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## RESEARCH OF THE PROPERTIES OF PHOSPHATMOBILIZING BACTERIA PERSPECTIVE FOR INCREASING THE PRODUCTIVITY OF AGRICULTURAL GRAINS

Phosphorus is one of the main nutrients that ensure the formation of high and stable crop yields with a favorable quality of marketable products. One of the promising directions for improving the phosphorus nutrition of agricultural crops is biological phosphate mobilization with the help of soil microorganisms, which contributes to the transfer of sparingly soluble phosphorus compounds from the soil and fertilizers into a form accessible to higher plants. Aboriginal soil phosphate mobilizing bacteria were isolated from the rhizosphere soil of the northern region of the Republic of Kazakhstan. Of the five phosphate mobilizing bacteria: *Acinetobacter calcoaceticus* 2/5 pr, *Stenotrophomonas rhizophila* 1/7 c, *Pseudomonas koreensis* 4/5 kr, *Acinetobacter calcoaceticus* 1/5 pr, *Pseudomonas koreensis* 3/4 pr, three strains were selected: *Acinetobacter calcoaceticus* 2/5 pr, *Pseudomonas koreensis* 4/5 kr, *Acinetobacter calcoaceticus* 1/5 pr, with high soil phosphate mobilization activity. The properties of phosphate-mobilizing bacteria have been studied, their ability to stimulate plant growth and convert insoluble tricalcium phosphate  $\text{Ca}_3(\text{PO}_4)_2$  into a form accessible to plants has been established. The ability to phosphate mobilization and stimulation of plant growth determines the positive effect of the introduction of phosphate mobilizing bacteria on crop yields.

The selected strains of phosphate-mobilizing bacteria are promising as a basis for creating a complex biological fertilizer for a wide range of agricultural crops.

**Key words:** phosphate mobilization, soil, bacteria, growth stimulation, wheat seeds.

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### Ауыл шаруашылығы дақылдарының өнімділігін арттыру үшін перспективті фосфатмобилизациялаушы бактериялардың қасиеттерін зерттеу

Фосфор тауарлық өнімдердің қолайлы сапасымен жоғары және тұрақты ауыл шаруашылығы дақылдарының өнімін қалыптастыруды қамтамасыз ететін негізгі қоректік заттардың бірі болып табылады. Ауыл шаруашылығы дақылдарының фосформен қоректенуін жақсартудың перспективті бағыттарының бірі топырақ микроағзаларының көмегімен биологиялық фосфатты жұмылдыру болып табылады, бұл топырақтың және тыңайтқыштардың құрамындағы аз еритін фосфор қосылыстарын жоғары сатыдағы өсімдіктерге қолжетімді түрге көшіруге ықпал етеді. Қазақстан Республикасының солтүстік аймағының ризосфералық топырағынан аборигендік фосфатты жұмылдырғыш бактериялар бөлініп алынды. Бес фосфатты жұмылдыратын бактериялардан: *Acinetobacter calcoaceticus* 2/5 пр, *Stenotrophomonas rhizophila* 1/7 с, *Pseudomonas koreensis* 4/5 кр, *Acinetobacter calcoaceticus* 1/5 пр, *Pseudomonas koreensis* 3/4 пр, топырақ фосфатының жұмылдыру белсенділігі жоғары, үш белсенді штамм таңдалды: *Acinetobacter calcoaceticus* 2/5 пр, *Pseudomonas koreensis* 4/5 кр, *Acinetobacter calcoaceticus* 1/5 пр. Фосфатты мобилизациялаушы бактериялардың қасиеттері зерттелді, олардың өсімдіктердің өсуін ынталандыру және ерімейтін үшкальций фосфатын  $\text{Ca}_3(\text{PO}_4)_2$  өсімдіктерге қолжетімді түрге айналдыру қабілеті анықталды. Фосфатты мобилизациялау және өсімдіктердің өсуін ынталандыру қабілеті фосфатмобилизациялаушы бактерияларды енгізудің ауыл шаруашылығы дақылдарының өнімділігіне оң әсерін анықтайды.

Фосфатмобилизациялаушы бактериялардың таңдалған штамдары ауыл шаруашылығы дақылдарының кең ауқымдылығы үшін кешенді биологиялық тыңайтқыштарды құру үшін негіз ретінде перспективті болып табылады.

**Түйін сөздер:** фосфорды жұмылдыру, топырақ, бактерия, өсуді ынталандыру, бидай тұқымдары.

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### **Исследование свойств фосфатмобилизирующих бактерий, перспективных для повышения урожайности сельскохозяйственных культур**

Фосфор относится к основным питательным элементам, обеспечивающим формирование высоких и устойчивых урожаев сельскохозяйственных культур с благоприятным качеством товарной продукции. Одним из перспективных направлений улучшения фосфорного питания сельскохозяйственных культур является биологическая фосфатмобилизация с помощью почвенных микроорганизмов, способствующая переводу труднорастворимых соединений фосфора из почвы и удобрений в доступную для высших растений форму. Из ризосферной почвы северного региона Республики Казахстан выделены аборигенные почвенные фосфатмобилизирующие бактерии. Из пяти фосфатмобилизирующих бактерий: *Acinetobacter calcoaceticus* 2/5 пр, *Stenotrophomonas rhizophila* 1/7 с, *Pseudomonas koreensis* 4/5 кр, *Acinetobacter calcoaceticus* 1/5 пр, *Pseudomonas koreensis* 3/4 пр, отобраны три активных штамма: *Acinetobacter calcoaceticus* 2/5 пр, *Pseudomonas koreensis* 4/5 кр, *Acinetobacter calcoaceticus* 1/5 пр, с высокой активностью мобилизации фосфатов почвы. Изучены свойства фосфатмобилизирующих бактерий, установлена их способность стимулировать рост растений и переводить в доступную для растений форму нерастворимый трикальцийфосфат  $Ca_3(PO_4)_2$ . Способность к фосфатмобилизации и стимуляции роста растений определяет положительное влияние внесения фосфатмобилизирующих бактерий на урожайность сельскохозяйственных культур.

