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Prevalence and Fungicidal Activity of Endophytic Micromycetes of Plants in Kazakhstan

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***Abstract*—**Endophytic microorganisms are presented in plants of different families growing in the foothills and piedmont plains of Trans-Ili Alatau. It was found that the maximum number of endophytic micromycetes is typical to the *Fabaceae* family. The number of microscopic fungi in the roots reached $(145.9\pm5.9)\times10^3$ CFU/g of plant tissue; yeasts - $(79.8\pm3.5)\times10^2$ CFU/g of plant tissue. Basically, endophytic microscopic fungi are typical for underground parts of plants. In contrast, yeasts more infected aboveground parts of plants. Small amount of micromycetes is typical to inflorescence and fruits. Antagonistic activity of selected micromycetes against *Fusarium graminearum, Cladosporium* sp.*, Phytophtora infestans* and *Botrytis cinerea* phytopathogens was detected. Strains with a broad, narrow and limited range of action were identified. For further investigations Rh2 and T7 strains were selected, they are characterized by a broad spectrum of fungicidal activity and they formed the large inhibition zones against phytopathogens. Active antagonists are attributed to the *Rhodotorula mucilaginosa* and *Beauveria bassiana* species.

***Keywords*—**Endophytic micromycetes, fungicidal activity, prevalence.

I. Introduction

CROP yield is one of the main criteria for assessing the effectiveness of individual methods of technology for their cultivation and production in general. The yield is determined by many factors; conditionally they can be classified into the following groups: external (exogenous not regulated by plant), own (endogenous factors and the system of self-regulation, such as the hormonal system of plants) and factors operating within the plant, but are not actually plant – they are endophytes [1].

Currently biocontrol direction is developing, associated with the use of specific microorganisms living inside plant tissues and received naming endophyte. Studies of a number of authors have shown that mushrooms and a variety of bacteria can be endophytes; many of them show high antagonistic activity against pathogenic microorganisms. These and other results of the studies allowed starting practical developments in the field of microbiopreparations based endophytic on microbes [2].

A prospect of using endophytes seems are very large. Their application for biocontrol fits in biological (agrolandscape, ecological) agriculture. Efficiency of the use of endophytes can be probably that once infiltrating the tissues of plants, they contribute to long-term the plant organism confrontation pests and pathogens, possibly throughout the growing season.

Search of endophytes capable to protect plants from diseases and other adverse environmental factors, the study of the properties of these microorganisms are an important issue.

All endophytes can infect the seed or plant in the fall under the influence of abiotic and external environmental factors. Endophytes gain a competitive advantage over the rhizosphere and philosphere organisms. They are characterized by a stable power supply, pH, humidity, a certain protection against numerous competitors. The energy that plants spend on providing endophyte's biomass pays the benefits that provide micromycetes for plant health. This suggests that endosphere - no accident, but a special natural niche, created specifically to each plant [3].

Micromycetes are able to infect not only the entire plant, but some of its shoots. Micromycetes are capable to apical growth and spread in the direction of growth of the meristem. It is known that endophytic fungi are not rigidly associated with certain plant organs and can be develop differently depending on any change in the plant and/or to the environment [4].

Yeast species, whose number on the surface of the fruit exceeds their number inside are typical epiphytes and possess such adaptations to the epiphytic lifestyle as carotenoid pigmentation or powerful polysaccharide capsule, which serve as protection from adverse environmental factors. This suggests that the development of endophytic yeast can also be seen as a manifestation of the adaptive strategy of avoidance.

Herbal plants are the favorite habitat of endophytic organisms. These are *Alternaria, Fusarium* and other species which can be latent pathogens. While plants infected with endophytic microorganisms, may not have the visual differences with uninfected individuals. It is known that endophytes can propagate vertically, confirming what was the discovery of their hyphae and spores inside the seeds. This is especially common way to transfer endophytes inhabiting herbaceous plants. It should be noted that seed's infectiousness of meadow grasses can reach 72%. At the same time, from the infected seeds plant can be developing without having endophytic microorganisms. This phenomenon is attributed to the fact that under the influence of various factors viability of endophytes is reducing and seed viability is preserving [5].

