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ISOLATION AND IDENTIFICATION OF PROMISING STRAINS OF BACILLUS SUBTILIS

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Abstract. The article presents data on the isolation and study of the physiological and biochemical properties of Bacillus subtilis cultures. Samples of Chernozem soils of wheat fields were used as materials for selecting crops. 10 isolates with antimicrobial properties of B. subtilis were isolated from the samples. Microscopic observation of these isolates showed that they are gram-positive, rod-shaped, endospore-forming bacteria. According to the cultural-morphological and physiological-biochemical characteristics, the isolated isolates were identified as Bacillus subtilis. Work was carried out to study the antagonistic activity of isolated Bacillus subtilis strains to 2 test strains: Alternaria alternata and Fusarium avenaceum. Based on the data obtained, it was noted that Bacillus subtilis strains EU7, EU14, EU22, EU31 and EU34 have antagonistic activity against Alternaria alternata and Fusarium avenaceum. Thus, promising strains for biocontrol of alternarious and Fusarium diseases were obtained.

Introduction.

In recent years, there has been increased interest in studying the biological control of phytopathogens using beneficial plant microorganisms, especially bacteria, as evidenced by the exponential growth of the global market for biopesticides from $800 million. US $2.8 billion in 2014. United States at present [1]. Many bacterial genera, such as Paenibacillus, Pseudomonas, Burkholderia, Lysobacter, and Bacillus, have been described as symbiotic microorganisms with biocontrol capability [2–4]. This group of microbes inhibits the development of disease by preventing the formation of phytopathogens, due to the production of extracellular inhibitory molecules such as lytic enzymes, toxins, siderophores, biosurfactants and activation of plant protection signals [2, 5, 6]. Recent studies show that bacteria have been successfully used to fight fungal diseases in several economically important crops, such as maize [7], common beans [8], and soybeans [9], while reducing the severity of the disease (>60 %) and improving the phytosanitary condition of plants.

Currently, in Kazakhstan, chemical preparations based on ciproconazole, imazalil, tebuconazole, benomil, tiram, fludioxonil and other antimicotic drugs are mainly used to combat pathogens of grain crops. The use of chemical fungicides in agriculture has a number of disadvantages: the formation of resistant races of pathogens, toxicity to warm-blooded mammals and humans, and inhibition of rhizosphere microorganisms.

Biological fungicides based on soil microorganisms do not have similar disadvantages. Pretreatment of seeds before sowing with bacterial preparations that have fungicidal activity allows to reduce the damage of crops at the initial stages of cultivation. Improvement of the soil and prevention of infection of plants at the early stages of development are possible with the direct introduction of microorganisms into the soil that synthesize fungicidal substances. In this regard, the isolation of b strains. subtilis, antagonistic activity in relation to alternarious and Fusarium fungi are important in the development of biocontrol agents. Most bacteria of the genus Bacillus (including B. subtilis) are not dangerous to humans and are widely distributed in the environment.

Research materials and methods.

The research materials used were microorganisms with antimicrobial activity of the genus Bacillus.

The following nutrient media were used in the work:

Potato-dextrose agar, (g / l): potato broth-200; dextrose-20; agar-agar-20; pepton-10.

Soil sampling was performed in accordance with GOST 28168-89 [10]. The antagonistic activity of microorganisms in relation to phytopathogenic fungi was studied on potato-dextrose agar using the agar block method [13]. Phytopathogenic strains taken from the collection of microbial strains of the national center of biotechnology branch in Stepnogorsk were used as test organisms: Fusarium graminearum-the causative agent of wheat ear Fusarium, Fusarium oxysporum-the causative agent of wheat root rot Fusarium. Quantitative accounting of microorganisms was carried out by the method of serial dilutions, by the method of direct counting in the Goryaev chamber, and by the method of Koch seeding [14].

Results.

