Volume 2, Issue 10, October - 2024
ISSN (E): 2938-3781

Web of Agriculture: Journal of Agriculture and Biological Sciences
webofjournals.com/index.php/8

STUDY OF RHIZOSPHERE BACTERIA ABILITY TO MOBILIZE P2O5 FROM HARD-TO-ACCESS SOIL PHOSPHATES

Zakiryaeva Saidakhon I.
Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, Tashkent, Uzbekistan

Abstract
In this paper, the ability of 12 strains of wheat rhizosphere bacteria genus Enterobacter, Rahnella, Pantoea, Pseudomonas and Bacillus to mobilize phosphate was investigated. The strains were found to efficiently release P2O5 from tricalcium phosphate. The maximum release of P2O5 from Ca3(PO4)2 in the nutrient medium was observed on the 4th day of the experiment, ranging from 11.0 to 22.5 mg P2O5/100 ml. Phosphate-mobilizing activity was accompanied by a decrease in pH of the medium. The pH varied from the initial 7.1±0.01 to the final 4.1±0.02 during 4 days of incubation.

Keywords: Phosphorus, mobilization, rhizosphere bacteria, phosphate-mobilizing microorganisms, cultivation, optical density, pH value.

Introduction

Phosphorus is the second most important macronutrient after nitrogen for proper functioning of plants [1, 2]. Phosphorus plays a vital role in all aspects of plant growth and development, and its deficiency can significantly retard plant development. Although soils contain phosphorus in the form of organic and inorganic compounds, most of this element remains in an inactive form and is therefore unavailable to plants. Due to the fact that many farmers are unable to use expensive phosphorus fertilizers to eliminate phosphorus deficiency, alternative methods of solving this problem are relevant. Phosphate-solubilizing microorganisms are a group of beneficial microorganisms capable of hydrolyzing organic and inorganic insoluble phosphorus compounds, transforming them into a soluble form easily assimilated by plants. These microorganisms provide an environmentally friendly and cost-effective approach to eliminate phosphorus deficiency in soil and optimize its uptake by plants [3]. However, the availability of soluble forms of phosphorus to plants in soil is limited because it is present in the form of insoluble calcium, iron and aluminum phosphates [4-6]. Most soils contain significant amounts of phosphorus, but most of it is bound to the mineral components of the soil and is unavailable to plants. In soils with low content of total phosphorus, its amount can be increased by phosphorus fertilizers, but such soils do not retain the added phosphorus. About 75-90% of chemical phosphorus applied to the soil is bound in the form of metallocation complexes and firmly fixed in the soil layer, resulting in long-term environmental impact [2].

Phosphorus in soil solution is represented in the form of insoluble inorganic and organic phosphorus. Its circulation in the biosphere can be characterized as "sedimentary" because it is not exchanged with the atmosphere and, unlike nitrogen, has no available atmospheric sources. Therefore, phosphorus deficiency significantly limits the growth and yield of agricultural crops

Volume 2, Issue 10, October - 2024
ISSN (E): 2938-3781

Web of Agriculture: Journal of Agriculture and Biological Sciences
webofjournals.com/index.php/8

[4]. The phosphorus content of soil is about 0.05% [2]. Although soil test values are usually higher, most of the phosphorus (about 95-99%) is in the insoluble form of phosphate [7].

Plant cell can assimilate phosphorus in several forms, but the main part is absorbed in the form of phosphate ions, mainly HPO_4^{2-} or $H_2PO_4^-$, depending on soil pH [4, 8-10]. The main source of inorganic phosphorus in agricultural soils is phosphorus fertilizers. About 70-90% of phosphorus applied to the soil with fertilizers is bound to cations and transferred to insoluble forms [4]. Phosphorus is immobilized by cations such as Ca^{2+} in calcareous or neutral soils to form calcium phosphate ($Ca_3(PO_4)_2$), and Al^{3+} and Fe^{3+} in acidic soils to form aluminum phosphate ($AlPO_4$) and iron phosphate ($FePO_4$) [8, 9]. These insoluble forms of phosphorus are not available to plants. However, the accumulated phosphate in agricultural soils is sufficient to provide maximum crop yields worldwide for about 100 years [4] if it could be mobilized and converted into soluble forms by phosphate-mobilizing microorganisms. This raises the need for an alternative but economically accessible technology capable of providing plants with the required amount of phosphorus [3].

