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SPECIFIC RHIZOBACTERIA PROTECT WHEAT PLANTS FROM PHYTOPATHOGENIC FUNGI.

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Abstract

Pure cultures were isolated from the rhizosphere of wheat plants grown in different regions of Uzbekistan. Of these rhizobacteria, 21 strains were selected for their ability to mobilize phosphate. The antagonistic and antifungal activity of selected strains of rhizobacteria against 6 phytopathogenic fungi of wheat plants was studied. For this purpose, strains of phytopathogenic wheat fungi were used as test cultures: Fusarium graminearum, F. oxysporum, F. tricinctum, F. avenaceum, B. sorokiniana and B. spicifera.

Key words

antagonism, rhizosphere, culture, strain, phosphorus mobilization.

Introducrition

Products that influence the growth and development of phytopathogenic fungi, which are considered secondary metabolites of wheat rhizosphere bacteria, are antibiotics, phytohormones, enzymes, siderophore substances, which, with their active antagonistic properties, protect plants from various pathogens. These active strains of rhizobacteria serve as biofungicides that increase seed fertility, making it possible to limit the use of various chemicals in the development and increase the productivity of agricultural products, as well as clean up soil damaged by them over the years.

The diverse group of bacteria that colonize rhizosphere habitats are called rhizobacteria. One of the main mechanisms of antagonism is that bacteria directly affect plant pathogens or increase plant resistance to disease by strengthening the immune system. The antagonistic activity of rhizosphere microorganisms is associated with the production of secondary metabolites that suppress the growth and reproduction of phytopathogenic bacteria and fungi [1]. They directly or indirectly affect plant growth [2].

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The rhizosphere is a thin layer of soil considered to be directly adjacent to the roots of a plant, and is formed under the influence of root exudates. The rhizosphere is one of the "hot spots" of microbial activity in the soil and one of the most complex loci in terms of diversity and interspecific relationships [3, 4]. Bacteria that do not form spores are often found in the rhizosphere: pseudomonads, mycobacteria, radiobacteria, etc. Bacteria create physiologically active substances for plants, break down residual substances and, in turn, affect higher plants. Rhizosphere bacteria use substances from plant roots [5]. There are many microbes on the surface of the soil, and as you descend, their number decreases. Microorganisms are more numerous in the 10-15 cm layer, since there is no direct sunlight, sufficient nutrition and moisture. There are fewer of them in deep layers, since the soil acts as a natural filter and does not transfer bacteria to groundwater [5]. The soil at a depth of 4-5 meters can be practically sterile [6].

Further metabolic activity of these bacteria in the rhizosphere is the transport of mineral nutrients and activation of their absorption by plant roots. Plant growth promoting rhizobacteria include bacteria that live in the rhizosphere and promote plant health, which in turn promotes plant growth. Soil organisms extract inorganic nutrients from organic reserves sufficient to support rapid plant growth, and such soil is naturally fertile.

Rhizosphere bacteria, in turn, also affect plants: some enhance their development, others inhibit and lead to a slowdown in development. Thanks to the stimulating effect of rhizosphere bacteria, organic substances in the soil are converted into mineral nutrients (for example, Azotobacter and Tuganak bacteria improve soil structure by synthesizing and releasing biotin, vitamins B6, B1, heteroauxin). Organisms living in the rhizosphere use root secretions and sugars and metabolize them to form organic carbohydrates [7]. The effect of rhizobacteria on plants is divided into two types: direct and indirect. A direct effect is any mechanism that directly increases plant growth by providing nutrients or producing growth regulators that protect the plant from infection, or any mechanisms that promote healthy cord growth under environmental stress are indirect mechanisms. Indirect effects include two important mechanisms: induction of systemic plant resistance to phytopathogens (biotic stress) and protection from harmful environmental conditions (abiotic stress) [8]. The number of antagonist microorganisms in the rhizosphere can be increased artificially; they play an important role in the fight against certain pathogens in the soil [9]. The rhizosphere is somewhat different in its properties from "free" soil. The humidity there is high, the reaction of the environment is also slightly different, usually the soil is acidic or

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alkaline, and the rhizosphere is closer to neutral, the amount of organic matter and the solubility of some minerals, such as Fe and Mn compounds, are also high.

Materials and methods

Cultivation of wheat rhizobacteria strains on a liquid nutrient medium and determination of the number of cells.

