Isolation of Phosphate Solubilizing Microorganism (PSMs) From Soil

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ABSTRACT

Plants acquire phosphorus from soil solution as phosphate anion. It is the least mobile element in plant and soil contrary to other macronutrients. It precipitates in soil as orthophosphate or is absorbed by Fe and Al oxides through legend exchange. Phosphorus solubilizing bacteria play role in phosphorus nutrition by enhancing its availability to plants through release from inorganic and organic soil P pools by solubilization and mineralization. Principal mechanism in soil for mineral phosphate solubilization is lowering of soil pH by microbial production of organic acids and mineralization of organic P by acid Phosphatase. Use of phosphorus solubilizing bacteria as inoculants increases P uptake. These bacteria also increase prospects of using phosphatic rocks in crop production. Greater efficiency of P solubilizing bacteria has been shown through co-inoculation with other beneficial bacteria and mycorrhiza. This article incorporates the recent developments on microbial P solubilization into classical knowledge on the subject.

Keywords: Soil phosphorus, Solubilization, Mineralization, Organic acids, Soil pH, Bacillus, Pseudomonas.

INTRODUCTION

Phosphorous is essential for growth and productivity of plants. It plays an important role in plants in many physiological activities such as cell division, photosynthesis, and development of good root system and utilization of carbohydrate. Phosphorous deficiency results in the leaves turning brown accompanied by small leaves, weak stem and slow development. In ancient times the use of animal manures to provide phosphorous for plant growth was common agricultural practice. Organically bound phosphorous enters in soil during the decay of natural vegetation, dead animals and from animal excretions. At that time role of micro flora on soil fertility was hardly understood [1].

Assimilation of phosphate from organic compounds by plants and microorganisms take place through the enzyme "phosphatase" which is present in a wide variety of soil microorganisms.
Plant can absorb phosphate only in soluble form. The transformation of insoluble phosphate into soluble form is carried out by a number of microbes present in the soil. A large fraction of soil microbes can dissolve insoluble inorganic phosphates present in the soil and make them available to the plants [2].

Phosphorus (P) is sequestered by adsorption to the soil surface and precipitation reaction with soil cations, particularly iron, aluminium and calcium. Therefore, a large amount of P fertilizer has been used to increase plant growth, which is likely to cause negative impact in respects to both environment and economy. Insoluble phosphate compounds can be solubilized by organic acids and phosphatase enzymes produced by plants and microorganisms. For example, PSB have been shown to enhance the solubilization of insoluble P compounds through the release of organic acids and phosphatase enzymes [3].

Plants acquire phosphorus from soil solution as phosphate anion. It is the least mobile element in plants and soil contrary to other macronutrients. In plants, phosphorous increases the strength of cereal straw, promotes flower formation and fruit production, stimulates root development, and is essential for seed formation. Adequate P fertilization may improve the quality of fruits, vegetables, and grain crops and increase their resistance to diseases and adverse conditions. It is essential for the development of meristematic tissues, in stimulation of early root growth and in hastening plant maturity. Because of the negative charge of phosphate ions, they are quickly absorbed after weathering of clays or detritus particles, forming insoluble forms of aluminum, calcium, or iron phosphates, all unavailable to mangroves. Fungi and bacteria have the ability to solubilizing these compounds [4].

Isolation of Microbial Strains
A considerable number of bacterial species are able to exert a beneficial effect upon plant growth. Mostly they are associated with the plant rhizosphere, so they are called as rhizobacteria. This group of bacteria has been termed plant growth promoting rhizobacteria, and among them are strains from genera such as Alcaligenes, Acinetobacter, Arthrobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Paenibacillus, Pseudomonas, Rhizobium, and Serratia. They are used as biofertilizers or control agents for agriculture improvement, and there are numerous researchers for the area with the agricultural environment conservation. Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crops. Phosphorus is an essential element for plant development and growth making up about 0.2% of plant dry weight. Plants acquire P from soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca²⁺, Mg²⁺, Fe³⁺, and Al³⁺, depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants. As the results, the amount available to plants is usually a small proportion of this total. Several scientists have reported the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate [5].

Occurrence of Phosphate Solubilizing Bacteria
High proportion of PSM is concentrated in the rhizosphere, and they are metabolically more active than from other sources. Usually, one gram of fertile soil contains 101 to 1010 bacteria, and their live weight may exceed 2,000 kg ha⁻¹. Soil bacteria are in cocci (sphere, 0.5 µm), bacilli (rod, 0.5–0.3 µm) or spiral (1-100 µm) shapes. Bacilli are common in soil, whereas spirilli are very rare in natural environments. The PSB are ubiquitous with variation in forms and population in different soils. Population of PSB depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities. Larger
populations of PSB are found in agricultural and rangeland soils. In north of Iran, the PSB count ranged from 0 to 107 cells g-1 soil, with 3.98 % population of PSB among total bacteria.

Mechanisms of Phosphorus Solubilization
Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively. Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms. Phosphate solubilization takes place through various microbial processes/mechanisms including organic acid production and proton extrusion.

General sketch of P solubilization in soil is shown in Figure 1. A wide range of microbial P solubilization mechanisms exist in nature, and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi. Phosphorus solubilization is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms. Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils. The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and keto glutamic acids in addition to lowering the pH of rhizosphere. The pH of rhizosphere is lowered through biotical production of proton / bicarbonate release (anion / cation balance) and gaseous (O2/CO2) exchanges.