Isolation of microorganisms with antagonistic activity was performed from samples of black earth soils of wheat fields (Akmola region, Kenes village). As a result of the work performed, 23 isolates with activity were identified. Identification of isolates was performed based on morphological, cultural and physiological characteristics using The "Bergi bacteria Determinant". All the isolates formed completely white, rounded, smooth and shiny colonies. Microscopic observation of these isolates has shown that they are gram-positive, rod-shaped endospore-forming bacteria. According to physiological and biochemical characteristics, the isolated isolates were identified as Bacillius subtilis.

Also, 23 isolates with antimicrobial activity were isolated from soil samples. Of these, 10 showed clear antagonistic activity against Alternaria alternata and Fusarium avenaceum According to cultural-morphological and physiological-biochemical characteristics, the isolated isolates were identified as Bacillius subtilis.

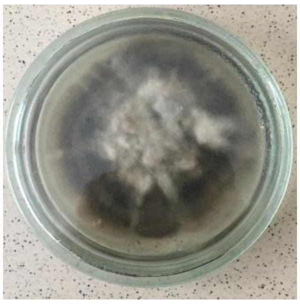
The antagonistic activity of the isolated Bacillus subtilis strains that showed the highest solubilizing ability was studied for 2 test strains: Alternaria alternata and Fusarium avenaceum.by the method of agar blocks. The results are presented in tab. 1. The results presented in table 1 show that Bacillus subtilis strains EU7, EU14, EU22, EU31 and EU34 have antagonistic activity against Alternaria alternata and Fusarium avenaceum.

Table 1 – Antagonistic activity of strains against Alternaria alternata and Fusarium avenaceum on potato-dextrose agar

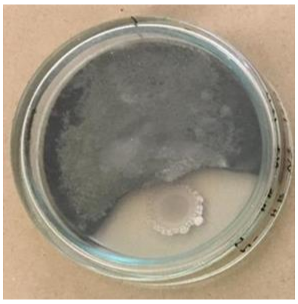
№	The strain	Inhibited the distance against strains	
		Alternaria alternata	*Fusarium avenaceum*
1	Bacillus subtilis EU4	+	++
2	Bacillus subtilis EU7	++	++
3	Bacillus subtilis EU10	+	+
4	Bacillus subtilis EU14	+++	++
6	Bacillus subtilis EU19	+	+
7	Bacillus subtilis EU22	+++	+++
8	Bacillus subtilis EU27	+	+
9	Bacillus subtilis EU31	+++	+++
10	Bacillus subtilis EU34	++	+++

The figure shows in vitro tests for the antagonistic activity of the EU22 isolate against Alternaria alternata and Fusarium avenaceum.on potato-dextrose agar on the 5th day of incubation at 28 °C.

Note. "+" – low-sensitivity, transparent zone from mushroom growth (1 mm); "++" – sensitive, transparent zone from mushroom growth (1–3 mm); "+ + + " – highly sensitive, transparent zone from mushroom growth (>3 mm)

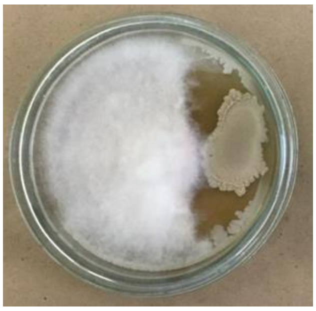


a) alternaria alternata control



b) strain EU22 against

c) fusarium avenaceum control



d) strain EU22 against

Figure 1

Fusarium avenaceum.

Picture. In vitro tests for antagonistic activity of the as29 strain against F. graminearum and F. oxysporum

Conclusion.

As a result of this work, the physiological and biochemical properties of Bacillus subtilis crops were isolated and studied from samples of black earth soils of wheat fields. According to cultural-morphological and physiological-biochemical characteristics, the isolated isolates are identified as Bacillus subtilis.. As a result, 10 of the isolated 23 isolates showed the highest activity. Work has also been carried out to study the antagonistic activity of isolated Bacillus subtilis strains to 2 test strains: Alternaria alternata and Fusarium avenaceum. Based on the data obtained, Bacillus subtilis strains EU7, EU14, EU22, EU31 and EU34 have antagonistic activity against: Alternaria alternata and Fusarium avenaceum.

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