ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (PhD) in the educational program 8D05105 - «Biotechnology»

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on the thesis: "Creation of a biopreparation based on immobilized rhizobacteria in various sorbents"

General description of the work. The dissertation is devoted to the selection of an optimal technology for immobilizing promising rhizobacteria on natural sorbents for the development of biopreparation. This formulation is expected to enhance the stability of the bacterial inoculant, promote its effective functioning in the rhizosphere, improve soil agrochemical properties, and consequently stimulate the growth and development of the model plant, maize.

Rhizobacteria were screened for their plant growth-promoting potential, and their biological properties were studied. The optimal conditions for immobilizing rhizobacteria on sorbents were determined.

The effect of the developed biopreparation on the growth and development of maize, as well as on the agrochemical properties of the soil, was investigated. The effectiveness of the biopreparation was evaluated based on the morphometric and physiological parameters of maize. The scientific and practical foundations for the application of immobilized rhizobacterial formulations in agriculture to enhance plant biological productivity were established.

Relevance of the research topic. Conventional intensive application of mineral fertilizers and pesticides leads to a decline in soil fertility, disruption of nutrient balance, soil acidification, and environmental pollution. These issues are particularly relevant for agricultural lands in Kazakhstan, where soil degradation and reduced productivity are observed.

In this context, the development of environmentally safe, biologically based approaches to enhancing plant productivity is of particular importance. A promising direction is the use of plant growth-promoting rhizobacteria (PGPR), which can improve plant mineral nutrition, produce phytohormones, suppress phytopathogens, and increase plant resistance to biotic and abiotic stress factors. However, free bacterial cells do not always survive well after soil application, due to their low tolerance to adverse environmental conditions and competition with the indigenous microbiota. One effective strategy to improve the stability and viability of PGPR is their immobilization on natural sorbents.

Biochar and vermiculite are considered promising carriers, as they possess a highly developed porous and layered structure, high sorption activity, and the ability to retain moisture and nutrients. Their use for rhizobacterial immobilization may not only help preserve bacterial viability but also enable controlled release of bacteria into the soil, thereby enhancing positive interactions within the "plant-microorganism-soil" system.

The aim of the work: Development of a biopreparation based on rhizobacteria immobilized on natural sorbents to restore soil fertility and stimulate

the growth and development of the model plant, maize, as a component of an environmentally sustainable agricultural system.

To achieve this aim, the following tasks were solved:

1. Isolate rhizobacterial strain, screen them for plant growth-promoting properties and identify promising strains;
2. Determine the chemical composition and structural characteristics of four natural sorbents;
3. Optimize the immobilization conditions for the most promising rhizobacterial strains to develop sorbent-bacteria biocomposites;
4. To evaluate the effect of sorbent-rhizobacterial compositions on the agrochemical properties of the soil, as well as on the vegetative and morphometric parameters of the model plant - maize under greenhouse conditions;
5. Substantiate the composition and develop a technology for producing a biological preparation based on immobilized rhizobacteria and the Vermiculite-Miscanthus composite.

The object of study:

The rhizobacterial strains were isolated from the rhizosphere of winter wheat cultivar "Almaly" (*Triticum aestivum L.*), triticale cultivar "Taza" (×*Triticosecale Wittm.*), and maize hybrids "Turan 480SV" and "KazZP 200" (*Zea mays L.*), grown in the fields of the Kazakh Research Institute of Agriculture and Crop Production. Vermiculite, biochar from wheat straw, biochar from miscanthus, and biochar derived from sewage sludge were used as carrier sorbents for immobilization. Under greenhouse conditions, the three-way maize hybrid Ceklad 235 (*Zea mays L.)* was used as the model plant.

Research methods:

During the dissertation research, experiments were carried out under laboratory conditions using microbiological, molecular-genetic, physicochemical, and statistical methods: sequencing of a 16S rRNA gene fragment, physical adsorption, energy-dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), and analysis of variance (ANOVA).

