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Isolation and screening of phosphate solubilizing bacterium from soil

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ABSTRACT

In this study a bacterial strain was isolated from rhzhosphere soil and screened for phosphate solubilization property. The morphological, biochemical characteristics of bacterial strain was identified as *Psedomonas* spp. The bacterial isolate showed maximum similarity (0.632) with *Pseudomonas putida* biotype A. A comparative study was carried out to select better medium for phosphate solubilization with bacterial isolate in two media Pikovskay's and National Botanical Research Institutes Phosphate growth Medium. Maximum phosphate solubilization 204 µg/ mL, 170 µg/ mL were at 28^0 in 16th day of incubation in two media respectively.

INTRODUCTION

Phosphorus (P) is one of the three major essential nutrients for plant growth, the other two being nitrogen (N) and potassium (K). Phosphorus plays many vital roles in crop yields and referred as the "king-pin" in Indian agriculture. Compared with the other major nutrients, phosphorus is by far the least mobile and unavailable to plants in most soil conditions. To circumvent phosphorus deficiency, phosphate-solubilizing microorganisms (PSM) could play an important role in a more ecofriendly and environmentally sustainable manner[9,14,20,24,26]. *Pseudomonads* are known for their plant growth promoting property and they are the most studied phosphate-solubilizers[26]. Phosphorus compounds in Indian soils are predominantly inorganic that are chiefly locked as $Ca_3(PO4)_2$ (Tricalcium Phosphate) The group of phosphate solubilizing microorganisms dissolving $Ca_3(PO4)_2$ appears to have an implication in Indian agriculture. The major microbiological means by which insoluble phosphorus compounds mobilized is by the production of organic acids[3-5,11,15,22,25]. The aim of present work was to isolate Phosphate solubilizing bacterium from rhizosphere soil and to test phosphate solubilization from insoluble sources (TCP) and comparison of activity on two liquid media of Pikovskay's medium (PKVS) and National Botanical Research Institutes Phosphate growth Medium (NBRIP).

MATERIALS AND METHODS

Collection of soil

Soil sample was collected from the roots of groundnut plants growing in and around the fields of Tirupati, Chittoor Dt, India and the samples were transferred to the laboratory in sterile polythene bags[2]. Roots were thoroughly washed with tap water for two minutes to remove the loosely adhering soil particles followed by washing with sterile 0.85% saline.

Isolation of bacterial strain

The bacterial strain was isolated from rhizosphere soil and the roots were macerated in 0.85% saline using a sterile mortar and pestle[17]. Serial dilutions of the root homogenate and soil (10% soil in sterile distilled water) samples were plated on *Pseudomonas* specific medium (Hi-media Laboratories, Mumbai, India). The isolate was designated as Ps1. The isolate was purified and maintained on *Pseudomonas* agar slants at 4 ^{0}C and in 10% glycerol at 20 ^{0}C. All the experiments were conducted after raising fresh cultures.

Identification of bacterial strain

Bacterial genus-level identification was carried out by subjecting the bacterial isolate to cultural (oxygen requirement), morphological (colony morphology and pigmentation), microscopic (Gram staining), biochemical (utilization of carbon sources and enzyme activity), tests following standard procedures[13,16]. Identification by FAME analysis conducted by Microbial Type Culture Collection & Gene Bank, Institution of Microbial Technology (IMTECH, Chandigarh), India.

Estimation of phosphate solubilization

The bacterial isolate was evaluated for the ability to solubilize $Ca_3(PO4)_2$ in two different phosphate solubilizing media both qualitatively and quantitatively. Qualitatively the plates were incubated at 28±1^{0}C for 12 days and observed regularly for solubilization zone. Solubilization index (SI) was calculated according to the ratio of the total diameter (colony + halo zone) and colony diameter[6]. Quantitative estimation of tricalcium phosphate solubilization in broth was carried out at 28^{0}C using Erlenmeyer flasks (250 mL) containing 100 mL of Pikovskaya and NBRIP broth inoculated with 1 mL of bacterial suspension (3·105 cells/mL); uninoculated controls were used in each case. Every experiment was conducted in triplicate, Erlenmeyer flasks were incubated on rotary shaker at 180 rpm for 20 days. The growth medium was withdrawn aseptically at 2-day intervals from each flask and centrifuged (10,000 rpm, 10 min). The supernatant was analyzed for inorganic P (Phosphorus) content by Fiskie and Subbarao method[8]. The pH of the supernatant was measured in each case. All the data are an average of three replicates. The data was statistically analyzed by Independent sample T tests with SPSS, 13.55 modules. The ingredients of the two media are as follows: Pikovskay's medium PKVS[25] (per liter): glucose, 10g; $Ca_3(PO_4)_2$, 5g; $(NH)_2SO_4$ 0.1g; $MgSO_4.7\ H_2O$, 0.1g; KCl, 0.2g, Yeast extract, 0.5g;$MnSO_4.H_2O$, trace; $FeSO_4\ H_2O$ trace; and National Botanical Research Institutes Phosphate growth Medium NBRIP[18] (per litre): glucose, 10g; $Ca_3(PO_4)_2$, 5g; $(NH)_2SO_4$ 0.1g; $MgCl_2.6\ H_2O$ 5g; ; $MgSO_4.7\ H_2O$, 0.25g; KCl, 0.2g.

