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Extraction of Microorganisms in the Cotton Rhizosphere and the Study of Their Practical Significance

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Abstract: This article provides the research results on that in the conditions of Khorezm region's dry climate and saline soils, pure cultures of bacteria involved in the mobilization of mineral phosphates were isolated, morphologically analyzed and found to belong to the genus Bacillus as gram-negative rods. Bacterial activity was monitored – solubility activity of water-soluble phosphate and antagonistic properties of soil-borne fungi such as Alternaria alternata Rhizoctonia solani, Fusarium solani Verticillium dahlia were studied. When cotton seeds were processed with the association of bacteria found to be active and encapsulated with composts, high yields of cotton were obtained in the bacterial-treated variants. The results obtained are important for the conditions of saline soils and can be used to prevent phosphorus deficiency in crops and to obtain high yields from cotton.

Keywords: rhizobacteria, mineral phosphates, phosphate mobilization, cotton, saline, antagonism, fungus.

Introduction

Similar to other organisms, plants coexist with many organisms in nature. These organisms can be fungi and bacteria that live in the soil. It is known that the life of a plant and its fertility depend on how microorganisms live in and around its body [2;8]. The plant controls the number of microorganisms that live around its roots. Due to the activity of these microorganisms, nutrients are easily absorbed by plants. At the same time, physiologically active substances that control the growth and development of various plants are synthesized by microorganisms and create conditions for normal plant nutrition [12]. In the rhizosphere, the number of microorganisms is 100 times greater than in soils where its root divisions do not reach. [15].

Complex of bacteria living in the rhizosphere varies depending on the plant species and averages 66 species. The most common types include Pseudomonas, Bacillus, Bacterium, Chromobacterium, mycobacterium, Mycococcus, Micrococus, Pseudobacterium, Sarcina, Promyxobacterium, Azotomanos, Lactobacterium [2; 4; 5; 6; 10; 11]. Cases of isolation of the genera Fusarium, Alternaria and Cladosporium were also recorded. On a number of individuals recorded a mixed infection, as we isolated B. tenella with Mucor sp.; B. tenella with Penicillium sp.; and B. tenella with Alternaria sp [1].The distributional geography of microorganisms depends on a complex of agroecological factors, such as humidity, temperature, substrate type, acidity, soil salinity, etc [7]. Associations of some specific microorganisms that dominate the soil develop [3]. Associative groups of certain bacteria convert organic and mineral phosphates, which are difficult to assimilate for plants, into easily digestible forms, and at the same time have the properties of increasing disease resistance [11]. Among the bacteria known to have such properties, almost 50% are

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rhizosphere bacterial strains [3; 9]. An in-depth study of these bacteria will enable the cultivation of ecologically clean products through the creation of biopreparations in agriculture and their application to crops. In the conditions of Khorezm region, where the ecological situation is complex, we studied the possibility of creating a biopreparation by extracting pure cultures of bacteria in the rhizosphere of cotton plants.

Methods

The research was conducted in cotton fields in the South Aral Sea region of Uzbekistan. During the study of the cotton root rhizosphere in selected fields, samples were obtained by means of a modified modification substantiated on the D.G.Zvyagintsev [14] method. In determining the number of microorganisms in the samples, we prepared a suspension from the soil, diluting it repeatedly and planting it in nutrient media. Microorganisms involved in the mobilization of mineral phosphates were studied in the Pikovsky nutrient medium.

Determination of the number of microorganisms that mobilize mineral phosphates in the soil was carried out in fields with different mechanical composition and different levels of salinity. The amount of microorganisms belonging to different physiological groups was studied in soils with the most common average sandy mechanical composition in the conditions of Khorezm region. In order to determine the number of microorganisms belonging to different physiological groups, MPA (Meat peptone agar-agar), Chapek, Vinogradsky, Giltay, Ashby environments were used. The MPA nutrient medium was delivered readily.

After enumerating colonies of microorganisms grown from suspensions planted in Pikovsky nutrient media, the areas of calcium phosphate dissolved around their colonies were studied. Calcium phosphate-dissolved microorganisms were extracted in more areas around the colonies. Their morphological properties were studied using an immersion 1800x lens of a microscope. The studied microorganisms were stored at 5–

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70^0C in the refrigerator in a nutrient medium of the potato-agar. Among bacteria, the most acid-forming microorganisms were studied and their cultures were numbered. 2 cultures were found to be active and propagated in liquid Chapek medium and their effect on seed germination was studied in vegetative containers under laboratory conditions. Dissolution of calcium phosphates in cultures of Pikovsky in 50 ml volumetric flasks of cultures was studied.