Microorganisms play a key role in the natural phosphorus cycle. For many years, the possibility of using phosphate-mobilizing microorganisms as biofertilizers to improve the efficiency of agriculture has been studied [3]. Phosphate-mobilizing microorganisms are a group of useful microorganisms capable of hydrolyzing organic and inorganic phosphorus compounds from insoluble forms. These phosphate-mobilizing microorganisms include bacterial strains of the genera *Pseudomonas, Bacillus, Rhizobium, Enterobacter, Achromobacter, Agrobacterium, Micrococcus* and others.The use of phosphate-mobilizing bacteria can significantly increase the availability of phosphate to plants. These bacteria play a key role in breaking down difficult-to-solubilize forms of phosphate, converting them into plant-available compounds. The use of soil and rhizosphere microorganisms capable of converting difficult to soluble forms of phosphate into plant-available compounds can effectively address this problem.

Therefore, the aim of the study was to investigate the ability of wheat rhizobacteria to mobilize phosphorus from mineral compounds difficult to access for plant nutrition (tricalcium phosphate).

Materials and methods of research

Active cultures of rhizobacteria, previously isolated from the rhizosphere of wheat growing in Kashkadarya, Syrdarya, Andijan regions and Karakalpak Republic [11], served as objects of the study.

The study of growth intensity and $Ca_3(PO_4)_2$ mobilization by rhizobacteria was carried out in liquid medium - peptone water with glucose. The medium was inoculated with a suspension of each culture with an initial titer of 10^6 CFU/mL. Rhizobacteria were cultured under batch conditions at 29° C for 4 days on rocker (240 rpm). Tricalcium phosphate (2 g per 1 L) was added to the medium as a source of phosphorus. The activity of cultures to mobilize $Ca_3(PO_4)_2$ was judged by the number of viable cells and accumulation of P_2O_5 in the culture fluid. Their concentration in the medium was determined according to the method of N.B. Sergeeva [12]. The number of viable cells was estimated by the optical density (OD_{600}) of the cultures. In parallel, changes in pH of the nutrient medium were studied. The amount of P_2O_5 was determined on a spectrophotometer V-5100 China. All experiments were carried out in 3-fold repetition.

Statistical processing of experimental data was carried out by standard methods of calculating errors, averages, confidence intervals, standard deviations. All calculations and mathematical

Volume 2, Issue 10, October - 2024
ISSN (E): 2938-3781

analyses were performed using Microsoft Excel 2007 [13].

Results and their discussion

It was found that the studied rhizobacterial cultures differed significantly in their ability to mobilize P_2O_5 from $Ca_3(PO_4)_2$. *Pantoea agglomerans* 20 showed active mobilization of P_2O_5 from $Ca_3(PO_4)_2$on the second day of the experiment. Thus, for 2 days of cultivation strain *P. agglomerans* 20 mobilized up to 5.7±0.12 mg P_2O_5/100 ml, pH value of nutrient medium decreased from 7.1±0.01 to 5.5±0.02, optical density (OD_{600}) of the culture was 1.27±0.02. The cultures genus of *Rahnella* released P_2O_5 from $Ca_3(PO_4)_2$ to 1.1-2.5 mg P_2O_5/100 ml, genus of *Pseudomonas* to 2.1-2.6 mg P_2O_5/100 ml, genus of *Enterobacter* to 1.8-2.2 mg P_2O_5/100 ml, genus of *Bacillus* to 1.2-3.7 mg P_2O_5/100 ml on the second day of the experiment.