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II. Materials and Methods

A. Research Materials

Collection of plants was carried out in the period from May to September in the foothills and piedmont plains of Trans-Ili Alatau. The following plant families were used: *Asteraceae* (Compositae) - *Achillea millefolium* (Yarrow), *Centaurea squarrosa* (Cornflower splayed), *Acroptilon repens* (Gorceac repens), *Artemisia lercheana* (White wormwood), *Artemisia annua* (Sweet wormwood), *Xanthium strumarium* (Common cocklebur) species; *Poaceae* (Cereals) - *Festuca pratensis* (Meadow fescue), *Poa annua* (annual bluegrass), *Hordeum vulgare* (Barley) Arna cultivar, *Avena sativa* (Oats) Kazakh 70; *Fabaceae* (Legumes) - *Medicago sativa* (Alfalfa) Semirechenskaya cultivar, *Glycine max* (Soybean) Almaty cultivar, *Melilotus officinalis* (Yellow sweet clover); *Lamiaceae* (Labiatae) - *Salvia deserta* (Sage desert); *Chenopodiaceae* (Goosefoot) *Chenopodium botrys* (Pigweed fragrant).

B. Isolation of Microorganisms from the Roots, Leaves, Stems, Inflorescences and Fruits

Sterilization of aboveground and underground plant surfaces was done according to the following scheme: 3 minutes in 70% ethanol, 12 min in a 5% solution of sodium hyperchloride, 1min in 70% ethanol. Then thoroughly they were washed 5 times with sterile distilled water. Plant tissue (1g) was homogenized in a mortar with 10ml of sodium phosphate buffer (pH 7.4). The suspension was diluted and seeded on an agar medium [6].

For isolation micromycetes potato dextrose agar (PDA) and Saburo medium were used. The number represents the number of colony forming units (CFU) followed by recalculation of the 1 g of plant tissue

C. Determination of Fungicidal Activity by the Agar Blocks Method

As test objects phytopathogenic fungi *Fusarium graminearum, Cladosporium* sp.*, Phytophtora infestans* and *Botrytis cinerea* were used. Strains of endophytic micromycetes were grown on agar medium tubes for 5 days. An aqueous suspension with a conidia titer of 10^6/ml was prepared, 0.1ml was plated onto the surface of the lawn PDA and grown for 4-5 days and then the pitch of the formed blocks of 8 mm in diameter were excised. Phytopathogenic fungi are cultured on an agar medium for 5-7 days. Cultures grown from aqueous suspensions prepared with a titer of 104 conidia/ml, 0.1ml of the suspension was plated on a lawn surface PDA in Petri dishes. On the surface of Petri dishes sown by phytopathogenic fungi blocks with endophyte micromycetes cultures were placed. An antagonistic activity was judged by the lack of growth of the phytopathogen zone [7].

D. Molecular Identification of Micromycetes

Identification of fungi and yeasts was carried out by determining the nucleotide sequence of the direct ITS region. Chromosomal DNA isolation procedure was performed by the method of Kate Wilson. The DNA concentration was measured spectrophotometrically using a NanoDrop spectrophotometer at a wavelength of 260 nm. After quantifying, the DNA concentration was normalized to 30 ng/ml. Amplification of ITS region fragment was performed with the universal primer ITS4-5'-TCCTCCGCTTATTGATATGC-3', ITS5-5'-GGAAGTAAAAGTCGTAACAAGG-3' in a total volume of 30 microliters. PCR amplification program included a long denaturation 95°C for 7 minutes; 35 cycles: 95°C - 15 s, 52°C 30 s, 72°C - 30 s; 7 min final elongation at 72°C.

Purification of PCR products from unbound primers was performed enzymatically by using Exonuclease I and alkaline phosphatase. Sequencing reactions were performed using BigDye ® Terminator v3.1 Cycle Sequencing Kit (Applide Biosystems) according to the manufacturer's instructions followed by separation of the fragments on an automated genetic analyzer 3730xl DNA Analyzer (Applide Biosystems). Nucleotide sequences were identified in the GeneBank algorithm BLAST. The constructions of phylogenetic trees were performed using the method of using the nearest neighbor (Neiighbor-Joining NJ).

