Isolation and selection of nodulating bacteria from the rhizosphere of legume (mash - Vigna radiata) and bean (Phaseolus lunatus) plants and to enhance specific properties through induced mutagenesis methods

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Abstract. The aim of the research is to create a collection of PGPR properties of bacteria for legume plants, as well as to increase their growth—stimulating activity by methods of induced mutagenesis.In this article, studies have been conducted on the isolation of microorganisms from the rhizosphere of legumes. And also, to increase the PGPR growth-stimulating properties of rhizosphere microorganisms using methods of physical and chemical mutagenesis. A collection of active rhizosphere bacteria has been created from 4 strains of the genus *Bacillus megaterium*, *Arthrobacter globiformis, Arthrobacter crystallopoietes and Bacillus spp*. During the screening, bacteria with PGPR properties *Bacillus megaterium, Arthrobacter globiformis,* and *Arthrobacter crystallopoietes* were isolated, as these strains stimulated the growth of legume plants.

1 Introduction

One of the main problems of the agro-industrial complex is the decrease in the fertility of the humus soil cover, and in this regard, there is a growing interest in a biotechnological product based on PGPR properties to bacteria. To determine the effect of PGPR properties of microorganisms, a variety of methods are used, based on biochemical, physiological and growth-stimulating processes in the studied crops. According to the literature, the rhizospheric PGPR properties of bacteria in the rhizosphere of legumes of masha and beans are still poorly studied [1-4].

2 Materials and methods

2.1 Isolation of rhizospheric bacterial strains

The PGPR properties of rhizospheric microorganisms that promote plant growth and development were isolated in a universal medium, peptone agar.

2.2 Induced mutagenesis

Mutagenesis methods were used to improve the PGPR properties of rhizospheric microorganisms. After mutagenesis, it is possible to increase the hereditary variability of PGPR microorganisms by tens and hundreds of times, which facilitates and accelerates the screening process of active strains of PGPR microorganisms.
The article presents the main stages of obtaining PGPR microorganisms by mutagenesis, the first one is the selection of the initial strain of microorganisms; pre-selection of natural strains of PGPR bacteria; preparation of strains of PGPR properties of microorganisms for mutagenic treatment; mutagenesis of PGPR properties of microorganisms; screening of promising mutant PGPR microorganisms with improved growth-stimulating properties. Ultraviolet rays with a wavelength of 255 nm were used for the physical mutagenesis of PGPR microorganisms. When treating PGPR strains of microorganisms with UV radiation using a UV lamp, radiation is given predominantly with a wavelength of 255 nm, test tubes with bacterial suspension of PGPR strains of microorganisms are placed in such a way as to ensure maximum isotropy of UV radiation in the sample during irradiation, for which, during irradiation of the liquid, it is stirred with a magnetic stirrer. Before irradiation, the lamp is kept on for 10 minutes. At a distance of 32 cm from the center of the lamp, the exposure time to achieve survival is from 30 to 150 seconds. Treatment with a mutagen solution using the example of a nitroso compound: A solution of N-nitroso- N-ethyl urea mutagen with a concentration from 0.02% to 1.0% is prepared 10-15 minutes before treatment, dissolving the suspension in a citrate buffer (5.5 + 0.1) pH. 3.0 ml of the prepared N-nitroso-N-ethyl urea mutagen solution is placed in a sterile tube, 3.0 ml of citrate buffer (5.5 + 0.1) pH is placed in another (control) tube. 3.0 ml of culture of the selected strain of rhizospheric microorganisms prepared for mutagenic treatment is added to both test tubes. The test tubes are gently shaken, mixing the mutagen with rhizospheric bacterial cells and cultured at 28 ± 1 0 C at the optimal temperature for culture. After a certain time (from 1 to 6 hours), 1 ml is taken from them and dilutions from 10-1 to 10-7 are prepared, and a citrate buffer (8.0 + 0.1) pH is used for

dilution of 10-1 (for inactivation of the mutagen), and for subsequent dilutions a sterile physiological solution is used [5].
Identification and selection of promising mutants with improved properties. After treatment of the suspension, survival is calculated, isolated colonies are isolated from Petri dishes seeded with a mutagen-treated suspension, on which the number of CFU does not exceed 150. To do this, CFU/ml is determined by the cup method (1 ml of dilutions from 10-5 to 10-7) in a mutagen-treated and control suspension of cells of the selected rhizosphere strain. Colonies are isolated only if the survival rate was from 1 to 50%.

