### ANNOTATION

General characteristics of the dissertation work. The work is devoted to the study of the peculiarities of the distribution of endophytic microorganisms of medicinal plants, the search for new strains, the characterization of their growthstimulating and biocontrol properties for the development of effective microbiological preparations used in crop production.

# **Relevance of the research topic.**

Medicinal plants are a valuable source of biologically active compounds that contribute to their survival in the natural environment, including protective functions against biotic and abiotic stresses. These biologically active secondary metabolites synthesized by medicinal plants can also have a strong effect on microbial communities associated with plants and their physiological functions. Moreover, plants depend on their microbiome for specific properties and activities, including growth promotion, nutrient production, induced systemic resistance and resistance to stress factors. In this regard, associations of plants with beneficial microorganisms attract the attention of scientists not only as an object for studying the fundamental foundations of the interaction of various organisms, but also from the point of view of their possible use in the practice of environmentally oriented crop production.

The main environmental factors limiting the productivity of agricultural plants are drought, soil salinization, temperature fluctuations, heavy metals, and phytopathogens. The adverse effects of stress are exacerbated by climate change and unpredictable weather conditions. Resistance to basic biotic and abiotic stresses is one of the main requirements for modern varieties of agricultural crops and technologies for their cultivation. For many agricultural crops, the problem of complex long-term resistance to stress factors still remains unresolved, therefore, in order to obtain satisfactory yields, it is necessary to use chemical plant protection products. All this encourages, based on the priorities of adaptive landscape farming, to search for the most environmentally friendly methods and means that reduce crop losses and stabilize plant productivity. Such methods include both the direct effect of biologics consisting of microorganisms and their metabolites, and an increase in the general nonspecific resistance of plants (immune status) to adverse factors of biotic and abiotic nature by induction of natural protective mechanisms.

The endophytes of medicinal plants contribute to the survival of the host plant and enhance the production of its bioactive metabolites. The use of endophytes as plant growth stimulants can help ensure stable yields of both host plants and other plants, including agricultural crops, and reduce problems associated with the effects of biotic and abiotic factors.

Endophytic microorganisms contribute to the growth and development of the host plant through the production of phytohormones, improved transport of water and nutrients, the action of biological defense mechanisms and the induction of systemic resistance to phytopathogens. Endophytes are of interest primarily as potential agents of the so-called systemic biocontrol, since they penetrate into plants. In addition, these microorganisms are able to influence the physiological processes of plants that determine resistance to the action of abiogenic environmental stress factors.

In this regard, research aimed at studying the ability of endophytic microorganisms to increase plant resistance to biotic and abiotic environmental factors is becoming particularly relevant.

The purpose of the study: experimental substantiation of the possibility of biotechnological use of endophytic microorganisms and the development of effective methods of their application.

### **Research objectives:**

1. Characterization of the quantitative composition and taxonomic structure of endophytic microorganisms of medicinal plants.

2. Screening of endophytic strains with biotechnologically valuable properties.

3. Determination of the ability of halotolerant bacteria to stimulate the growth of barley plants in saline soil conditions.

4. Development of a method for protecting apples from post-harvest infections using polyhydroxyalkonoate (PHA) endophytic strains.

5. Preparation of a biological product for pre-sowing seed treatment based on endophytic microorganisms and their BAM in conditions of phytopathogenic load.

**The object of research**: strains of endophytic microorganisms isolated from 11 medicinal plants (Peppermint (*Méntha piperíta*), Sage officinalis, Chicory (*Cichórium intybus*), Echinacea purpurea, Nettle dioecious (*Urtíca dióica*), Oregano (*Oríganum vulgare*), Medicinal wormwood (*Artemisia abrotanum*), Marsh iris (*Iris pseudacorus*), Naked licorice (*Glycyrrhiza glabra*), Medicinal melissa (*Melissa officinalis*), Garlic (*Allium satívum*)).

**Subject of research:** characterization of growth-stimulating and biocontrol properties of endophytic microorganisms and their BAS with subsequent development of effective methods of their application.

**Research methods:** modern microbiological, biochemical, molecular biological, physico-chemical and vegetative methods are used in the work. Statistical data processing was carried out using the licensed Statistica software package version 10.0.

# Scientific novelty of the research results:

The quantitative composition and taxonomic structure of endophytic microorganisms of 11 medicinal plants of Kazakhstan has been characterized for the first time. It was shown that in the structure of endophytic microbial communities, representatives of the genera *Pseudomonas* and *Bacillus*, among fungi *Penicillium* and *Aspergillus*, occupied the largest share among bacteria.

Original results have been obtained that have proven the positive effect of medicinal plant endophytes on agricultural crops due to the synthesis of auxins, the effect on the activity of antioxidant enzymes and proline content, increased availability of nutrients, pronounced antifungal activity and halotolerance.

For the first time, an innovative environmentally friendly method of protecting apples from post-harvest infections using PHA as a biocontrol agent against *P. expansum* has been proposed.

For the first time, compositions based on domestic strains of endophytes, as well as their metabolites, were created, and effective ways of using them to improve crop growth were developed.

The practical significance of the research is associated with the creation of an extensive collection of effective strains of agricultural endophytes, which is a valuable biological resource for research. These strains have a high potential for use in the composition of drugs to solve individual and complex tasks in the field of agricultural development and environmental protection. For 2 strains (*Pseudomonas flavescens* D5 and *Bacillus aerophilus* A2), patent for inventions No. 37123 and utility model patent No. 9024 was obtained. A number of observations and patterns identified during the study can be used as practical recommendations for the development of biological products based on microorganisms. The developed compositions based on endophytes and their metabolites can be used to improve the growth of crops, including in conditions of salinization and phytopathogenic load.