III. Results

A. Prevalence of Endophytic Microflora in Plants of Different Families

It is known that endophytic microorganisms are distributed in organs and tissues of plants unevenly [8]. The main mechanism of forming endophytic microflora is contamination of plants through breaks of the epidermis, resulting in growth of lateral roots [9]. Therefore, the richest flora is detected in the roots of which s microorganisms migrate (mainly by conducting vessels) in the aerial organs. The richness of root microflora is caused by the fact that mycorrhizal fungi also are endophytes and many types can be found in tubers and bulbs of plants [3]. It is noted that the majority of endophytic micromycetes colonizing shoots microflora similar to roots. At the same, roots microflora is plentiful and flora of leaf has great biological diversity - in the leaves occurs more species of endophytic microorganisms than in the roots [4].

The prevalence of endophytic micromycetes in various organs of plants belonging to the *Asteraceae, Poaceae, Fabaceae, Lamiaceae, Chenopodiaceae* families is investigated.

The differences in the prevalence of endophytes from different plant species within the same family were observed. For example, among the *Asteraceae* family, the most heavily infected plants species were *Achillea millefolium* and *Artemisia lercheana*. Number of microscopic fungi in the roots of these plants was $(26.7\pm1.1)\times10^3$ and $(45.9\pm2.1)\times10^3$ CFU / g of plant tissue, respectively; yeasts - $(31.5\pm1.2)\times10^2$ and $(35.6\pm1.5)\times10^2$ CFU/g of plant tissue, respectively (see Tables I and II).

TABLE I
NUMBER OF E ENDOPHYTIC MICROSCOPIC FUNGI ISOLATED FROM PLANTS BELONGING TO DIFFERENT FAMILIES

Plant Species	Number, $\times 10^3$ CFU/g of plant tissue			
	roots	shoots	leaves	inflorescences and fruits
Asteraceae family (Compositae)				
Achillea millefolium (Yarrow)	26,7±1,1	9,5±0,3	7,2±0,2	3,6±0,1
Centaurea squarrosa (Cornflower splayed)	-	-	-	---
Acroptilon repens (Gorceac repens)	-	-	-	---
Artemisia lercheana (White Wormwood)	45,9±2,1	15,3±0,6	10,8±0,3	6,6± 0,2
Artemisia annua (Sweet wormwood)	23,3±0,7	8,1±0,3	6,7±0,2	-
Xanthium strumarium (Common cocklebur)	-	-	-	-
Poaceae family (Cereals)				
Festuca pratensis (Meadow fescue)	27,3±1,2	9,1±0,3	7,4±0,2	5,2 ± 0,1
Poa annua (Annual bluegrass)	17,6± 0,5	8,6± 0,1	6,7±0,1	-
Hordeum vulgare (Barley) Arna cultivar	8,8±0,3	3,7±0,1	2,4±0,1	-
Avena sativa (Oats) Kazakh 70	25,9±1,1	8,7±0,3	6,4±0,2	3,1±0,1
Fabaceae family (Legumes)				
Medicago sativa (Alfalfa) Semirechenskaya cultivar	145,9±5,9	41,8±1,5	36,4±1,5	12,5±0,4
Glycine max (Soybean) Almaty cultivar	121,4±5,1	65,7±2,9	56,4±2,5	-
Melilotus officinalis (*Yellow sweet clover*)	136,4±4,8	-	-	-
Lamiaceae family (Labiatae)				
Salvia deserta (Sage desert)	12,8±0,4	4,4±0,1	-	-
Chenopodiaceae family (Goosefoot)				
Chenopodium botrys (Pigweed fragrant)	4,7±0,1	-	-	-

