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SEARCH FOR MICROMYCETES – ANTAGONISTS OF PHYTOPATHOGENIC MICROORGANISMS

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Summary. The antagonistic activity of 11 non-phytopathogenic isolates of microscopic fungi was studied. According to the results of the studies, isolates of microscopic fungi TV, T4 and 6G2, active against phytopathogenic bacteria, inhibiting the growth of mycelium of phytopathogenic fungi, were selected for further research in order to include phytoprotective action in biological preparations.

Microscopic fungi TV and T4 were identified as representatives of the genus *Trichoderma,* micromycete 6G2 was identified as representative of the genus *Penicillium*, subgenus *Eupenicillium.*

Keywords: *Micromycetes, antagonists of phytopathogens, plant protection*.

Introduction. The modern phytosanitary situation in countries with intensive agriculture contributes to an increase in the level of infestation of agricultural plants with pathogenic microorganisms. Resistance, adaptability and pathogenicity of plant pathogens are constantly increasing. This situation requires using of a large amount of pesticides. Such a strategy leads to a decrease in the safety of obtained crops and a deterioration of the environmental situation.

One of the ways out of this situation is to reduce the doses of chemical pesticides and to apply biological plant protection products. In particular, recently the attention of researchers has been attracted by the possibility of using microscopic fungi as part of biological products for plant growing.

Micromycetes are widespread in nature, which is explained by their greater resistance to changing environmental conditions compared to other microorganisms. They can produce antimicrobial metabolites; have the ability to hyperparasitism, the ability to stimulate the formation of induced resistance of the host plant to the phytopathogen. In addition, competition for living space

between virulent and avirulent strains in the rhizosphere of plant roots plays an important role in reducing the level of plant damage [1]. Thus, fungi are promising agents for combating phytopathogens, soil and plant's rhizosphere is their natural habitat.

The purpose of this work is to search for new isolates of microscopic fungi, promising for inclusion in biopreparations with phytoprotective action.

Materials and methods. *Strains.* Eleven isolates of non-phytopathogenic microscopic fungi were used. They were isolated from the forest litter of pine and mixed forests, last year's cones, acorns and from the root zone of blueberries. Also strains of phytopathogenic bacteria *Erwinia carotovora atroseptica* 3-2, *E. carotovora* 330, *E. aroidea* 348-1, *E. aroidea* 3, *Pseudomonas seringa* 345, *Ps. syringae* 510, *Ps. syringae* S-10, *Clavibacter* sp. (Kiev), *Clavibacter* sp. (Mogilev), *Clavibacter* sp. MVF (Minsk Vegetable Factory) and strains of phytopathogenic fungi *Fusarium culmorum, F. semitectum, Botrytis cinerea* from the collection of microorganisms of the Research Laboratory of Molecular Genetics and biotechnology of BSU were used.

Methods

The method of "agar block located in the center of the cup" was used to study the antagonistic activity of micromycetes in relation to phytopathogenic bacteria [2].

The method of "counter cultures" was used to study the antagonistic activity in relation to phytopathogenic fungi. Tested fungus culture and the culture of a phytopathogenic fungus were sown on the agarized potato medium with an injection at a distance of 5 cm from each other. A phytopathogenic fungus grown on the same medium without the studied micromycetes was used as a control. Fungus cultures were cultivated at 26 °C. The results were taken into account when the mycelium diameter of the phytopathogenic fungus in the control reached a diameter of 8 cm. Radius of the mycelium of the phytopathogen was measured in the experiment by the shortest distance to the oncoming culture of tested fungus (R1) and radius of the mycelium of the phytopathogen that grew in the direction opposite to tested fungus (R2). The degree of inhibition of phytopathogen growth (I) was calculated by the formula:

$$I = (1-R_1/R_2) \times 100\%$$

The study of the structure of conidia, conidiophores and hyphae mycelium of the isolated strains was carried out using a Micromed 3 U3 microscope with a camera. Fixation of the obtained results was performed using the ToupViev program. Identification of micromycetes was carried out according to the "Determinant of microscopic soil fungi" by M.A. Litvinov [3].

Results and discussions. The ability of 11 isolates of non-phytopathogenic micromycetes isolated from natural sources to inhibit the growth of phytopathogenic bacteria was evaluated with potato blackleg (*E. carotovora* subsp. *atroseptica, E. carotovora*), mucosal bacteriosis of cabbage (*E. aroidea),* bacteriosis of wheat *(Ps. syringae), bacterial cancer of tomatoes (Clavibacter* sp.). The results are presented in Table 1.

