



## Phosphate solubilizing activity of *Pseudomonas fluorescens* PSM1 isolated from wheat rhizosphere

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**Abstract:** A study was designed to screen and analyze the efficient phosphate solubilizing bacteria (PSBs) from wheat rhizosphere. Five biovars of *Pseudomonas fluorescens* (PSM1, PSM2, PSM3, PSM4 and PSM5) were isolated from wheat rhizosphere and *Bacillus megaterium* MTCC 8755 procured from microbial type culture collection (MTCC) Chandigarh, India. The *P. fluorescens* biovar PSM1 was observed to be most efficient phosphate solubilizer. Inoculation of *P. fluorescens* PSM1 and *B. megaterium* MTCC 8755, alone and in combination, caused the highest phosphate solubilization at pH 5. At this pH, maximum phosphate solubilization was observed with *B. megaterium* MTCC 8755 inoculation (8.2 mg mL<sup>-1</sup>) on sixth day of incubation with *P. fluorescens* PSM1 (8 mg mL<sup>-1</sup>) on seventh day of incubation and with dual bacterial treatment (10.5 mg mL<sup>-1</sup>) on the fourth day of incubation. A correlation coefficient of linear regression equation of phosphate solubilization with pH indicated that pH value of the medium was directly correlated with tricalcium phosphate solubilization. The study will help in choosing soil pH specific PSB inoculant for optimizing plant growth.

**Keywords:** Phosphate solubilizing bacteria, *Pseudomonas fluorescens*, Rhizosphere

### INTRODUCTION

Phosphorus is an essential plant macronutrient making up to 0.2 % of the plant dry weight. It is a component of key molecules like nucleic acids, phospholipids, adenosine triphosphate, and consequently plants cannot grow without a reliable supply of this nutrient. Due to excessive use of chemical fertilizers in past few decades the global problem of "over phosphatization" of soil has come under notice (Corbridge, 1985). Only 10-25% of P introduced in the form of chemical fertilizers is utilized by plants. Plants can absorb only inorganic phosphates, and concentration of inorganic phosphates in soil is very low because most of the phosphorus in soils is present in insoluble forms. Most of the applied phosphorus transforms into insoluble hard to reach for plant forms through adsorption or precipitation. Insoluble phosphates include tricalcium-phosphate, hydroxyapatite, fluorapatite, aluminum phosphate, iron phosphate, bone meal and rock phosphates. It has been estimated that there is almost 40 million tons of phosphatic rock deposit in India (Roychoudhury and Kaushik, 1989) and this material can be used as a cheap source of phosphate fertilizer for crop production.

Rhizosphere is a crucial zone for screening efficient

phosphate solubilizing bacteria (PSBs). Plant growth promoting rhizobacteria (PGPR) is one of the classes of beneficial bacteria inhabiting the rhizosphere (Kloepper *et al.*, 1989). The effect of PGPR on plant growth can be mediated by direct mechanisms like phosphate solubilization, production of auxins, gibberellins, cytokines or by supplying biologically fixed nitrogen. Many soil microorganisms surviving in rhizosphere have the capacity to solubilize insoluble inorganic phosphate through phosphatase activity. Rhizosphere of many plants have already been screened for isolating PSBs (Krishnaveni, 2010; Maheswar and Sathiyavani, 2012; Prasanna *et al.*, 2011) In present study, five *P. fluorescens* biovars isolated from wheat rhizosphere along with *B. megaterium* MTCC 8755 were checked for phosphate solubilizing activity at four different pH values.

### MATERIALS AND METHODS

The biovars of *P. fluorescens* were isolated from wheat rhizosphere from Balawala region of Dehradun, India (30° 25'49" N, 78°11'26" E). The wheat plants were carefully uprooted and the soil attached to the roots was collected and serially diluted. The dilutions were spread on King's - B agar (King *et al.*, 1954) plates, incubated for 24 h at 28°C and observed for the devel-

opment of colonies. *Bacillus megaterium* MTCC 8755 was procured from Microbial Type Culture Collection (MTCC), Chandigarh, India for reference purpose.