Отобранные штаммы фосфатмобилизирующих бактерий перспективны в качестве основы для создания комплексного биологического удобрения для широкого спектра сельскохозяйственных культур.

**Ключевые слова:** мобилизация фосфора, почва, бактерия, стимуляция роста, семена пшеницы.

#### **Abbreviation**

PMB – phosphate mobilizing bacteria;

PCR – polymerase chain reaction;

DNA – deoxyribonucleic acid;

rRNA – ribosomal ribonucleic acid.

#### **1. Introduction**

Phosphorus is one of the most important mineral elements in plant life which can absorb it only in an inorganic form, mainly in the form of phosphate ions [1,2,3]. Despite the high content of total phosphorus in the soil, its bioavailability is a limiting factor in the growth, development and productivity of plants; this phenomenon is called by many authors as the “phosphorus paradox” [4, 5]. Between the phosphoric solution, there is a significant concentration gradient of neo-organic orthophosphate and the cytoplasm of plants. In the first concentrations of

the phosphate ions, it rarely exceeds 10  $\mu$ M, while in plants the cytoplasmic concentration is probably about 10 mM of phosphate ions [6,7].

In agriculture, the problem of a shortage of available phosphorus in the soil is solved by regular application of mineral fertilizers. However, only 10-15% of the applied phosphorus fertilizers are assimilated by plants and most of them pass into a form that is difficult for plants to access or are washed out with groundwater [8]. An alternative to the non-ecological use of mineral phosphorus fertilizers to increase the yield of agricultural crops is the biological mobilization of phosphates from insoluble compounds through the use of phosphate-mobilizing microorganisms capable of converting insoluble phosphates from fertilizers and soil into a soluble form [9-17].

Grain farming is the main branch of agriculture in Kazakhstan. In recent years, the total sowing of grain crops has occupied over 80% of the sown area

of agricultural crops. The average seed yield is on average 10-13 c/ha. The country produces about 13.5 – 20.1 million tons of seed, which is a low figure in comparison with other countries. One of the reasons for this is the lack of phosphorus nutrition in plants. To solve this problem, we tried to isolate effective phosphate-mobilizing bacteria for creating a biological product.

The purpose of the study is the isolation of phosphate-mobilizing bacteria from the rhizosphere of plants, the selection and production of active strains that have a positive effect on the development of agricultural plants.

## 2. Materials and methods

### 2.1 Isolation of phosphate-mobilizing bacteria.

The object of the study was microorganisms isolated from the rhizosphere soil of agricultural plants in the Akmola region Republic of Kazakhstan. Soil samples for microbiological analyzes were taken from the root and root zones of plants in compliance with the rules of asepsis and placed in sterile parchment bags. The average soil sample was obtained by mixing three soil samples taken according to the “envelope principle”. Isolation of microorganisms from soil was carried out by sowing on elective nutrient media, that dissolve calcium orthophosphates were detected on solid nutrient media of Pikovskaya – M1 [18], Muromtsev – M2 [19], and NBRIP (National Botanic Research Institutes phosphate growth medium) with tricalcium phosphate – M3 [21].

Active strains were selected from colonies forming zones of phosphate dissolution. The culture medium prepared in this way was poured 25 ml into Petri dishes and, after the agar had solidified, 0.1 ml of soil suspension from dilutions  $10^{-4}$  –  $10^{-7}$  was inoculated superficially. The dishes were incubated at 28°C for 3 – 10 days. Colonies were taken into account, around which large zones of calcium phosphate dissolution were formed.

### 2.2 Identification of phosphate mobilizing bacteria

The identification of PMB strains was carried out by determining the direct nucleotide sequence of the 16S rRNA gene fragment, followed by determining the nucleotide identity with the sequences deposited in the international database GeneBank. Extraction of DNA followed by PCR and sequencing. Genomic DNA of strains was isolated using the Genomic DNA Purification Kit (Fermentas, Germany) according to the manufacturer’s instructions. The statistical significance of phylogenetic

reconstructions was assessed by the “jackknife” method (“Bootstrap”) by constructing alternative trees [22,23].

The identification of microorganisms was also carried out on the basis of comparing data on the composition and ratio of peptides. The peptides were obtained by hydrolysis of proteins in the colonies of the studied microorganism, with similar data of typical strains from the Maldi Biotyper database (Bruker Daltonic Germany) by mass spectrometry on MALDI-TOF, Microflex LT. The instrument was calibrated using a commercial protein mixture (Bruker Daltonics). On the basis of these data, the similarity (belonging to the same species and genus) of the studied organism with the organisms presented in the database was estimated [24,25].

### 2.3 Activity estimation of phosphate-mobilizing strains.

The evaluation of phosphorus mobilization of motile forms in a liquid medium under the action of the studied bacteria was carried out according to the method [19]. Sterile medium was poured into 100 ml flasks and inoculated with bacterial cultures. A non-inoculated medium served as a control. Then the flasks were incubated on a shaker for 15 days at a speed of 180 revolutions per minute at 28°C. The mobilization activity of mobile phosphorus was determined on the 3, 5, 7, 10 and 15 days after the start of the experiment. The concentration of phosphates in the solution was determined by the colorimetric method using the yellow phosphomolybdenum complex [26].