TABLE 1 : Morphological, biochemical characteristics of bacterial isolate.

Characterization of Pseudomonas putida Biotype A	
Morphological	
Gram's Staining	-
Motility	+
Biochemical	
Oxidase	+
Catalase	+
Indole	-
Methyl red	-
Voges Proskauer	-
Citrate	+
Urease	-
Fluorerescent Pigment	+
Growth At	
4^0 C	+
41^{0}C	-
Characterization	
Arginine dihydrolase	+
Lecithinase	-
Gelatin hydrolysis	-
Starch hydrolysis	-
Denitrification	-
Levan formation	-
Utilization of	
Fructose	+
Sucrose	-
Galactose	-
Arabinose	-
Trehalose	-
L-Tryptophan	-
Valine	+
D-alanine	+

+Positive; - negative for the above reactions

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RESULTS

The phosphate solubilizing bacterial strain was isolated from rhizosphere soil and isolate was identified based on biochemical tests and found to be aerobic, fluorescent Gram-negative rod, fluorescent, pigmented motile bacteria. Biochemical tests find positive for oxidase, catalase and citrate utilization negative for Indole, Methyl Red, Voges Proskauer, Urease tests. Able to grow at 4^0C no growth at 41^0C, positive for the production of Arginine Dihydrolase enzyme and showed negative reactions for lecithinase, gelatin, starch hydrolysis, denitrification and levan formation from sucrose. Utilization patterns of sugars and amino acids find that the isolate utilize fructose, alanine and valine as sole nitrogen source (TABLE 1). The isolate showed a maximum similarity 0.632 with *Pseudomonas putida* biotype A based on Fatty Acid Methyl Ester analysis results Microbial Type Culture Collection Centre; Institution of Microbial Technology, Chandigarh, India

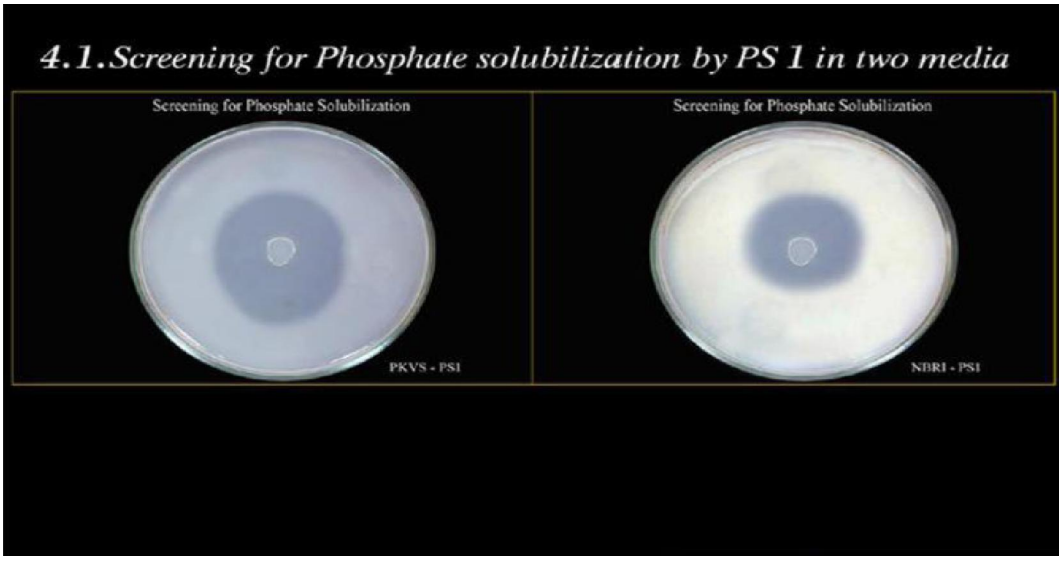

Figure 1 : Screening of *Pseudomonas* (Ps1) for phosphate solubilization.