The qualitative estimation of phosphate solubilization by bacteria was done using Pikovskaya's agar medium (HiMedia) containing tricalcium phosphate. The bacterial cultures were point inoculated on Pikovskaya's agar. Appearance of clear zone around bacterial colony in otherwise opaque medium (due to calcium phosphate) confirmed the solubilization process. After two days of incubation the diameter of colony, zone of clearance and solubilization index was determined by the formula mentioned below.

$$\text{Solubilization index} = A/B$$

Where,

A = Diameter (colony + halo zone) and B = Colony diameter

The bacteria were identified by the method described by Bergey's manual of determinative bacteriology (Bergey and Holt, 2000).

Quantitative estimation of liberated phosphorus from bacterial biovars was done (Subba Rao, 1982) at different pH values (5, 6, 7 and 8). In culture medium the pH of 5 and 6 was maintained using sodium citrate buffer (Dawson *et al.*, 1986) and pH 7-8 using sodium barbital buffer (Lundblad and Macdonald, 2010). A 10 mL culture containing broth was withdrawn in sterilized Eppendorf® tubes and centrifuged at 120 rpm for 20 min at 4°C. The pellet was discarded and one mL of supernatant was withdrawn and used for estimating free phosphorus. In a separate experiment, phosphate solubilization on Pikovskaya's broth medium (HiMedia) by *P. fluorescens* PSM1 and *B. megaterium* MTCC 8755, alone and in combination, was quantitatively estimated in unbuffered medium with initial neutral pH (Prasanna *et al.*, 2011). The final pH of the medium was recorded after eight days of treatment. All the observations were done in five replications. Regression studies were performed using Statistical Package for the Social Sciences (SPSS 15) software.

## RESULTS AND DISCUSSION

Phosphate solubilization is done by monocarboxylic acids like acetic acid, formic acid, gluconic acid and glycolic acid and by tricarboxylic acids like citric acid. Some of the other uncommon acids like trans-aconitic acids, propionic, isobutyric, isovaleric, ketobutyric, itaconic, isocaproic, malonic acids (Park *et al.*, 2010); monocarboxylic hydroxyl acids like lactic acid and succinic acid (Yoshikawa *et al.*, 1993), carboxylic acids like 2-keto gluconic acid and maleic acid; monocarboxylic acids like oxalic acid (Gulati *et al.*, 2010) and dicarboxylic hydroxy acids like malic acid (Kang *et al.*, 2012) are secreted by various phosphate solubilizing bacteria.

Microorganisms often export organic acids as anions (Netik *et al.*, 1997). Organic acids contribute to the lowering of solution pH as they dissociate in a pH de-

pendent equilibrium into their respective anion(s) and proton(s). Organic acids buffer solution pH and continue to dissociate as protons are consumed by the dissolution reaction (Welch *et al.*, 2002).

All the six bacterial isolates *viz.*, *P. fluorescens* PSM1, PSM2, PSM3, PSM4, PSM5 and *Bacillus megaterium* MTCC 8755 solubilized phosphate from Pikovskaya's medium. On the basis of solubilization index (Table 1) the best phosphate solubilizing biovar, *P. fluorescens* PSM1 along with *B. megaterium* MTCC 8755 was selected for further study. Similar studies have already been performed on rhizosphere of foxtail millet (Krishnaveni, 2010), rice (Prasanna *et al.*, 2011) and groundnut (Maheswar and Sathiyavani, 2012).

