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Biodiversity of Micromycetes Isolated from Soils of Different Agricultures in Kazakhstan and Their Plant Growth Promoting Potential

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Abstract**—**The comparative analysis of different taxonomic groups of microorganisms isolated from dark chernozem soils under different agricultures (alfalfa, melilot, sainfoin, soybean, rapeseed) at Almaty region of Kazakhstan was conducted. It was shown that the greatest number of micromycetes was typical to the soil planted with alfalfa and canola. Species diversity of micromycetes markedly decreases as it approaches the surface of the root, so that the species composition in the rhizosphere is much more uniform than in the virgin soil. Promising strains of microscopic fungi and yeast with plant growth-promoting activity to agricultures were selected. Among the selected fungi there are representatives of *Penicillium bilaiae, Trichoderma koningii, Fusarium equiseti, Aspergillus ustus*. The highest rates of growth and development of seedlings of plants observed under the influence of yeasts *Aureobasidium pullulans, Rhodotorula mucilaginosa*, *Metschnikovia pulcherrima.* Using molecular - genetic techniques confirmation of the identification results of selected micromycetes was conducted.

Keywords**—**Agricultures, biodiversity, micromycetes, plant growth-promoting microorganisms.

I. Introduction

AGRICULTURE is one of the key sectors of the economy of Kazakhstan. Promising area is the cultivation of crop agriculture for fodder forage for intensively developing livestock farming and for solving the problem of overproduction of wheat crop.

Legume-grass mixtures comprise up to 50% of the planted acreage. The share of legumes is 25-30%, including alfalfa - 10-15%, sainfoin - 15-20%, clover - 5-10%. Studies held in recent years have shown promise for cultivation of alfalfa, sainfoin and canola as green fodder. For example, summer crops of rape form forage mass -100-200kg/ha, they are less damaged by pests and vegetate until snowfall. The frozen mass can be eaten by cattle, sugar content after frost increases in twice.

One of the important areas of modern research is to improve the productivity of plants. This rate depends on the species and growth conditions of plants. Great influence on the growth and development of plants has soil microorganisms. It should be noted that some of them are phytopathogens, which have a negative impact, and microorganisms - antagonists that have a positive impact on a plant [1], [2].

There are many biological functions of microorganisms aimed for the implementation of the biosynthesis of various metabolites. Potential target of agricultural biotechnology are PGPM (Plant Growth-Promoting Microorganisms), widely used for the development of biological products to stimulate growth and improve plant productivity. Application of PGPM is an attractive alternative to chemical fertilizers, allowing reducing pollution because they are isolated mostly from natural plant habitat. However, the effectiveness of the action of growth stimulating microorganisms depends on the plant species, the conditions of their growth and many other factors [3], [4].

The positive impact of associative microorganisms to plants includes mediated stimulation of plant growth due to displacement and suppression of soil pathogens by producing compounds inhibiting pathogens and directly stimulation due to the synthesis of various exometabolites by microorganisms that directly penetrate the plant and have an impact on the biochemical processes [5]-[7]. Plant Growth-Promoting Micromycetes (PGPM) include representatives of many species belonging to the genera *Aureobasidium, Aspergillus, Penicillium, Trichoderma, Fusarium* and other. Products of their life activity could intensify metabolism, increase germination, accelerate the development, improve the accumulation of reserve substances and influence the nature of the biochemical processes that explains the successful application of biological products manufactured on the basis of these microorganisms to protect plants from disease, preserving and enhancing the crop is environmentally safe products of high quality, no harmful effects on humans and animals [8]-[11].

II. Materials and Methods

220 isolates of micromycetes isolated from dark chernozemt soils under different agriculture (alfalfa, melilot, sainfoin, soybean, and rapeseed) of Almaty region of Kazakhstan were studied.

Determining the number and species composition of the complex of soil micromycetes is done by standard methods of

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sowing soil dilutions on solid nutrient medium. The identification was carried out after isolating pure cultures of micromycetes using modern determinants of r the groups and genera of fungi [12]-[15].

Determination of plant growth-promoting activity was carried out by the locks of seed. The test cultures have been previously grown in a Saburo liquid nutrient media on a shaker. Filtered culture fluid was poured into cups with 10ml, 20 seeds were selected, soaked for 24 hours. For controlling, seeds were soaked in tap water and sterile medium. As the test seeds, seeds of alfalfa *Medicago sativa* L. were used. After daily soaking, seeds were laid out on the damp cotton wool with the filter paper in Petri dishes. All dishes were wetted with an equal number of cups of tap water. The seeds were germinated for 10 days. The number of germinated seeds, stems and roots of a length in the experiment and control were counted. Seeds that were not treated with culture supernatant of micromycetes served as a control option [12], [16].

