Study of the properties of culture fluids of strains of bacteria of the genus azotobacter

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Abstract. Soil microorganisms of the genus Azotobacter are characterized by a number of positive effects on plants, among which the determining ones are the ability to fix molecular nitrogen of the atmosphere, the synthesis of substances of hormonal nature, as well as vitamins and substances of antibiotic nature. Due to the high biological activity, Azotobacter seem promising for use in plant growing. In this regard, the prospects of conducting work revealing the possibility of using new strains of microorganisms of the genus Azotobacter in the practice of plant growing and biological farming for the creation of bacterial preparations are quite obvious.

1 Introduction

One of the current areas of development of ecological farming is the creation of microbial biotechnologies that promote the intensification of agricultural production and the preservation of soil fertility. Microbiological factors are of great importance for the modern farming system, the use of which makes it possible to significantly increase soil fertility and the degree of realization of the genetic potential of cultivated plants. A necessary condition for the development of ecological farming is the creation of methods and technologies for the formation, maintenance and effective functioning of highly integrated microbial-plant systems that combine the beneficial properties of both plants and microorganisms. From this point of view, the creation of multicomponent systems in the soil that reproduce optimal natural agrophytocenoses and ensure high sustainability of agriculture is promising. Research aimed at creating highly productive agrophytocenoses by selecting active complementary partners (plant + microorganisms) is relevant for crop production [1-5].

Increasing the yield of agricultural crops largely depends on their provision with mineral nutrition elements, primarily nitrogen. The economic and environmental crisis, the decline in the quality of plant products, and the decline in natural soil fertility are causing increasing attention to biological farming, the essence of which lies in the use of the potential of natural ecosystems, in particular nitrogen-fixing microorganisms [5-12].

One of the most relevant applications of biotechnology in this area is the production of various types of bacterial fertilizers, since the soil microflora has a direct impact on its fertility and, as a result, on plant productivity. Soil microorganisms improve the structure of the soil during growth, accumulate nutrients in it, mineralize various organic compounds,

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turning them into easily digestible plant nutrition components. To stimulate these processes, various bacterial fertilizers are used, enriching the rhizosphere of plants with useful microorganisms. Microorganisms used to produce bacterial preparations help supply plants not only with mineral nutrition elements, but also with physiologically active substances (phytohormones, vitamins, etc.) [13-17].

It has now been proven that the use of bacterial fertilizers not only helps to increase the yield of valuable crops, but also significantly reduces the environmental impact of chemical compounds - mineral fertilizers and plant protection products, which allows for more efficient use of limited land resources and less effort to restore them [18-21].

2 Materials and Methods.

The strains of Azotobacter chroococcum A-8A and A-21 isolated from saline soils of the Bukhara region were used in the work. These microorganisms were grown in Ashby medium. The number of viable cells in the suspension was determined by seeding from tenfold dilutions on Ashby agar medium with counting the grown colonies (CFU/ml).

The experiment was conducted in three repetitions for each variant of seed treatment.

3 Results and Discussion

The first stage of the work was the isolation of Azotobacter strains from samples of saline soils of the Bukhara region. The results are presented in Tables 1 and 2. The presence of typical colonies of Azotobacter strains was revealed in 2 samples isolated from saline soils of the Bukhara region.

At the next stage, the cultural characteristics - morphological features of colonies and cells, physiological and biochemical characteristics of the isolated strains were studied in order to confirm their belonging to the genus Azotobacter and certain species. The results are given in Tables 1 and 2. It was established that the isolated bacterial strains from various soil samples, according to key morphological and physiological and biochemical characteristics, belonged to the genus Azotobacter and the species Azotobacter chroococcum (Table 1).

Table 1. Characteristics of isolated Azotobacter strains

Sample №	Mobility	Pigment	Use of carbohydrates		Cysts	Capsules
			starch	beckons		
8	+	dark brown	+	+	+	+
21	+	dark brown	+	+	+	+

Table 2. Morphological features of Azotobacter colonies

Sample №	Size, mm	Form	Surface	Gloss/Transparency	Color	Diffusion of pigment into the medium
8	2-3	Round with a smooth edge	Smooth	Shiny cloudy	Dark brown	-
21	3-5	Round with wavy edge	Smooth	Shiny cloudy	Dark brown	-

Determination of the sensitivity of isolated bacteria to antibiotics. The next stage of the work was to determine the levels of antibiotic resistance of the Azotobacter chroococcum strains isolated by us. In setting this task, we proceeded from the following well-known concepts that in nature, microorganisms exist in close associations with each other and in the process of evolution, certain groups of microorganism species inhabiting the soil and other natural substrates have developed various mechanisms of adaptation to environmental conditions and to its inhabitants. This led to the colonization of various ecological niches by microbial communities. In these communities, microorganisms are linked by energy chains and experience mutual influence. The relationships between community members are complex and dynamic due to constant changes in environmental conditions and the physiological variability of the microorganisms themselves. The relationships are regulated by the production of either substances stimulating the viability of the associate or inhibiting viability. This regulation is necessary in natural conditions to maintain the number and optimal species composition of the population. The production of antibiotic substances by natural microflora in natural conditions affects associates in different ways - they either die if there are no adaptive mechanisms, or overcome the adverse effects.