2.3 Testing of PGPR properties of collectible crops

The objects of research were promising mutant strains of *Bacillus megaterium*, *Arthrobacter globiformis*, and *Arthrobacter crystallopoietes*. Legume seeds were treated with PGPR culture liquid and microorganisms. The assessment of growth-stimulating activity was determined by a test for the ability of mutant strains to stimulate plant growth. Inoculated plants were cultivated under the conditions of the Binder KBF 720 continuous plant growth chamber (Germany) at a temperature of 24 ° C, humidity of 60% and constant illumination of 14200 lux for 20 days.

2.4 Primary identification of PGPR properties of microorganisms

The primary identification was carried out by the phenotypic characterization of PGPR properties of microorganisms according to the Bergey determinant [6].

2.5 Identification of PGPR properties of microorganisms was determined by the PCR method

The molecular identification of PGPR properties of microorganisms was determined by PCR analysis [7].

2.6 Statistical analysis

The collected information was analyzed using R (3.3.1), SPSS 16.0 and GraphPad Prism 5.0 with an average probability of 5%. For repeated measurements, the analysis of variance was obtained using the Duncan criterion.

3 Results and Discussion

3.1. Isolation and breeding of P PGPR bacteria from the rhizosphere of legume crops of *Vigna radiata* and *Phaseolus lunatus* plants and increasing the activity of isolated isolates by induced mutagenesis

The selection of rhizospheric PGPR properties of microorganisms was carried out from the rhizosphere of legumes in the conditions of Southern Kazakhstan in the fields of the Manshuk farm. 20 isolates of rhizosphere microorganisms were isolated from the rhizosphere of masha, 33 isolates of rhizosphere microorganisms were isolated from the rhizosphere of beans.

3.2 Effect of induced mutagenesis on PGPR properties of isolates of rhizospheric microorganisms

It was established at a distance of 50 cm from the center of the lamp, the exposure time of survival of 5 minutes of isolates of rhizospheric microorganisms on three strains is 1%.
When treated with a chemical mutagen after a time of 6 hours, the survival rate of isolates of rhizospheric microorganisms on three strains is 1%. In many cases, induced mutagenesis is not only of scientific interest, but also makes it possible to practically obtain a biological product based on stable valuable mutants with improved PGPR properties.

3.3. Testing PGPR properties of collectible crops

The objects of research were promising mutant strains of *Bacillus megaterium, Arthrobacter globiformis, and Arthrobacter crystallopoietes*. Legume seeds were treated with PGPR culture liquid and microorganisms. The assessment of growth-stimulating activity was determined by a test for the ability of mutant strains to stimulate plant growth.

Treatment of legume seeds with mutant strains of *Bacillus megaterium, Arthrobacter globiformis, and Arthrobacter crystallopoiete*s increased seed germination by 30% compared to the control variant (treated with sterile water).

The maximum result was established in the variant using *Bacillus megaterium* – the length of the shoot and root was 12.8 mm (27.0%) and 10.0 mm (15.9%).

3.4. The phenotypic characterization of bacterial isolates was carried out in accordance with the recommendations of Bergey

Cultural and morphological features: Gram-positive. Aerobes or facultative anaerobes. Straight sticks, 0.5 -1.8 microns in size, with rounded or "chopped off" ends, often arranged singly, in pairs or in chains. Sporulation: Forms endospores, oval or sometimes spherical or cylindrical endospores, highly resistant to many unpleasant influences. No more than one spore forms in a cell. Endospores in young cultures are rod-shaped; the filament does not form; Sporulation is not suppressed in the air atmosphere. Division type: simple. Mobility: Mobile due to peritrichial flagella. The ratio of elevated temperature, salinity pH varies greatly. Chemoorganotrophs; fermentation or respiratory type metabolism. When cultivating the strain on MPA and potato agar for a day at (29 ± 10C), folded colonies of viscous consistency, dark beige color form. Physiological and biochemical features: Physiological and biochemical signs: It forms acid without gas from glucose, arabinose, xylose, maltose, lactose, mannitol, sucrose. Hydrolyzes polysaccharide and gelatin, does not hydrolyze urea. Catalase positive. When storing a pure culture on mowed MPA agar at a temperature (+4 ± 10C), the consistency and color of the microorganism does not change for 5 months.