The theoretical significance of the study. The results obtained deepen and expand knowledge about the composition and properties of endophytic communities of medicinal plants, which is an important issue of the ecology of endophytes and the functioning of terrestrial ecosystems. The study of the mechanisms of the positive effect of endophytic microorganisms on plants is of paramount importance for understanding the processes underlying the stimulation of agricultural growth, and also provides a fundamental platform for developing strategies for their application. Since the research is at the intersection of biotechnology, microbiology, biochemistry and agrobiology, the results obtained may have an impact on the development of these fields of science in both fundamental and applied aspects.

### The main provisions of the dissertation submitted for defense:

The endophytic microbiome of medicinal plants is a promising source of effective strains with biotechnologically valuable properties.

- Endophytic halotolerant strains of *Ps. flavescens* D5 and *Lysinibacillus sp.* S1 has a protective effect on plants under conditions of salt stress.

- New microbial PHAs with high antimicrobial activity are potential agents of biocontrol of post-harvest diseases.

- The use of the developed compositions from endophytic strains and their metabolites is an effective method of pre-sowing seed treatment, which has a significant stimulating effect on the growth and development of agricultural crops.

#### Main conclusions:

The results of the study allow us to draw the following conclusions:

1. The main components of communities of endophytic bacteria of medicinal plants were various species of the genera *Pseudomonas* and *Bacillus*, representatives of the genera *Penicillium* and *Aspergillus* predominated among fungi. The level of colonization and the coefficient of isolation of endophytic bacterial strains was 6.67 to 60% and 0.07 to 0.83, and for fungi these indicators were 3.33 to 43.33% and 0.03 to 0.57, respectively. The quantitative distribution of microorganisms in plant organs

was expressed in a sequence: roots, stems, leaves, flowers. The greatest colonization by endophytes was typical for peppermint and chicory.

2. As a result of large-scale screening of 386 bacterial and 160 fungal isolates, 6 strains were selected: *Pseudomonas flavescens* D5, *Bacillus aerophillus* A2, *Serratia proteamaculans* B5, *Peribacillus simplex* B9, *Pseudomonas putida* D7, *Lysinibacillus sp.* S1, possessing a complex of valuable biotechnological properties: the ability to produce IAA and PHA, antagonistic activity against the phytopathogens *Fusarium solani* and *Fusarium oxysporum*, the ability to mobilize inorganic phosphorus, halotolerance.

3. Halotolerant strains *Ps. flavescens* D5 and *Lysinibacillus sp* S1 have been found to be endophytic. exerted a complex of beneficial effects on plants under conditions of salt stress, improving their growth and strengthening the protective mechanisms of plants. An increase in the morphometric parameters of plants was shown: the biomass of shoots by 8-30%, the biomass of roots by 7-20%. The treated plants showed a 41% increase in chlorophyll content and a 1.5–2.2-fold decrease in proline levels compared to the control. An increase in the activity of enzymes of the antioxidant system in inoculated plants was noted: catalase by 1.4–7.4 times, ascorbate peroxidase by 1.3–3.1 times, guaiacol peroxidase by 1.2–2.4 times.Treatment with bacterial strains contributed to maintaining high levels of Na ions and reducing the level of Ca ions under salt stress.

4. An innovative environmentally friendly method has been developed to protect apples from post-harvest infections caused by *P. expansum* using microbial PHAs. A nutrient medium has been proposed to increase the PHA yield (up to 5.9 g/l) based on the waste of the fat and oil industry – soapstock. IR spectroscopic and thermogravimetric analysis of PHA samples was carried out, and their antifungal activity was studied. The use of PHA as a biocontrol agent was most effective when applied 24 hours before infection of *P. expansum* fruits (preventive treatment). It was shown that PHA caused a 2-fold decrease in morbidity on the 10th day compared with control fetuses. The weight loss of fruits treated with PHA was 2 times lower than that of control apples.

5. A method of pre-sowing seed treatment using a biopreparation based on an association consisting of 5 biocompatible strains: *Pseudomonas flavescens* D5, *Bacillus aerophillus* A2, *Serratia proteamaculans* B5, *Pseudomonas fluorescens* D7 and *Lysinibacillus* sp. S1 and the polymer component (pullulane, pectin and PHA in a ratio of 2:1:0.1). The influence of the developed biopreparation on the growth of barley under conditions of phytopathogenic load is estimated. It was shown an increase in the energy of germination and germination of seeds, an improvement in the morphometric parameters of plants: the length and biomass of shoots by 8-30%, the length and biomass of roots by 7-20%. The positive effect of the biopreparation was expressed in an increase in the content of Chl *a* in the leaves by 1.4–2.1 times, Chl *b* by 2-2.4 times, as well as the total content of Chl (a + b) by 1.6–2.2 times, in a decrease in the level of proline by 3.7 times. Pre–sowing treatment of seeds led to an increase by 2.4 times, guaiacol peroxidase by 2.7 times.

The main content of the dissertation is reflected in 12 printed works, including 3 articles in journals indexed in the Web of Science and Scopus databases, 2 articles in republican scientific journals included in the list of National Scientific Journals of the Ministry of Internal Affairs of the Republic of Kazakhstan, 2 patents of the Republic of Kazakhstan, 1 article in the materials of an international conference, 4 abstracts in the materials of international conferences.

The author's personal contribution consists in carrying out the bulk of theoretical and experimental research, analyzing, interpreting and formatting the results obtained, and preparing the manuscripts of publications.

**The volume and structure of the dissertation.** The dissertation is presented on 119 pages and consists of notations and abbreviations, an introduction, a review of literature, materials and methods, results and discussions, a conclusion, a list of used sources from 234 titles. The work contains 19 tables, 27 figures and 2 applications.