### 2.4 Biological activity of phosphate-mobilizing microorganisms.

For inoculation of seeds, 3-day-old cultures grown on a nutrient medium SPB were used [21]. The study of the effect of seed inoculation was carried out with phosphate-mobilizing microorganisms: (*Acinetobacter calcoaceicus* 2/5 pr (option 2 in the 5<sup>th</sup> sample of wheat rhizosphere soil), *Stenotrophomonas rhizophila* 1/7 c (option 1 in the 7<sup>th</sup> sample of beet rhizosphere soil), *Pseudomonas koreensis* 4/5 kr (option 4 in the 5<sup>th</sup> sample of maize rhizosphere soil), *Acinetobacter calcoaceicus* 1/5 pr (option 1 in the 5<sup>th</sup> sample of wheat rhizosphere soil), *Pseudomonas koreensis* 3/4 pr (option 3 in the 4<sup>th</sup> sample of wheat rhizosphere soil),. The density of the bacterial suspension is  $10^9$  cells / ml. To assess the effect of phosphate-mobilizing microorganisms on seed germination, the objects of study were the seeds of the “Rubin” radish variety (*Raphanus sativus* var. *Radicula*), the “Turkestan” cotton seeds (*Gossypium hirsutum* L.) and the “Astana 2” wheat

varieties (*Triticum aestivum* L.). The seed surface was sterilized with a 10% sodium hypochlorite solution for 20 min, after which the seeds were washed with 70% ethanol and three times with sterile distilled water.

Observing the rules of asepsis, the inoculated seeds were placed on a moistened filter paper in Petri dishes. As a control seeds soaked in sterile water and sterile medium were used for the cultivation of bacteria. Incubation was performed at 28°C. The experiment was repeated three times. All parameters were measured at least three times.

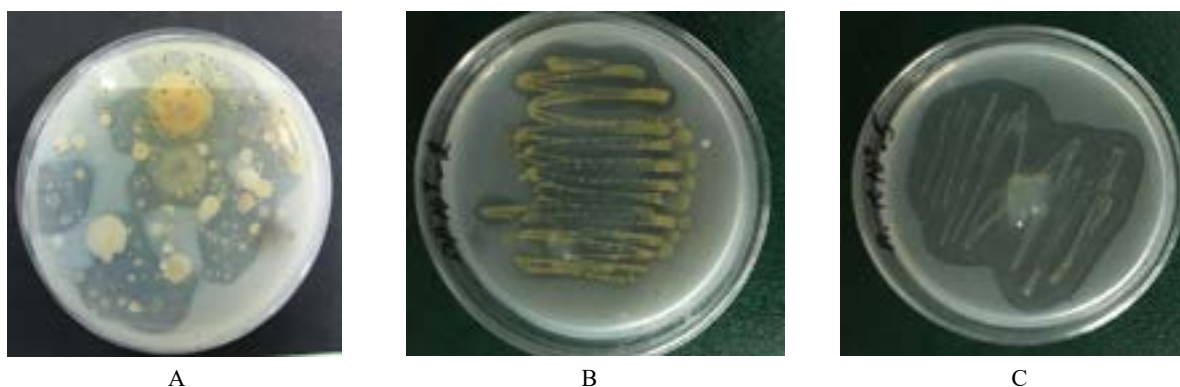
Static data processing was performed using Microsoft Office Excel 2007 spreadsheets. The signifi-

cance of differences was assessed by the Student's criterion for  $p \leq 0.05$ .

### 3. Results and discussion

#### 3.1 Isolation of active phosphate-mobilizing bacteria

During the study, 130 bacterial strains were isolated, of which 28 were pure cultures of phosphate-mobilizing bacteria. It was noted, that when isolating phosphate-mobilizing bacteria from soil samples on M1 and M3 media, clearly visible zones of phosphate dissolution were observed (Figure 1).



Zones of dissolution around bacterial colonies A – M1 (Pikovskaya medium); B – M2 (Muromtsev medium); C – M3 (NBRIP medium), on the 5th day of cultivation

**Figure 1** – Zones of phosphate dissolution

At the next stage, the ability of bacteria to mobilize phosphate from various insoluble compounds was assessed. The highest phosphate-dissolving activity have bacterial isolates *Acinetobacter calcoaceticus* 2/5 pr, *Stenotrophomonas rhizophila* 1/7c, *Pseudomonas koreensis* 4/5 kr, and *Pseudomonas koreensis* 3/4 pr and *Acinetobacter calcoaceticus* 1/5 pr with the diameter of the dissolution zones

ranged from 15 mm to 30 mm. On M2 medium, the appearance of clearing zones was not observed even when the most active microorganisms were cultivated for more than 7 days (Table 1).

Thus, the glucose-ammonium medium M1 and M3 medium can be used as alternatives to M2 medium, when isolating soil phosphate-mobilizing microorganisms.

