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SCREENING OF STRAINS OF BACTERIA OF THE GENUS AZOTOBACTER AND STUDY OF BROAD-SPECTRUM CHARACTERISTICS

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Abstract. *The study was designed to screen individual Azotobacter genera bacteria strains for a wide spectrum of properties and several physiological properties related to plant growth during experiments. In the experiments, the antagonistic properties against five different pathogens were tested.*

Studies were conducted against isolates of these five types of pathogens F oxysporum f sp vasinfectum, Fusarium oxysporum f sp lycopersici, Rhizoctonia solani. It showed a positive result in broad-spectrum biocontrol of isolated strains resistant to the development of five different pathogens.

In the extended antagonist test, these strains were observed together with bacteria of the Azotobacter genus as a biological control against major plant pathogens such as F oxysporum f sp vasinfectum, Fusarium culmorum, Fusarium graminearum, Fusarium oxysporum f sp lycopersici, Rhizoctonia solani.

Keywords. *Fusarium graminearum, Fusarium oxysporum, F. oxysporum f. sp. Lycopersici, R. solani, Azotobacter chroococcum, Antifungal analysis, nitrogen fixation, pathogen, PGPR.*

1. Introduction

Cultivation of crops by biological method is one of the urgent issues of today's agriculture, which is important not only for obtaining organic products, but also for improving soil quality and limiting the development of plant pathogens (1,4). As a solution, the use of plant growth-promoting rhizobacteria (PGPR) among beneficial soil microorganisms gives high results, because they can stimulate plant growth and provide biological control of plant diseases (3.4). Bacteria of the genus *Azotobacter* are being considered as a way to use biopesticides or biofertilizers, to reduce or supplement chemical pesticides, fertilizers (5,8).

As a method of biological control, some strains of bacteria of the *Azotobacter* genus show high results of antagonistic properties under the influence of metabolic products of biological control against pathogens (1,5,9).

Biological control with the help of antagonistic strains of bacteria of the genus *Azotobacter* showed antagonistic properties against many plant pathogens (for example, *F. oxysporum f. sp. vasinfectum, Fusarium culmorum, Fusarium graminearum, Fusarium oxysporum f. sp. lycopersici, Rhizoctonia solani, spp*.) (9,11).

However, most studies have focused on a single target pathogen. As plant growth promoting agents (PGPR), bacteria of the genus *Azotobacter* are often involved in nitrogen fixation, increased available phosphorus, production of plant growth regulators (indole-3-acetic acid (IAA), gibberellins and cytokinins) one or more common features can be observed such as synthesis. As a result of the transformation of insoluble phosphorus in the soil into soluble

phosphorus (HPO_4^{2-} or $H_2PO_4^-$), it plays an important role in supplying the plant with phosphorus (14).

IAA synthesis can increase root growth and root length, resulting in a larger root surface area, which allows the plant to take up more soil nutrients (9,12,14). It synthesizes small molecule proteins and antifungal compounds, chelates the Fe+3 cation in the rhizosphere, restricts the vital activity of phytopathogenic microflora (11,13). Biosurfactants synthesized by bacteria of the genus *Azotobacter* have been associated with improved soil quality by improving soil quality, increasing nutrient availability for PGPR, and eliminating plant pathogens (16).

Various environmental factors can affect the growth of *Azotobacter* bacteria strains and change their effect on the plant.

Promotion of research is an enabler of their biological cultivation of crops. This study was conducted to screen a large number of pathogen strains for their broad-spectrum biocontrol activity and several PGP markers. The objectives of this study were to: 1) screen 9 *Azotobacter* genera bacteria strains for phenotypes related to the biological control of multiple plant diseases and 2) multiple beneficial effects related to growth and development. testing strains showing broad-spectrum biocontrol activity for markers.

2. Materials and methods

2.1. Isolation of strains of bacteria of the genus *Azotobacter* from the rhizosphere of wheat.

In the isolation of *Azotobacter chroococcum* bacteria, soil samples were taken from the rhizosphere of wheat, 10 g samples of root parts were mixed in 90 ml of liquid nutrient medium, and collective cultures were obtained. In addition, soil samples were simultaneously mixed with solid Ashby medium (Sucrose-20, K_2HPO_4-0.2, $MgSO_4 \cdot 7H_2O$-0.2, NaCl-0.2, K_2SO_4-0.1, $CaCO_3$-10, dist. water-1 l, agar-20 , pH-6.9) in an incubation thermostat at 28^oC and monitored for one week.