Identification of fungi and yeast cultures of by molecular - genetic techniques included: DNA extraction, qualitative and quantitative analysis of DNA, amplification of ITS fragments, the determination of its nucleotide sequence, information analysis - determination of identity with the sequences deposited in the international database GeneBank, and phylogenetic trees with the nucleotide sequences of the reference strains [17]. PCR was performed with universal primers ITS4-5'-TCCTCCGCTTATTGATATGC-3 ', ITS5 5'-GGAAGTAAAAGTCGTAACAAGG-3'.

III. Results

A. Determining the Number and Species Diversity of Micromycetes under Agriculture

During the study of micromycetes population isolated from soils under different agricultures some characteristics were identified (see Table I). Differences in the number of micromycetes depending on the plant species are negligible. The number of yeasts ranged from 132.3 ± 4.6 to 167.4 ± 2.1 thousand of KOE / g of soil, microscopic fungi - from 423.2 ± 17.4 to 544.8 ± 15.6 tys.KOE / g of soil. The greatest number of micromycetes was characteristic of soils under alfalfa and rapeseed.

Another striking feature of the distribution of micromycetes is the prevalence of fungi and relatively small amounts of yeast and yeast-like organisms in all experimental variants. It is shown, for example, that the number of fungi in the rhizosphere of alfalfa was 544.8 ± 15.6 thousand of KOE / g soil, yeast 3.4 times less – 160.2 ± 6.4 thousand of KOE / g soil.

It is known that the rhizosphere is a layer of soil, feeling the effects of root exudates, which differ in their physical and chemical properties from the rest of the soil. Soil particles are more structured. In this regard, the process of the movement of roots and microorganisms is improving; moisture keeps better, supported by more constant temperature. Rhizosphere - an area rich in food, due to secretions of roots (by ekzoosmos), which is found sugars, amino acids, vitamins, auxins, phosphatids and various flavorings. The total number of fungi is as well as other microorganisms in the rhizosphere more than outside the rhizosphere [18], [19]. In the root zone of plants, there were significantly more micromycetes than in virgin soil. For example, the amount of yeasts in the root zone of agricultures amounted to 167.4 ± 2.1 thousand of KOE / g of soil, while in the virgin soil of their number was equal to 79.8 ± 3.2. Similar results were obtained in the study of the quantitative composition of microscopic fungi. The relevant data are shown in Table I.

TABLE I
NUMBER OF MICROMYCETES SOWN WITH VARIOUS AGRICULTURE IN KAZAKHSTAN

Variant	Number of micromycetes (in thousands of .KOE on a 1 g of soil)	
	yeasts	microscopical fungi
Alfalfa	160,2±6,4	544,8±15,6
Melilot	137,1±6,3	423,2±17,4
Sainfoin	145,4±3,2	507,4±16,1
Soybean	132,3±4,6	458,3±19,3
Rapeseed	167,4±2,1	520,9±18,2
Virgin soil	79,8±3,2	249,7±6,6

Set of species that can be isolated from soil is varied. Microscopic fungi isolated from the soils, for a number of morphological and cultural characteristics have been attributed to two classes: *Zygomycetes* (represented by single genus - Mucor) and *Deuteromycetes* (5 genera identified - *Aspergillus, Penicillium, Fusarium, Cladosporium, Trichoderma*). Yeast and yeast-like fungi were identified as members of five genera: *Aureobasidium, Lipomyces, Rhodotorula, Metschnikovia, Trichosporon*. Among which, the only *Lipomyces and Metschnikovia* belong to askomycetes yeast, all others are Deuteromycetes.

Community of soil micromycetes was assessed by the frequency of occurrence of representatives of various sorts. Frequency of occurrence (%) was defined as the percentage of samples in which the particular species, from the total number of analyzed samples. In the frequency of fungal species in the samples were ranked in 3 groups:

1. Dominant (frequency of occurrence is more than 60%).
2. Common (30% to 60%).
3. Rare (less than 30%) [18], [19].

Data on the structure of communities of micromycetes sown by various agricultures is presented in Table II. Composition of micromycetes in virgin soil was characterized by high frequency of occurrence (60-85%) of species such as: *Trichoderma koningii, Penicillium commune, Penicillium bilaiae, Fusarium equiseti, Aspergillus ustus, Lipomyces starkey, Rhodotorula mucilaginosa*. Other typical eurytopic kinds of micromycetes having a wide distribution area were representatives of *Penicillium, Fusarium, Metschnikovia, Aureobasidium* genera.