TABLE II
NUMBER OF E ENDOPHYTIC YEASTS ISOLATED FROM PLANTS BELONGING TO DIFFERENT FAMILIES

Plant Species	Number, $\times 10^2$ CFU/g of plant tissue			
	roots	shoots	leaves	inflorescences and fruits
Asteraceae family (Compositae)				
Achillea millefolium (Yarrow)	31,5±1,2	51,4±1,7	12,2±0,3	7,1±0,2
Centaurea squarrosa (Cornflower splayed)	-	24,5±1,1	-	4,5±0,1
Acroptilon repens (Gorceac repens)	25,9±1,1	42,7±1,1	-	-
Artemisia lercheana (White Wormwood)	35,6±1,5	58,6±2,5	12,3±0,4	7,2±0,1
Artemisia annua (Sweet wormwood)	27,9±0,4	41,4±1,1	7,4±0,2	3,6±0,1
Xanthium strumarium (Common cocklebur)	12,9±0,2	19,5±0,3	-	-
Poaceae family (Cereals)				
Festuca pratensis (Meadow fescue)	22,6±0,7	36,8±1,3	9,7±0,2	5,3±0,2
Poa annua (Annual bluegrass)	28,3±1,2	38,1±1,4	11,4±0,2	6,4±0,2
Hordeum vulgare (Barley) Arna cultivar	29,4±1,2	41,4±1,5	17,8±0,5	13,6±0,1
Avena sativa (Oats) Kazakh 70	-	34,5±1,5	15,3±0,6	9,6±0,4
Fabaceae family (Legumes)				
Medicago sativa (Alfalfa) Semirechenskaya cultivar	79,8±3,5	96,4±3,9	18,7±0,4	12,4±0,4
Glycine max (Soybean) Almaty cultivar	34,6±1,3	49,5±2,1	14,7±0,3	8,6±0,3
Melilotus officinalis (*Yellow sweet clover*)	45,8±1,8	58,6±2,6	21,9±0,5	16,4±0,6
Lamiaceae family (Labiatae)				
Salvia deserta (Sage desert)	5,9±0,1	8,6±0,3	1,4±0,05	3,3±0,1
Chenopodiaceae family (Goosefoot)				
Chenopodium botrys (Pigweed fragrant)	1,8±0,05	2,7±0,1	-	-

The greatest number of microscopic fungi and yeasts was characteristic for the plants of *Fabaceae* family. Thus, among the family members number of microscopic fungi in the roots ranged from $(121.4\pm5.1)\times10^3$ to $(145.9\pm5.9)\times10^3$ CFU/g of plant tissue, and the number of yeasts $(34.6\pm1.3)\times10^2$ and $(79.8\pm3.5)\times10^2$ CFU/g of plant tissue. Then in descending order the representatives of *Asteraceae*, *Poaceae*, *Lamiaceae* and *Chenopodiaceae* families are arranged.

Microscopic fungi were absent in plants of *Centaurea squarrosa, Acroptilon repens* and *Xanthium strumarium* species of *Asteraceae* family. Yeasts were not detected in the roots of *Centaurea squarrosa* species of *Asteraceae* family and *Avena sativa* Kazakh70 of *Poaceae* family (see Tables I and II).

Despite the fact that most rich endophytic microflora can be detected in the roots of the plants from which the microorganisms migrate to the surface organs, study of stems and leaves presents the big interest for the study of endophytes. It is known that in the aerial parts have their own pockets of infection: for example, microorganisms can enter the plant through the stomata, including in the composition of microflora of leaves and stems [10].

In the literature, there are conflicting data on the distribution of fungi in various plant organs. So, B. Schulz believes that more often endophytic colonization can be found in roots and it can be as inter as intracellular [1]. Conversely, S. K. Gond shows the predominance endophytic fungi in plant leaves [8].

Table I shows the results of analysis of various plants' organs infected by fungi. There was a slight decrease in the number of microscopic fungi in the stems in 3 times comparing to their content in the roots. The content of fungi in plant stems was $(3.7\pm0.1)\times10^3$ and $(65.7\pm2.9)\times10^3$ CFU/g of plant tissue depending on the plant species. Most infected stems belonged to *Medicago sativa* Semirechenskaya cultivar and *Glycine max* Almaty cultivar belonging to the *Fabaceae* family.

Comparing to the number of fungi detected in plant roots, their number in leaves was reduced in six times averagely. This index was from $(2.4\pm0.1)\times10^3$ to $(56.4\pm2.5)\times10^3$ CFU/g of plant tissue depending on the plant species. Most infected were leaves of *Glycine max* Almaty cultivar.

Number of endophytic fungi in the inflorescences and fruits was negligible. Depending on the plant species the index was

from $(3.1\pm0.1)\times10^3$ CFU/g of plant tissue at the *Avena sativa* Kazakh 70 to $(12.5\pm0.4)\times10^3$ CFU/g of tissue from the *Medicago sativa* Semirechenskaya cultivar plant. In n the inflorescences and fruits of the most mushrooms were not found.