Strains of rhizosphere bacteria were grown on a liquid peptone nutrient medium at a temperature of 280°C for 3-5 days at a speed of 220 rpm using the submersible cultivation method. The titer of cultivated rhizobacteria strains was determined by dilution method. In this case, 1 ml of the suspension of grown strains was taken with a pipette and thoroughly mixed with 9 ml of water in a sterilized test tube. This process was continued serially, diluted to 1:1,000,000 and repeated. 1 ml of liquid from a test tube was inoculated into the GPA nutrient medium in a Petri dish in triplicate and incubated in a thermostat at 28°C for 24 hours [10].

The composition of the peptone cold nutrient medium (g/l) is as follows:

Peptone - 10 g/l

Glucose - 20 g/l

NaCl - 500 mg/l

Water - 1 l

pH - 6.8±0.2

Isolation of leaf pathogenic fungi

Leaf pathogenic fungi strains Fusarium graminearum, F. oxysporum, F. tricinctum, F. avenaceum, Bipolaris sorokiniana, and B. spicifera were obtained from the Microorganisms Collection of the Institute of Genetics and Experimental Plant Biology of the Academy of Sciences of the Republic of Uzbekistan. The strains of phytopathogenic fungi were cultured in Chapek cold nutrient medium for 7 days at a temperature of 28°C.

The composition of the Chapek cold nutrient medium is as follows:

Glucose - 20 g

Sodium nitrate - 2.0 g

Potassium dihydrogen phosphate - 1.0 g

Magnesium sulfate + 7H2O - 0.5 g

Potassium chloride - 0.5 g

Calcium carbonate - 3 g

Water - 1000 ml

Studying the antagonistic activity of rhizobacterial strains against leaf pathogenic fungi

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The antagonistic and antifungal activities of phosphate-mobilizing rhizobacterial strains were investigated against six leaf pathogenic fungi. For this purpose, as a trial culture, strains of leaf pathogenic fungi were used: Fusarium graminearum, F. oxysporum, F. tricinctum, F. avenaceum, B. sorokiniana, and B. spicifera. The rhizobacterial strains were incubated for 5 days at a temperature of 28°C in peptone cold nutrient medium. The fungal strains were cultured for 7 days in Chapek cold nutrient medium. Suspensions of fungal spores with a titer of 105 CFU/ml were prepared.

The "well" method (colony method) was used to determine the antibiotic production characteristics of rhizobacteria and their ability to suppress the growth of various pathogenic fungi in the leaf. This method is based on the diffusion of antagonistic substances produced by microorganisms into the cold nutrient medium. For this purpose, the bacterial suspension was inoculated onto agar in a Petri dish containing Chapek nutrient medium. A thick agar culture containing the test culture was placed on the agar where the antagonistic substance was present. If the rhizobacteria have antagonistic properties, a sterile zone is formed around the agar culture.

Morpho-physiological characteristics of phosphate and potassium mobilizing rhizobacterial strains were studied, and their identification was performed using MALDI TOF mass spectrometry.

Fosfat parchalovchi rizobakteriyalarning faol kulturalarining kultural-morfologik va fiziologik-biokimyoviy xususiyatlarini umumiy qabul qilingan usullar yordamida amalga oshirildi Bergey [11].

The active cultures of phosphate-solubilizing rhizobacteria were subjected to comprehensive cultural-morphological and physio-biochemical characterization using accepted methods outlined by Bergey [11]. The identification of active rhizobacterial cultures was determined using MALDI TOF (Matrix Assisted Laser Desorption/Ionization Time-of-Flight) mass spectrometry. MALDI is a technique that involves ionization of molecules through the use of a matrix and laser light. Vitek MS (MALDI-TOF) spectral analysis directly from bacterial cells, without the need for lengthy sample preparation, was employed for bacterial identification.

Research Results and Analysis

The antagonistic activity of soil rhizobacteria against phytopathogenic fungi was investigated under laboratory conditions. Phytopathogenic fungi, including F. graminearum, F. oxysporum, F. tricinctum, F. avenaceum, Bipolaris sorokiniana, and B. spicifera, were cultured on 2% GPA agar medium. Subsequently, agar plugs with a diameter of 5 mm containing the fungal pathogens were prepared.