Scientific novelty of the research:

For the first time, the scientific basis for the development of a biological preparation based on a vermiculite-miscanthus biochar biocomposite with immobilized rhizobacterium *Bacillus thuringiensis AS31* was established.

The most effective combinations of rhizobacterial strains and natural sorbents were identified, providing high microbial viability and a pronounced plant growth-promoting effect.

For the first time, a relationship was established between the physicochemical properties of natural sorbents, the survival of immobilized rhizobacteria, changes in the functional characteristics of the soil, and the growth of the model plant - maize.

Scientific and practical significance of the research:

Three new plant growth-promoting rhizobacterial strains were isolated from the plant rhizosphere, identified, and deposited in the international GenBank database: *Stenotrophomonas indicatrix AS8, Pantoea agglomerans AS23*, and *Bacillus thuringiensis AS31.*

The novel active strain *Bacillus thuringiensis AS31* was registered in the Republican Collection of Microorganisms (RCM) under accession number B-RKM 1248. A patent of the Republic of Kazakhstan for a utility model № 11603, "Strain *Bacillus thuringiensis AS31* as a preparation against phytopathogens," was granted for this strain.

Methods for immobilizing rhizobacteria on natural sorbents - biochar and vermiculite - were improved.

The environmental and agronomic advantages of using immobilized rhizobacteria for restoring soil fertility and enhancing the resilience of agroecosystems were experimentally substantiated.

Provisions for defense:

– The biotechnological potential of the three new rhizobacterial strains is defined by a complex of growth-promoting and biocontrol traits, including IAA and siderophore synthesis, phosphate and zinc solubilization, ammonia production, and antagonistic activity.

– The physicochemical, surface, and porous characteristics of natural sorbents determine their capacity for efficient immobilization of bacterial cells. The most effective biological object for immobilization is the strain *Bacillus thuringiensis AS31*, for which optimal process parameters have been established.

– Immobilization of rhizobacteria on natural sorbents enhances the stability of the bacterial inoculant, promotes its effective functioning in the rhizosphere, improves the agrochemical properties of the soil, and consequently stimulates the growth and development of the model plant - maize.

– The "Vermiculite-Miscanthus" biocomposite is a promising carrier matrix for *Bacillus thuringiensis AS31* and ensures increased efficacy of the biopreparation compared with individual sorbents, supporting its potential for practical application.

The conducted research enabled the following conclusions to be drawn:

1. Promising rhizobacterial strains, *Stenotrophomonas indicatrix* AS8, *Pantoea agglomerans* AS23, and *Bacillus thuringiensis* AS31, were identified. The strains produced indole-3-acetic acid (IAA) at 43.1-48.5 mg/L, siderophores at 1.33-3.6 mg/L, and ammonia at 22-25 mg/L, solubilized phosphate and zinc (SI 2.3-4.0), and exhibited antagonistic activity against the phytopathogenic fungi *Fusarium graminearum, F. solani, F. oxysporum, Alternaria alternata*, and *Pythium aphanidermatum.*

2. The elemental composition and surface and porous characteristics of four sorbent carriers were determined:

Wheat-straw biochar was characterized by a high content of Si, Ca, and K and a well-developed mesoporous structure, with a specific surface area of 17 m^2/g and pore size of 2-5 nm. Vermiculite contained Mg, Si, and Al and exhibited a layered mineral structure with a well-developed mesoporous system; its specific surface area was 23.24 m^2/g, and the total pore volume was 54.96 mm^3/g. Akmeko biochar had a specific surface area of 59 m^2/g and a micropore volume of 17 mm^3/g. Miscanthus biochar contained Ca, Si, K, S, and P and was characterized by the highest specific surface area (174 m^2/g) and micropore volume (54 mm^3/g). Among the studied

sorbents, Miscanthus biochar exhibited the most developed surface area and porous structure, indicating its high sorption potential and suitability as a carrier for bacterial immobilization.