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TABLE II
STRUCTURE OF MYCROMECETES COMMUNITY BY FREQUENCY OF OCCURRENCE OF REPRESENTATIVES

Variant	Dominant	Common	Rare
Alfaalfa	*Penicillium bilaiae* *Penicillium anatolicum* *Fusarium equiseti* *Aspergillus ustus* *Aureobasidium pullulans*	*Trichoderma koningii* *Penicillium aculeatum* *Fusarium oxysporum* *Rhodotorula mucilaginosa*	*Cladosporium herbarum* *Penicillium crustosum* *Metschnikovia pulcherrima*
Meliot	*Penicillium bilaiae* *Aureobasidium pullulans*	*Trichoderma koningii* *Penicillium aculeatum* *Fusarium equiseti* *Fusarium oxysporum* *Rhodotorula mucilaginosa*	*Cladosporium herbarum* *Penicillium crustosum* *Metschnikovia pulcherrima*
Sainfoin	*Penicillium bilaiae* *Aureobasidium pullulans*	*Trichoderma koningii* *Penicillium aculeatum* *Fusarium equiseti* *Fusarium oxysporum* *Rhodotorula mucilaginosa*	*Cladosporium herbarum* *Penicillium crustosum* *Metschnikovia pulcherrima*
Soybean	*Penicillium bilaiae* *Aureobasidium pullulans*	*Trichoderma koningii* *Penicillium aculeatum* *Fusarium equiseti* *Fusarium oxysporum* *Rhodotorula mucilaginosa*	*Cladosporium herbarum* *Penicillium crustosum* *Metschnikovia pulcherrima*
Rape	*Penicillium bilaiae* *Fusarium equiseti* *Aspergillus ustus* *Aureobasidium pullulans*	*Trichoderma koningii* *Penicillium restrictum* *Penicillium aculeatum* *Fusarium oxysporum* *Rhodotorula mucilaginosa*	*Cladosporium herbarum* *Penicillium crustosum* *Metschnikovia pulcherrima*
Virgin soil	*Trichoderma koningii* *Penicillium commune* *Penicillium bilaiae* *Aspergillus ustus* *Lipomyces starkeyi* *Rhodotorula mucilaginosa*	*Penicillium restrictum* *Penicillium aculeatum* *Fusarium oxysporum* *Aureobasidium pullulans* *Metschnikovia pulcherrima*	*Cladosporium herbarum* *Mucor circinelloides* *Penicillium crustosum* *Penicillium anatolicum* *Trichosporon porosum*

Generic diversity of micromycetes markedly decreases as it approaches the surface of the root, so that the species composition in the rhizosphere is much more uniform than in the virgin soil outside the rhizosphere. A characteristic feature is the identification of *Fusarium equiseti* species in the root zone of agriculture, while in the virgin soil this type was not found. In the variants with alfalfa and canola *Fusarium equiseti* is dominant, in other cases, the incidence of type of agriculture does not exceed 55% of this type is related to the typical. It was also revealed that the yeast *Aureobasidium pullulans*, being a virgin soil typical representative, is dominated in the root zone of agriculture. The relevant data are shown in Table II.

B. Primary Screening of Micromycetes with Plant Growth-Promoting Activity

Plant growth-promoting activity is a common feature of microorganisms of different taxonomic groups. 220 strains of microscopic fungi and yeast, previously isolated from the root zone of different agricultures were collected.

It was found that among 170 strains of microscopic fungi, plant growth-promoting activity in relation to agricultures was clearly expressed in representatives of *Penicillium bilaiae* (9 strains out of 16) and *Trichoderma koningii* (7 of 18). Some strains of *Fusarium equiseti* (4 of 13) and *Aspergillus ustus* (4 of 17) also had plant growth-promoting effect (Table III).

