RESEARCH PAPER | Agriculture | Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X


# Isolation and Characterization of Phosphate Solubilizing Pseudomonas Sp. from Rhizospheric Soil of Rice

KEYWORDS: Phosphate solubilization, Rhizobacteria, Fluorescent pseudomonas, Macronutrients

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**ABSTRACT** *Phosphorus (P) is an essential macronutrient for the growth of plants. However , in most soils a large portion of phosphorus becomes insoluble and therefore, unavailable to plants. Thirty one Pseudomonas sp. were isolated from rhizospheric soil of rice. Out of thirty one nine isolates were able to solubilize Ca3(PO4)2 on Pikovskya's agar medium. A total six sp. of phosphate solubilizing fluorescent pseudomonads which showed fluorescence under UV radiation (λ=365nm) viz. Pseudomonas aeruginosa, P. fluorescens, P. putida and P. plecoglossicida and three species of phosphate solubilizing non-fluorescent pseudomonas were isolated. Isolate MGR38 was tentatively identified as Pseudomonas fluorescens which showed 83.33% efficiency in phosphate solubilization and the other isolate MGR31, MGR37, MGR39, MGR40, MGR41, MGR42, MGR43 and MGR44 were showed 33.33, 57.14, 50, 50, 16.16, 40, 60 and 80 % efficiency of phosphate solubilization respectively.*

## INTRODUCTION

Phosphorus is one of the most essential macronutrients of plants and its deficiency is a severe constraint to crop production. Plants absorb only inorganic form of phosphorus and the level of inorganic phosphorus in soil is very low normally 1ppm or less (Goldstein,1994). Most of the phosphorus in soil is present in insoluble mineral forms such as hydroxyapatites, oxyapatites and appetites. Rice field soils possess considerable accumulation of phosphorus due to regular application of chemical phosphate fertilizers and a large proportion of the applied fertilizer is converted in to insoluble form and become unavailable to plants (Rodriguez & Fraga, 1999). Soil microorganisms have the ability to solubilize insoluble phosphates and to improve soil health and fertility Jaharamma, Badri, and Sakthivel (2009). Phosphate solubilizing microbes have been reported for plant growth promoting and enhancing yield Kapoor, Mishra, and Kuhkreja (1989); Rodriguez and Fraga (1999). Microorganisms play an important role in the availability of soil phosphate to the root system and enhance the mobilization of phosphate in soil (Richardson, 2001). Production of organic acids and the enzymes phosphatase and phytase by fluorescent pseudomonads seems to be the main cause of phosphate solubilization Jaharamma et al. (2009). Considering the plant health and crop productivity it is important to study the diversity of phosphate solubilizing Pseudomonas sp. as these native strains may be used as bioinoculants for sustainable agriculture which will be cost effective and ecofriendly.

## MATERIALS AND METHODS

### Sample collection

Rhizospheric soil samples of rice (Oryza sativa L.) were collected in pre sterilized polythene bags from Sone beel wet land, Assam,India. Samples were stored at 40C within 24h of collection before being processed.

### Isolation of Pseudomonas species

Root samples were shaken vigorously to remove loosely adhering soil. 10g of tightly attached soil with roots were taken in a conical flask containing 90ml of 0.1M $MgSO_4.7H_2O$ buffer and kept for 10min on a rotary shaker at 150 rpm. Resulting suspensions were tenfold diluted, 0.1 ml aliquots of $10^{-6}$ dilution was inoculated on sterilized King B agar medium King,Ward,and Pandey (1954) and incubated for 2-3days at 28$^0$C. After incubation, fluorescent Pseudomonads were identified under UV light for few seconds at 365 nm and colonies exhibited bluish green fluorescence were picked up and streaked on sterilized King B agar plate to obtain pure cultures. Stock cultures were made in Luria Bertani (LB) broth containing 50% (w/v) glycerol and stored at -86$^0$C.