In contrast to the number of microscopic fungi, number of yeasts in stems exceeds the amount in the roots. Most likely, this is because the yeast is more adapted to life in the liquid and fine environment, rich in readily available carbon sources, while filamentous fungi may benefit from growth on solid surfaces [1]. Indeed, yeast - the most common inhabitants of natural substrates, characterized by a high content of readily accessible nutrients (sugars, sugar alcohols, organic acids, etc.). In such substrates, populations of certain types of yeast can reach very high values of strength.

According to the data, the number of endophytic yeasts in shoots ranged from $(2.7\pm0.1)\times10^2$ CFU/g of plant tissue in *Chenopodium botrys* species to $(96.4\pm3.9)\times10^2$ CFU/g of plant tissue in *Medicago sativa* Semirechenskaya cultivar species. The stems of *Medicago sativa* Semirechenskaya cultivar, *Artemisia lercheana*, *Melilotus officinalis* were most infected in descending order.

There is evidence that the developing yeasts on plants, as well as most of phytopathogenic fungi may lead endophytic lifestyle, i.e. not grow on the leaf surface and to leave in the extracellular space and inside the tissue sheet, such as *A. pullulans* [11]. We have found that number of yeasts, isolated from leaves was significantly lower than that extracted from plant stems. Thus, the number of yeasts was ranged from $(1.4\pm0.05)\times10^2$ CFU/g of plant tissue in *Salvia deserta* to $(21.9\pm0.5)\times10^2$ CFU/g of plant tissue in *Melilotus officinalis*.

Amount of endophytic yeasts in inflorescences and fruits were lower than in the leaves. *Melilotus officinalis* and *Hordeum vulgare* Arna cultivar were most infected, from which $(16.4\pm0.6)\times10^2$ and $(13.6\pm0.1)\times10^2$ CFU/g of plant tissue was identified respectively (see Table II).

Thus, it was revealed that underground parts (roots) are rich mainly by microscopic endophytic fungi. In contrast, yeasts more infected underground parts and in particular the stems of the plant.

B. Fungicidal Activity

Among the fungi that live in the soil, there are a number of pathogenic species that can cause plant diseases and influence their productivity. These fungi cause diseases with different manifestations of symptoms. It may be damping off of plants, young plants rot, root rot, wilting of plants [12], [13].

Phytopathogenic fungi in soils are very diverse taxonomically, physiologically and biochemically. The most common disease that causes harmful fungi is root rot. The disease occurs in all areas of agriculture and affects all cultivated plants, as well as wild. Common root rot pathogens are members of the *Fusarium, Bipolaris* genera - *B. sorokiniana, F. graminearum, F. avenae, F. oxysporum, F. solani*. They cause disease in spring cereals. Root rot of winter cereals – ophiobolesus and is caused by *Ophiobolus graminis* fungus. Bean root rot caused by the *Pythium debarianum, P. ulmorumy* and *F. oxysporum*, in particular its specialized forms. Rot of young plants are caused by *Bipolaris, Drechslera, Rhizoctonia, Thielaviopsis* species. They may be responsible for the death of the seed and sprouts as a result of almost complete destruction of their vegetative tissues. On weakened seedlings as secondary parasites appear *Alternaria, Cladosporium, Botrytis* and even saprotrophic *Penicillium* species [202]. The main causative agents of wilting plants, carrying the name of wilt - fungi of the *Fusarium* and *Verticillium* genera, ubiquitous in all soil - climatic zones. Fungi penetrate into the plant from the soil in the tissues and proliferate to roots and stems. Proliferation of mycelium occurs mainly by conducting tissues, resulting in their destruction and blockage. As a consequence, the metabolism of plants disturbs and wilting symptoms appears [12], [13].

It is known that among the endophytic microorganisms searching of cultures with antagonistic activity can be done; the mechanism of this activity may be varied. Among the isolated endophytic micromycetes cultures screening of strains with fungicidal activity was held. As test objects *Fusarium graminearum, Cladosporium* sp., *Phytophtora infestans* and *Botrytis cinerea* phytopathogenic fungi were used. It is known that these phytopathogens cause diseases of economically important plants, causing significant economic damage [12]-[15]. Representatives of the *Fusarium* and *Cladosporium* genera cause diseases of crops; *Botrytis cinerea* - rot of sugar beet; *Phytophtora infestans* affect leaves, stems and tubers.