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Suspensions of local rhizobacterial strains, grown for 5 days in liquid GPB medium, were inoculated onto the agar plugs. The bacterial cultures and their antagonistic and antifungal activities against the fungal pathogens were recorded in Table 1 and Figures 1-2.

All tested phosphate-solubilizing rhizobacterial strains exhibited varying degrees of antagonistic activity against the studied soil phytopathogens. Culture №25 demonstrated antagonistic activity against all six tested phytopathogens (50-80%). Cultures №22 and №24 exhibited high activity against four out of six phytopathogens, namely F. graminearum, F. tricinctum, B. sorokiniana, and B. spicifera, inhibiting their growth by 80-100%. Cultures №10 and №14 demonstrated high antagonistic activity against F. graminearum and B. spicifera, inhibiting their growth by 90-100%. Antagonistic activity against other phytopathogens ranged from 30% to 70%.

Phosphate-solubilizing rhizobacteria's antagonistic activity against soil phytopathogenic fungi

Table 1

Rhizobacterial Culture №	Fusarium graminearum	Fusarium oxysporum	Fusarium tricinctum	Fusarium avenaceum	Bipolaris sorokiniana	Bipolaris spicifera
1	80%	70%	40%	-	10%	40%
3	30%	-	-	-	-	-
4	-	40%	-	-	-	-
9	20%	-	-	-	-	-
10	100%	70%	40%	-	60%	90%
14	100%	60%	30%	-	40%	90%
16	70%	-	-	-	-	-
17	90%	60%	80%	-	50%	50%
7	90%	90%	70%	-	100%	30%
8	40%	50%	40%	-	-	-
18	90%	50%	80%	-	50%	50%
19	50%	-	30%	40%	-	-
21	20%	-	-	-	-	-
23	100%	20%	80%	-	100%	90%
28	40%	-	-	-	-	-
29	80%	60%	20%	80%	-	-
31	60%	-	30%	60%	40%	80%
25	60%	50%	50%	50%	50%	80%
20	40%	60%	-	-	-	-
24	100%	30%	80%	-	100%	100%
26	80%	50%	40%	-	100%	90%
30	70%	60%	60%	-	100%	90%

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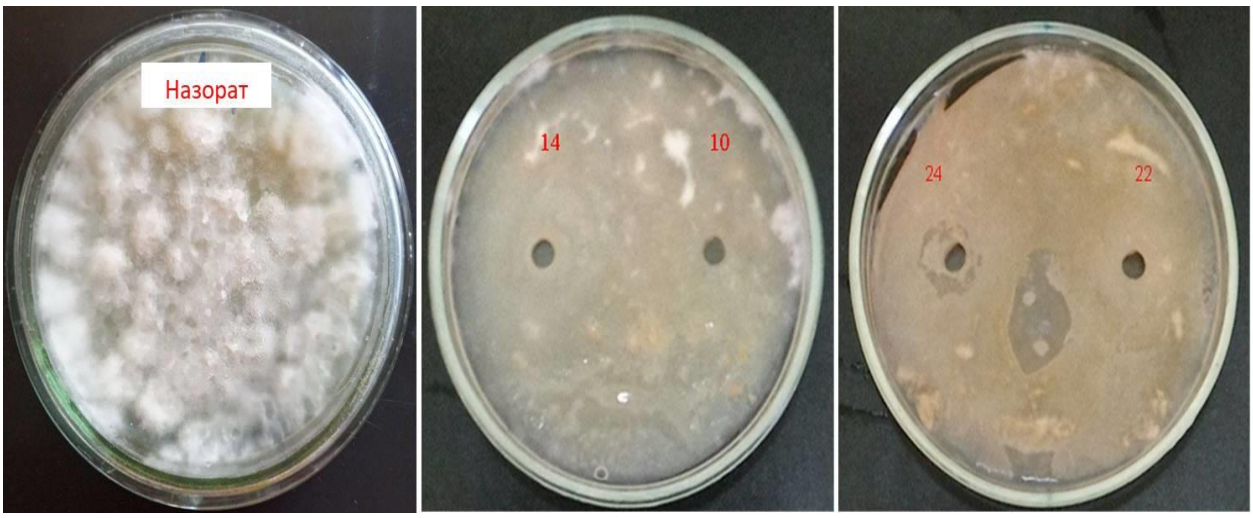

Figure 1. Phosphate-solubilizing rhizobacterial cultures №10, 14, 22, and 24's antagonistic activity (10 days) against Fusarium graminearium.

