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SEARCH FOR ISOLATES OF ENDOPHYTIC BACTERIA-ANTAGONISTS OF PHYTOPATHOGENS

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Summary. Antagonistic activity of 20 isolates of non-phytopathogenic endophytic bacteria isolated from adult plants of lavender, thyme, St. John's wort and triticale against phytopathogenic microorganisms was studied in laboratory conditions. Nine isolates of endophytic bacteria antagonists of phytopathogens were selected. The ability of selected endophytic antagonist bacteria to protect spring wheat plants of the "Sudarynya" variety from diseases was evaluated in a small-plot experiment under conditions of a natural infectious background. According to the experimental results, endophytic bacteria Tr-2, D-2.1 and L-2 were proposed for research to create new phytoprotective biological preparations.

Keywords: *endophytic bacteria, antagonists of phitopathogens, plant protection, biotechnology.*

Introduction. A modern and environmentally safe way to increase the productivity of agricultural plants is application of biological preparations. Endophytic bacteria has become a new promising direction in this area. They are able to colonize the internal tissues of plant without causing its diseases and without adversely affecting its development. Being in the endosphere of plants, these microorganisms have an advantage over organisms living in the rhizosphere and phyllosphere due to stable pH, humidity, nutrient flow and lack of competition from a large number of microorganisms. This allows them to be less dependent on external environmental factors and at the same time to exhibit a complex of economically useful properties directly in plant tissues [1].

Bacterial endophytes colonize the same ecological niches as phytopathogenic microorganisms, therefore they are considered as a promising agent of biocontrol of phytopathogens. Plant protection with the help of endophytic bacteria is usually associated with their competition with pathogenic microflora for nutrients and a suitable niche for colonization; with their

synthesis of various metabolites with antibiotic activity, with their ability to trigger the formation of systemic resistance in plants to adverse environmental factors of biogenic and abiogenic origin. Therefore, the study of endophytic bacteria is very relevant and may allow us to obtain highly effective phytoprotective preparations based on these microorganisms in the future [2].

The purpose of this work is to find endophytic bacteria with antagonistic properties and promising for inclusion in phytoprotective biological products.

Materials and methods. *Strains*

Isolated endophytic bacteria from leaves, stems, roots of triticale plants, Marshall thyme, lavender, and St. John's wort were used in this work. 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 strain of phytopathogenic fungi *Fusarium oxysporum* from the collection of microorganisms of the Research Laboratory of Molecular Genetics and biotechnology of BSU were used.

Methods

The antibacterial activity of studied endophytic bacteria's isolates was evaluated using the method of perpendicular strokes [3].

To determine the antifungal activity of isolates of endophytic bacteria, the bacterial culture was sown on a nutrient agar with a line crossing the cup at a distance of 2 cm from the center and cultured at 28°C for 48 hours. After that, mycelium and spores of the phytopathogenic fungus *F. oxysporum* were injected into the center of the cup (at a distance of 2 centimeters from the bacterial line). The results were taken into account on the 6th day of phytopathogen cultivation. To assess the antagonistic activity of studied bacteria, the radius of the mycelium *F. oxysporum* was measured along the shortest distance to the bacterial line (R_1) and the radius of the mycelium that grew in the opposite direction from the bacterial culture (R_2). The degree of inhibition of fungal growth by bacteria (I) was calculated by the formula:

$$I = (1 – R_1/R_2) \times 100\ \%$$

The study of the phytoprotective properties of endophytic bacteria was carried out in small-scale experiments in the conditions of natural infectious

background of the botanical garden of the biological Faculty of BSU. The research was carried out on spring-planted wheat ("Sudarynya" variety).

To prepare the working solution, endophytic bacteria were cultured in a liquid mineral medium M9 at 28 ° C for 48 hours. This bacterial suspension was diluted with tap water 100-fold. Treatment with endophytic bacteria was carried out when plants were planted in the ground (soaking seeds in 1% working solution for 3 hours), in the tillering phase (root watering) and in the tube phase (root watering). The consumption of the working solution is 1.2 l/m^2. Plants treated with water were used as a control.

The number of dead plants was taken into account during the growing season. The assessment of plant and seed diseases was carried out after harvesting wheat plants. The following indicators were determined: the spread of the disease or the number of affected plants in crops and the development of the disease or the degree of infestation [4].

Results and discussions. *Evaluation of antagonistic activity of endophytic bacteria on phytopathogenic microorganisms in laboratory conditions.*

The antagonistic properties of 20 isolates of endophytic bacteria were evaluated in this study. The ability of studied microorganisms to suppress of phytopathogenic bacteria growth was evaluated using pathogens of the following diseases of agricultural plants: potato blackleg (*E. carotovora* subsp. *atroseptica, E. carotovora*), cabbage mucosal bacteriosis (*E. aroidea*), wheat bacteriosis (*Ps. syringae*), bacterial tomato cancer (*Clavibacter* sp.). To assess the antifungal activity of endophytic bacterial strains, the phytopathogenic fungus *F. oxysporum*, which affects the vascular system of plants and causes a number of diseases (wilting), was used. Results are presented in Table 1.

During the experiment, it was found out that endophytic bacteria of eight tested isolates (D-2.1, D-3, Z-1, Z-2, Z-4, L-1, L-2 and E-33) have the ability to inhibit the growth of phytopathogenic bacteria. Ten out of twenty strains demonstrated antagonistic activity towards *F. oxysporum*. The degree of inhibition of phytopathogen growth by the tested strains ranged from 64.5% (B-2) to 44.5% (L-1).

