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In Vitro Dissolution of Inorganic Phosphates Using Certain Root Zone bacteria Associated with the Roots of Different Plants

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Abstract

Degradation, mobilization nutrient, mineralization, solubilization, nitrogen fixation, and growth hormone synthesis are among the soil processes that are mediated Via (PGPR). As result 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 P-solubilizing bacteria. The availability From the soil Soil phosphorus around roots differs substantially based Upon the plantspecies 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 grown in Kirkuk, Erbil .In overall, there were (128)isolates Associated with plant growth-promoting rhizobacteria in Kirkuk &123) isolates in Erbil (though only 54 of these isolates Evaluated for different plant growth-promoting traits were selected and detected. A total of 54 isolates were investigated In vitro phosphate solubilization by these isolates in legumes. According to Bergey's Manual, the overall percentages of isolates were Pseudomonas spp (34%), Enterobacter spp (31%), Legionella beijerinckiaspp (10%), Bacillus spp (8%), Nitrobacterspp (8%), Nitrosomonasspp (3%), Paenibacillus (3%), Actinomycetes (1%), Frankia (1%), Mycxobacteriaspp (1%), Closttridumspp (1%), Actinobacillus (1%).

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

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1. INTRODUCTION

Pseudomonas one of the essential macronutrients is Phosphorus is that are needed for 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 [1-3]. There are certain soils, as much as about 75% of applied phosphate fertilizer may become immobilized or fixed, making it unavailable to the plant crystalline phase precipitation [4, 5]. Bacteria that solubilise phosphate are capable of converting insoluble phosphates to soluble forms [6-8].They have Utilized to enhance the dissolution of re precipitated soils for improved crop growth [9]. (PSB) are the ones able to transform fixed phosphorus into an available form of the greatestcrucial elements to promote plant growth and is considered Recognized as most essential nutrient after nitrogen in soils. Many part 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 enhance release of bound soil phosphorus and applied phosphates, causing increased harvest output. Positive effect of giving phosphorus to plants promotes the formation of deeper and more plentiful roots [12]. Microorganisms solubilize inorganic insoluble phosphate to acids and chelating oxoacids produced from sugars. Multiple methods such as Hydrogen ion concentration reduction through Production of acids, ion chelation, and ion exchange activities in growth medium acquire acted as reported of contribute to phosphate solubilization by (PSMs). The microorganisms that dissolve phosphates are key to enhance 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, Enterobacter spp and*. 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 for several environmental stress factors. One of the major effective methods for achieving sustainable agricultural objectives for to 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 fertilizers of chemical and pesticides should be limited, while simultaneously increasing matters of organic content of the soil [15-16].

2. RESOURCES AND METHODOLOGY

Extraction of Rhizosphere Bacteria

The different plants of legume with earth sticking 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 4°C. The plants were gathered from every location on the same day and mm in thickness layer of the zone roots soil remained attached it after the majority the soil was softly extracted from the roots using a spatula. Before being diluted (0.1 M. of $MgSO4.7H2O$./L.), After being separated from the shoots, the roots and the rhizosp here soil they were attached to were put in containers with 0.1 M $MgSO4.7H2O$/L. for 30 minutes, 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 incubator48 hours at 27 C^0. Tryptic soy agar was used to purify the randomly chosen colonies [17].

Determination of PSMs

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

3. RESULTS

The identification of potential phosphate-solubilizing rhizobacterial species from diverse legume plants a total of 251 bacterial strains were obtained from the roots of various legume plants cultivated in Kirkuk and Erbil. The number of plant growth-promoting rhizosphere bacteria isolated from Kirkuk was 128, while 123 isolates 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 legumes plants were purified through successive streak culturing on NB medium and their phosphate solubilizing potential was assessed using an agar plate assay.ans they were *Pseudomonas spp (34%), Enterobacter spp (31%), Legionella beijerinckiaspp (10%), Bacillus spp (8%), Nitrobacterspp (8%) , Nitrosomonasspp (3%), Paenibacillus (3%), Actinomycetes (1%), Frankia (1%), Mycxobacteriaspp (1%), Closttridumspp (1%), Actinobacillus (1%)*.illustrate in (table 1):

Table 1: The frequency of each isolated bacterium ()

ISOLATES STRAINS	FREQUENCY %
Pseudomonas spp	34
Enterobacter spp	31
Legionella beijerinckiaspp	10
Bacillus spp	8
Nitrobacterspp	8
Nitrosomonasspp	3
Paenibacillus	3
Actinomycetes	1
Frankia	1
Mycxobacteriaspp	1
Closttridumspp	1
Actinobacillus	1