With increasing time of rhizobacteria cultivation in liquid medium the amount of P_2O_5 increased, and on the fourth day of the experiment active mobilization of P_2O_5 from $Ca_3(PO_4)_2$ was shown by strains *Pseudomonas chlororaphis* 21, *P. agglomerans* 20 and *Bacillus* sp. 34. Thus, during 4 days of cultivation, the strains released up to 22.5-21-20.7 mg P_2O_5/100 ml, respectively, and the pH values of the nutrient medium decreased from 7.1±0.01 to 4.1-4.2-4.4, the optical density (OD_{600}) of the cultures was 1.93-1.54-1.84 optic units. In Rahnella aquatilis strain 3, the amount of P_2O_5 released from $Ca_3(PO_4)_2$ was up to 17.0±0.21 mg P_2O_5/100 ml, and the pH values of the nutrient medium decreased from 7.1±0.01 to 4.6±0.02, the optical density (OD_{600}) of the culture was 1.63 optic units. *Enterobacter cloacae* 9 strain mobilized up to 16.5±0.19 mg P_2O_5/100 ml, the pH values of the nutrient medium decreased from 7.1±0.01 to 4.8±0.02, the optical density (OD_{600}) of the culture was 1.86 optic units (Fig. 1.).

The main mechanism of soil phosphorus solubilization is a decrease in soil pH due to microbial production of organic acids or proton release [8, 14-15]. The production of organic acids in combination with pH lowering by microbial action contributes to phosphorus solubilization [16].

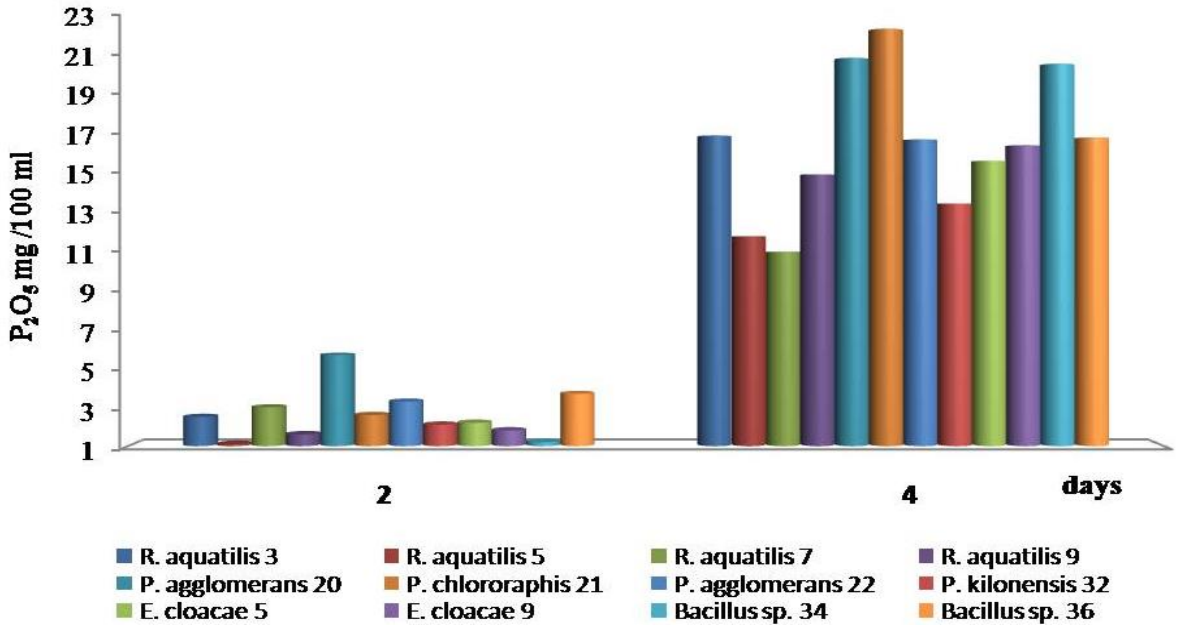

Figure 1. Mobilization of P_2O_5 from $Ca_3(PO_4)_2$ by wheat Rhizobacteria

Volume 2, Issue 10, October - 2024
ISSN (E): 2938-3781

When soil pH increases, divalent and trivalent forms of inorganic phosphorus such as HPO_4^{-2} and HPO_4^{-3}occur. Organic and inorganic acids produced by phosphate-mobilizing microorganisms dissolve insoluble soil phosphate by chelating cations and competing with phosphate for adsorption sites in the soil [7, 17].