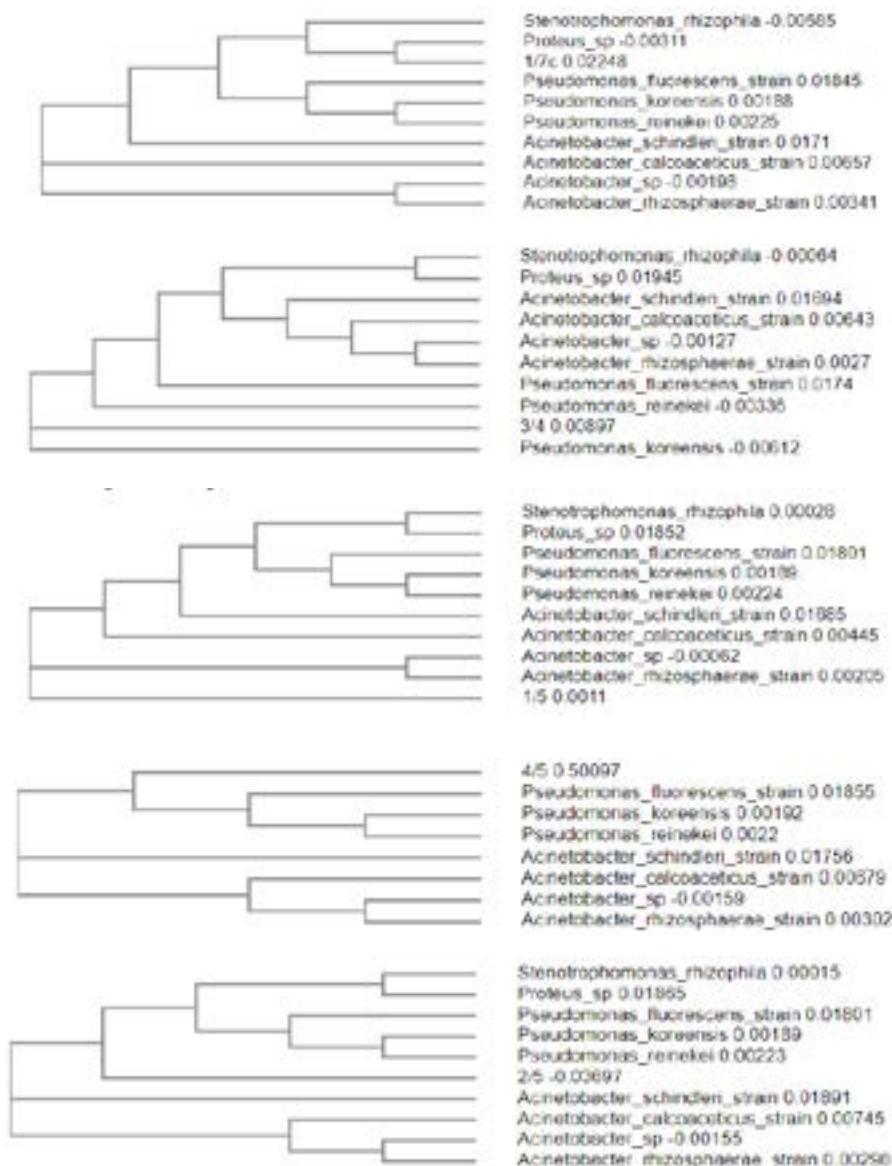
**Table 1** – Phosphate-mobilizing activity of bacteria on various media according to the diameter of the phosphate dissolution zones

Strain name of bacterial culture	Diameter of $\text{Ca}_3\text{PO}_4$ dissolution zones in different media, d = mm		
	M1	M2	M3
<i>Acinetobacter calcoaceticus</i> 2/5 pr	15	00	25
<i>Stenotrophomonas rhizophila</i> 1/7c	17	00	19
<i>Pseudomonas koreensis</i> 4/5 kr	30	00	30
<i>Pseudomonas koreensis</i> 3/4 pr	25	02	30
<i>Acinetobacter calcoaceticus</i> 1/5 pr	23	01	28

*Identification of phosphate-mobilizing bacteria.*

When carrying out the genetic identification of strains 1/5 pr, 2/5 pr 3/4 pr, 4/5 kr and 1/7 c, a nucleotide sequence of 660 bp was obtained based on the analysis of the 16S rRNA gene nucleotide sequence after the removal of terminal fragments, which was identified in Gene Bank using the BLAST algorithm. When identified using the international database NCBI, the strains identified had the maximum iden-

tity (97.66 – 99.86%). However, taking into account the literature data indicating the presence of nucleotide sequence errors in such international banks as the Gene Bank and the Ribosomal Database Project (RDP-II), a phylogenetic tree was additionally built with the 16S rRNA nucleotide sequences of the gene for reference strains of this species (NCBI National Center for Biotechnology Information, USA) (Figure 2).



Phylogenetic tree showing phylogenetic relationships between strains 1/7 c, 3/4 pr, 1/5 pr, 4/5 kr and 2/5 pr and typical strains of related species.

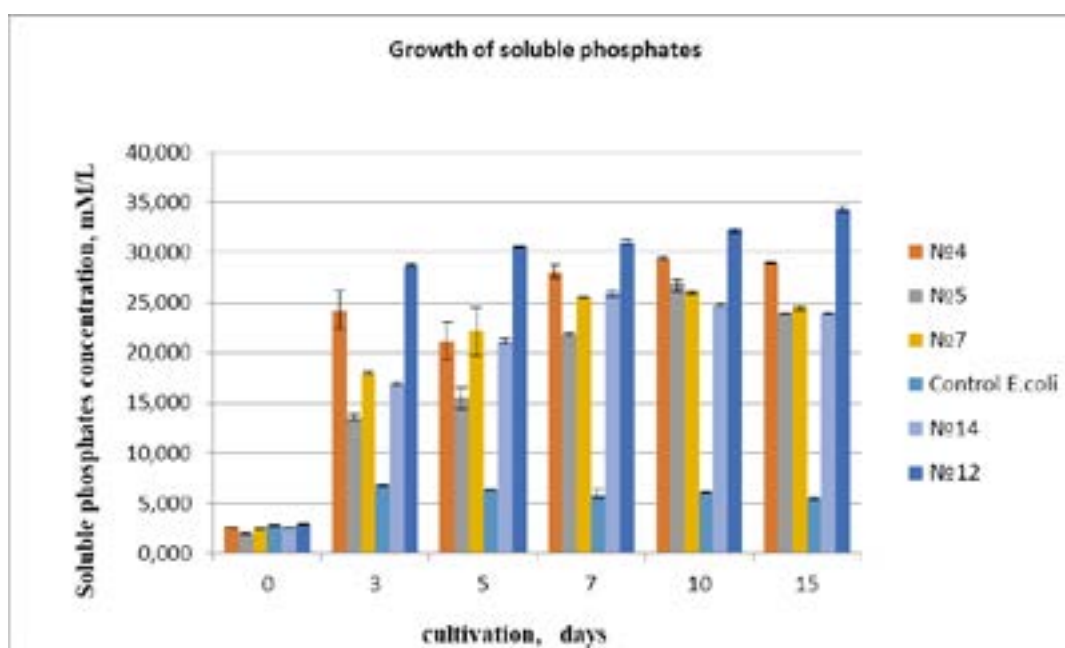
**Figure 2** – Phylogenetic tree based on the analysis of the 16S rRNA

