Tsanev Institite of Molecular Biology, BAS,

member of scientific jury, appointed by the ordinance №РД-38-473/24.07.24 from the Rector of SU ‘‘St. Kl. Ohridski’’

Concerning: PhD thesis of Gloria Biserova Georgieva at the Department of ‘‘General and Industrial Microbiology’’ in Faculty of Biology, SU ‘‘St. Kl. Ohridski’’ for the award of the educational and scientific degree "Doctor" (PhD) in the field of Higher education 4. Natural Sciences, Mathematics and Informatics, professional area 4.3 Biological sciences (Microbiology), entitled ‘‘Physiological and biochemical characteristics of the plant - microbial symbiosis of representatives of the genus *Pseudomonas*’’ with Scientific supervisor Assoc. Prof. Dr. Trayana Nedeva.

Plant–microbe–microbe (specifically bacteria) interactions in the rhizosphere and their influence on rhizosphere microbiomes and impact plant health, represent an attractive direction in microbial ecology in the recent years. Due to the severe climate change effects on plants and rhizosphere biology, there is a growing interest in stress-resilient plant growth-promoting microorganisms (PGPM) and their application to induce stress tolerance mechanism in plants. Considering the multifarious activities of plant growth promoting rhizobacteria (PGPR), they contribute to the improvement of soil structure, health, fertility and functioning with directly or indirectly support plant growth under both normal and stressed conditions. The use of PGPR for so-called rhizosphere engineering is also promising, as well as their widespread application for the development of eco-friendly sustainable agriculture and the conservation of ecosystems. It is very important in this direction to search for and identify local strains with a pronounced potential for stimulating plant growth, as well as to demonstrate this potential through model systems with economically significant plants.

The above-mentioned highlights the relevance and significance of Gloria Georgieva's dissertation, which presents a comprehensive and in-depth study of bacterial strains from the genus Pseudomonas aimed at exploring the phenomenon of plant-microbe symbiosis and demonstrating their PGP potential.

Georgieva's dissertation is written on 215 pages and contains 69 figures, 10 tables and 347 cited references. It is structured according to generally accepted requirements and contains sections: Introduction (1 page); Literature review (45 pages); Purpose and tasks (2 pages); Materials and Methods (20 pp.) Results and Discussion (86 pp.); Conclusions (2 pages); Contributions (1 page) and Appendix (9 pages).

The abstract is written on 58 pages, accurately reflects the content of the dissertation and the contributions of the conducted research.

The literature review offers an in-depth analysis of the current state of knowledge on the issue of dissertation work. It identifies six main points related to the role of microorganisms in the soil - rhizosphere microorganisms that stimulate plant growth, the role of microorganisms in the phyllosphere, PGPR of the genus Pseudomonas, plant-microbial symbiosis, plant immune response to biotic and abiotic stress and the role of microorganisms in it; application of PGPR as biological control agents in conventional agriculture. The way the literature review is structured and the analytical presentation of the information show that Gloria Georgieva is familiar with the problem in detail. In the review (p. 15), Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria are referred to as types, but they should correctly be designated as phyla.

The formulated working hypothesis is based on the expected PGP potential of bacteria belonging to the genus Pseudomonas and suitable experimental procedures proving this potential have been selected. They are summarized in a very comprehensive and informative scheme.

The goal of the dissertation is convincingly argued in the light of the literature review and it is "to select and study bacterial strains belonging to the genus *Pseudomonas* to reveal the phenomenon of plant-microbe symbiosis and demonstrate their PGP potential". To achieve this goal, a total of three tasks and eight sub-tasks have been formulated.

In the experimental part - section "Materials and methods" experimental methods and the conducted data processing are described in detail and correctly, which allows their correct reproduction. During the work on the dissertation, the doctoral student used a wide range of methods: traditional methods for cultivating and monitoring the growth of microorganisms; biochemical methods for determining enzyme activities; analytical chemical, chromatographic and mass spectrometric; genetic methods for the identification of bacterial and fungal strains; plant tests and derivation of vessel experiments; methods for monitoring the cultivation processes of bacterial cultures and lyophilization.

In the "Results and Discussion" section, the results are described in detail, richly illustrated and discussed in full agreement with the clearly stated objective and the properly defined tasks to be performed.

More significant scientific results and research contributions in the dissertation could be summarized as follows:

Fundamental contributions are related to the genetic identification and characterization of strains *Pseudomonas chlororaphis* 1S4 and *Pseudomonas yamanorum* 1046, and their

physiological-biochemical screening proved growth stimulation of cereal/cereal/legume crops and ornamental plants.

Scientifically-applied contributions are related to 1) construction of a prototype series of fresh and dry cultures of *Pseudomonas chlororaphis* 1S4 and *Pseudomonas yamanorum* 1046 with potential for application as biofertilizers or biocontrol agents and 2) the application of a cost-effective approach to replace the synthetic inducer L-tryptophan with tryptophan of microbial origin (*E. coli* K12) to effectively induce indolyl-acetic acid production. The obtained results suggest future research and optimization of the biotechnological processes for the production of phytohormones.

At the end of the dissertation, 8 main conclusions are drawn and 4 contributions of an original scientific and scientifically-applied are formulated, and one contribution of a confirmatory character, which faithfully and accurately reflect the main results of the conducted experimental studies.

On the topic of the dissertation, one scientific publication is published in the journal BioRisk with quartile Q3, and two articles, accepted for publication in the journal Acta Microbiologica Bulgarica with quartile Q4. In the three publications, Gloria Georgieva is the first and corresponding author, which is a testimony to her leading participation and contribution in the researches carried out and their writing.
The doctoral student is participated in one national and three scientific forums with international participation.

Conclision:

The research in Gloria Georgieva's dissertation is conducted at a high scientific level, and the results obtained are either new to science or contribute to clarifying the plant-microbial symbiosis between bacteria and model plant systems.

The PhD student has mastered a wide variety of experimental and theoretical methods, as well as the ability to present her research to the scientific community.

The dissertation meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the regulations for its application, including of the internal rules of the Faculty of Biology, SU "St. Kl. Ohridski". All this gives me reason to confidently give my positive vote for awarding the educational and scientific degree "Doctor" to Gloria Biserova Georgieva in the professional field: 4.3. Biological Sciences, scientific specialty Microbiology.

26.09.2024 Prepared by:

/Assoc. prof. G. Radeva, PhD/