ABSTRACT

dissertation for the degree of Doctor of Philosophy (PhD) in the specialty "6D070100-Biotechnology" Brazhnikova Yelena Valerievna
on the topic: " Micromycetes of agrocenoses and the possibilities of their application for growth promotion of agricultural crops"

General characteristics of the dissertation work. The work is devoted to the study of the distribution of micromycetes in agrocenoses, the investigation of the mechanisms of their beneficial effect on plants and the development of methods for using these microorganisms to improve the crop growth.

Relevance of the research topic. The agricultural sector is strategically important and occupies one of the leading places in the economy of the Republic of Kazakhstan. In 2022, the sown area of all agricultural crops amounted to 22.9 million ha. Modern technologies for the cultivation of agricultural crops include such techniques as: seed treatment, application of mineral fertilizers, and the use of pesticides. In 2022, 626.5 thousand tons of mineral fertilizers and 16.6 million liters of pesticides were applied on the sown areas of Kazakhstan. The use of mineral fertilizers and chemical plant protection products is environmentally unsafe, entails a violation of the biological balance in agrocenoses, pollution of agricultural products and groundwater.

In this regard, increasing the productivity of agricultural plants, reducing the amount of chemical agents used to stimulate and protect agricultural crops, as well as increasing the resistance and adaptation of plants to adverse conditions are the relevant directions both for the development of agriculture and for solving environmental problems. The microbiological approaches that are based on the use of the potential of endophytic and soil microorganisms appear to be particularly important and promising. It is known that microorganisms can provide a number of beneficial effects on plants, the main of which are: increasing the availability of nutrients and increasing the utilization of nutrients from fertilizers and soil; production of metabolites with hormonal and signaling functions; protective action on plants under stress conditions.

Thus, the development of preparations based on microorganisms and their metabolites is an important and relevant area of research. The use of such microorganisms seems to be an attractive alternative to chemical preparations promotes the development of intensive crop production and environmentally balanced agriculture.

Micromycetes are of particular interest because they are one of the main structural and functional components of biocenoses. They also have a number of advantages over other groups of microorganisms.

Despite the ubiquity of distribution, multifunctionality and significance of this group of microorganisms, information on soil and endophytic micromycetes of agrocenoses in Kazakhstan is very limited. Issues related to the mechanisms underlying the stimulating effect of domestic strains on the growth and development of plants remain poorly studied. There are no polyfunctional agricultural preparations based on domestic strains of micromycetes. Thus, the characterization

of micromycetes of agrocenoses, a comprehensive study of the processes that determine their positive effect on plants, as well as the development of methods for using these microorganisms are relevant and have important theoretical and practical significance.

The purpose of the study: substantiation of the possibility of using micromycetes to improve plant growth and the development of effective methods for the application of these microorganisms.

Research objectives:

1. Characteristics of the quantitative composition and taxonomic structure of soil and endophytic micromycete communities of agrocenoses.
2. Screening of micromycete strains with agronomically valuable properties that are promising for growth promotion and protection of agricultural crops.
3. Study of the direct mechanisms of the positive action of micromycetes on plants.
4. Determination of the mechanisms of the protective action of micromycetes on agricultural crops under the biotic and abiotic stresses.
5. Creation of a collection of effective strains of micromycetes for agricultural purposes.
6. Creation of compositions based on selected strains of micromycetes and development of methods for their use to improve plant growth.

Object of study. strains of micromycetes isolated from seven agricultural crops (soybean, barley, alfalfa, rapeseed, safflower, sweet clover, sainfoin) and soils of agrocenoses of these plants.

Research methods: modern microbiological, biochemical, molecular, physicochemical and vegetative methods. Statistical data processing was carried out using the licensed software package Statistica version 10.0 (TIBCO Software Inc., USA).

Scientific novelty of the research results:

For the first time, the quantitative composition and taxonomic structure of soil and endophytic micromycete communities of agrocenoses of seven agricultural crops of Kazakhstan was characterized.