Analysis of the nucleotide sequences of the 16S rRNA gene made it possible to assign strains 1/5 pr and 2/5 pr to the genus *Acinetobacter*, strains 3/4 pr and 4/5 kr to the genus *Pseudomonas*, and strain 1/7 c to the genus *Stenotrophomonas*. Strain 1/7 c had 99.58% identity with the *Stenotrophomonas rhizophila* strain. Strains 3/4 pr and 4/5 kr had 99.81% and 100% identity with the *Pseudomonas koreensis* strain, and strains 1/5 pr and 2/5 pr had 99.81% and 99.50% identity with the strain *Actnetobacter calcoaceticus*.

In order to assess the ability of PMB strains to mobilize phosphorus from insoluble soil phosphates, an experiment was performed with strains of *Acinetobacter calcoaceticus* 2/5 pr, *Stenotrophomonas rhizophila* 1/7 c, *Pseudomonas koreensis* 4/5 kr, *Pseudomonas koreensis* 3/4 pr *Acinetobacter calcoaceticus* 1/5 pr bacteri within 15 days on NBRIP medium. The content of soluble phosphates was determined in the culture liquid.

As a result of the studies, the phosphate-mobilizing ability of the strains was determined. With a constant level of soluble phosphate fraction in sterile control (3.1 mM/l), the increase in soluble phosphates by the biomass of the strain №14 – *Acinetobacter calcoaceticus* 1/5 pr and №7 – *Pseudomonas koreensis* 4/5 kr was – 25.5 mM/l, biomass of strain №5 – *Stenotrophomonas rhizophila* 1/7 c – 27.0 mM/l, biomass of strain №12 – *Pseudomonas koreensis* 3/4 pr – 34.3 mM/l, biomass of strain №4 – *Acinetobacter calcoaceticus* 2/5 pr – 30.0 mM /l (Figure 3).

Of the five PMB strains, the most active were strains №14 – *Acinetobacter calcoaceticus* 2/5 pr – 30.0 mM/l, №12 – *Pseudomonas koreensis* 3/4 pr – 34.3 mM/l, №5 – *Stenotrophomonas rhizophila* 1/7 c – 27.0 mM/l of soluble phosphates, respectively. Strains №14 – *Acinetobacter calcoaceticus* 1/5 pr – 25.0 mM/L and №7 – *Pseudomonas koreensis* 4/5 kr – 25.5 mM/l had a slightly lower activity.

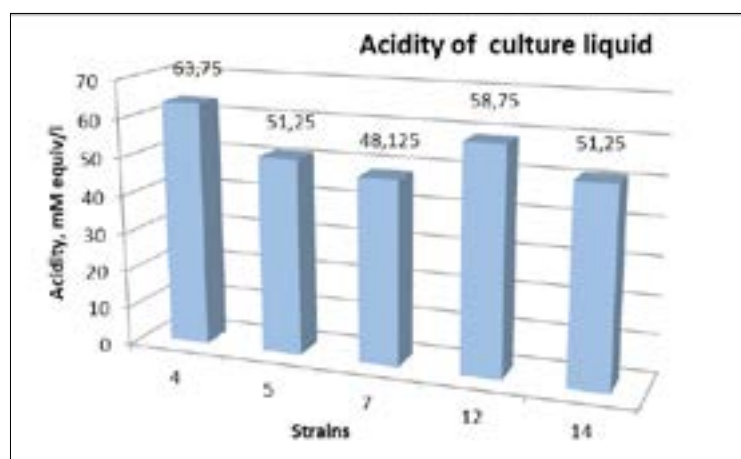


**Figure 3** – Dynamics of the formation of phosphorus mobile forms by phosphate-mobilizing bacteria. №4 – *Acinetobacter calcoaceticus* 2/5 pr; №5 – *Stenotrophomonas rhizophila* 1/7 c; №7 – *Pseudomonas koreensis* 4/5 kr; №12 – *Pseudomonas koreensis* 3/4 pr; №14 – *Acinetobacter calcoaceticus* 1/5 pr.

To explain the mechanisms of the phosphate-mobilizing ability, it was suggested that bacteria acidify the medium with organic acid.

Therefore, an experiment was carried out to determine the total acidity of the medium. The titrated solution of sodium hydroxide 0.025 M was prepared and determined the acidity of the envi-

ronment of these strains by acid-base titration of the indicator phenolphthaleine. The amount of total acidity directly correlates with an increase in the concentration of soluble phosphates and, in fact, with the effectiveness of the strain. The results were expressed in mMol equivalent of hydrogen ions (Figure 4).



