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Utilizing Specific Root Zone Bacteria Connected To Various Plant Roots, Inorganic Phosphates Can Be Dissolved In Vitro.

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Abstract
Degradation, mobilization of nutrients, solubilization, mineralization, nitrogen fixation, and growth hormone synthesis are among soil processes that are mediated via PGPR. As a result of developing organic acids, microorganisms with the ability to solubilize phosphates can change insoluble phosphates into soluble forms. Phosphorus deficit may be mitigated by the promising method of inoculating seeds with phosphate-solubilizing bacteria. The availability of the soil phosphorus around the roots differs substantially based upon the plant species and soil nutrition. Derived from 251 bacterial isolates were extracted starting at the roots of three different plants. Legumes such as chickpeas, lentils, and beans are grown in Kirkuk and Erbil. Overall, there were 128 isolates associated with PGPR at Kirkuk & 123 isolates at Erbil (though only 54 isolates evaluated of different plants of growth-promoting traits were selected and detected). A total of 54 isolates were investigated. In vitro phosphate solubilization of these isolates in legumes. By Bergey's, overall isolate percentages were Pseudomonas spp (34%), Enterobacter spp (31%), Legionella beijerinckia spp (10%), Bacillus spp (8%), Nitrobacter spp (8%), Nitrosomonas spp (3%), Paenibacillus (3%), Actinomycetes (1%), Frankia (1%), Myxobacteria spp (1%), Clostridium spp (1%), and Actinobacillus (1%).

Keywords: *Rhizosphere bacteria, phosphorus, Legume Plants , Pseudomonas*.

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I. Introduction

One of the essential macronutrients is Phosphorus is needed to achieve maximum yield of crops of agricultural importance. Most agricultural soils have large phosphorus reserves, much of which has accumulated through the regular application of phosphate fertilizers. [1], [2]. [3] There are certain soils in which as much as 75% of the applied phosphate fertilizer may become immobilized or fixed, unavailable to the plant due to the crystalline reprecipitation phase. [4], [5] Bacteria that solubilize phosphate are capable of converting insoluble phosphates to soluble forms [6], [7], [8]. They have utilized it to enhance dissolution to reprecipitate soils to improve the crop growth. [9]. (PSB) are the ones able to transform fixed phosphorus into an available form of the most crucial element to promote plant growth and is recognized as the most essential nutrient after nitrogen in soils. Many of the essential nutrients needed via the plant remain in a form that is not soluble in the soil [10]. Most of the inorganic phosphates added to soils as a fertilizer will be immobilized shortly after the fertilizer is applied. Because of this immobilization, phosphorus is considered unavailable to the plant. Several alternative strategies have been considered, such as releasing the insoluble and fixed form of phosphorous to make it available to soil through enhancement of phosphate availability [11]. For example, inoculation of soil seeds treated with phosphate-dissolving bacteria has been identified as enhancing the release of bound soil phosphorus and applied phosphates, causing increased harvest output. The positive effect of giving phosphorus to plants promotes the formation of deeper and more plentiful roots. [12]. Microorganisms solubilize inorganic insoluble phosphate to chelating oxoacids and acids produced off sugars. Multiple methods, such as hydrogen ion concentration reduction through output of acids, ion chelation, and ion trade activities within the growth medium, acted as reported to contribute to solubilization of phosphate by (PSMs). Microorganisms that dissolve phosphates are key to enhancing phosphorus availability to plants for the sustainable management of phosphorus fertilizers [13], [14]. Phosphate solubilizing bacteria can significantly facilitate phosphorus uptake and promote secretion of auxin hormones, consequently improving plant growth. The aforementioned soil bacteria community groups are important strains, particularly *Pseudomonas, Klebsiella, Bacillus,* and Enterobacter spp. The *Pseudomonas sp.* has a high rate of efficiency of phosphorus acquisition. The bacterium shows a recognized relevance as a biofertilizer due to its ecotype diversity & its ability to withstand several

environmental stress factors. One of the major effective methods for achieving sustainable agricultural objectives is increasing utilization of biofertilizer. Serving as biofertilizers, they are among the most preferred natural substances for enhancing microbial activity in the soil. To accomplish this goal, use of chemical fertilizers and pesticides should be limited, while simultaneously increasing matters of organic content of the soil [15] [16].