How do nitrogen-fixing bacteria react to the action of antibiotics? This issue is not discussed in modern literature. However, it is of undoubted interest for understanding the specifics of the ecology of nitrogen fixers and the mechanisms of their adaptation to adverse effects. The sensitivity of Azotobacter strains was determined to natural (producer microorganisms) and synthetic antibiotics using the paper disk method. The results of the study are presented in Table 3.

Table 3. Sensitivity and resistance to antibiotics of Azotobacter chroococcum strains

№	Name of the antibiotic	Azotobacter chroococcum strains		
		Sample 8	Sample 21	Reference strain
Growth inhibition zone, d (mm)				
1.	Carbenicillin	1-2	-	-
2.	Rifampicin	7-9	10	25
3.	Gentamicin	5-6	6	30
4.	Lincomycin	-	-	-
5.	Streptomycin	12-13	13	46
6.	Oleandomycin	9-10	7-9	30
7.	Levomycetin	6-7	8-9	22
8.	Fusidin	2-3	4-5	15-17
9.	Neomycin	4-5	8-9	20
10.	Kanamycin	10	10-11	32
11.	Ristomycin	5-6	1-2	-
12.	Polymyxin M	3-4	1-2	-
13.	Erythromycin	14-15	15	34
14.	Oxacillin	4-5	-	-
15.	Cephalexin	10	5-7	10
16.	Bacitracin	-	-	12
17.	Furazolidone	5	7-9	33
18.	Novobiocin	-	-	-

It was established that the strain Az. chroococcum pr 8 was resistant to 3 antibiotics (lincomycin, novobiocin and bacitracin) and sensitive to 15 others.

Resistance of the strain Az. chroococcum pr 21 was determined by five antibiotics of natural and synthetic origin (carbenicillin, lincomycin, oxacillin, bacitracin and novobiocin) and sensitive to 13 antibiotics.

Resistance of the reference strain was noted to 6 antibiotics (carbenicillin, lincomycin, ristomycin, polymyxin M, oxacillin and novobiocin) and sensitivity to 12 antibiotics.

Resistance to lincomycin, bacitracin and novobiocin was detected in both studied strains of Azotobacter.

Thus, based on the data obtained, it can be assumed that nitrogen-fixing bacteria Az. chroococcum have certain adaptation mechanisms that help maintain viability in unfavorable conditions of exposure to biotic factors.

Study of the effect of bacteria of the genus Azotobacter on seed germination and the formation of tomato sprouts.

Soil microorganisms of the genus Azotobacter are characterized by a number of positive effects on plants, among which the determining ones are the ability to fix molecular nitrogen of the atmosphere, the synthesis of substances of hormonal nature, as well as vitamins and substances of antibiotic nature. Due to high biological activity, bacteria of the genus Azotobacter seem promising for use in plant growing.

In this regard, it is quite obvious that it is promising to conduct work that reveals the possibility of using new strains of microorganisms of the genus Azotobacter in the practice of plant growing and biological farming to create bacterial preparations.

Taking this into account, the aim of our work was to investigate the ability of some bacteria of the genus Azotobacter to synthesize biologically active substances.

In the work, we used strains of Azotobacter chroococcum A-8 and A-21 isolated from saline soils of the Bukhara region. These microorganisms were grown in Ashby's medium. The number of viable cells in the suspension was determined by seeding from tenfold dilutions on Ashby's agar medium with counting the grown colonies (CFU/ml).

The culture liquid (CL) was freed from cells by centrifugation in a UPC-50 centrifuge for 30 minutes at 15 thousand rpm, thus obtaining a culture medium (CM). The effect of CL and CM on seed germination and seedling formation was determined by biotesting. The experiments involved tomato seeds, 100 of which were placed in plastic containers on filter paper pre-moistened with sterile tap water. Each experiment had three replicates (experiments were repeated three times). The KS and KS were diluted 1:10 and 1:100) with sterile tap water. Normally formed sprouts were counted on the 10th day.

We have studied the effect of the CL of bacteria of the genus Azotobacter on seed germination and the formation of tomato sprouts. Treatment of seeds of these plants with the CL of A. chroococcum A-8 significantly increases the above-mentioned indicators (Tables 6, 7): seed germination increases by 7.4%, and the length of sprouts - by 9.0-20.5%. Under the influence of undiluted CL of A. chroococcum A-8, the length of sprouts increases by 20%, when this liquid is diluted with water in a ratio of 1:10 - by 21.7%, and 1: 100 - by 17.3%. Apparently, a significant part of the influencing factor is in the CL, since it also significantly affects the studied indicator.