TABLE III
PLANT GROWTH-PROMOTING ACTIVITY OF MYCROMYCETES

Name of species	Total number of strains	Number of active strains
Penicillium bilaiae	16	9
Penicillium anatolicum	12	-
Penicillium commune	14	-
Penicillium restrictum	12	-
Penicillium crustosum	15	-
Penicillium aculeatum	9	-
Aspergillus ustus	13	4
Fusarium oxysporum	18	-
Fusarium equiseti	17	4
Cladosporium herbarum	16	-
Trichoderma koningii	18	7
Mucor circinelloides	10	-
Aureobasidium pullulans	15	8
Rhodotorula mucilaginosa	12	6
Metschnikovia pulcherrima	11	4
Lipomyces starkeyi	8	3
Trichosporon porosum	4	-

The stimulatory effect was detected at the earliest stages of plant development, from seed germination. Analysis of the germination test cultures in a variety of experience has shown that the best seeds ascended in versions with *Penicillium bilaiae* and *Trichoderma koningii*. The proportion of germinated seeds reached 99.6%. The length of the root and aerial part of seedlings increased by 27.4 - 65.2% and 19.4 - 64.7% respectively compared with the control. In experiments with *Fusarium equiseti* and *Aspergillus ustus* found that the length of the root and aerial parts of alfalfa seedlings also was

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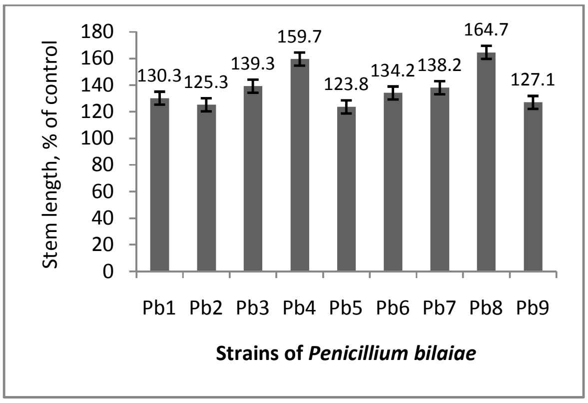
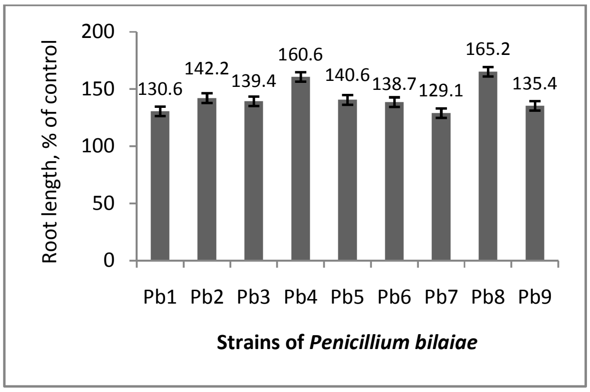
significantly greater than in the control. All other studied cultures showed minimal plant growth-promoting activity, but rather had an inhibitory effect on the growth and development of test cultures.

From 50 strains, 21 yeast cultures were characterized by plant growth-promoting ability. They are representatives of the general *Aureobasidium pullulans* (8 of 15 strains), *Rhodotorula mucilaginosa* (6 of 12), *Metschnikovia pulcherrima* (4 of 11) and *Lipomyces starkeyi* (3 of 8) (Table III). The analysis showed that the strains *Aureobasidium pullulans, Rhodotorula mucilaginosa* and *Metschnikovia pulcherrima* have pronounced phytostimulating effects. The highest rates of growth and development of seedlings were observed for strains of *Aureobasidium pullulans* A1*, Aureobasidium pullulans* A3 and *Rhodotorula mucilaginosa* Rh2*, Rhodotorula mucilaginosa* Rh6 and *Rhodotorula mucilaginosa* Rh8. The length of the stem and root parts of seedlings increased by 11.5 - 40.2% and 9.5 - 42.6%, respectively, compared with the control options. In strains *Metschnikovia pulcherrima* and *Lipomyces starkeyi* rates of germination and seedling length were somewhat lower. Significant inhibitory effect of the root and stem growth of *Trichosporon porosum* strain was observed, germination test plants deteriorated from 5.4 to 30.3%.

As an example, a diagram showing the plant growth-promoting activity of strains of microscopic fungi *Penicillium bilaiae* (Fig. 1) and yeast *Aureobasidium pullulans* (Fig. 2) on the following parameters: length of root and aerial parts of seedlings test culture is submitted.