It has been shown that the 10 cultures had the fungicidal activity (see Table III).

TABLE III
ANTAGONISTIC ACTIVITY OF MICROMYCETES

Strain	Zone of Inhibition, mm			
	Fusarium graminearum	*Cladosporium* sp.	*Phytophtora infestans*	*Botrytis cinerea*
T7	30,6±0,5	17,8±0,6	28,3±0,6	27,2±0,8
Rh2	29,9±0,6	21,5±0,3	25,6±0,8	19,2±0,2
Mp1	19,5±0,2	20,6±0,4	0	14,8±0,5
Mp2	25,1±0,8	0	18,5±0,8	0
Zh 4	21,7±0,5	0	0	20,4±0,6
AC4	26,2±0,9	0	22,8±0,8	0
OR 5	0	15,4±0,7	0	0
AN 1	0	0	0	12,6±0,5
U 3	0	10,5±0,2	0	0
Wh 3	11,4±0,3	0	0	0

They differed in the spectrum of fungicidal activity. Strains were revealed with a broad, narrow and limited range of action. Particular interest was the culture exhibiting fungicidal activity directly to the 4 pathogens, i.e. with a broad spectrum of action. These included T7 and Rh2 strains. Strains Mp1, MP2, Zh4 and AC4 has a narrow spectrum of action (against 2-3 pathogens). They all possess activity against *Fusarium graminearum*. OR5, AN1, U3 and Wh3 strains had limited activity spectrum: OR5 and U3 only against *Cladosporium sp*.; AN1 against *Botrytis cinerea*; Wh3 against *Fusarium graminearum*

Studied culture inhibited the growth of plant pathogens at varying degrees. Zone of growth inhibition was in the range of from 10.5±0.2 to 30.6±0.5 mm. The largest inhibition zones were typical to Rh2 and T7 strains against *Fusarium graminearum* (29.9±0.6 and 30.6±0.5 mm, respectively). It was noted that phytopathogen was depressed in a great degree that visually expressed in the formation of a rare spawn, pressed to the substrate (Fig. 1).

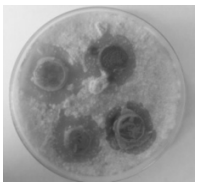

Fig. 1 Zone of inhibition of *Fusarium graminearum*

It is known that fungicidal effect can be manifested contact in the form of hyperparasitism and distant by synthesis of mycotoxins. In distant case, the abnormal growth and morphogenetic effects (reduced branching mycelium, suppression of sporulation) are observed [16]. Minimal activity was observed in AN1 (12.6±0.5 mm), U3 (10.5±0.2 mm), Wh3 (11.4±0.3 mm) strains. Relevant data are summarized in Table III.

C. Identification of Active Strains

Rh2 and T7 strains characterized by a broad spectrum of fungicidal activity and forming the largest zone of inhibition of phytopathogens were identified. Studying of specific characters of micromycetes using molecular biological techniques based on direct analysis of the nucleotide sequence ITS- region was performed.

Phylogenetic analysis of the nucleotide sequence of T7strain (in the figure labeled as Fungi-11) was combined into a single cluster with *Beauveria bassiana* (Fig. 2).

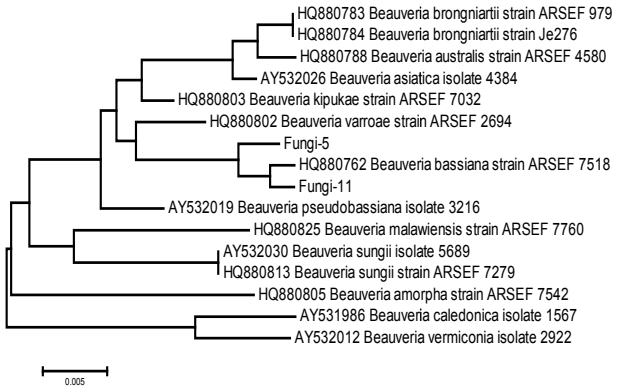

Fig. 2 Phylogenetic tree constructed on the basis of analysis of the fragment ITS region of *Beauveria* genus

Importantly, in the identification of nucleotide sequences in the GeneBank ITS region had a maximum strain part identity with *Beauveria bassiana*.