II. Resources And Methodology

Extraction of Rhizosphere Bacteria

The different plants of legumes with earth sticking to the zone of roots were gathered at random from Kirkuk on June 1st, 2018, and Erbil on June 2nd, 2018. During the journey to the lab, the samples were kept on ice and in sizable plastic bags. Before being processed within 48 hours, the samples were kept at 4ºC. The plants were gathered from every location on the same day, and a mm-thick layer of the zone roots soil remained attached after the majority of the soil was softly extracted off the roots using a turner. Before being diluted (0.1 M of $MgSO_4.7H_2O$/L), after being separated from the shoots, the roots and the rhizosphere soil they were attached to were put in containers with 0.1 M $MgSO_4.7H_2O$/L for 30 minutes, and they were shaken at 150 rpm in a rotary shaker. Tryptic soy agar (TSA) was covered with 100 microliter aliquots from the previous three dilutions, and agar plates were placed in an incubator for 48 hours at 27°C. Tryptic soy agar was used to purify the randomly chosen colonies [17].

etermination of PSMs

The plates of Pikovskaya's agar (PA), containing insoluble tricalcium phosphate (2.5 g), glucose (13 g), 0.6 g of ammonium sulfate, 0.3 g of sodium chloride (NaCl), 0.1 g of magnesium sulfate heptahydrate, 0.3 g of potassium chloride, 0.4 g of yeast extract, trace manganese sulfate, trace iron sulfate heptahydrate, and 15 g of agar, with pH 7.3 dissolved in 1 L of distilled water, were inoculated with each PSM with about 0.1 mL of culture stored within sterile distilled water and incubated for seven days. The solubilization index was calculated by employing the following method [18].

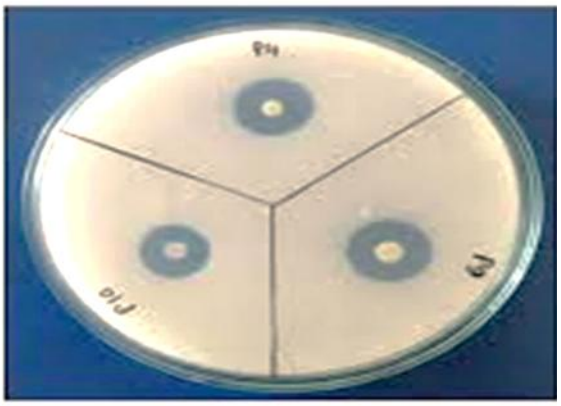
III. Results

The diagnosis of potential phosphate-solubilizing rhizobacterial species off diverse legume plants. A total of 251 strains of bacteria were obtained off the roots of various cultivated legume plants at Kirkuk and Erbil. The total of plant growth-promoting rhizosphere bacteria isolated from Kirkuk was 128, while isolates of 123 were obtained from Erbil. Out of the total isolates, 54 were selected and screened for their in vitro capacity to solubilize inorganic phosphate. The rhizosphere bacterial strains, derived from the roots of different legume plants, were purified through successive streak culturing upon NB medium, and their phosphate solubilizing potential was assessed using an assay agar plate, and those were *Enterobacter spp. (31%), Pseudomonas spp. (34%), Legionella beijerinckia spp. (10%), Bacillus spp. (8%), Nitrobacter spp. (8%), Nitrosomonas spp. (3%), Paenibacillus (3%), Actinomycetes (1%), Frankia (1%), Myxobacteria spp. (1%), Clostridium spp. (1%), and Actinobacillus (1%).* illustrate in table 1):

The frequency of each isolated bacterium (Table 1)

Isolates strains	Frequency %
Pseudomonas spp.	34
Enterobacter spp.	31
Legionella beijerinckia spp.	10
Bacillus spp.	8
Nitrobacter spp.	8
Nitrosomonas spp.	3
Paenibacillus	1
Actinomycetes	
Frankia	1
Mycxobacteria spp.	1
Clostridium spp.	1
Actinobacillus	1

Only ten isolates were chosen for additional research based on their ability to create a transparent area surrounding bacterial growth upon Pikovskaya's medium that is enriched with tricalcium phosphate, as illustrated in Figure 1:



(Figure 1) A halo zone indicating phosphate solubilization formed by PSMs on Pikovskaya's agar.

These ten microbial isolates respective phosphate solubilizing indices (PSI) were 2.98, 2.95, 2.19, 1.93, 1.64, 1.25, 1.24, 1.13, and 1.12. To find out how well the chosen isolates could solubilize phosphate in liquid culture, they further inoculated in Pikovskaya's liquid medium. The phosphate amounts that they were able to solubilize were then calculated. (Table 2)

(Table 2) Bacterial isolates' solubilization with tricalcium phosphate present in Pikovskaya's nutrient medium & broth assay

Isolates strains	Solubilized zone index in phosphate agar assay	Solubilized phosphate concentration in broth
Kirkuk (Chickpea1)	**1.13**	**47.5**
Kirkuk(bean 1)	**1.24**	**46.6**
Kirkuk (lintel)	**1.25**	**45.8**
Erbil (chickpea1)	**1.12**	**43.2**
Erbil(bean1)	**2.98**	**21.2**
Erbil(lintel 1)	**2.95**	**20.8**
kirkuk (chikpea2)	**2.19**	**20.7**
Kirkuk (bean 2)	**1.64**	**27.7**
Erbil (lintel 2)	**1.93**	**37.6**
Erbil (Chickpea2)	**1.13**	**47.5**