3.5. Identification of bacterial PGPR strains was carried out by PCR analysis

Molecular genetic analysis is an important step in the study of nodule bacteria, allowing a deeper understanding of their genetic properties. Identification of bacterial PGPR strains was performed by PCR analysis. The isolation of PGPR properties of *Bacillus megaterium, Artrobacter globiformis, and Arthrobacter crystallopoietes* strains from the rhizosphere of legumes shows their survival to this habitat (Fig. 1-3) [6-7]. Genus Artrobacter they are commonly found in the rhizosphere of legumes, these bacteria are known for their ability to stimulate plant growth through nitrogen fixation and the production of certain enzymatic substances to stimulate plant growth. These microorganisms live in symbiosis with legumes, contributing to their growth and assimilation of substances from the rhizosphere. The population of *Bacillus megaterium* as an *Artrobacter* fixes nitrogen from the atmosphere, promotes plant growth and the distinctive properties of this species they can still solubilize phosphates helping legume plants to obtain available phosphorus for growth.

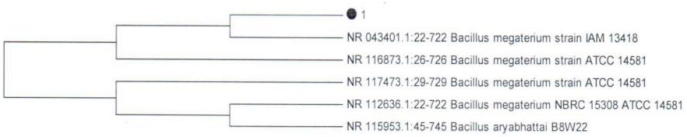

Fig. 1. *Phylogenetic* tree of *Bacillus megaterium* strain isolated from nodules of bean culture.

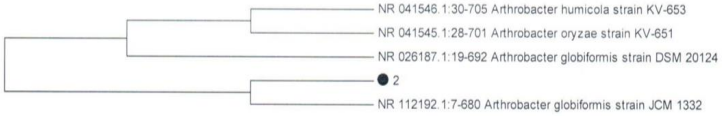

Fig. 2. *Phylogenetic* tree of the *Artrobacter globiformis* strain isolated from the nodules of the masha bean culture.

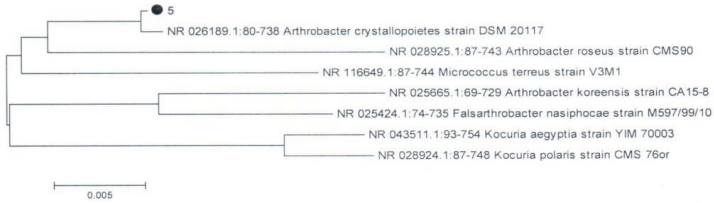

Fig. 3. *Phylogenetic* tree of the *Artrobacter crystallopoietes* strain isolated from nodules of the masha bean culture.

Thus, the collection of rhizospheric rhizobacteria properties of bacteria isolated from the rhizosphere of legumes and beans is a valuable biotechnological resource for the production of environmentally friendly organic fertilizers. As a result of the experiments, a collection of rhizosphere microorganisms was created, consisting of 3 strains of rhizobacterium bacteria isolated from beans and 1 strain of *Bacillus* cultures isolated from masha, 2 strains of *Artrobacter globiformis* and *Artrobacter crystallopoietes.*

4. Conclusion

In the conditions of Southern Kazakhstan, 20 isolates of rhizosphere microorganisms were isolated from the rhizosphere of masha in the fields of Amankeldi LLP, 33 isolates of rhizosphere microorganisms from the rhizosphere of beans.
It was established at a distance of 32 cm from the center of the lamp, the exposure time of survival of 150 sec of isolates of rhizospheric microorganisms on three strains is 1%.
When testing PGPR properties of collection crops with strains of *Bacillus megaterium*, *Arthrobacter globiformis, Arthrobacter crystallopoietes* increased seed germination by 30% compared to the control variant. The maximum result was established in the variant using *Bacillus megaterium* – the length of the shoot and root was 12.8 mm (27.0%) and 10.0 mm (15.9%).
A certain phenotypic characteristic of isolates in accordance with the recommendations of Bergey.
A phylogenetic tree constructed by comparing the 16S rRNA gene of the studied sample with sequences of reference strains hosted in the International Blast Database. The degree of homology with the nearest strain NR_026189.1 *Arthrobacter crystallopoietes* strain DSM 20117 was 99.3%, which allows the studied sample to be classified as *Arthobacter crystallopoietes.*

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