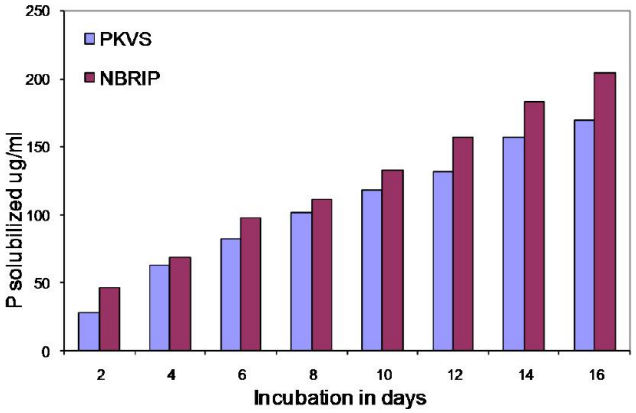

Figure 2 : Phosphate solubilization by *bacterial strain* in PKVS and NBRIP media.

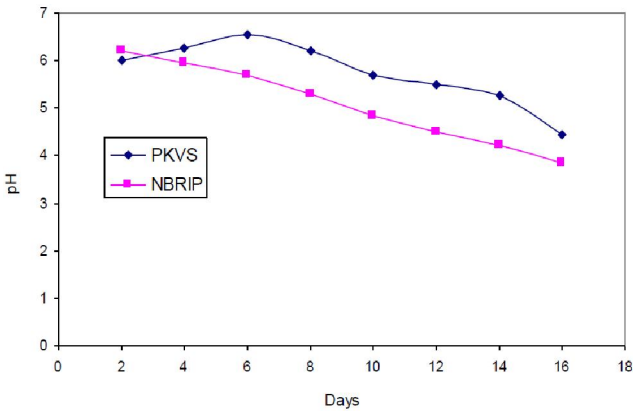

Figure 3 : Lowering of pH in two media (PKVS, NBRIP) by bacterial strain.

Screening of phosphate solubilizing bacterium

The bacterial strain was screened for phosphate solubilization by quantitavely and the bacterial isolate (Ps1) shown clear halo zones with solubility index (SI) of 17/6, 9/4 on PKVS and NBRIP media respectively after 12 days of incubation at 28 ^{0}C. (Figure 1). Quantitative estimation of phosphate solubilization in two media (PKVS, NBRIP) after incubation from 2 to 16 days (2 days intervals) is presented in Figure 2. The bacteria solubilized P in an efficiently increasing manner with reference to incubation days in both media. Maximal solubilization was seen in NBRIP medium (204μgmL^{-1} of P) on day 16, after which the solubilization declined gradually (Figure 3). The pH of the broth

was found to decline in each case due to bacterial activity, lowering of pH coincided with phosphate- solubilizing activity. The pH was lowered from control 7.8 to 3.85 and 4.45 on 16th day of incubation in NBRIP and PKVS media respectively. (Figure 3).

DISCUSSION

Phosphate solubilization ability of microorganisms was evaluated by two techniques in laboratory conditions. One method uses a precipitated phosphate agar plate assay that are used widely in the screening of P solubilizing microorganisms and the other uses a liquid media/culture broth[1,12,19,23]. The correlation between the SI on agar medium and Phosphate solubilization in liquid cultures of present results supported by the findings of Gupta *et al.*,[10] that the strain with small clear halo zone in NBRIP medium exhibited high solubilization in liquid NBRIP medium but the same isolate with more SI in PKVS agar has relatively less solubilization in liquid cultures. This shows that the plate technique is insufficient to detect all P solubilized as reported by Sangeetha Mehta and Nautiyal[23]. Over decades bacteria have been characterized and identified according to a few phenotypic characters such as morphology, pigmentation, reaction to dyes, the presence or absence of spores, nutritional requirements ability to produce acids from sugars. These simplified characterization methods are still the basis of classification. Besides the enzymatic and metabolic activities fatty acid composition profiles have been proposed as method for taxonomic identification in which fatty acids are methylated etherified and then analyzed by gas chromatography[7].

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