Mobilizing activity of phosphorus correlated well with the change in pH of the medium. The pH value of nutrient medium decreased from 7.1±0.01 to 5.6±0.02 by the second day of the experiment, and to 4.1±0.02 by the fourth day (Fig. 2).

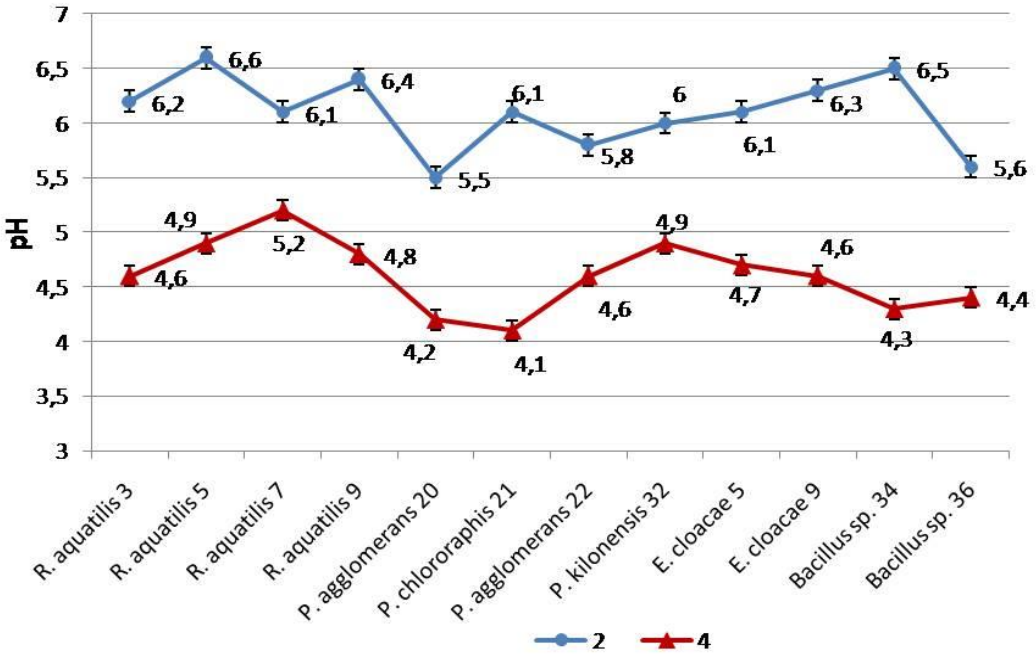

Figure 2. Variation of pH value of nutrient medium by rhizobacteria

Conclusion

One of the promising directions for improving phosphorus nutrition of plants is the use of indigenous phosphate-mobilizing microorganisms that provide biological mobilization of hard-to-access soil phosphates, as well as activation of low-grade phosphate rock. These microorganisms can efficiently release phosphorus, facilitating its uptake by plants and thus increasing yields and quality of agricultural products. In addition, the use of such microorganisms contributes to the environmental sustainability of agroecosystems, reducing the need for chemical fertilizers and minimizing the negative impact on the environment.

Thus, the isolated strains of wheat rhizosphere bacteria actively mobilized P_2O_5 from $Ca_3(PO_4)_2$. High mobilization was shown by *P. chlororaphis* 21, *P. agglomerans* 20, *Bacillus* sp. 34 and *Rahnella aquatilis* 3, and the amount of P_2O_5 released from $Ca_3(PO_4)_2$ was 22.5, 21, 20.7 and 17.0 mg P_2O_5/100 ml, respectively. These results offer potential prospects for the use of rhizobacterial strains as biofertilizers that can mobilize P_2O_5 from hard-to-reach soil mineral phosphates into plant-available forms. In addition, the use of these strains may help reduce agriculture's dependence on chemical fertilizers, which is an important step towards more sustainable agricultural practices. The study also highlights the need to further investigate the interaction of

Volume 2, Issue 10, October - 2024
ISSN (E): 2938-3781

these microorganisms with plants to optimize their use in agronomy and increase agricultural productivity.