Phosphate solubilization index is obtained by dividing the sum of colony and halo diameters by the diameter of colony

The suggestion is that the isolates (Erbil bean 1) produced the greatest halo zone (2.98), followed by(Erbil Lintel 1) (2.95). Also showed that the Kirkuk chickpea 2 and Kirkuk chickpea 1 isolates had 47.5 mg/L of Kirkuk phosphate, which was the most phosphate in the broth assay using Pikovskaya's liquid medium, followed by the Kirkuk bean 1 isolate (46.6 mg/L). It was also observed during the plate assay that the Kirkuk (lintel) isolate (20.7) showed limited phosphate clearance on Pikovskaya's agar. In contrast, the broth-based assay utilizing the Kirkuk Chikpea 2 isolate, which generated a comparatively large halo zone (2.19) in agar medium, demonstrated comparatively less phosphate solubilization in the broth experiment (20.8 mgL^{-1}) than Pikovskaya's liquid medium, which comparatively solubilized more (45.8 mgL^{-1}) tricalcium phosphate. This is in agreement with [19], who observed that isolates with prominent zones, which were clear on agar, sometimes exhibited little phosphate solubilization in liquid medium but maintained more efficiency in dissolving insoluble phosphate. The significant concentration of insoluble mineral phosphates was dissolved in broth culture by the extremely weak clear zone that some isolates created on agar plates, according to this investigation. A large number of isolates did not exhibit any visible clear area on the agar plates that made insoluble inorganic phosphates soluble in a liquid media, according to reports by [20, 21]. The results clearly show that the agar plate halo zone development is the criterion for screening phosphate-solubilizing rhizoplane bacteria.

IV. Discussion

According to the study, PGPR were significant for the plant growth soil functions such as phosphate solubilization, nutrient mobilization, and decomposition. The potential of PGPR to enrich soil fertility and promote plant growth is demonstrated by its capacity to transform insoluble phosphates into bioavailable forms

by producing organic acid. Beans, lentils, and chickpeas are examples of legumes that need effective nutrient intake for nitrogen fixation and general production, making this especially important. The identification and collection of 251 isolates of bacteria from the rhizosphere of three different leguminous plants in two separate locations—Kirkuk and Erbil—is an important part of the investigation. There was a minor variation in the total number of PGPR isolates between the two locations; Tanjaro had 128 isolates, whereas Chamchamal had 123. The necessity of screening to find the According to the study, PGPR were significant for the plant growth soil functions such as phosphate solubilization, nutrient mobilization, and decomposition. The potential of PGPR to enrich soil fertility and promote plant growth is demonstrated by its capacity to transform insoluble phosphates into bioavailable forms by producing organic acid. Beans, lentils, and chickpeas are examples of legumes that need effective nutrient intake for nitrogen fixation and general production, making this especially important. The identification and collection of 251 isolates of bacteria from the rhizosphere of three different leguminous plants in two separate locations—Kirkuk and Erbil—is an important part of the investigation. There was a minor variation in the total number of PGPR isolates between the two locations; Tanjaro had 128 isolates, whereas Chamchamal had 123. The necessity of screening to find the

V. Conclusion

Through a diverse set of biochemical activities, containing phosphate solubilization, biological nitrogen fixation", and growth hormone synthesis, rhizobacteria that promote plant growth (PGPR) are vital in improving soil fertility, according to the study. 251 bacterial strains from legumes cultivated in Kirkuk and Erbil were isolated and characterized, highlighting the variety of advantageous microbial communities in various soil types. Species from the genera *Pseudomonas*, *Enterobacter*, and *Legionella beijerinckia* dominated the 54 isolates that were chosen, indicating their capacity for phosphate solubilization. According to the results, introducing P-solubilizing bacteria into seeds may be a viable method of increasing phosphorus availability in nutrient-deficient soils. This study advances our knowledge of rhizosphere microbial diversity and how it applies to sustainable agriculture.

There is no conflict of interest

References

[1] Abd-Aalla, M. H. (1994). Phosphatases And The Utilization Of Organic Phosphorus By Rhizobium Leguminosarum Biovar Viceae. Letters In Applied Microbiology, 18, 294–296.

[2] Arpana, N., Kumar, S. D., & Prasad, T. N. (2002). Effect Of Seed Inoculation, Fertility And Irrigation On Uptake Of Major Nutrients And Soil Fertility Status After Harvest Of Late Sown Lentil. Journal Of Applied Biology, 12(1/2), 23–26.

[3] Brady, N. C. (1990). The Nature And Properties Of Soils (Pp. 351–380). Macmillan.