### Qualitative estimation of phosphate solubilization

To detect phosphate solubilizing bacteria, strains were spot inoculated on to Pikovskaya's agar medium (Pikovskya,1948) containing (per liter): 0.5g yeast extract, 10g dextrose, 5g $Ca_3(PO_4)_2$, 0.5g $(NH_4)_2SO_4$ 0.2g KCl, 0.1g $MgSO_4.7H_2O$, 0.0001g MnSO4.$H_2O$, 0.0001g $FeSO_4.7H_2O$ and 15g agar and incubated for 3- 4 days at 28$^0$C. Formation of clear zone around the colonies indicated phosphate solubilization by the bacteria. Diameter of clear zone around the colonies was recorded. Solubilization efficiency of bacterial isolates were calculated based on the following formula Nguyen, Yan, LeTacon, and Lapayrie (1992).

### Identification of bacterial isolates

Cultural and biochemical characterization was done by the methods of Cappuccino and Sherman (2004) and were tentatively identified by following Bergeye's Manual of determinative Bacteriology Holt, Krieg ,Sneath, Staley, and Williams (1994).

## RESULTS AND DISCUSSION

A total of thirty one Pseudomonas sp. were isolated from rhizospheric soil of rice. Out of thirty one nine isolates were phosphate solubilizer (Fig 1). Among them four were fluorescent pseudomonads which showed fluorescence under UV radiation ( λ= 365nm) and five were Pseudomonas sp. All these phosphate solubilizing isolate MGR31, MGR37, MGR38, MGR39, MGR40, MGR41, MGR42, MGR43 and MGR44 were tentatively identified as Pseudomonas sp. , P. aeruginosa, P. fluorescens, P. putida, P. sp, P. sp., P. plecoglossicida, P. sp., and P. sp. respectively on the basis of morphological (Table 1) and biochemical characteristics (Table 2). All the isolates were gram negative, rod shaped and were produced either yellowish green or light green or green pigment on Kings B agar medium. Among all the phosphate solubilizer the isolates MGR38 showed highest ( 83.33%) and MGR41 showed least (16.66%) efficiency in phosphate solubilization and the other MGR31, MGR37, MGR39, MGR40, MGR42, MGR43 and MGR44 was found 33.33, 57.14, 50, 50, 40, 60 and 80 % respectively (Fig 2). The isolate MGR38 produce enzyme like amylase, cellulase, protease and gillatinase. It has been reported that strains of Pseudomonas which produce protease, cellulase, pectinase, amylase and gillatinase solubilize inorganic phosphate and 18% of the

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

fluorescent pseudomonads are efficient phosphate solubilizer Naik, Raman,Badri Narayan, and Sakthivel (2008).

**Table 1.Morphological characteristics of the bacterial isolates**

| SL. NO | STRAIN NAME | SIZE | COLOUR | FORM | OPACITY | MARGIN | ELEVATION | FLURESCENCE |
|---|---|---|---|---|---|---|---|---|
| 1 | MGR31 | Small | Light green | Circular | Translucent | Entire | Flat | + |
| 2 | MGR37 | Small | Greenish yellow | Irregular | Translucent | Entire | Convex | +++ |
| 3 | MGR38 | Small | Green | Circular | Translucent | Undulate | Flat | +++ |
| 4 | MGR39 | Moderate | Light green | Circular | Translucent | Entire | Flat | - |
| 5 | MGR40 | Small | Light green | Circular | Opaque | Entire | Convex | - |
| 6 | MGR41 | Small | Yellowish green | Circular | Opaque | Entire | Convex | ++ |
| 7 | MGR42 | Small | Yellowish green | Circular | Translucent | Undulate | Convex | +++ |
| 8 | MGR43 | Moderate | Light green | Circular | Opaque | Entire | Raised | +++ |
| 9 | MGR44 | Moderate | Yellowish green | Circular | Opaque | Entire | Convex | + |

Note: +++ = high fluorescence, ++ = medium fluorescence, + = weak fluorescence, - = no fluorescence