The antagonistic properties of phosphorylating bacteria were studied on the example of fungi that cause fusarium wilt and rhizoctonia. We used the "a pit" method. For this, we placed a suspension of FMB in a pit on the agar where phytopathogenic fungi grow. As a result, it was observed that zones of fungal growth were formed around the pit [14].

Field experiments were carried out on the basis of generally accepted methods in Uzbekistan, in 4 repetitions and 6 variants, in field parts of the size 50 m^2, according to the following scheme. The seeds in the control variant were sown in water for 13 hours. The experiments were conducted in saline fields.

1. The seeds in the control variant were sown in water for 13 hours.

2. In the second variant, without fertilizer, the seeds were soaked in phosphorylating bacteria for 13 hours and encapsulated in biocompost.

3. In the third variant, with the application of 50% fertilizer ($N_{100}P_{70}K_{50}$), seeds were soaked in water for 13 hours.

4. In the fourth variant, with the application of 50% fertilizer ($N_{100}P_{70}$ K_{50}), seeds were soaked in phosphorylating bacteria for 13 hours and encapsulated in biocompost.

5. In the fifth variant, with the application of 100% fertilizer ($N_{200}P_{140}$ K_{100}), seeds were soaked in water for 13 hours.

6. In the sixth variant, with the application of 100% fertilizer ($N_{200}P_{140}$ K_{100}), seeds were soaked in phosphorylating bacteria for 13 hours and encapsulated in

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biocompost.

Phenological observations of the cotton were carried out in accordance with the methods adopted by the General Uzbek Cotton Research Institute.

The agrochemical composition of the field was studied in the laboratory conditions before the experiment. It was studied using generally accepted methods.

Table 1

Agrochemical composition of the field that the experiment conducted

Depth	**Humus**	**Total %**			**Easily assimilated ones, mg. kg**			
		Nitrogen	Phosphorus	Potassium	Ammonium	Nitrate	Phosphorus	Potassium
Agrochemical composition of saline washed field								
0 -30	0,91	0,047	0,190	1,40	17,8	6,3	37,2	360
30-60	0,80	0,045	0,149	1,53	15,3	5,0	10,4	288

Analysis of experimental data

The amount of humus in the soil was determined by the method of V.I.Tyurin, the total amount of nitrogen was determined by means of Keldal method and the amount of phosphorus was found out using Meshyakov method [13]. The results obtained are presented in Table 1.

In Khorezm region, mainly saline soils are prevalent, the soil salinity is washed away in autumn and spring, and the dry climate and very hot summer months lead to secondary salinization of soils. Given the prevalence of salinity in the region, samples were taken from the cotton rhizosphere growing in saline soils, and 21 acid-forming bacteria were isolated. According to the results of monitoring, two bacteria were extracted because of their strong antagonistic properties against active acid-forming and phytpathogenic fungi (Fig. 1) and their fish peptone agar-grown colonies were

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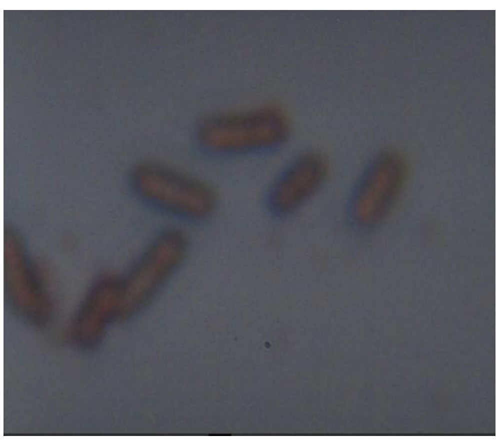
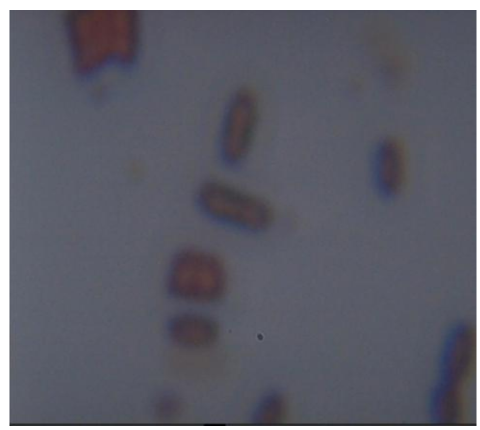
photographed. These bacteria were studied by gram staining and found to be susceptible to gram-negative bacteria. When the spores were stained, they were found to have spore-forming properties. Relationships to carbohydrates and amino acids were also analyzed and it was found that these bacteria belong to Bacillus sp, Baccilus subtillis and species.