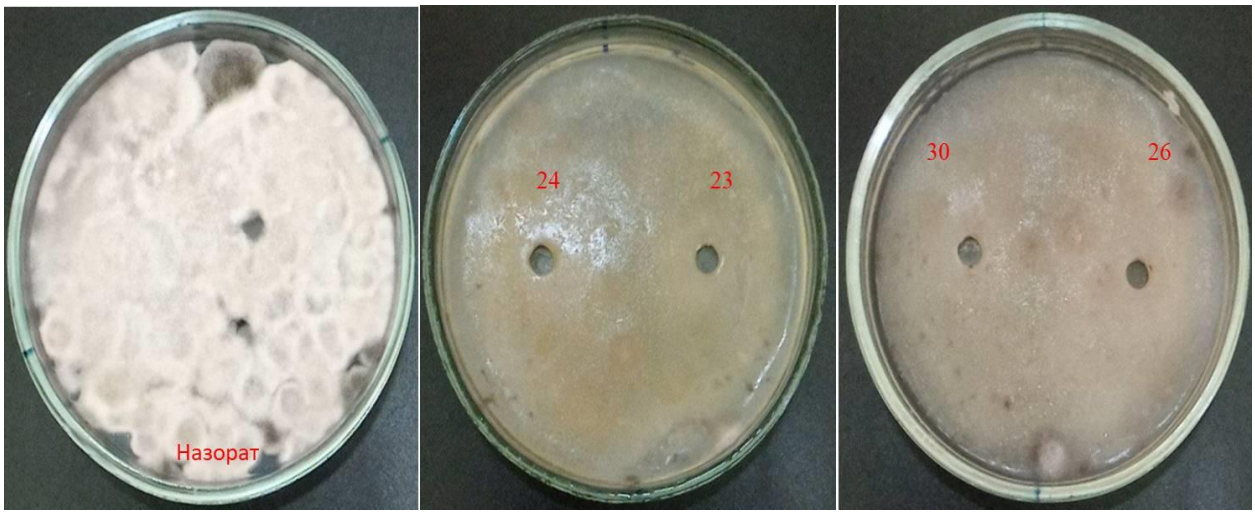

Figure 2. Phosphate-solubilizing rhizobacterial cultures №23, 24, 26, and 30's antagonistic activity against Bipolaris spicifera.

During the research, it was determined that local phosphate-solubilizing rhizobacterial cultures № 23, 24, 26, 30, 10, 14, 7, and 29 exhibited high activity as antagonists and antifungal agents against the soil phytopathogens F. graminearum, F. oxysporum, F. tricinctum, F. avenaceum, B. sorokiniana, and B. spicifera.

The identification results of the active rhizobacterial cultures using the MALDI-TOF MS method are presented in Table 2.

№	Strain №	Bacterial Species
1	1	Escherichia hermannii
2	3	Rahnella aquatilis
3	4	Rahnella aquatilis
4	7	Enterobacter cloacae

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5	8	Enterobacter cloacae
6	9	Rahnella aquatilis
7	10	Rahnella aquatilis
8	14	Rahnella aquatilis
9	16	Rahnella aquatilis
10	17	Rahnella aquatilis
11	18	Enterobacter cloacae
12	19	Pantoea agglomerans
13	20	Pseudomonas chlororaphis
14	21	Pantoea agglomerans
15	22	Bacillus cereus
16	23	Pseudomonas kilonensis
17	24	Pseudomonas kilonensis
18	25	Paenibasillus dendritiformis
19	26	Bacillus simplex
20	29	Bacillus megaterium
21	30	Pseudomonas kilonensis

In the following way, the morphological-cultural characteristics of the cultures isolated from the soil rhizosphere were investigated, and through the MALDI-TOF mass spectrometry method, active cultures belonging to the species Escherichia hermannii, Enterobacter cloacae, Rahnella aquatilis, Pantoea agglomerans, Bacillus cereus, Bacillus simplex, Bacillus megaterium, Pseudomonas chlororaphis, and Pseudomonas kilonensis were identified. The studied rhizobacterial strains exhibited varying degrees of antagonistic activity against soil phytopathogens. The obtained data indicate that local active strains of rhizobacteria can be utilized as biocontrol agents to protect plants from various fungal diseases.

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