**Table 2.Biochemical characteristics of the bacterial isolates**

| PARAMETERS | | MGR31 | MGR37 | MGR38 | MGR39 | MGR40 | MGR41 | MGR42 | MGR43 | MGR44 |
|---|---|---|---|---|---|---|---|---|---|---|
| Gram stain | | -, Rod | -, Rod | -, Rod | -, Rod | -, Rod | -, Rod | -, Rod | -, Rod | -, Rod |
| Motility | | + | + | + | + | + | + | + | + | + |
| FERMENTATION | Lactose | - | - | - | - | - | - | - | - | - |
| | Dextrose | - | + | + | + | - | - | - | - | - |
| | Fructose | - | - | - | + | - | - | - | - | - |
| ENZYME PRODUCTION | Catalase | + | + | + | + | + | + | + | - | - |
| | Amylase | _ | _ | _ | - | - | _ | + | - | - |
| | Protease | + | + | _ | _ | + | - | + | + | + |
| | Gillatinase | + | + | _ | _ | + | + | _ | + | + |
| | Cellulase | - | + | _ | _ | - | - | - | - | - |
| | Pectinase | - | _ | + | + | - | - | - | - | - |
| | Urase | - | + | - | - | - | | - | - | - |
| | Oxidase | + | + | + | + | + | + | + | + | - |
| $H_2S$ Production | | + | - | - | - | + | - | + | + | - |
| Indole Production | | + | - | _ | - | + | - | + | + | + |
| Citrate Utilization | | + | + | + | + | + | + | + | + | + |
| $NO_3$ Reduction | | - | + | + | + | + | + | + | - | - |
| MR-Reaction | | - | _ | + | - | + | - | - | - | + |
| VP-Reaction | | + | _ | - | - | - | - | - | - | + |
| Arginine Hydrolysis | | + | + | + | + | + | + | + | + | + |
| Lipid Hydrolysis | | - | + | + | + | + | + | + | + | - |
| Casein Hydrolysis | | - | + | + | + | + | + | + | + | - |
| GROWTH AT | 4°C | + | _ | - | + | + | + | _ | + | + |
| 41°C | - | + | _ | - | - | - | - | - | - | |
| Tantative identification | | P.sp. | P.aeruginosa | P.fluorescens | P.putida | P.sp. | P.sp. | P.plecoglossicida | P.sp | P.sp. |

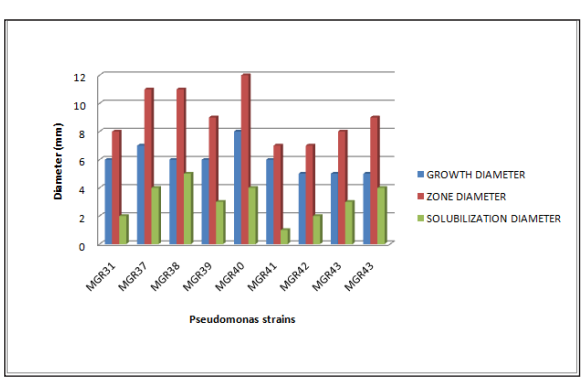


**Fig.1: Qualitative estimation of Phosphate solubilization**

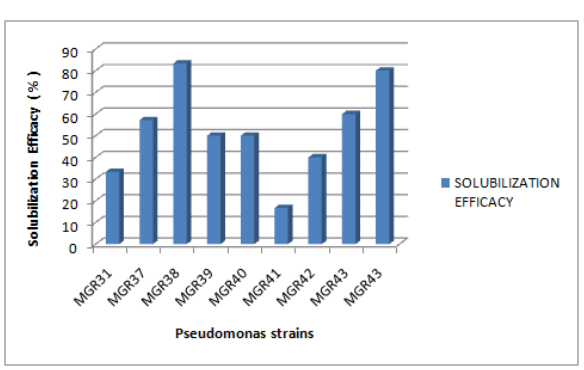


**Fig.2: Phosphate solubilization efficiency of Pseudomonas strains**

Volume : 3 | Issue : 9 | Sept 2013 | ISSN - 2249-555X

## CONCLUSION

Soil fertility is one of the most important factor for crop production . The excess use of chemical pesticides in agricultural fields minimizes the solubility of chemical fertilizers and making it unavailable to plants. Plant growth and yield is often limited by insufficient phosphate availability. Our study revealed that genus Pseudomonas play an important role in phosphate solubilization . Considering the phosphate solubilizing efficiency it may be used as bioinoculants for sustainable agriculture.

**Acknowledgement:**
The authors are thankful to the Head, Department of Life science & Bioinformatics, Assam University, Silchar, India for providing laboratory facilities.

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