For the first time, it was shown for the first time that the main components of micromycete communities were filamentous fungi of the genera *Penicillium*, *Aspergillus* and *Fusarium*, representatives of the genera *Aureobasidium*, *Rhodotorula* and *Metschnikowia* dominated among yeasts.

Original results have been obtained showing that the positive effect of micromycetes on plants is due to the improvement of phosphorus nutrition, the production of metabolites with hormonal and signal functions, and the protective effect is provided by the synthesis of hydrolytic enzymes and compounds with antimicrobial activity, the absorption and detoxification of heavy metals.

For the first time in micromycetes of the species *Talaromyces pinophilus*, *Beauveria bassiana* and *Metarhizium robertsii*, ACC-deaminase activity was revealed and the stimulating effect of these ACC-utilizing strains on plant development under abiotic and biotic stress conditions was established.

For the first time ccompositions based on domestic strains of micromycetes and their metabolites were obtained and effective methods for their use to promote plant growth were developed.

The practical significance of the study is associated with the creation of an extensive collection of effective strains of micromycetes for agricultural purposes, which is a valuable biological resource for research. These strains have a high potential for use as part of preparations for solving individual and complex problems in the field of agricultural development and environmental protection.

The most effective 7 strains (*Aspergillus* sp. D1, *B. bassiana* T7, *B. bassiana* T15, *M.robertsii* An1, *Metschnikowia pulcherrima* MP2, *Penicillium bilaiae* Pb14 and *T. pinophilus* T14) were deposited in the RSE "Republican Collection of Microorganisms" (RKM) (Astana, Republic of Kazakhstan) and/or in the Russian Collection of Agricultural Microorganisms (RCAM) (St.-Petersburg, Russian Federation). Patents for inventions No. 34305 and No. 34350 were obtained for 2 strains (*B.bassiana* T7 and *P. bilaiae* Pb14).

A number of observations and regularities identified during the study can be used as practical recommendations for the development and use of biological products based on microorganisms in crop production.

The practical significance of the work is due to the prospects for using the developed compositions based on micromycetes and their metabolites to stimulate the growth of agricultural crops both under favorable and stressful conditions. The most effective variant of using the studied compositions is recommended - seed priming by soaking in filtrates in combination with inoculation of strains into the soil.

Theoretical significance of the study. The obtained results deepen and expand knowledge about the composition and properties of soil and endophytic micromycete communities in agrocenoses, which is an important issue in the ecology of micromycetes and the functioning of terrestrial ecosystems. The study of the mechanisms of positive action of micromycetes on plants is of paramount importance for understanding the processes that underlie the promotion 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 obtained results may have an impact on the development of these areas of science in both fundamental and applied aspects.

The main provisions of the dissertation submitted for defense:

- Soil and endophytic micromycete communities are a promising source of effective strains for agricultural purposes.

- The isolated strains of micromycetes improve phosphorus nutrition of plants, carry out biocontrol of phytopathogens and have a protective effect on plants when exposed to heavy metals.

- The application of the created compositions based on strains of micromycetes and their metabolites improves the growth and development of crops under both favorable and stressful conditions.

Main results and conclusions:

1. It is shown that the amount of filamentous fungi in the soils of agrocenoses is 1-3 orders higher than the content of yeast. The predominance of micromycetes in the upper layers of the soil (0-10 cm) was established. It was revealed that the level of colonization and the isolation coefficient of endophytic fungal strains were 3.0-3.3 times and 2.7-4.0 times higher than those for yeast, respectively**.** The quantitative distribution of micromycetes in plant organs was expressed as follows: roots > stems > leaves. It was shown that the main components of filamentous fungal communities were various species of the genera *Penicillium*, *Aspergillus* and *Fusarium*, and among yeasts - representatives of the genera *Aureobasidium*, *Rhodotorula* and *Metschnikowia*.