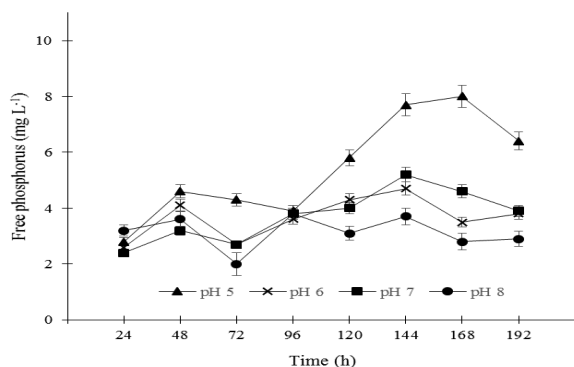
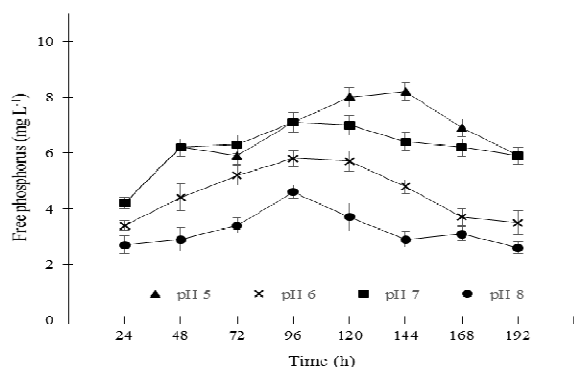
Quantification of phosphate solubilization by two isolates (*P. fluorescens* PSM1 and *B. megaterium* MTCC 8755) along with their composite inoculation was done up to eight days in Pikovskaya's broth at 30°C at four pH values: pH 5, 6, 7 and 8 (Figs. 1-3). Level of phosphate solubilized by *B. megaterium* MTCC 8755 was found maximum (8.2 mg mL<sup>-1</sup>) at pH 5 on sixth day of treatment and minimum (2.6 mg mL<sup>-1</sup>) with pH 8 on eighth day of treatment. With *P. fluorescens* PSM1 treatment the level of free phosphorus was maximum at pH 5 (8 mg mL<sup>-1</sup>) on seventh day of treatment and minimum (2.6 mg mL<sup>-1</sup>) at pH 8 on third day of treatment. With dual bacterial treatment the free phosphorus concentration was highest (10.5 mg mL<sup>-1</sup>) with pH 5 on the fourth day of treatment and lowest (2.9 mg mL<sup>-1</sup>) with pH 8 on eighth day of treatment. Similar findings have previously been reported from dual inoculation with other microorganisms (Aftab Afzal, 2008; Hassani *et al.*, 2015; Najjar *et al.*, 2012). The increased phosphate solubilization with dual bacterial treatment could be due to different modes of phosphate solubilization by two bacteria.

After attaining the peak of phosphate solubilization a decline in free phosphate concentration was seen with all the three inoculation treatments. Similar observation has previously been reported on *Penicillium* sp. GL-101 (Kang *et al.*, 2002). This effect could be due to accumulation of secondary metabolites which might hinder the overall growth of bacteria in the medium leading to slower solubilization of phosphate or could be due to the deficiency of nutrients in culture medium (Gand and Gaur, 1989). Phosphate solubilization by *P. fluorescens* PSM1 was comparable with *B. megaterium* MTCC 8755. Both the bacteria demonstrated maximum phosphate solubilization with pH 5 and minimum with pH 8 (Figs. 1 and 2). This observation was in agreement with previous findings on microbial phosphate solubilization (Gand and Gaur, 1989; Marra *et al.*, 2015).

In a separate experiment of phosphate quantification using non-buffered medium with neutral pH, a linear regression of dissolved phosphorus by final pH of the medium was observed with *B. megaterium* MTCC 8755, *P. fluorescens* PSM1 and co-inoculation treat-