[4] Goldstein, A. H. (1986). Bacterial Solubilization Of Mineral Phosphates: Historical Perspective And Future Prospects. American Journal Of Alternative Agriculture, 1, 51–57.

[5] Gupta, R. P., Vyas, M. K., & Pandher, M. S. (1998). Role Of Phosphorus Solubilizing Microorganisms In P-Economy And Crop Yield. In B. D. Kaushik (Ed.), Soil–Plant–Microbe Interaction In Relation To Nutrient Management (Pp. [Pages Not Specified]). Venus Printers & Publishers.

[6] Halder, A. K., Mishra, A. K., & Chakarbarthy, P. K. (1991). Solubilization Of Inorganic Phosphate By Bradyrhizobium. Indian Journal Of Experimental Biology, 29, 28–31.

[7] Hynes, R. K., Leung, G. C. Y., Hirkala, D. L. M., & Nelson, L. M. (2008). Isolation, Selection And Characterization Of Beneficial Rhizobacteria From Pea, Lentil And Chickpea Grown In Western Canada. Canadian Journal Of Microbiology, 54, 248–258.

[8] Illmer, P., & Schinner, F. (1995). Solubilization Of Inorganic Calcium Phosphate Solubilization Mechanisms. Soil Biology And Biochemistry, 27, 257–263.

[9] Leyval, C., & Barthelin, J. (1989). Interactions Between Laccaria Laccata, Agrobacterium Radiobacter And Beech Roots: Influence On P, K, Mg And Fe Mobilization From Mineral And Plant Growth. Plant And Soil, 17, 103–110.

[10] Peix, A., Rivas-Boyero, A. A., Mateos, P. F., Rodríguez-Barrueco, C., Martínez-Molina, E., & Velázquez, E. (2001). Growth Promotion Of Chickpea And Barley By A Phosphate Solubilizing Strain Of Mesorhizobium Mediterraneum Under Growth Chamber Conditions. Soil Biology And Biochemistry, 33, 103–110.

[11] Ravina, M. D., Acea, M. J., & Carballás, T. (1992). Seasonal Fluctuations In Microbial Populations And Available Nutrients In Forest Soil. Biological Fertility Of Soils, 16, 198–204.

[12] Richardson, A. E. (1994). Soil Microorganisms And Phosphorus Availability. In C. E. Pankhurst, B. M. Doube, V. V. S. R. Gupta, & P. R. Grace (Eds.), Soil Biota: Management In Sustainable Farming Systems (Pp. 50–62). CSIRO.

[13] Sharma, A. K. (2002). Bio-Fertilizers For Sustainable Agriculture (P. 456). Agrobios (India).

[14] Shekhar, N. C., Bhaclauriay, S., Kumar, P., Lal, H., Mondal, R., & Verma, D. (2000). Stress Induced Phosphate Solubilization In Bacteria Isolated From Alkaline Soils. FEMS Microbiology Letters, 182, 291–296.

[15] Sudhakara, R. M., Kumar, S., Babita, K., & Reddy, M. S. (2002). Biosolubilization Of Poorly Soluble Rock Phosphates By Aspergillus Tubingensis And Aspergillus Niger. Bioresource Technology, 84, 187–189.

[16] Sundara, B., Natarajan, V., & Hari, K. (2002). Influence Of Phosphorus Solubilizing Bacteria On The Changes In Soil Available Phosphorus And Sugarcane And Sugar Yields. Field Crops Research, 77, 43–49.

[17] Vincent, J. M. (1982). Enumeration And Determination Of Growth. In J. M. Vincent (Ed.), Nitrogen Fixation In Legumes (Pp. [Pages Not Specified]). Academic Press.

[18] Viverk, K., & Singh, K. P. (2001). Enriching Vermicompost By Nitrogen Fixing And Phosphate Solubilizing Bacteria. Bioresource Technology, 76, [Page Missing].

[19] Yadav, K. S., & Dadarwal, K. R. (1997). Phosphate Solubilization And Mobilization Through Soil Microorganisms. In K. R. Dadarwal (Ed.), Biotechnological Approaches In Soil Microorganisms For Sustainable Crop Production (Pp. 293–308). Scientific Publishers.
[20] Young, C. C. (1990). Effects Of Phosphorus-Solubilizing Bacteria And Vesicular Arbuscular Mycorrhizal Fungi On The Growth Of Tree Species In Subtropical Tropical Soils. Soil Science And Plant Nutrition, 36, 225–231.
[21] Young, C. C., Juanag, T. C., & Guo, H. Y. (1986). Vesicular Arbuscular Mycorrhiza Inoculation On Soybean Yield And Mineral Phosphorus Utilization In Subtropical–Tropical Soils. Plant And Soil, 95, 245–254