**Figure 4** – Influence of the total acidity of the environment on the phosphate-mobilizing activity of phosphate mobilizing strains

It was found that five promising phosphate-mobilizing strains also produced more organic acids. Strains №4 – *Acinetobacter calcoaceticus* 2/5 pr, №5 – *Stenotrophomonas rhizophila* 1/7 c, №7 – *Pseudomonas koreensis* 4/5 kr, №12 – *Pseudomonas koreensis* 3/4 pr and №14 – *Acinetobacter calcoaceticus* 1/5 pr, which acidity approached 50 mMeq/L, which directly correlates with their phosphate-mobilizing ability. From five PMB strains, the most active were strains №4 – *Acinetobacter calcoaceticus* 2/5 pr – 63.75 mMequiv/L, №5 – *Stenotrophomonas rhizophila* 1/7 c – 51.25 mMequiv/L, №14 – *Acinetobacter calcoaceticus* 1/5 pr – 51.25 mMeq/L and №12 – *Pseudomonas koreensis* 3/4 pr – 58.75 mM eq/L, strain №7 – *Pseudomonas koreensis* 4/5 kr – 48.125 mMeq/L had a slightly lower activity. Selected phosphate-mobilizing bacterial strains are a potential basis for creating bacterial fertilizers to improve phosphorus nutrition of agricultural plants.

#### *Testing the effect of phosphate-mobilizing bacteria on agricultural crops in laboratory conditions.*

Active strains of PMB were selected to study the growth-stimulating effect on seed germination, growth and development of agricultural crops seedling in laboratory experiments. The selected isolates stimulate the germination of the most studied crops. A higher germination rate compared to the control was revealed in variants of the experiment with seed treatment with strains of *Acinetobacter calcoaceticus* 2/5 pr, *Stenotrophomonas rhizophila* 1/7 c, *Pseudomonas koreensis* 4/5 kr, *Pseudomonas kore-*

*ensis* 3/4 pr and *Acinetobacter calcoaceticus* 1/5 pr (-80%), while in the control the germination rate was only 20-30% (Figure 5).

Based on the data obtained, it can be seen that under the influence of pre-sowing treatment of cotton seeds with strains of phosphate-mobilizing rhizobacteria, a stimulating effect on the growth and development of cotton plants was observed. In all variants of the experiment, the green and dry weight of cotton plants was greater than in the control. At the same time, the wet weight of one plant increased 4.0 – 4.5 times in comparison with the control variant. It was shown that the maximum accumulation of green mass of plants was observed when cotton seeds were treated with *Pseudomonas koreensis* 4/5 kr, *Pseudomonas koreensis* 3/4 pr and *Acinetobacter calcoaceticus* 1/5 pr strains, which have the highest positive effect on plant development (Figure 6).

Thus, bacterization of cotton seeds by phosphate-mobilizing rhizosphere bacteria has a complex effect on plants. At the stage of manifestation of the first roots, the mineral nutrition of the seedling improves due to a more developed root system, and on the other, due to an increase in the concentration of phosphorus compounds available for plants in the rhizosphere and rhizoplane (phosphate-mobilizing activity of rhizosphere bacteria). This promotes the development of stronger seedlings and gives a start for further growth and development of plants. After germination, inoculated cotton seedlings accumulate biomass faster due to a well-developed root system and photosynthetic apparatus.

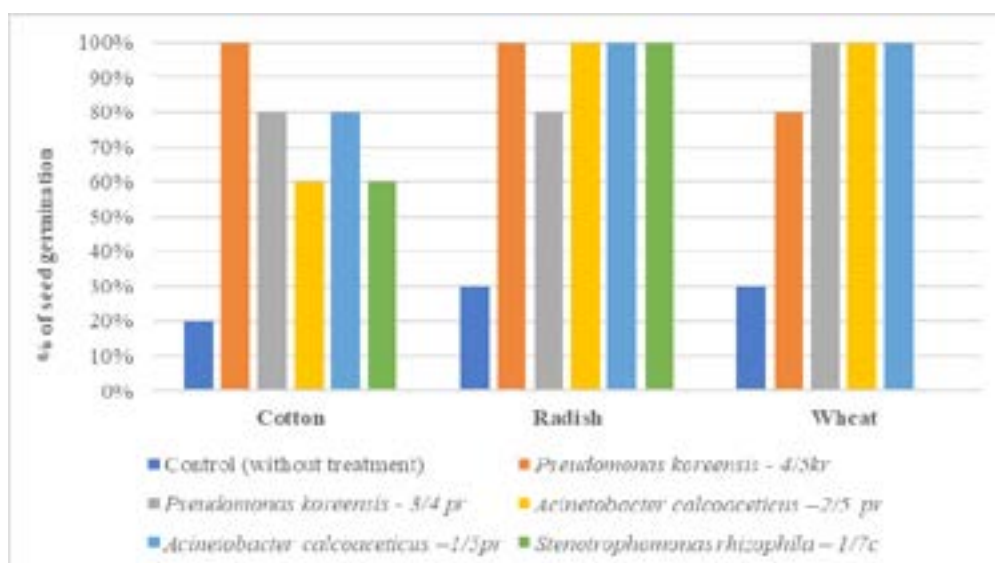


Figure 5 – Growth-stimulating effect of crops on the germination of cotton, radish and wheat seeds.