2.2. Antifungal analysis

Bacterial isolates tested for antifungal activity were fully cultured in the appropriate nutrient medium. Test fungi were grown on Saburo dextrose agar (SDA), (per liter of distilled water: 40 g dextrose, 10 g peptone, 20 g agar). A diluted fungal spore suspension (0.1 ml, 10 5 CFU/ml) was inoculated onto Mueller Hinton agar (distilled water 1/l - 300 g beef broth, 17.5 g casein hydrolyzate, 1.5 g starch, 20 g agar, pH 7.2). Nutrient medium was used as organic control and 100 μg/ml antifungal antibiotic, nystatin, as comparison. Nutrient media were incubated in a thermostat at 28 ± 2 °C for 5-6 days. Antifungal activity indicator of *Fusarium oxysporum, R. solani, Fusarium culmorum, Fusarium graminearum, F. oxysporum f. sp. lycopersici* was evaluated by measuring the growth limitation zone against fungi.

2.3. Research on selected strains

Nine elite strains showing broad-spectrum antibiotic activity were selected to test properties related to plant growth: nitrogen fixation, phosphate solubility, IAA synthesis, and biomass formation were studied. Bacterial strains of the genus *Azotobacter* were purified in *Ashby's* medium for 2 days and then grown in Lysogeny broth (LB) for 48 hours. Each of these tests was repeated three times.

2.3.1. Nitrogen fixation

In the course of research, the nitrogen nitrogenase activity of bacteria of the genus Azotobacter is determined using the acetylene-reductase method. In this case, 10 ml of samples

are taken from strains grown in liquid *Ashby's* medium and placed in 10 ml vials. After sealing the vials with rubber stoppers, acetylene is injected using a syringe. Due to the active nitrogenase in the studied system, acetylene is reduced to ethylene. The formed ethylene was detected by a gas chromatograph LXM-80 with a flame-ion detector. Results were expressed in nanomoles.

2.3.2. Studying the solubility of poorly soluble phosphates

The study of quality dissolving properties of hard-soluble phosphates was conducted at the Shukhrat Umid servis farm belonging to "Art Soft Holding" LLC (Limited Liability Company) in the Pop district of Namangan region. Calcium phosphate ($Ca_3(PO)_4$) compound was obtained as an inorganic source of phosphate (Nautiyal, 1999). 10 ml of culture was placed on the surface of the medium and the development of a clear zone around the culture indicated the solubility of phosphate.

2.3.3. IAA synthesis during the growth and development of *Azotobacter* strains

Bacterial strains belonging to the genus Azotobacter were used in the research (3,7). The synthesis of IAA of these bacterial strains was studied by growing them in medium with L-tryptophan. A bacterial strain belonging to the genus Azotobacter was observed to produce IAA in culture fluids containing 0.5, 1, 1.5, 2, 2.5 and 3.0 mg/ml tryptophan.

2.3.4 Study of the effect of *A. chroococcum* on the growth and development of wheat during research.

"Aleksevich" and "Davr" wheat varieties were selected as plant sources for research. Seeds were first sterilized with 70% ethanol and then with 30% NaCl solution for 30 minutes. Sterilized seeds were repeatedly washed in normal tap water. Washed seeds were grown in petri dishes with moist filter paper for one day. Grown seed samples were placed in a 60 ml test tube containing 0.4% agar nutrient medium. 1-week-old cultures of Azotobacteria were used for seed inoculation [1,8]. The density of the bacterial suspension is 108 cell/ml. The plants were grown in a greenhouse at a temperature of 25^{o}C, under a light intensity of 1500 lk. After the plants were grown for 1 month, the plant samples were dried at room temperature for 7 days. Experiments were performed in triplicate.

Experiments in field conditions were carried out in the field of "Shukhrat Umid servis" farm belonging to "Art Soft Holding" LLC (Limited Liability Company) in Pop district of Namangan region based on the following scheme: 1) non-inoculated control option; 2) experimental variant inoculated with suspensions of azotobacter strains with a titer of 10^8 cell/ml for one week. The cultivated area is 10 m^2, the seed rate is 300 g per 10 m^2. The distance between the rows is 60 cm. The seeds were inoculated with bacterial suspensions before sowing. The seeds were sown at a depth of 3-5 cm at the end of September and harvested next year, at the beginning of June. Experiments were performed in triplicate.

3. Results

3.1. Morphology of local bacterial strains belonging to the genus *Azotobacter*

Two types of morphologically different colonies were isolated in Ashby nutrient medium. Morphological-physiological, biochemical (cell shape, formation of capsule mucus, staining according to *Gram's* reaction, catalase indicator) characteristics of the colonies showed that they belong to the genus *Azotobacter*.