The appearance of Bacillus sp colonies

The appearance of Baccilus subtillis colonies



Gram staining of Bacillus sp



Gram staining of Baccilus subtillis

Figure 1. Some morphological features of rhizosphere bacteria

These bacteria were propagated in the laboratory and their antagonism against the pathogens Rhizoctonia sola, Fusarium sola, Verticillium dahliae, Alternaria alternata was studied. In this case, the diameter of the lysis zone around the phytopathogenic fungus culture was determined by the percentage of the total area of

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the lysis zone when the middle part of the petri dish was filled with a phosphor-mobilizing bacterial suspension. (Table 2) In this case, when the antagonism of Bacillus subtillis was studied against the phytopathogenic fungus Rhizoctonia solani, it formed a 65 mm lysis zone and accounted for 70%.

Table 2

Inhibition of the development of phytopathogenic fungi by phosphorylating bacteria

Cultures of rhizobacteria	Inhibition of the development of phytopathogenic fungi							
	Rhizoctonia solani		Fusarium solani		Verticillium dahliae		**Alternaria alternata**	
	d, mm	**%**	d, mm	**%**	d, mm	**%**	d, mm	**%**
Bacillus subtillis	**65**	**70**	**60**	**66**	**40**	**44**	**50**	**55**
Bacillus sp	**60**	**70**	**60**	**66**	**25**	**30**	**15**	**16**

In the study of the antagonism of Fusarium solani against the fungus, the lysis zone was 60 mm and accounted for 66%. The antagonism of Verticillium dahlia was slightly less than that of the fungus 40 mm: 44%. Alternaria alternata was 50 mm relative to the alternate fungus and accounted for 55%. When Bacillus sp was studied, similar results were obtained for the fungi Rhizoctonia solani, Fusarium solani. Verticillium dahlia was observed to be less atogenic than Bacillus subtillis compared to Alternaria alternata fungi.

Bacterial calcium phosphate solubility activity was studied. (Table 3) In this case, less soluble phosphates in water were dissolved in the early stages of Bacillus subtillis - 14 days after inoculation of bacteria than in Bacillus sp. After 21–28 days,

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the amount of water-soluble phosphates in the Bacillus subtillis culture medium was moderate compared to the Bacillus sp culture medium. It was observed in the experimental results that the amount of soluble phosphates, which accelerated the formation of acid, which can be explained by the change in pH, also increased. No solubility of phosphates was observed in the control variants in which bacteria were not cultured. When bacteria isolated from the conditions of Khorezm region were cultured in Pikovsky nutrient medium for the dissolution of calcium phosphate, it was observed that a lysis zone was formed around 100% of the colonies of these bacteria, indicating the dissolution of calcium phosphate.

Table 3

Dissolution of $Ca_3(PO_4)_2$ in bacterial suspension (mg/50 ml in 50 ml suspension)

Variants	**After 7 days**			**After 14 days**			**After 21days**			**After 28 days**		
	The number of bacteria, mln	pH	P_2O_5	The number of bacteria, mln	pH	P_2O_5	The number of bacteria, mln	pH	P_2O_5	The number of bacteria, mln	pH	P_2O_5
Control	0	6,6	0,46	0	6,7	0,5	0	6,6	6,9	0	6,6	6,9
Bacillus subtillis	7,6	5,3	22,0	38,0	5.1	52,0	35,0	4,8	46,0	32,0	4,8	27,0
Bacillus sp	7,6	5,2	25,0	34,0	5,0	54,5	38,0	4,7	42,0	28,5	4,8	25,0

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The seeds were mixed with the studied bacteria in a 10^6-titre suspension of the bacterial association in a sheltered place from the sun until they were completely covered with compost made from straw (Fig. 2). The purpose of covering the top of the bacterized seeds with compost is to keep them in a dry climate without losing a certain amount of moisture, along with protecting them from sunlight. The climate of Khorezm region is dry and moisture plays an important role in the development of biological processes in the soil.

