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Research Article

Phosphate solubilisation by the Isolates of Fluorescent pseudomonads

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Abstract

Field soils possess considerable accumulation of phosphorous due to regular application of chemical phosphate fertilisers and a large proportion of the applied fertilisers are converted into insoluble form and become unavailable to plants. Interest has been focused on the inoculation of phosphate solubilising microorganisms into the soil so as to increase the availability of native, fixed phosphorous and to reduce the use of fertilisers. Fluorescent pseudomonads solubilise di-calcium phosphate and tricalcium phosphate.The present study focuses on the phosphate solubilising ability of the 22 strains of fluorescent pseudomonads isolated from rhizosphere soils of plants of coastal districts of Andhra Pradesh, India. All the 22 isolates were reported to solubilise phosphates.

Key words: Rhizosphere soil- Fluorescent pseudomonads- Tricalcium phosphate- phosphate solubilisation.

INTRODUCTION

Bacteria are the predominant microorganisms that can solubilise phosphate compared to fungi and actinomyces. Bacteria belonging to *Mesorhizobium, Rhizobium, Klebsiella, Acinetobacter, Enterobacter, Erwinia, Achrobacter, Micrococcus, Pseudomonas* and *Bacillus* isolated from different soils have been reported as efficient phosphate solubilisers.

Sustainable agriculture could be promoted by harnessing soil microbes, in particular fluorescent pseudomonads, to mobilise soil inorganic phosphate and increase bioavailability for plants. In particular, soil microorganisms are effective in releasing phosphorous from organic pools of total soil phosphorous by mineralisation and from inorganic complexes through solubilisation.

The mechanism of mineral phosphate solubilisation by phosphate solubilising bacteria (PSB) strains is associated with the release of low molecular weight organic acids, which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms.

MATERIALS AND METHODS

Estimation of Total cell protein

Total cell proteins were estimated by Lowry method (Lowry *et al.* 1951). An aliquot (0.5 ml) of the bacterial suspension was mixed with 0.5 ml of 1 N NaOH and kept in boiling water for 10 min. After cooling, 5 ml of copper carbonate reagent (50 ml of 2% sodium carbonate, 1 ml of 0.5 % copper sulphate and 1 ml of 1% sodium potassium tartarate) were added. The solution was allowed to stand at room temperature for 10 min. Then 0.5 ml of diluted (1:1 dilution with water) Folin Phenol reagent was added with vigorous shaking. After 30 min. the absorbance of the coloured solution was read at 660 nm against reagent blank. Bovine serum albumin was used as standard.

Quantification of Phosphate solubilisation

The cultures were grown in 100 ml of Pikovskaya's broth and centrifuged at 10000 rpm for 10 min and an aliquot of 1ml of the supernatant was mixed with

10 ml of chloromolybdate solution. Then 0.25 ml of chlorostannous acid was added and made up to 50 ml with distilled water. The resulting blue coloured solution was read at 600 nm against a reagent blank.

Composition of Pikovskaya's medium

Glucose	-	10.0 g
MgSO4 7H2O (2.5%)	-	10.00 ml
CaCl2 (1%)	-	10.00 ml
Tricalcium phosphate	-	5.0 g
Distilled water	-	1000 ml
pH	-	7.00
Agar	-	18.00 gm

Preparation of Chlorostannous acid

An amount of 2.5 g of $SnCl_2.2H_2O$ was dissolved in 10 ml of conc. HCl and made upto 100 ml with distilled water. This solution was prepared fresh for each set of experiment.

Preparation of Chloromolybdic solution

To 15 g of Ammonium molybdate in 400 ml of warm distilled water, 342 ml of conc. HCl was added and cooled. Then the volume was made to 1000 ml with distilled water.

RESULTS AND DISCUSSION

Solubilisation of inorganic phosphorus by the isolates of fluorescent *Pseudomonas*

All the 22 isolates were able to solubilise the inorganic phosphorus, which were identified by the formation of haloes on Pikovskaya agar medium. The amount of phosphorous relesased from tricalcium phosphate ranged from 315.46 µg of Pi/mg protein to 50.14 µg of Pi/mg protein (Table 1). Among the 22 isolates, *P. fluorescens* Os25 showed highest level of phosphate solubilisation (315.46 µg of Pi/mg protein, Plate 1). This was followed by the isolate *P. fluorescens* Ae1, which produced 260.16 µg of Pi/mg protein. However, *P. aeruginosa* So17 exhibited the least phosphate solubilisation property (50.14 µg of Pi/mg protein). The data is depicted in Fig.1.

The average phosphate solubilisation by the isolates was 159.72 µg of Pi/mg protein.

These findings are similar to the results observed by Nautiyal *et al*. (2000). They found that the phosphate solubilisation exhibited by the four most efficient fluorescent pesudomonads isolates from the alkaline soils of tropical India, NBRI2601, NBRI3246, NBRI0603 and NBRI4003 were, 450, 290, 250, and 200 µg/mL respectively. Megha *et al*. (2007) reported that the amount of Pi released from tricalcium phosphate by the 52 strains isolated from the forest soils of Western ghats in Pikovskaya's broth at 15 DAI ranged from 1.78 to 15.44 per cent.