Table 1. Antibacterial activity of isolates of non-phytopathogenic micromycetes

Strain of phytopathogenic bacteria	Analyzed micromycetes										
	6G2	7G1	7G9	7G15	TV	T4	T5	Zh-1	L/S	2-b	9-b7
Clavibacter sp. MVF	-	-	-	-	-	-	-	-	-	-	-
Clavibacter sp. (Mogilev)	+	-	-	-	-	-	+	-	-	-	-
Clavibacter sp. (Kiev)	-	-	-	-	-	-	+	-	-	-	+
E. aroidea atroseptica	-	-	-	-	-	-	-	-	-	-	+
E. aroidea 348-1	-	+	-	-	-	-	-	-	-	-	+
E. aroidea atroseptica 9	+	+	-	-	-	-	-	-	-	-	+
E. carotovora 330	+	+	-	-	+	+	-	+	-	-	+
E. carotovora atroseptica 3-2	+	+	-	-	+	+	-	+	-	-	+
Ps. syringae 345	-	-	-	-	+	+	-	-	-	-	+
Ps. syringae S-10	-	-	-	-	+	+	-	-	-	-	-

As can be seen from the data presented, four studied fungal isolates – 7G9, 7G15, L/S, and 2-b did not show antagonistic activity against

phytopathogenic bacteria. All of tested micromycetes showed antibacterial activity against phytopathogens.

Micromycetes of isolates 6G2 and T5 showed antagonistic activity against *Clavibacter* sp. (Mogilev); fungi of isolates T5 and 9-b7 growth of *Clavibacter* sp. (Kiev) suppressed; growth of *E. aroidea atroseptica* suppressed only micromycete 9-b7. Micromycetes of isolates 7G1 and 9-b7 are active against *E. aroidea* 348-1; isolates 6G2, 7G1 and 9-b7 are active against *E. aroidea atroseptica* 9. Phytopathogenic strains *E. carotovora* 330 and *E. carotovora atroseptica* 3-2 were more sensitive to the influence of the studied fungal cultures. Six micromycetes – TV, T4, 6G2,7G1, Zh-1 and 9-b7 inhibited the growth of these phytopathogens. Micromycetes of isolates TV and T4 possessed the ability to inhibit the growth of *Ps. syringae* 345 and *Ps. syringae* S10, in addition, micromycete 9-b7 inhibited the growth of *Ps. syringae* 345. None of the microscopic fungi studied had the ability to inhibit the growth of bacteria of the *Clavibacter* sp. strain (MVF).

As can be seen from the data presented in Table 2, isolate 9b7 had the greatest antibacterial activity, suppressing the growth of bacteria of seven phytopathogenic strains. Micromycetes of isolates TV, T4, 6G2 and 7G1 inhibit the growth of four, isolate Zh-1 – three, isolate T5 of two strains of phytopathogenic bacteria.

Cultures of phytopathogenic fungi, *F. culmorum*, the causative agent of fusarium of wheatear of rye and barley, *F. semitectum*, the causative agent of fusarium of wheat grain and *B. cinerea*, the causative agent of gray rot of vegetable crops were used to study the antifungal activity of non-phytopathogenic micromycetes's isolates. The results obtained during the experiments are presented in Table 2.

Table 2. The ability of the studied micromycetes to inhibit the growth of phytopathogenic fungi (counter mycelium method)

Isolate	Inhibition of mycelium growth (I),%		
	F. semitectum	*F. culmorum*	*B. cinerea*
Control (pathogen growth without antagonists)	0	0	0
6G2	0	22	18
7G1	0	0	0
7G9	0	0	0

7G15	0	0	0
TV	28	33	50
T4	18	29	13
T5	15	0	0
Zh-1	0	0	0
L/S	0	0	0
2-b	0	39	0
9b7	0	0	0

The antifungal activity of micromycetes was evaluated by the degree of inhibition of phytopathogen's growth in the area of action of antifungal metabolites of tested fungi compared with the growth of phytopathogen in the area where the action of metabolites is absent. Antifungal activity was taken into account on the fifth day of growth of *B. cinerea* and on the seventh day of growth of *F. semitectum* and *F. culmorum*. In the control variant, phytopathogens grew in the absence of the tested fungal cultures. As can be seen from the presented data, micromycetes of isolates 6G2, TV, T4, T5 and 2-b had the ability to inhibit the growth of mycelium of phytopathogens.