Figure 2. Seeds that have been bacterized and covered with compost

It was taken into account that humid microorganisms and seeds provide moisture in the conditions of the desert zone, where the climate is dry, and protect from bactericidal sunlight during involuntary sowing. Seeds were sown in special drills. Rhizosphere microorganisms were analyzed for variants during each growth period of the plant. (Table 4) Experimental results showed that the number of bacteria that grow if the meat peptone agar is high during the seedling and cocoon formation phase of the cotton. MPA-Meat peptone agar was higher in the number of bacteria growing than in the control variant or in the variants used in phosphorbacteria in the 3-4 leaf extraction phase. This process was observed to be higher in the control variant as opposed to in the variants in which fungi were used. The number of actinomycetes and cellulose decomposers in the rhizosphere of cotton decreased to the bactericidal variants before the seedling phase. During the phases of flowering and the formation of cotton balls, a massive decrease in their number was observed. An increase in the

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number of bacteria belonging to another group studied was observed.

Table 4

Distribution of agronomically important microorganisms in the root system of cotton by its growth phases in the unwashed saline field

Variants	Physiological groups of microorganisms					
	MPA	Fungi	Actinomycetes	Nitrifier	Cellulose breakers	Ashby
Sprouting 3- 4 leaves						
Control	1350	60	81	53	60	100
FMB	1490	55	110	55	30	480
50% fertilizer	1953	12.5	92	55	60	300
50%+ FMB	1420	51	96	76	77	450
100% fertilizer	1490	86	82	102	30	510
100% fertilizer + FMB	1319	45	96	180	32	510
Seedling phase						
Control	1400	87	65	100	55	150
FMB	2800	66	96	200	28	520

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50% fertilizer	2120	58	90	150	55	380
50%+ FMB	1950	47	86	200	53	720
100% fertilizer	1950	95	71	200	30	600
100% fertilizer + FMB	2860	60	85	220	28	680
Flowering phase						
Control	1300	89	110	50	10	92
FMB	2450	57	270	72	32	680
50% fertilizer	1490	58	270	87	25	420
50%+ FMB	1420	42	360	92	53	740
100% fertilizer	1740	71	320	110	30	740
100% fertilizer + FMB	2560	51	830	102	55	940
Phase of cotton ball formation						
Control	2400	79	120	28	100	86
FMB	4260	82	380	180	200	620

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50% fertilizer	3300	90	260	55	100	360
50%+ FMB	5600	118	360	76	180	640
100% fertilizer	3600	77	330	96	200	420
100% fertilizer + FMB	4700	89	380	180	300	620

During the flowering phase, the number of cellulose-degrading bacteria increased only in the bacterized variants. There was also a small increase in the number of bacteria growing in the Ashby nutrient medium.

The effect of FMB on cotton yield was studied (Fig. 5) in which an increase in yield was observed with the use of phosphorylating bacteria. In all the variants studied, the application of FMB yielded high in its unused 50% fertilizer and 100% fertilizer applied variants.

Figure 5. Influence of FMB on cotton yield

Mineral fertilizers were not used in the control and FMB variant. At the same time, only in the variant using FMB, the yield increased by 2.7 centner. When ($N_{100}P_{70}K_{50}$) -50% fertilizer was used, the yield increased by 2.2 centner compared to the usage of FMB with 50% fertilizer. When (N_{200} P_{140} K_{100}) -100% fertilizer was used, the yield was 2.4 centner higher than when compared to the variant using FMB applied with 100% fertilizer.

Conclusion

Bacteria that mobilize active mineral phosphates in the cotton rhizosphere of saline soils of Khorezm region are gram-negative bacteria that belong to the genus Bacillus, they belong to the genus Baccilus sp. Baccilus subtillis.

They form a lysis zone of 66-70% of Rhizoctonia sola, Fusarium sola phytopathogenic fungi. They form a lysis zone of 44–55% and 16–30%, respectively, of Verticillium dahlia, Alternaria alternata phytopathogenic fungi.

They were observed to increase the water solubility of phosphates as a result of the acidic conditions of Rn in aqueous solution.

It was observed that the number of rhizosphere microorganisms in the growth phases of cotton increased with the number of microorganisms of agrobiological importance in the variants using phosphobilizing bacteria. An increase in cotton yield was observed in the variants using FMB.

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