2. As a result of a large-scale screening of 848 soil and endophytic micromycetes, strains with agronomically valuable properties were selected: 9 isolates with pronounced antagonistic activity against 3 phytopathogens (*F. graminearum*, *P. infestans* and *A. alternata*); 14 strains with polyresistance to 3 heavy metals (cadmium, lead, zinc); 12 micromycetes capable of mobilizing both organic and inorganic P; 10 cultures producing phytohormone IAA.

3. It has been established that the direct mechanisms of the positive effect of micromycetes on plants are due to the improvement of phosphorus nutrition and the production of metabolites with hormonal and signaling functions. Five strains of micromycetes (*P. bilaiae* Pb14, *P. bilaiae* C11, *P. rubens* EF5, *T. pinophilus* T14, and *Aspergillus* sp. D1) with high (up to 86%) phosphate-mobilizing activity were identified. The main mechanisms of P mobilization have been revealed: a decrease in the pH of the medium, the production of organic acids, and the activity of acid and alkaline phosphatases. These strains increased the availability of P in the soil by 15–31% and increased its uptake by barley by 13–35%. Ten strains of micromycetes synthesizing auxins, abscisic and salicylic acids have been identified. IAA concentration ranged from 1,2 to 627,6 ng/mL

4. The mechanisms of the protective action of micromycetes were determined. A strain of *M. robertsii* An1 was revealed, which has a pronounced antagonistic activity (RII of phytopathogens was 41.5 - 51%). The most significant mechanisms of the antagonistic action of the M. robertsii An1 strain were established: chitinase (0.23 U) and glucanase (3.42 U) activities, synthesis of soluble non-volatile (destructins A and E, hydroxyanthraquinones) and volatile compounds with antifungal properties. Five strains (*B. bassiana* T7, *B. bassiana* T15, *Rh. mucilaginosa* RH2, *Rh. mucilaginosa* MK1, and *M. pulcherrima* MP2) were found to be multiresistant to HM and capable of extracting 37–59% of cadmium from the medium. It was established that four strains (*M. robertsii* An1, *B. bassiana* T15, *B. bassiana* T7 and *T. pinophilus* T14) with high ACC-deaminase activity (0.95 - 2.73 μM α-KB/mg protein/h), improve the growth of barley plants under conditions of phytopathogenic load and soil contamination with cadmium.

5. An extensive collection of strains of micromycetes for agricultural purposes has been created. The most effective 7 strains *Aspergillus* sp. D1, *B.bassiana* T7, *B.bassiana* T15, *M. robertsii* An1, *M. pulcherrima* MP2, *P. bilaiae* Pb14 and *T. pinophilus* T14) were selected for the development of compositions and deposited in domestic and foreign collections of microorganisms

6. Two versions of the compositions were created: 1) a composition of micromycete filtrates containing biologically active substances; 2) composition of spore suspensions of filamentous fungi and supernatant of yeast strain *M. pulcherrima* MP2. Three methods of application of the obtained compositions have been developed: 1) seed priming by soaking in filtrates, 2) inoculation of a spore suspension of micromycetes into the soil immediately after sowing the seeds, 3) seed priming photosynthetic pigments. A beneficial effect of micromycetes on 7 types of agricultural crops has been established, which is expressed in an increase in germination and energy of seed germination, an increase in the morphometric parameters of plants and an effect on photosynthetic pigments.

Publications. The main content of the dissertation is reflected in 14 printed works, including 2 articles in journals indexed in the Web of Science and Scopus databases, 3 articles in republican scientific journals included in the list of KKSON MES RK, 2 patents of the RK, 1 article in the materials of the international conference, 6 theses in the materials of international conferences.

The author's personal contribution lies in the implementation of the bulk of theoretical and experimental research, analysis, interpretation and presentation of the results obtained, preparation of manuscripts for publications.

The volume and structure of the dissertation. The dissertation is presented on 136 pages. Contains 30 tables, 26 figures, 3 appendices, and a list of references from 210 items.