In the present study the concentration of phosphorous released into the media varied from strain to strain which could be a consequence of phosphorous precipitation of organic metabolites as reported earlier by Babenko *et al*. (1984). An alternative explanation could be the difference in the rate of phosphorous release and uptake. Phosphorous release is a complex phenomenon and depends on many factors such as nutritional, physiological and growth conditions of the culture (Reyes *et al*. 1999).

During mineral phosphate solubilisation, decrease of pH in the culture medium was observed. A significant decline in the pH of the culture medium by phosphate solubilisers *P. aeruginosa*, *P. plecoglossicida*, *P. mosselii* was observed during mineral phosphate solubilisation due to the microbial production of organic acids was reported earlier by Illmer and Schinner (1995). In their study on phosphate solubilisation, Suresh *et al*. (2010) reported that due to the microbial production of organic acids by fluorescent pseudomonads, the pH decreased from 5.9 to 4.9.

Phosphate assimilation from organic compounds by microorganisms takes place through the enzyme phosphatase and the phosphorous which is released as a by product, provides the cell with essential nutrients for plant growth and development (Goldstein, 1994). Prasanna *et al*. (2011) suggested that the efficiency of isolated phosphate solubilising strain can be determined by measuring the phosphatase activity on the phosphorous substrate.

Solubilisation of inorganic phosphorus by the isolates revealed that *P. fluorescens* Os25 showed maximum in phosphate solubilisation. Solubilisation of minerals such as phosphorus is one of the mechanisms involved in direct enhancement of growth by plant growth promoting rhizobacteria. Thakuria *et al*. (2004) reported that a better phosphate solubilising isolate of PGPR induced better rice yield. Phosphate solubilising *P. fluorescens* Pf-110 and Pf-173 were found to promote plant growth in pea, than others (Negi *et al*. 2005).

In agreement with these reports, the phosphate solubilising property of fluorescent *Pseudomonas* of this study is one among the several mechanisms to stimulate plant growth.

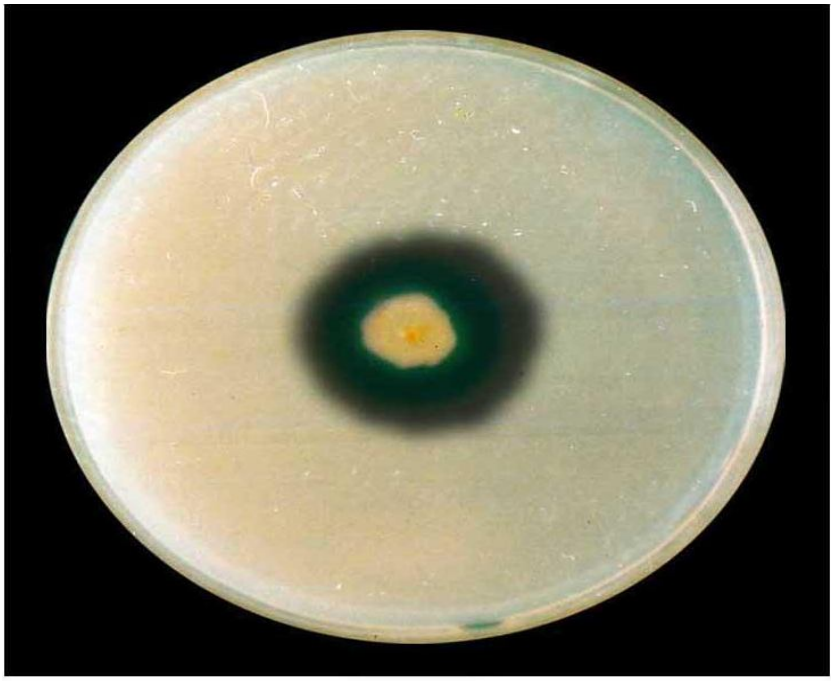
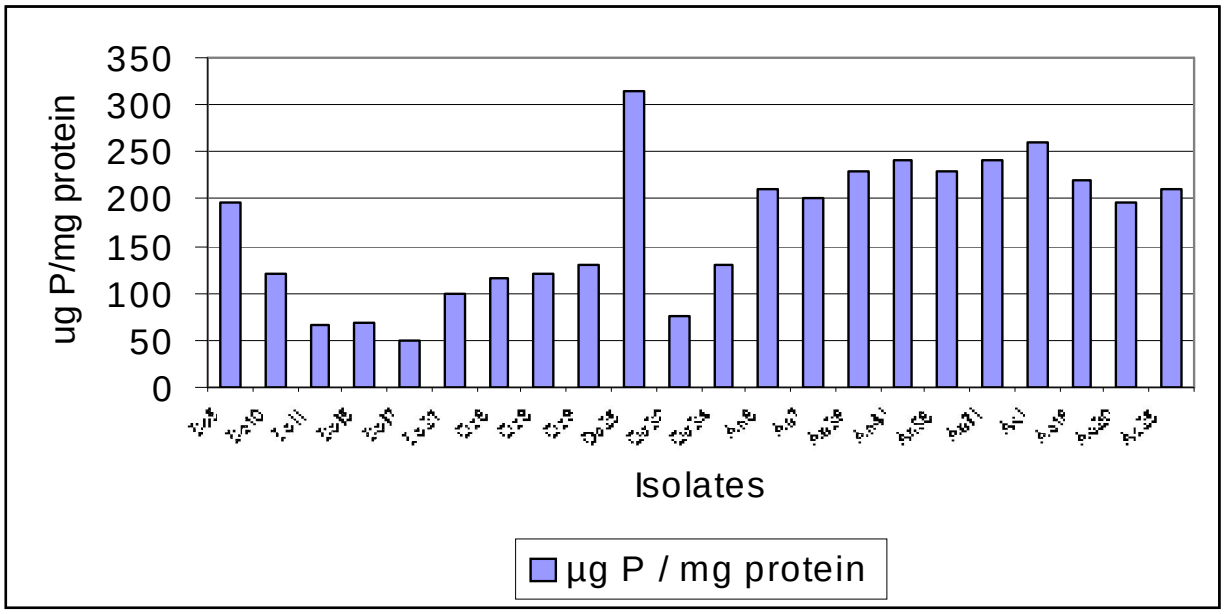