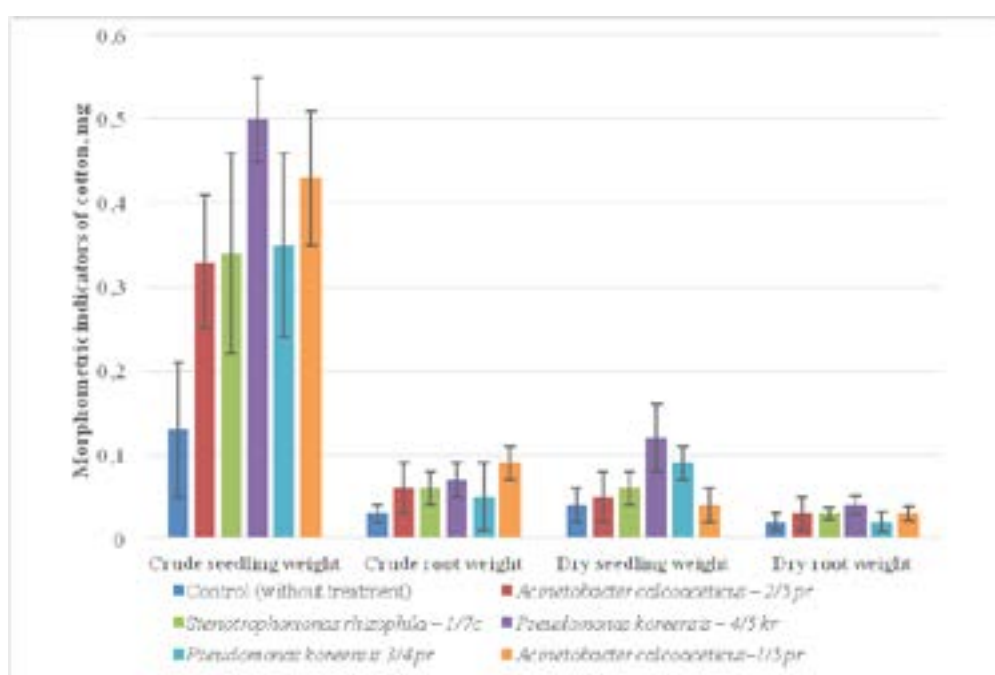


Figure 6 – Influence of strains of phosphate-mobilizing bacteria on the biomass of a cotton plant variety “Turkestan”

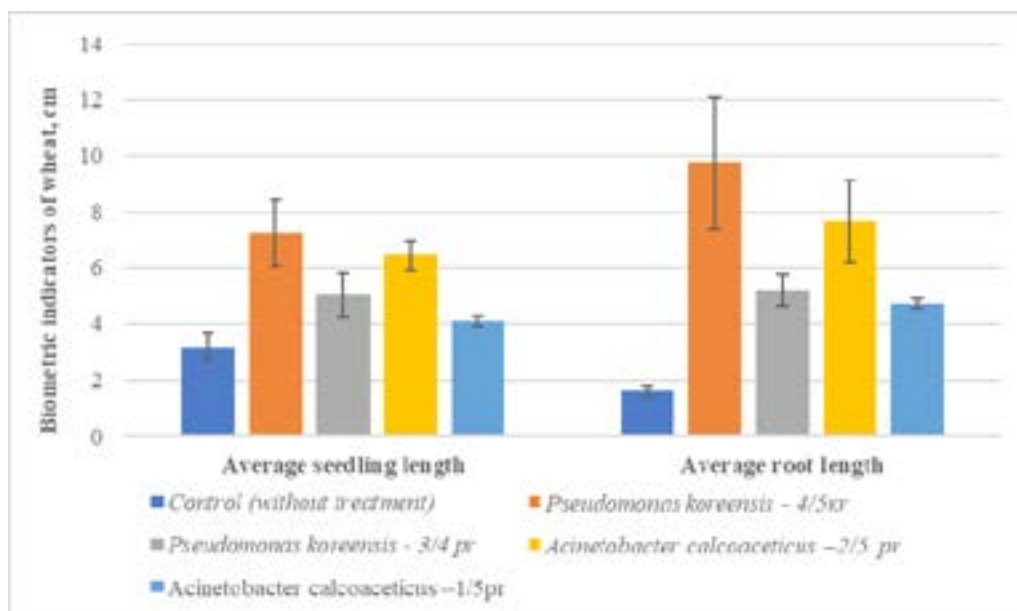
In laboratory studies with wheat seedlings, it was shown that the strains of *Acinetobacter calcoaceticus* 2/5 pr, *Stenotrophomonas rhizophila* 1/7 c, *Pseudomonas koreensis* 4/5 kr, *Pseudomonas koreensis* 3/4 pr and *Acinetobacter calcoaceticus* 1/5 pr exhibit growth promoting activity, in addition to phosphate stimulating. Bacterization of wheat seeds with the strains *Acinetobacter calcoaceticus* – 2/5 pr and *Pseudomonas koreensis* – 4/5 kr accelerated

seed germination. At the stage of the first roots appearance, the mineral nutrition of the seedling improves, on the one hand, due to a more developed root system. On the other hand, due to an increase in the concentration of phosphorus compounds in the rhizosphere and rhizoplane for plants. This promotes the development of stronger seedlings and gives a start for further plant growth and development (Figure 7).



The effect of the selected strains of phosphate-mobilizing bacteria on wheat seedlings was expressed in a change in root morphology: an increase in the number and length of roots by 2.5-3.0 times. The most active stimulants were isolates of *Acin-*

*netobacter calcoaceticus* 2/5 pr and *Pseudomonas koreensis* 4/5 kr, a statistically significant increase in root length was obtained on average by 74.5% – 100% and wheat seedlings on average 88.4% – 100%, respectively.



Data on the effect of PMB on the biometric parameters of wheat seedlings of the "Astana-2" variety (after 21 days of laboratory microvegetation experiment)

**Figure 7** – The influence of phosphate-mobilizing bacteria strains on the growth of seedlings and root formation of spring wheat variety "Astana – 2"

Thus, the data obtained made it possible to establish that the strains of rhizosphere bacteria are able not only to dissolve trisubstituted calcium orthophosphate, but also to actively stimulate the growth of wheat plants.