Effect of PSB on Crop Production
Phosphate rock minerals are often too insoluble to provide sufficient P for crop uptake. Use of PSMs can increase crop yields up to 70 percent. Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock. Higher crop yields result from solubilization of fixed soil P and applied phosphates by PSB. Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation. Pseudomonas spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop. Phosphate solubilizing bacteria enhanced the seedling length of *Cicer arietinum* while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield.
MATERIALS AND METHODS

Collection of soil samples:
Soil samples were collected from neighboring places like mango, park, alovera, graveyard, pea soils from the cultivated areas. Samples were collected from the selected sites at a depth of 15cm from 6 different points within the area. The samples were then air-dried, powered and mixed well to represent a single sample. The sample was then taken for the study. Soil samples were also collected from the rhizosphere of five different types of medicinal plants, *Aloevera, Bauhinia variegata, Cannabis sativa, Lantana camara* and *Mentha viridis* collected from different physico-chemical properties were determined such as soil moisture, soil pH Table-1

<table>
<thead>
<tr>
<th>S.NO</th>
<th>SOURCE /PLACE OF SOIL</th>
<th>SAMPLE TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mango soil</td>
<td>Roots</td>
</tr>
<tr>
<td>2.</td>
<td>Park soil</td>
<td>Rizosphere</td>
</tr>
<tr>
<td>3.</td>
<td>Graveyard</td>
<td>Rizosphere</td>
</tr>
<tr>
<td>4.</td>
<td>Alovera</td>
<td>Roots</td>
</tr>
</tbody>
</table>

Preparations of Medium:

Table No. 2. Ingredients of PVK medium

<table>
<thead>
<tr>
<th>Composition</th>
<th>Gram/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>10</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>0.5</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>0.5</td>
</tr>
<tr>
<td>Magnesium Sulphate(MgSO₄.7H₂O)</td>
<td>0.1</td>
</tr>
<tr>
<td>Calcium Phosphate (Ca₃(PO₄)₂)</td>
<td>5</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.2</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2</td>
</tr>
<tr>
<td>MnSO₄.2H₂O</td>
<td>0.002</td>
</tr>
<tr>
<td>FeSO₄.7H₂O</td>
<td>0.002</td>
</tr>
<tr>
<td>Agar</td>
<td>1.5</td>
</tr>
</tbody>
</table>

According to the literature the Pikovskaya’s agar medium (PVK) was found to be as selective media for the isolation of PSM. pH was maintained at 7. The composition of the medium was as given in table no2.

Isolation of PSM:-
PSM were isolated from each sample by serial dilution and spread plate method. One gram (1g) of soil sample was dispersed in 9 ml of autoclaved distilled water and was thoroughly shaken. 1 ml of the above solution was again transferred to 9 ml of sterile distilled water to form 10⁻² dilution. Similarly 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷ and 10⁻⁸ serials were made for each soil sample. 0.1ml of each dilution was spread on Pikovskaya’s agar medium (PVK) containing insoluble Tricalcium phosphate and incubated at 27 - 30°C for 7 days. Colonies showing halo zones were picked and purified by 5 times subculture method on Pikovskaya’s (PVK) agar medium for studying colony morphology. [7]

Morphological Characterization
Morphological characteristics of isolates viz. shape, size, elevation, surface form, margins and surface texture, color were observed for their characterization. [8]
Gram staining
The isolate was characterized for its gram staining characteristics as per the following standard procedure: Take the smear on the glass slide with the help of inoculating loop let it be air dry. After this with the help of flame fix it with the heat. Add crystal Violet for 30 seconds. Wash it with the distilled water, let it be dry. After that add Gram’s iodine for 60 seconds. Wash it with 95% Ethyl alcohol, Add safranin for 30 seconds after this wash it with the distilled water. Air dry it with the help of blotting paper. Observe in the microscope. The pink colonies will show the gram negative bacteria and the purple colonies will show the gram positive bacteria.

RESULTS AND DISCUSSION

Isolation of PSB
Out of four Samples, only one isolate exhibiting halozone was found (as shown in figure 2) showing the capability of P solubilization was obtained from graveyard sample of 10^{-6} dilution.[9]

This Isolate had the morphological features like colorless colonies which did not produce pigment, cells were gram negative, rod shaped and on the basis of biochemical reactions it was found to be *Pseudomonas fluorescens*. Upon gram staining, it was found as gram negative characteristics. Isolate produced slimy, white colonies with irregular margins, cells were gram positive and on the basis of biochemical reactions this isolate was identified as *Bacillus megaterium*.

The PVK medium was used in the present study because it act as specific isolation medium for PSM isolation due to the presence of Calcium Triphosphate which is known for halozone formation (3). Our results were found to be similar to reference (4) who also noted the morphological features like rod shape and white colonies with irregular margins gram negative.

CONCLUSION
A total of four samples were collected from different places like mango, park, alovera, graveyard soils. But we get these isolates were obtained from graveyard soils. A clear halozones was evident surrounding the colony on the PVK medium. On the basis of morphological characteristics like rod shaped and gram negative characteristic, isolate was confirmed as
bacteria. On comparison of our result with literature, the isolate was suspected to belong with *Pseudomonas fluoresces*. Further in vivo study using any cereal crop like gram is required to check its efficiency of P solubilizing.

**REFERENCES**