Plate 1 : Solubilisation of Tricalcium phosphate by the isolate *P.fluorescens* Os25

Table 1 : Solubilisation of Tricalcium phosphate by the isolates of fluorescent pseudomonads

Isolates	µg P / mg protein
So5	195.16
So10	120.16
So11	65.46
So15	69.14
So17	50.14
So27	100.15
Os6	115.16
Os8	120.16
Os9	130.17
Os25	315.46
Os32	75.15
Os34	130.45
An6	210.16
An7	200.16
An36	230.16
An41	240.45
An56	230.16
An71	240.17
Ae1	260.16
Ae16	220.25
Ae20	195.45
Ae25	210.49

Fig. 1. Solubilisation of Tricalcium phosphate by the isolates of fluorescent pseudomonads



REFERENCES

1. Babenko YS, Tyrygina G, Grigoryev EF, Dolgikh LM. and Borisova TI.. Biological activity and physiologo-biochemical properties of bacteria dissolving phosphates. Microbiologiya**. 1984;**53: 533-539.
2. Goldstein AH. Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous mineral phosphates by gram-negative bacteria,. *In*: Phosphate in microorganisms: cellular and molecular biology. (Eds.) Torriani-Gorini, A., Yagil, E. and Silver, S. ASM Press, Washington, DC. 1994; 197-203.
3. Illmer P and Schinner F. Phosphate solubililizing microorganisms under non-sterile conditions. Bodenkultur. 1995;46: 197-204.
4. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ.. Protein measurement with the folin phenol reagent. J. Biol. Chem. 1951;193: 265-275.
5. Megha YJ, Alagawadi AR and Krishnaraj PU. Multiple Beneficial Functions of Fluorescent Pseudomonads of Western Ghats of Uttara Kannada District**.** Karnataka J. Agric. Sci. *2007;*20(2): 305-309.
6. Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal R. and Verma D. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol Lett. 2000;182: 291-296.
7. Negi YK, Garg SK, and Kumar J. Cold-tolerant fluorescent *Pseudomonas* isolates from Garhwal Himalayas as potential plant growth promoting and biocontrol agents in pea. Curr. Sci. *2005;* 89: 2151-2156.
8. Prasanna RB, Jansi Rani Reddy MS. and Vijay Krishna KK. Isolation of siderophore producing strains of rhizobacterial Fluorescent Pseudomonads and their biocontrol against rice fungal pathogens. Int. J. Appl. Biol. and Pharmaceutical Technol. 2010;1(1): 134.
9. Reyes I, Bernier L, Simard RR. and Antoun H. Effect of nitrogen source on solubilization of different inorganic phosphates by an isolate of *Pencillium rugulosum* and two UV-induced mutants. FEMS Microbiol. Ecol. 1999;28: 281-290.
10. Suresh A, Pallavi P, Srinivas P, Praveen Kumar V, Jeevan Chandra S. and Ram Reddy S. Plant growth promoting activities of fluorescent pseudomonads associated with some crop plants. African J. Microbiol. Res.2010; 4(14): 1491-1494.
11. Thakuria D, Talukdar NC, Goswami C, Hazarika S, Boro RC. and Khan MR. . Characterization and screening of bacteria from rhizosphere or rice grown in acidic soils of Assam. Curr. Science.2004; 86: 978-985.