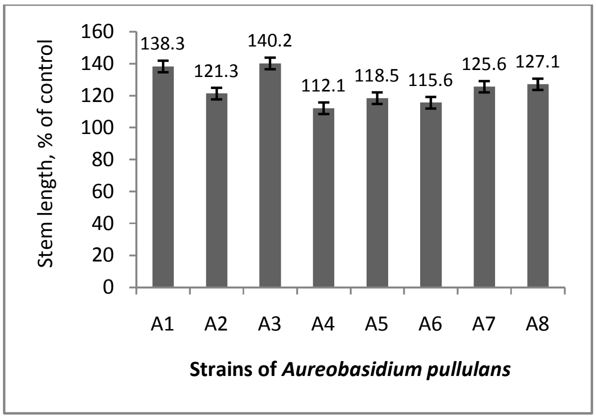
(a)



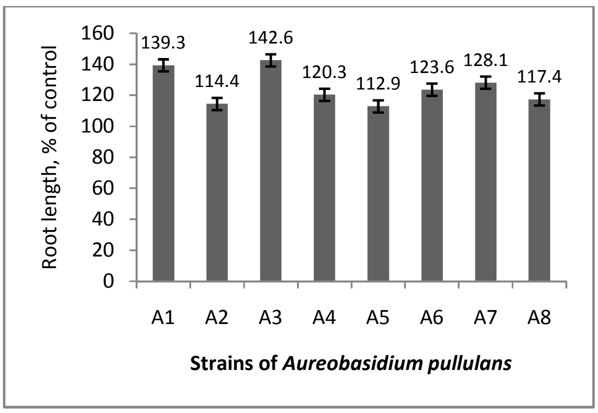
(b)

Fig. 1 Plant growth-promoting activity of *Penicillium bilaiae* strain (a) stem length, (b) root length

The data show that among the microscopic fungus *Penicillium bilaiae* plant growth-promoting activity signified in strains and Pb4 Pb8. Among the yeast *Aureobasidium pullulans* the most active strains were A1 and A3.



(a)



(b)

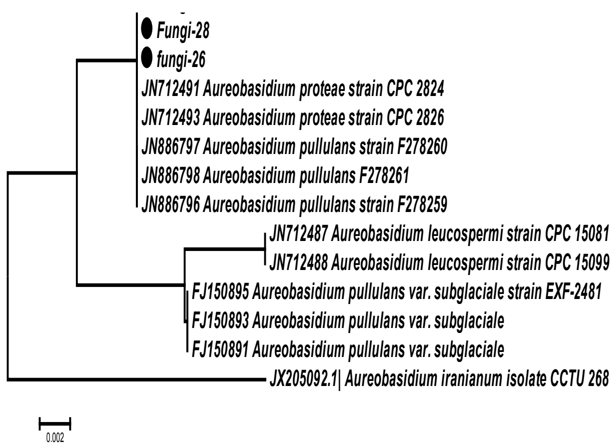
Fig. 2 Plant growth-promoting activity of *Aureobasidium pullulans* strains (a) stem length, (b) root length

Strains of microscopic fungi and yeasts with a strong plant growth-promoting activity to agricultures were selected. Among fungus, the strains are *Penicillium bilaiae* Pb4*, Penicillium bilaiae* Pb8*, Trichoderma koningii* Tk1*, Trichoderma koningii* Tk6*, Fusarium equiseti* Feq2*, Aspergillus ustus* Au3. The highest rates of growth and development of seedlings were observed for strains of *Aureobasidium pullulans* A1*, Aureobasidium pullulans* A3*, Rhodotorula mucilaginosa* Rh2*, Rhodotorula mucilaginosa* Rh4*, Rhodotorula mucilaginosa* Rh5*, Metschnikovia pulcherrima* Mp2*, Lipomyces starkeyi* L1 and *Lipomyces starkeyi* L2.