Rh2 yeast strain (in the figure labeled as fungi 30) is in the same clade with *Rhodotorula mucilaginosa* (Fig. 3). During identifying at Gene Bank, ITS region nucleotide sequence of this strain had a maximum identity with *Rhodotorula mucilaginosa*.

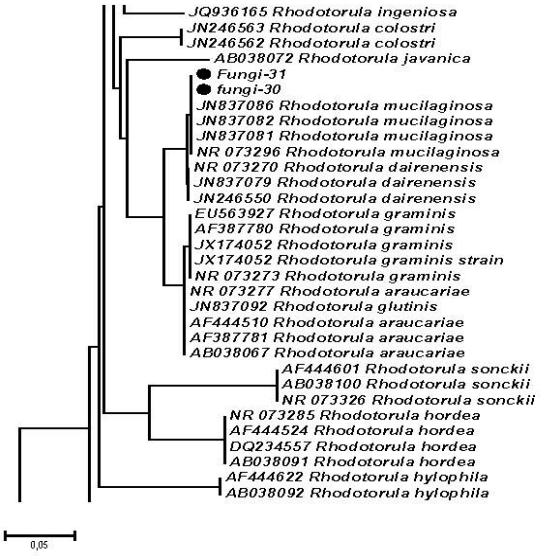

Fig. 3 Phylogenetic tree constructed on the basis of analysis of the fragment ITS region of *Rhodotorula* genus

IV. Conclusion

Endophytic micromycetes and higher plants evolutionarily developed mutualistic relationship. Plants provide microorganisms by food and stable habitat and endophytes emit various metabolites positively affecting the lives and functioning of plants. In this context, the study of endophytic micromycetes in neglected regions causes particular scientific and practical interest.

The differences in the spread of microscopic fungi and yeast plants in Kazakhstan, belonging to different families and species were revealed. It was found that microscopic endophytic fungi prevaile in the subterranean parts of the plants, whereas the yeasts more infect aboveground parts of plants and in particular the stems. The greatest number of endophytic microorganisms is typical to the *Fabaceae* family. It was found that endophytes occur in wild plants and in agriculture. Number of endophytic micromycetes was slightly higher in agriculture.

Search and selection of new strains - antagonists of pathogenic fungi is an important task of modern biotechnology, because of the need of constant updating plant diseases biocontrol means and for prospective producers of antifungal compounds screening. One of the groups of microorganisms, characterized by a large variety of antagonists species are micromycetes. Many other useful properties, including the secretion of extracellular enzymes, amino acids, polysaccharides, phytohormones, phosphates and the ability to dissolve other strains allows to use strains as plant protection products and probiotics [4], [16].

It is shown that 10 strains have the fungicidal activity against phytopathogenic fungi cultures. Among them, strains with wide, limited and narrow spectrum of fungicidal activity

were revealed. T7 (*Beauveria bassiana*) and Rh2 (*Rhodotorula mucilaginosa*) strains are the most promising, because they showed fungicidal activity directly against 4 pathogens and formed the largest zone of inhibition. These results are consistent with literature data. It is known that representatives of *Rhodothorula* have some antagonist activity. Such is the efficiency of *Rhodotorula glutinis to Penicillium expansum* and *Botrytis cinerea* [17]. It is known that the endophytic *Beauveria bassiana* can colonize a large range of plant species. *Beauveria bassiana* strains produce secondary metabolites such as beauvericin, beauveroides, bassianolides, oosporein, cyclosporin A and oxalic acid with antibacterial, antifungal and insecticidal activities. *Beauveria bassiana* is effective against soil pathogens such as *Pythium, Rhizoctonia* and *Fusarium* [18], [19].

These studies are an initial step in the study of endophytic micromycetes of plants in Kazakhstan. In future, isolation and characterization of antifungal ekzometabolites playing a key role in the antagonistic activity of these strains will be conducted.

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