REFERENCES

1. Santana E.B., Marques E.L.S., Dias J.C.T. Effects of phosphate solubilizing bacteria, native microorganisms and rock dust on Jatropha curcas L. growth //Genetics and Molecular Research, 2016.vol. 15, 4.
2. Sharma S.B., Sayyed R.Z., Trivedi M.H., Gobi T.A. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils // Springer Plus, 2013. vol. 2, P. 587.
3. Kalayu G. Phosphate solubilizing microorganisms: Promising approach as biofertilizers // International Journal of Agronomy. 2019. (4917256):7. DOI:10.1155/2019/4917256.
4. Walpola B.C., Yoon M. Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: a review //African Journal of Microbiology Research, 2012.vol. 6, P. 6600–6605.
5. Mehrvarz S., Chaichi M.R., Alikhani H.A. Effects ofphosphate solubilizing microorganisms and phosphoruschemical fertilizer on yield and yield components of barely (Hordeumvulgare L.) // American-Eurasian Journal of Agricultural and Environmental Science, 2008. vol. 3, P. 822–828.
6. Alam S., Khalil S., Ayub N., Rashid M. In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere // International Journal of Agricultural Biology, 2002.vol. 4, P. 454–458.
7. Pradhan N., Sukla L.B. Solubilization ofinorganic phosphate by fungi isolated from agriculture soil //African Journal of Biotechnology, 2005. vol. 5, P. 850–854.
8. Kumar A., Kumar A., Patel H. Role of microbes in phosphorus availability and acquisition by plants // International Journal of Current Microbiology and Applied Sciences, 2018. vol.7, no.5, P. 1344–1347.
9. Satyaprakash M., Nikitha T., Reddi E.U.B., Sadhana B., Vani S.S. A review on phosphorous and phosphate solubilising bacteria and their role in plant nutrition // International Journal of Current Microbiology and Applied Scences, 2017.vol. 6, P. 2133–2144.
10. Mahidi S.S., Hassan G.I., Hussain A., Faisul-Ur-Rasool Phosphorus availability issue-its fixation and role of phosphate solubilizing bacteria in phosphate solubilization-case study // Research Journal of Agriculture Science, 2011, vol. 2, P. 174–179.
11. Zakiryaeva S.I., Shakirov Z.S., Khamidova K.M., Normuminov A.A., Azatov F.R. Search for phosphate-mobilizing bacteria in the soils of Uzbekistan // Universum: Chemistry and Biology. Moscow. 2021. No. 9 (87). P. 5-11.
12. Sergeeva N.V. Influence of bacteria that decompose $Ca_3(PO_4)_2$ on the development of corn // Published by the Academy of Sciences of the Moldavian SSR. Chișinău, 1962. No. 7. P. 79-88.
13. Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J., Higgins D.G. Clustal W and Clustal X version 2.0. Bioinformatics, 2007. vol. 23. P. 2947-2948.
14. Carrillo A.E., Li C.Y., Bashan Y. Increased acidification in the rhizosphere of cactus seedlings induced by Azospirillum brasilense // Naturwissenschaften. 2002. 89, P. 428-432.

Web of Agriculture: Journal of Agriculture and Biological Sciences
webofjournals.com/index.php/8

Volume 2, Issue 10, October - 2024
ISSN (E): 2938-3781

15. Yousefi A., Khavazi K., Moezi A., Rejali F., Nadian H., Phosphate solubilizing bacteria and arbuscularmycorrhizal fungi impacts on inorganic phosphorus fractions and wheat growth // World Applied Sciences Journal, 2011. vol. 15, P. 1310–1318.

16. Selvi K.B., Paul J.J.A., Vijaya V., Saraswathi K. Analyzing the efficacy of phosphate solubilizing microorganisms by enrichment culture techniques // Biochemistry and Molecular Biology Journal, 2017. vol.3, P. 1-7.

17. Khan A., Jilani V., Akhtar1 M.S., Naqvi S.M.S., Rasheed M. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production // Journal of Agricultural and Biological Science, 2009. vol. 1, P. 48–58.

Web of Agriculture: Journal of Agriculture and Biological Sciences
webofjournals.com/index.php/8