Only ten isolates were chosen for additional research based on their ability to create a transparent area surrounding bacterial growth on Pikovskaya's medium enriched with tricalcium phosphate illustrate in (figure 1):

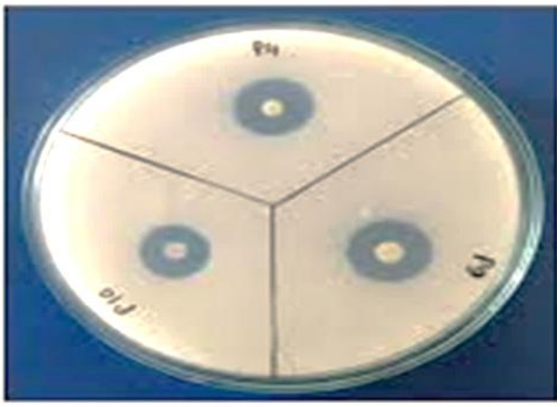

Figure 1: A halo zone indicating phosphate solubilization formed by PSMs on Pikovskaya's agar.

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 were further inoculated in Pikovskaya's liquid medium. The amounts of phosphate 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 colony diameter suggested that the isolate (Erbil bean 1) produced greatest halo zone (2.98) after that (Erbil Lintel 1) (2.95). Also showed that the (Kirkuk chikpea2 and Kirkuk Chickpea1) isolates were (47.5mgL-1) of Kirkuk phosphate was the most phosphate in the broth assay using Pikovskaya's liquid medium, Thereafter Kirkuk(bean 1 isolate (46.6 mgL-1).It was also observed during the plate assay that Kirkuk (lintel) isolate (20.7) Showed limited phosphate clearance on Pikovskaya's agar ;In contrast, the broth-based assay utilizing 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 media, which solubilized comparatively more tricalcium phosphate (45.8 mgL-1). This is in agreement with [19], who observed that isolates with prominent clear zones on agar sometimes exhibited low phosphate solubilization in liquid medium but maintained high efficiency in dissolving insoluble phosphate.

A significant concentration of insoluble mineral phosphates were 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 no visible clear area on the agar plates that made insoluble inorganic phosphates solublein a liquid media, according to reports by [20] and Leyval and [21] the results clearly show that the agar plate halo zone development is the criterion for screening phosphate-solubilizing rhizoplane bacteria.

4. DISCUSSION

According to the study, PGPR (plant growth-promoting rhizobacteria) are significant for plant growth soil functions such phosphate solubilization, nutrient mobilization, and decomposition. The potential of PGPR for enhance 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 collecting of 251 bacterial isolates 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 most advantageous strains is highlighted by the fact that only 54 isolates were chosen based on their characteristics that promoted plant growth. The microbial diversity linked to legumes can be understood by identifying bacterial species using Bergey's Manual. Given that *Pseudomonas spp (34%), Enterobacter spp (31%), Legionella beijerinckiaspp (10%), Bacillus spp (8%), Nitrobacterspp (8%), Nitrosomonasspp (3%), Paenibacillus (3%), Actinomycetes (1%), Frankia (1%), Mycxobacteriaspp (1%), Closttridumspp (1%), Actinobacillus (1%)*.are two surprising bacterial taxa that raise concerns regarding their ecological significance in the rhizosphere. Even while these genera aren't typically linked to promoting plant growth, their existence may suggest relationships that need more research. Likewise, it is interesting to note that *Nitrobacterspp (8%)* are present because they are usually found in high-salinity habitats; their presence may indicate a possible adaption mechanism in the soil conditions under study. Phosphate-solubilizing bacteria have the potential to improve legume yield, according to the study. By introducing these bacteria into seedlings, phosphorus deficiency—a prevalent problem in many agricultural soils—may be lessened. Nevertheless, more investigation is required to evaluate these isolates' effectiveness in field settings, their relationships with plants, and their long-term effects on soil microbiomes. Furthermore, these isolates' molecular characterisation and genome sequencing may shed more light on their functional characteristics and phosphate solubilization methods.

5. 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.

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8. CONFLICT OF INTEREST

The authors declare no conflict of interest.

9. INFORMED CONSENT

Include a statement confirming informed consent was obtained from participants, if applicable.

10. ETHICAL STATEMENT

There is no confirm

11. AUTHOR CONTRIBUTION

I did all these (concept, design, data collection, analysis, and writing).

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