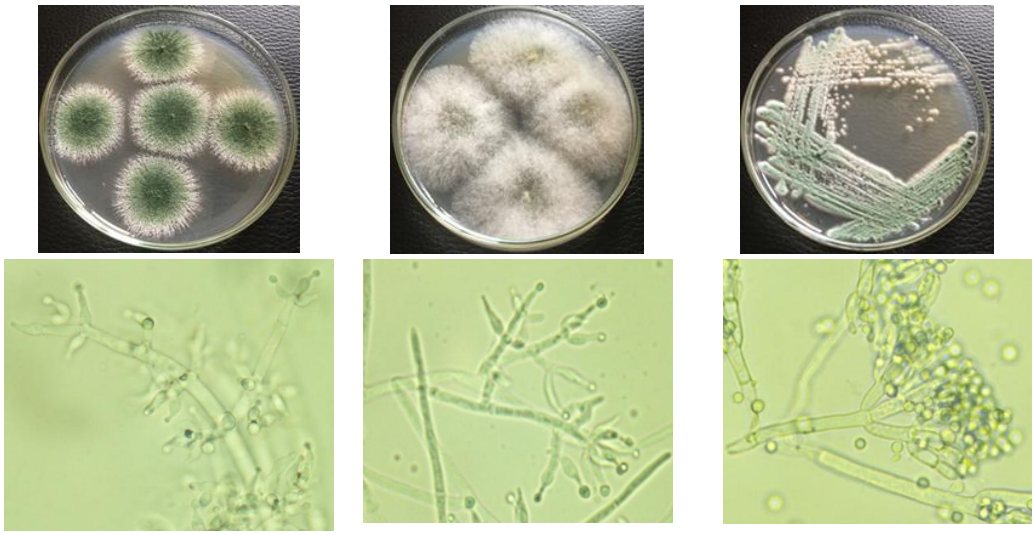
The highest antagonistic activity in the experiment was observed with TV isolate micromycete. This micromycete suppressed the growth of all tested phytopathogenic fungi. Inhibition of pathogen's mycelium growth was 28%, 33% and 50% for *F. semitectum*, *F. culmorum* and *B. cinerea*, respectively. This strain showed the highest degree of inhibition of pathogen mycelium growth in the experiment – 50% (for *B. cinerea*). The T4 isolate also suppressed the growth of all three tested phytopathogenic strains, but its activity was lower than the TV isolate showed, and ranged from 13% (*B. cinerea*) to 29% (*F. culmorum*). Micromycete 6G2 inhibited the growth of *F. culmorum* by 22% and the growth of *B. cinerea* by 18%. The degree of inhibition of *F. semitectum* growth by T5 isolate was the lowest in the experiment and amounted to 15%, and isolate 2-б inhibited the growth of *F. culmorum* by 39%.

Thus, the greatest antifungal activity in the experiment was registered by isolates 6G2, TV and T4. These micromycetes also showed antibacterial activity against four strains of phytopathogenic bacteria out of ten tested. Therefore, they were selected for further research.

At the final stage of the work, a microscopic study of the structure of spores and mycelium of fungi isolates TV, T4 and 6G2, which demonstrated the widest range of antagonistic activity in the experiments, was carried out.

According to the results of studies of the structure of conidia, conidiophores, hyphae of mycelium and the appearance of mycelium according to the "Determinant of microscopic soil fungi" by M.A. Litvinov, the identification of micromycetes was carried out. The genus of the studied fungi has been determined.

Based on the conducted studies, micromycete TV is identified as a representative of the genus *Trichoderma*, presumably *Trichoderma viride*, micromycete T4 is assigned to the genus *Trichoderma*, presumably the species *Trichoderma koningi*, and micromycete 6G2 is assigned to the genus *Penicillium*, subgenus *Eupenicillium*. The results of the research are shown in the figure.



micromycete TV micromycete T4 micromycete 6G2

Figure – The appearance of the mycelium and the microscopic structure of the conidiophores of micromycetes TV, T4 and 6G2.

The antagonistic micromycetes isolated and studied in the course of the conducted research are conserved in the collection of microorganisms of the Research Laboratory of Molecular Genetics and biotechnology of BSU and will be used in research and development of phytoprotective biologicals.

Conclusions. Micromycetes *Trichoderma* sp.TV, *Trichoderma* sp T4 and *Penicillium* sp 6G2, isolated from natural sources, have antagonistic activity against phytopathogenic bacteria and fungi. They are promising for further

research and development of biological preparations with phytoprotective action.

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