Table 4. The influence of the culture liquid of bacteria of the genus Azotobacter on the germination of tomato seeds of the Said variety

Variant of seed treatment with culture liquid	Length of sprouts	
	mm	% to control
Control (water)	57,5	100
A. *chroococcum 21 A*	60,0	104,3
A. *chroococcum 21 A(1:10)*	60,0	104,3
A. *chroococcum 21 A(1:100)*	61,6	107,1
A. *chroococcum 8- A* 3,0x108 cells/ml	64,3	111,8
A. *chroococcum 8 A* (1:10)	69,3	120,5
A. *chroococcum 8 A* (1:100)	62,7	109,0

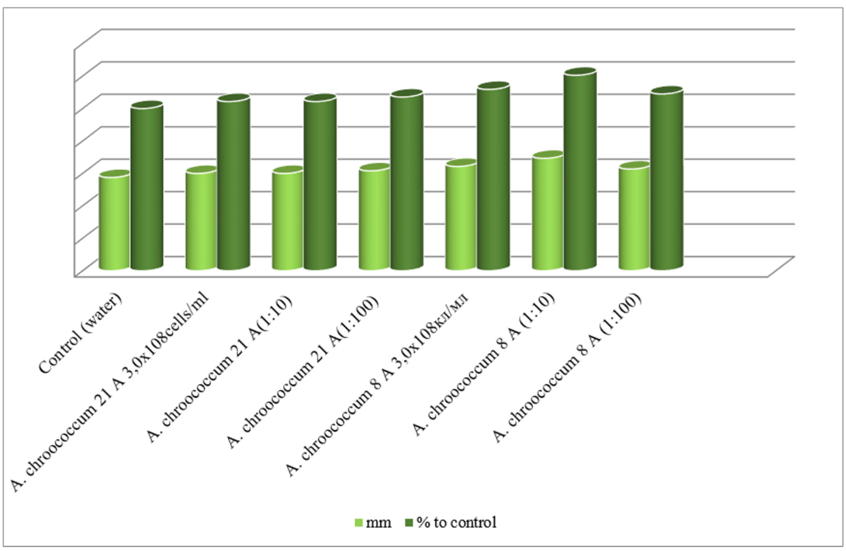

Figure 1. The effect of the culture liquid of bacteria of the genus Azotobacter on the germination of tomato seeds of the Said variety

Table 7. The influence of the culture liquid of bacteria of the genus Azotobacter on the germination of tomato seeds of the Said variety

Variant of seed treatment with culture liquid	Seed germination	
	Number of seeds, pcs.	% to control
Control (water)	90,8	100
A. chroococcum 21 A 3,0x108 cells/ml	88,8	97,8
A. chroococcum 21 A(1:10)	91,0	100,2
A. chroococcum 21 A(1:100)	90,2	99,3
A. chroococcum 8- A 3,0x108 cells/ml	92,5	101,9
A. chroococcum 8 A (1:10)	94,0	103,5
A. chroococcum 8 A (1:100)	94,8	107,4

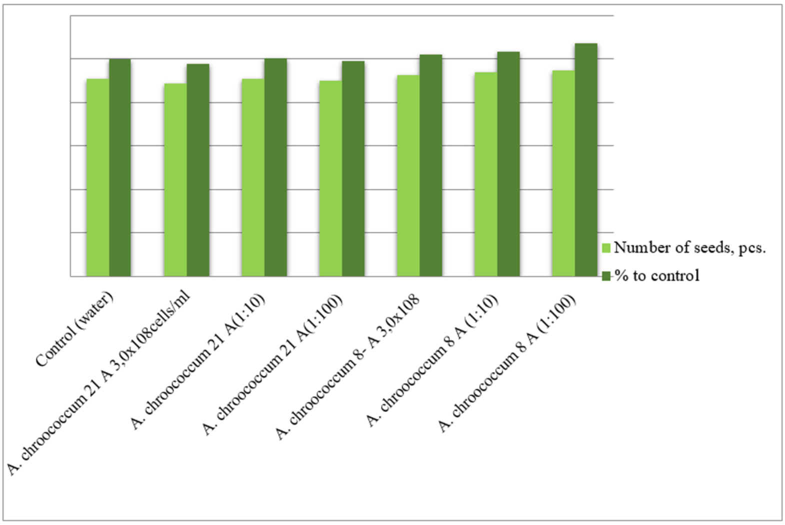

Figure 2. Diagram of the effect of culture liquid of bacteria of the genus Azotobacter on the germination of tomato seeds of the Said variety

The experiment was conducted in three repetitions for each variant of seed treatment.

According to the data obtained, A. chroococcum 21-A showed a result in two cases lower than the control by 2.2% and 0.7%. At 10 dilution, the result is higher than the control by 0.2%. A. chroococcum strain 8-A showed itself in seed germination significantly better, increasing the germination rates of tomato seeds by 1.9-7.4%. The best indicator was observed for A. chroococcum 8-A at the hundredth dilution.

4 Conclusion

According to literary data, the main activating effect of the use of Azotobacter as a bacterial fertilizer is associated not so much with its nitrogen-fixing ability, but with the production of various biologically active substances, in particular indole-3-acetic acid (IAA).

Based on the results we obtained from screening Azotobacter strains isolated from the saline soil of the collector for the synthesis of biologically active substances, in particular, the synthesis of IAA, the Azotobacter strain A. chroococcum A-8 has a high capacity for the synthesis and accumulation of 1.4•10-4 mg/ml of indoleacetic acid in 1 ml.

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