3. The highest immobilization efficiency, 83.3%, was achieved for *Bacillus thuringiensis* AS31. The initial bacterial suspension concentration used for immobilization was 10^7 CFU/mL, and the immobilization duration was 18 h.

4. The effectiveness of sorbent-bacterial composites based on *Bacillus thuringiensis AS31* was established. According to the principle of dual functionality, vermiculite and Miscanthus biochar were selected as promising sorbent carriers. Vermiculite contributed to a pronounced increase in maize shoot and root biomass by 27.5% and 104.8%. Miscanthus biochar was characterized by a well-developed porous structure and high specific surface area, providing favorable conditions for bacterial cell immobilization. Its application increased maize shoot and root biomass by 15.4% and 48.2%, respectively, and increased soil P, K, Ca, and Mg contents by 7.9%, 9.2%, 17.8%, and 1.7%, respectively. These results confirm the potential of vermiculite and Miscanthus biochar as sorbent carriers for the development of an effective microbial biofertilizer.

5. Based on a comprehensive assessment of the physicochemical characteristics of the sorbents, increases in soil macronutrient contents, and greenhouse experiment results, the composition of the "Vermiculite-Miscanthus" biocomposite was substantiated. Based on the obtained results, a technological scheme for producing the immobilized "Vermix" biofertilizer was developed. The main technological parameters were established: a 50:50 ratio of vermiculite to Miscanthus biochar, an initial bacterial suspension concentration of 10^7 CFU/mL, a 1:3 (v/w) ratio of bacterial suspension to biocomposite, and an immobilization duration of 18 h.

Personal contribution of the dissertation candidate to the creation of the results of the scientific work proposed for defense:

The author independently conducted literature analysis, formulated objectives and research tasks, performed experiments, processed and analyzed data, wrote scientific articles and the dissertation text, and prepared the manuscript.

The connection of the dissertation work with scientific research:

The dissertation research was carried out within the framework of the following research projects: AR23490702, "Development of a Technology for Producing Biochar for the Sustainable Conversion of Biomass Waste into Value-Added Products" (2024-2026), and the NATO SPS Multi-Year Project G6094 «Mitigation of Climate Change through Miscanthus × giganteus Phytotechnology Applied to Military Sites» (2023–2027).

Approbation, approval of the results of the work and personal contribution of the author:

The results of the dissertation research were presented and discussed at the following international scientific conferences.:

– International Scientific and Practical Conference "2nd International Conference on Sustainable Energy and Green Technology (ICSEGT 2025)", May 29-31, 2025, Baku, Azerbaijan.

– International Scientific and Practical Conference “RemTech Europe: International Conference and Exhibition on Land and Water Remediation Markets and Technologies”, September 15-19, 2025, Ferrara, Italy.

– International Scientific and Practical Conference “ICESICS 2025: International Conference on Environmental Sustainability: Innovations, Challenges and Solutions”, October 6-8, 2025, Almaty, Kazakhstan.

– International Scientific and Practical Conference “IABEP: International Conference of the International Association for Biochar, Environment and Pollution”, October 13-15, 2024, Belgrade, Serbia.

Publications:

The main results of the dissertation research have been published in 15 scientific publications, including 2 articles in national peer-reviewed scientific journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Republic of Kazakhstan; 3 articles in international scientific journals indexed in the Scopus and Web of Science databases: *Microorganisms* (percentile - 84, Q1), *International Journal of Plant Biology* (percentile - 70, Q2), and *International Journal of Biology and Chemistry* (percentile - 30, Q3); 8 conference abstracts published in the proceedings of national and international scientific conferences; and 2 articles published in the proceedings of international scientific conferences held in Kazakhstan and abroad.

Structure of the dissertation:

The dissertation is presented in 174 pages of main text and consists of normative references, definitions, symbols and abbreviations, an introduction, a literature review, materials and methods, results and discussion, and conclusions. The dissertation includes 342 references, 11 tables, 56 figures, and 2 appendices.