Nitrogen-free solid *Ashby* medium was used to isolate azotobacteria present in the obtained soil samples. As a result of the experiments, bacterial colonies morphologically consistent with azotobacteria grew in almost all nutrient media (colony color, mucus production). As a result of

replanting, 9 bacteriologically pure *Azotobacter* cultures were isolated. Bacteriologically pure cultures formed 2-10 mm diameter colonies of dark-brown, yellow and light brown color with specific shiny mucus. The resulting cultures were numbered based on the area where the soil sample was taken.

3.2. Antifungal activity of test isolates

Antifungal activity of *Azotobacter chroococcum* strain against *Fusarium oxysporum, R. solani, F. culmorum, F. graminearum, F. oxysporum f. sp. lycopersici*. Experiments were conducted against phytopathogens. *Fusarium oxysporum, R. solani, F. culmorum, F. graminearum* fungi using SDA (Table 3).

Table 3.2.1.

Antifungal activity of strains in experiments

Test isolates	Media used	Zone size				
Empty cell	Empty Cell	*F.oxysporum*	*R. solani*	*F. culmorum*	*Fusarium graminearum*	*F.oxysporum f. sp. lycopersici*
A. chroococcum	SDA	31.50±0.50	21.00±1.00	16.67±0.58	17.67±0.58	16.50±0.50
Bacillus subtilis	SDA	32.33±0.58	21.00	16.50±0.71	19.00	17.83±0.76
A. chroococcum + Bacillus subtilis	SDA	33.00±2.00	22.00	18.00±1.00	19.50±0.50	18.67±0.58

Antifungal activity of strains *A. chroococcum* with a diameter of 16.50 to 31.50 mm was observed in the experiments. *Bacillus subtilis* and *A. chroococcum* + strains of *Bacillus subtilis* when used together resulted in the growth restriction of a relatively larger pathogen. The result of this is that the antagonistic properties of *Bacillus subtilis* bacteria are known, but it has been studied that the use of *Azotobacter* genera bacteria strains gives good results in limiting the development of pathogens.

3.3. Testing the activity of strains in vitro

During the research, each selected strain was observed with a decrease in the activity of acetylene-reductase under conditions of nitrogen fixation under cell stress conditions, phosphate solubility was studied by forming soluble complexes with metal ions, and IAA synthesis was studied with an increase in the amount of tryptophan in culture fluids. Recent studies have revealed changes in phenological characteristics such as biomass, root branching and formation of lateral roots, weight of 1000 seeds and productivity of cultivars under the influence of strains.

3.3.1. Studying the effect of *Azotobacter chroococcum* strain on the solubility of difficult soluble phosphates

Phosphorus-synthesizing microorganisms play important roles in three major components of the soil *P* cycle (ie, dissolution–precipitation, sorption–desorption, and mineralization–immobilization).

Inorganic *P* solubilization by *P*-solubilizing microorganisms occurs mainly through organic acid production:

(1) by lowering *pH* or (2) by increasing the chelation of cations bound to P (3) by competing with P for adsorption sites in the soil. (4) P is synthesized by forming soluble complexes with metal ions associated with insoluble *P* (*Ca, Al, Fe*).

During the studies, it was observed that the decrease in the *pH* value of the environment led to the synthesis of organic acids by *P*-decomposing microorganisms.

Organic acids are mainly considered as products of microbial metabolism through oxidative respiration or fermentation of organic carbon sources. In scientific studies, as a result of anion exchange of phosphate with acid anion, heavy forms of mineral phosphorus are dissolved, or *Fe, Al* and *Ca* ions bound with phosphorus are chelated by bacteria of the *Azotobacter* genus. At the time of research, monovalent anionic phosphate $H_2PO^-_4$ is the main soluble form of inorganic phosphate, which usually occurs at low *pH*, but as the *pH* of the soil environment increases, divalent and trivalent forms of phosphorus (HPO^-_2 and HPO^-_3) occur. arrival was studied.

In the course of research, it was found that as a result of the synthesis of organic acid by phosphorus-synthesizing strains, the cells and the environment around them become an acidic environment, which not only causes a decrease in the kinetic forms of phosphorus, but also the limitation of the kinetic forms of other substances necessary for the plant. In addition, the release of *P* ions from the *P* mineral by replacing the phosphate-bound cation with H^+ was studied. Phosphate-solubilizing microorganisms produce a variety of organic and inorganic acids that dissolve by lowering the *pH*.