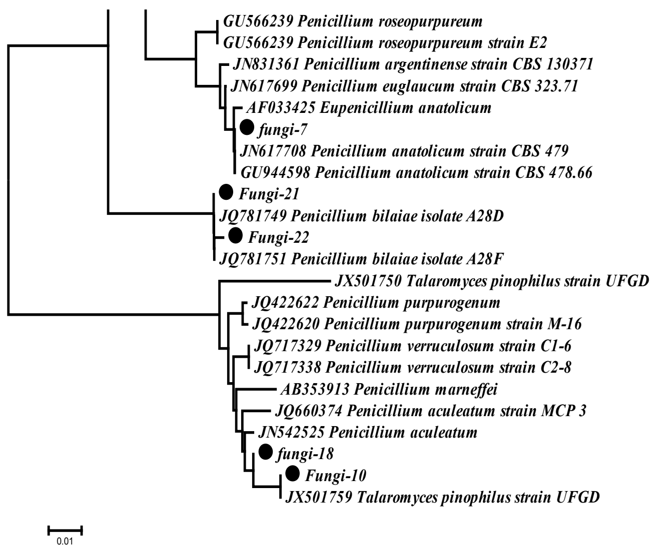
C.Phylogenetic Analysis Micromycetes Using Molecular Genetic Techniques

To confirm the identification of active strains selected using traditional techniques, studies using molecular genetic techniques were done. Fig. 3 in the form of dendrograms represents the results of phylogenetic analysis of nucleotide sequences of ITS region of *Penicillium bilaiae* Pb4*, Penicillium bilaiae* Pb8*, Aureobasidium pullulans* A1*, Aureobasidium pullulans* A3 strains.

During phylogenetic analysis, ITS regions of A1 and A3 yeast strains have been merged into a single cluster with JN886797 *Aureobasidium pullulans*. It is important to note that the identification of a Gene Bank nucleotide sequence of ITS region of these strains had maximum identity with *Aureobasidium pullulans* (Fig. 3).



(a)



(b)

Fig. 3 (a) Dendrograms constructed on base of ITS region fragment analysis of *Aureobasidium sp.* groups, (b) *D*endrograms constructed on base of ITS region fragment analysis of *Penicillium sp* groups

Based on the results of phylogenetic analysis of the ITS region, Pb4 strain was combined into a single cluster with JQ781749 *Penicillium bilaiae*, and strain Pb8into a cluster with JQ781751 *Penicillium bilaiae*. While identifying a Gene Bank, ITS region nucleotide sequences of these strains also had the highest identity with *Penicillium bilaiae* (Fig. 3).

IV. Conclusion

Numerous studies have shown that microorganisms can significantly stimulate the growth, development and metabolism of plants and in correct using used, biotechnology can significantly increase yield and improve quality. This is due to their ability to produce growth substances and antibiotics, leading to the immunization of plants and increase the absorptive activity of the roots. Currently, plant growth by microorganisms is a direct application of microbial activators, and the use of microbial metabolites varying treatment. Spores and metabolites of micromycetes penetrate the seed during germination, which resulted in the activation of biochemical processes in germinating seeds. According to the literature making spore material, biomass, and cell culture fluid metabolites of micromycetes in rhizosphere significantly activates many enzymes of plants - invertase, catalase, amylase, urease, increases the intensity of the oxidation-reduction processes, photosynthesis, and nutrient uptake of root system [5]-[10].

In the study of biodiversity of micromycetes it was shown that their composition in soils under agricultures was characterized by a high incidence of different types, depending on the plant. Dominant species are *Trichoderma koningii, Penicillium commune, Penicillium bilaiae, Fusarium equiseti, Aspergillus ustus, Aureobasidium pullulans, Rhodotorula mucilaginosa.*

Plant growth-promoting activity of the dominant and common species of micromycetes was studied. Active strains of micromycetes, which can be used to create a biological product to improve soil fertility and productivity of forage crops were selected. Creating microbial products of complex action of containing the composition of the different groups of microorganisms and the typical representatives of the dominant soil and rhizosphere microorganisms to alter approaches to technology for growing forage crops have a great importance for Kazakhstan. To improve soil fertility in Kazakhstan is appropriate to use microbial preparations of domestic production, since they are based on microorganisms adapted to local soil and climatic conditions.

These data show that the ability of Plant Growth-Promoting fungi to the forage crops was clearly expressed in representatives of *Penicillium bilaiae, Trichoderma koningii, Fusarium equiseti and Aspergillus ustus*. Strains of *Aureobasidium pullulans, Rhodotorula mucilaginosa* and *Metschnikovia pulcherrima* yeast cultures had a phytostimulating action.

These studies are the initial phase of the study of plant growth-promoting microorganisms of agriculture in Kazakhstan. It is further planned to study fungicidal activity of these microorganisms, the ability to transform xenobiotics, increasing the availability of nutrients, produce substances with phytohormonal nature and other biologically active compounds.

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