Evaluation of the biological activity of phosphate-mobilizing strains showed that they have growth-stimulating properties.

As a result of the data obtained, it was found that all the studied crops had the ability to increase the germination of radish plant seeds by 80 – 100% compared to the control. It was also noted that as a result of seed treatment with phosphate-mobilizing rhizobacteria, there is a significant stimulation of the embryonic roots and shoots (Figure 8).

The research results show that under the action of biologically active substances of bacteria, the growth force of seeds increases, which is manifested in the length increase of the embryonic roots and shoots. The greatest length of seedlings and roots was established when seeds were treated with phos-

phate-mobilizing microorganisms *Acinetobacter calcoaceticus* 2/5 pr, *Pseudomonas koreensis* 4/5 kr and *Acinetobacter calcoaceticus* 1/5 pr etc. The length of seedlings increased by 5.6 cm, germ roots by 5.2 cm, compared with control. The studied agronomically valuable microorganisms based on phosphate-mobilizing rhizobacteria had a positive effect on the growth of radish plants from the earliest stages of seed inoculation.

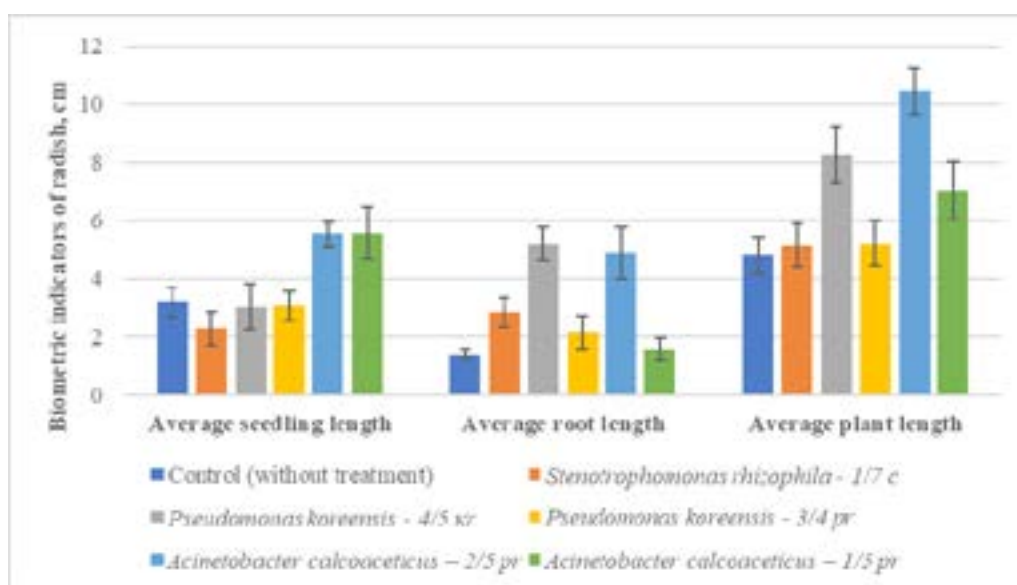
Based on the results of the data obtained, the most promising phosphate-mobilizing strains of soil microorganisms were selected for further research.

Thus, the experiment made it possible to determine the strains of rhizosphere bacteria most active in terms of their ability to phosphate mobilization, which was expediently tested in field experiments as a basis for bacterial fertilizers.

To assess the effect of phosphate-mobilizing bacteria on the yield of spring wheat, field experiments were carried out on southern carbonate chernozem soil.

On the crops of spring wheat variety “Shortandinskaya 2012”, the effectiveness of phosphate-mobilizing bacteria on the provision of southern carbonate chernozem soil with phosphorus and the dose of phosphorus fertilizers was studied. The dependence of the yield of spring wheat on the content of mobile phosphorus in the southern calcareous chernozem soil has been established. The introduction of drug 1 (based on phosphate-mobilizing rhizobacteria) against the background

without the introduction of  $P_2O_5$  made it possible to obtain reliable increases in yield – 2.26 t/ha of grain, respectively. When the soil was saturated with mobile phosphorus on average up to 123.5 kg/ha, the effect of bacterization of spring wheat decreased, the increase in grain was 2.18 t/ha. The use of preparation 1 (based on phosphate-mobilizing rhizobacteria) was effective only without the addition of  $P_2O_5$ , the increase from seed bacterization averaged 2.28 t/ha of grain.



**Figure 8** – The influence of strains of phosphate-mobilizing bacteria on the seedlings growth and root formation of the “Rubin” radish variety

Accordingly, the effectiveness of the use of phosphate-mobilizing rhizobacteria on spring wheat was established, depending on the application of  $P_2O_5$  in the southern-calcareous chernozem soil. The introduction of  $P_2O_5$  with Preparation 1 (phosphate-mobilizing bacteria) when the soil is supplied with mobile phosphates is impractical.

## Conclusion

Modern trends in the creation, development and promotion of “organic” farming technologies imply the replacement of chemicals used in intensive agricultural production with biological ones. In this regard, the creation of biological products based on effective native phosphate-mobilizing microorganisms is an actual task of modern agriculture.

Thus, based on the data obtained on the properties of new strains of phosphate-mobilizing bac-

teria, the degree of phosphate mobilization and the ability to stimulate plant growth and development, the selected phosphate-mobilizing bacterial strains is a potential basis for a new bacterial fertilization to improve the phosphorus nutrition of agricultural crops. Improving the mineral nutrition of plants contributes to an increase in yields and an improvement in the quality of agricultural products.

## Conflict of interests

The authors declare no conflicts of interest.

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