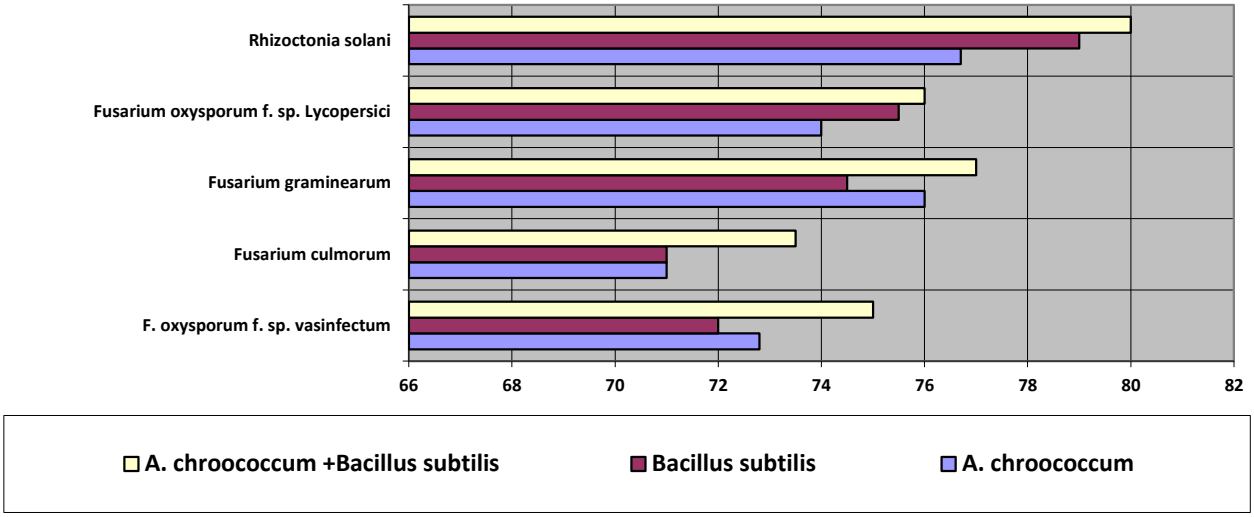
3.2. Antagonistic properties of selected strains

The research was continued with the study of antagonistic properties of cultured strains of bacteria belonging to the genus *Azotobacter*. In this case, bacteria belonging to the genus *Azotobacter* showed the development of more than 73% of isolates of R. solani; 65% of the strains inhibited the growth of *Fusarium oxysporum* subspecies; 54% strains.

Seven of these *Azotobacter* bacterial strains were antagonistic to the growth of all 9 pathogens, and 9 strains were observed to limit the growth of each pathogen. Bacterial identification showed that the bacterial strains belonging to the genus *Azotobacter* belonged to the genus *Azotobacter: Pseudomonadaceae* (*A. chroococcum*) and *Bacillales* (*Bacillus subtilis*).

During the research, each strain was tested for antagonistic activity. 9 strains based on *Azotobacter* bacteria produced pathogen inhibition zones when each pathogen was tested in 3 replicates, a total of 15 for each pathogen.

However, *A. chroococcum + Bacillus subtilis* showed a strong antagonistic property against pathogens tested together. *A. chroococcum* strains showed moderate inhibitory properties to the tested pathogen.



3.2.1. Determination of plant growth promoting properties of selected strains.

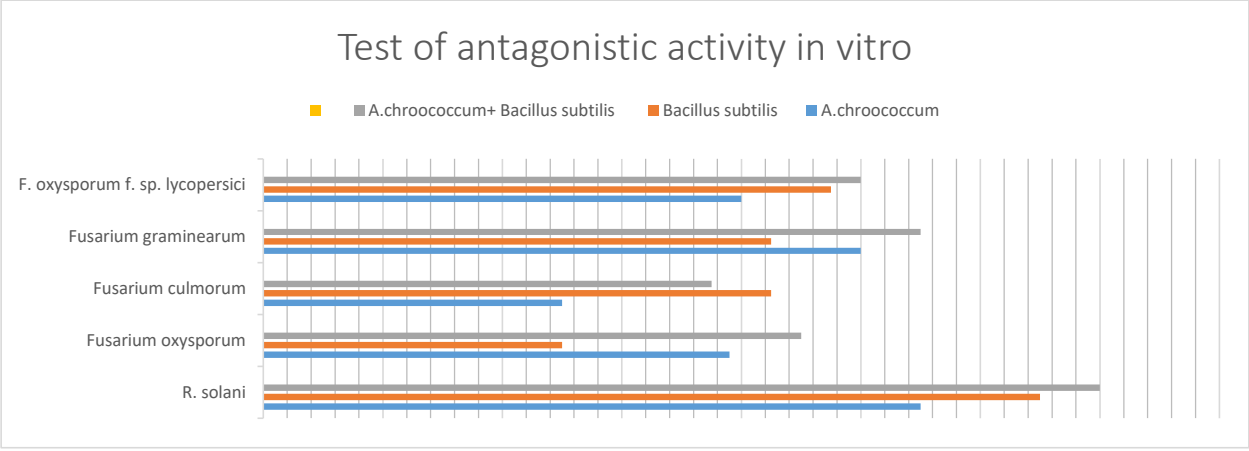
3.2. Test of antagonistic activity in vitro