According to the results of the conducted studies, endophytic bacteria of isolates D-2.1, D-3, Z-2, and E-33, which showed antagonistic activity against several strains of phytopathogenic bacteria, isolates B-2, D-1, D-5, and Tr-2, inhibiting mycelium growth of *F. oxysporum* more than 60% higher than the

bacteria of the L-2 isolate, which have simultaneously shown antibacterial properties on two strains of bacteria-phytopathogens and antifungal properties, were selected for further work.

Investigation of the ability of endophytic bacteria to exhibit phytoprotective properties against spring-planted wheat.

In small-scale experiments, the ability of endophytic antagonist bacteria to protect spring-planted wheat from diseases was tested. The experiment was carried out under conditions of a natural infectious background. During the experiment, the development of stem brown rust was registered on wheat plants; the defeat of seeds by a complex of diseases was noted. The development of brown stem rust was noted in all analyzed wheat plants. Thus, the prevalence of the disease both in the experiment and in the control is 100%.

Table 1 - Evaluation of antagonistic activity of endophytic bacteria

Isolate	Phytopathogenic bacteria growth inhibition	*F. oxysporum* mycelium growth inhibition (I), %
B1	no inhibition	50,0
B2	no inhibition	64,5
D-1	no inhibition	62,3
D-2.1	*Ps. syringae* S-10, *Clavibacter* sp. (Mogilev)	0
D-3	*Clavibacter* sp. (Mogilev)	0
D-5	no inhibition	62,3
D-6	no inhibition	0
Z-1	*Clavibacter* sp. (Kyiv)	49,0
Z-2	*Ps. syringae* S-10	0
Z-3	no inhibition	53,4
Z-4	*Ps. syringae* S-10	55,6
L-1	*Clavibacter* sp. (MVF)	44,5
L-2	*Clavibacter* sp. (Mogilev), *E. aroidea 3*	58,1
S-1	no inhibition	0
T-1	no inhibition	0
T-2	no inhibition	55,6
Tr-1	no inhibition	0
Tr-2	no inhibition	60,3
R-13	no inhibition	0
E-33	*Ps. syringae* 510, *Clavibacter* sp. (MVF); *E. aroidea 3*	0

The results about the degree of development of stem brown rust and the defeat of wheat seeds by a complex of diseases are presented in Table 2. It should be noted that in the control and experiment with endophytic bacteria of isolates B-2, D-1, D-5, Z-2 and E-33, the death of plants during vegetation was noted, reached in some cases to 20% of the total number of plants. Such plants are assigned the highest score of defeat on the accounting scale.

According to the results of the experiment, the effect of treatment of wheat plants with endophytic bacteria on the degree of development of stem brown rust was noted. Treatment of plants with endophytic bacteria of isolate L-2 reduced the degree of disease development in the experiment compared with the control by 37.2% (38.8% in the experiment, 76.0% in the control).

Treatment of plants with bacteria of isolate D-2.1 reduced the degree of disease development by 30.7% compared with the control, with bacteria of isolate Tr-2 – by 21.4% compared with the control. Wheat treatment with bacteria of isolates D-3 and D-5 had a slight effect on the degree of development of brown stem rust in plants (a decrease in this parameter compared to the control by 8.1% and 8.7%, respectively).

It should be noted that in the case of treatment of plants with bacteria of isolate L-2, the defeat of wheat seeds by a complex of diseases decreased by 1.78 times compared to the control (9.8% in the control and 5.5% in the experiment), bacteria of isolate Tr-2 – by 2.09 times (9.8% in the control and 4.7% in the experiment), bacteria isolate D-3 – 3.5 times (9.8% in the control and 2.8% in the experiment).

According to the results of the conducted experiments, endophytic bacteria of isolates Tr-2, D-2.1 and L-2 are proposed for further research in order to include phytoprotective action in biological preparations.

Table 2. Study of the ability of endophytic bacteria to exhibit phytoprotective properties on spring-planted wheat ("Sudarynya" variety, small-scale experiment, 2022)

Experiment option	Degree of development of the disease (brown stem rust), %	Defeat of seeds by a complex of diseases, %
Control (water treatment)	76,0	9,8
B-2	81,3	5,8
D-1	82,0	6,3
D-2.1	45,8	10,3
D-3	67,9	2,8
D-5	67,3	8,0
Z-2	72,0	9,2
L-2	38,8	5,5
Tr-2	54,6	4,7
E-33	76,0	11,8

Conclusions:

Endophytic bacteria of the Tr-2 isolate showed high antagonistic activity against *F. oxysporum* (the degree of mycelium growth inhibition was 60.3%). In small-scale experiments, the treatment of wheat plants with these bacteria reduced the degree of development of stem brown rust by 21.4% compared with the control and reduced the defeat of wheat by a complex of diseases by 2.09 times compared with the control.

Endophytic bacteria of isolate D-2.1 had antagonistic activity against pathogens of wheat bacteriosis and bacterial cancer of tomatoes, and also had the ability to reduce the growth rate of *F. oxysporum*. In small-scale experiments, the treatment of wheat plants with these bacteria reduced the degree of development of stem brown rust by 30.2% compared to the control.

Endophytic bacteria of isolate L-2 had antagonistic activity against the causative agent of bacterial cancer of tomatoes and soft rot of rhizomes. In small-scale experiments processing of wheat plants these bacteria reduced the degree of development of stem brown rust by 30.2% compared to the control.

These endophytic bacteria will be further investigated in order to include phytoprotective properties in biological products.

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