Overall, more than 77% of the *A. chroococcum* strain was observed to be strongly restricted to *R. solani*; 72% of *Bacillus subtilis* inhibited the growth of *Fusarium oxysporum* species; 79% of strains were *R. solani*, 71% *Fusarium culmorum*, 74.5% *Fusarium graminearum*, 75.5% *F. oxysporum f. sp. lycopersici* inhibited the growth of pathogens.

In addition, a combined 75% of all tested *A. chroococcum + Bacillus subtilis* inhibited the growth of *Fusarium oxysporum* species; 80% of strains were *R. solani*, 73.5% *Fusarium culmorum*, 77% *Fusarium graminearum*, 76% *F. oxysporum f. sp. lycopersici* was observed strong antagonistic properties of pathogens.

Table 3.2.

Indicator of antagonistic activity during the study.



Conclusion

It can be concluded from the conducted experiments that along with mineral nitrogen, phytohormonally active strains dramatically increase the growth, development and productivity of plants by synthesizing phytohormones, including IAA and gibberellin-like substances, and restricting phytopathogens to Azotobacter strains. In addition, the biopreparation prepared on the basis of A. chroococcum K1 strain does not have a negative effect on microbial populations in the plant rhizosphere. *Azotobacter* strains selected in the studies can be widely used for the purposes

of soil bioremediation, increasing the yield of corn and other agricultural crops grown in different soils of Uzbekistan.

REFERENCES

1. Pattaev A. A. ORGANIC CONTROL OF POTATOES //Ekonomika i sotsium. – 2021. – №. 8. – S. 185-188.
2. Abdugafurovich R. B., Abdusattorovich P. A. IMPORTANCE OF EPSS SYNTHESIZED BY MICROORGANISMS IN SOIL SALINITY AND PRODUCTIVITY //ResearchJet Journal of Analysis and Inventions. – 2021. – T. 2. – №. 04. – S. 306-310.
3. Pattaeva M.A, Pattaev A.A, & Rasulov B.A. (2021). STUDY OF THE PHYSICOCHEMICAL PROPERTIES OF EPS SYNTHESIZED BY THE RH.RADIOBACTER STRAIN AND THE BIOSORPTION ACTIVITY OF NaCl SALT UNDER CONDITIONS OF DIFFERENT SALINITY. *Innovative Technologica: Methodical Research Journal*, *2*(05), 32–35. https://doi.org/10.17605/OSF.IO/J8UVX
4. Pattayev Akmaljon Abdusattorovich. (2021). SYNTHESIS OF METABOLITES OF THE GENUS FUNGUS FUSARIUM OXYSPORUM f.sp. VASINFECTUM. *JournalNX - A Multidisciplinary Peer Reviewed Journal*, *7*(12), 269–273. https://doi.org/10.17605/OSF.IO/MD869
5. Abdusattarovich P. A., 2020. – T. 1. – №. 5. Antifungal properties of diazotrophic bacteria // International journal of discourse on innovation, integration and education. – (S. 331-334.)
6. Kanmani P, Lim ST (2013a) Synthesis and structural characterization of silver nanoparticles using bacterial exopolysaccharide and its antimicrobial activity against food and multidrug resistant pathogens. Process Biochem 48:1099–1106.
7. Расулов Б.А. Бактерии рода *Азотобастер* - продуценты фитогормонов в условиях закисления: Дис. канд. биол. наука. - Ташкент: Институт микробиологии АН РУз, 2010. - 120 с. Rayllo I.A. Грибы рода фузариум. М.: Изд-во АН СССР. 1950. 456 с.
8. Билай В.И. Фузарии. Киев: Наукова думка. 1977. 439 с.
9. Паттаева М.А., *Aspergillus* авлодига мансуб термофил ва термотолерант замбуруғларнинг ўсимликларни ўсишини фаоллаштирувчи хусусиятлари // Биология фанлари бўйича фалсафа доктори (PhD) диссертацияси автореферати. – Тошкент, 2019. – 45-б.
10. Соловева А.И. Метод создания провокационного фона. в кн .: «Сборник научных работ. Вредители и болезни хлопчатника и другие культуры »// СоюзНИХИ, - Ташкент, 1951. - С. 151-158.