**Table 1.** Phosphate solubilization index test.

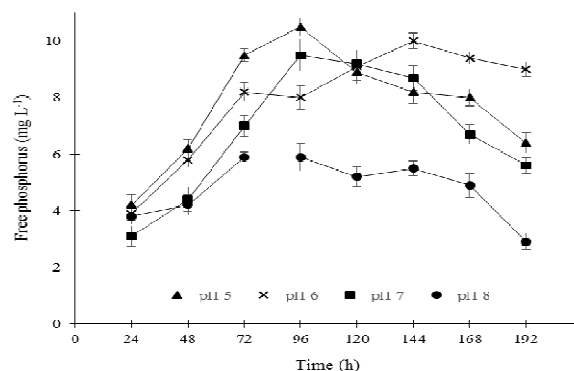
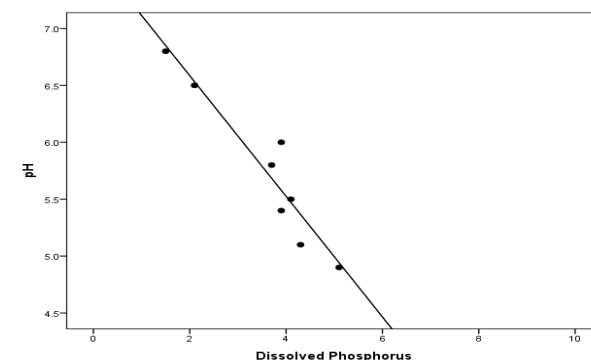
Isolate	Colony diameter (mm)	Halo zone diameter (mm)	Solubilization index
<i>P. fluorescens</i> PSM1	3.3	10.7	3.24
<i>P. fluorescens</i> PSM2	3.7	10.1	2.72
<i>P. fluorescens</i> PSM3	4.0	10.0	2.50
<i>P. fluorescens</i> PSM4	4.1	10.5	2.56
<i>P. fluorescens</i> PSM5	3.8	9.9	2.60
<i>B. megaterium</i> MTCC 8755	3.9	9.8	2.51

**Fig. 1.** Change of free phosphate concentration by *P. fluorescens* PSM1 at various pH levels with time courses. Vertical bars represent standard error (n = 5).**Fig. 2.** Change of free phosphate concentration by *B. megaterium* MTCC 8755 at four pH levels with time courses. Vertical bars represent standard error (n = 5).

ment of *B. megaterium* MTCC 8755 and *P. fluorescens* PSM1 (Figs. 4-6). The strongest regression of phosphate solubilization ( $R^2$  linear = 0.902) with pH of medium was obtained with *P. fluorescens* PSM1 treatment. A sharp decline in pH was observed with increase in phosphate solubilization.

## Conclusion

All the isolates maximally solubilized phosphate at pH 5 and minimally at pH 8. The *P. fluorescens* PSM1 was found to be the most efficient phosphate solubilizer. A strong regression of phosphate solubilization by final pH of the medium was obtained in all the treatments. The findings of this experiment explore the possibility of harnessing *P. fluorescens* on commercial scale. This isolate might prove useful in agricultural practices in the soils with acidic pH. Further study is needed in the field of bioformulation development for

**Fig. 3.** Change of free phosphate concentration by composite inoculation of *P. fluorescens* PSM1 and *B. megaterium* MTCC 8755 at four pH levels with time courses. Vertical bars represent standard error (n = 5).**Fig. 4.** Regression of dissolved phosphorus ( $\text{mg L}^{-1}$ ) by final pH of the medium with *P. fluorescens* PSM1 treatment ( $Y = -0.53x + 7.64$ ;  $R^2$  linear = 0.902). The regression coefficient is significant at  $P < 0.05$ .

extending bacterial shelf life, enhancing bacterial-plant specificity and effect on rhizosphere ecology.

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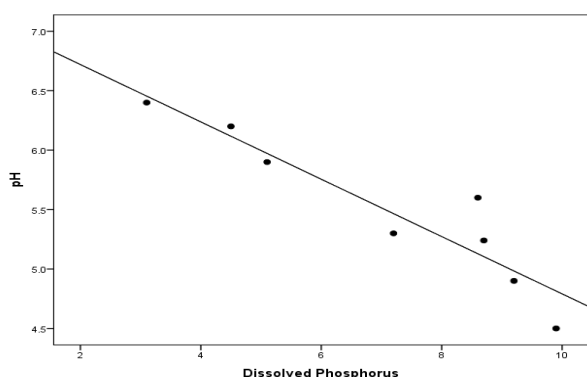


Fig. 5. Regression of dissolved phosphorus ( $\text{mg L}^{-1}$ ) by final pH of the medium with *B. megaterium* MTCC 8755 treatment ( $Y = -0.24x + 7.2$ ;  $R^2$  linear = 0.867). The regression coefficient is significant at  $P < 0.05$ .

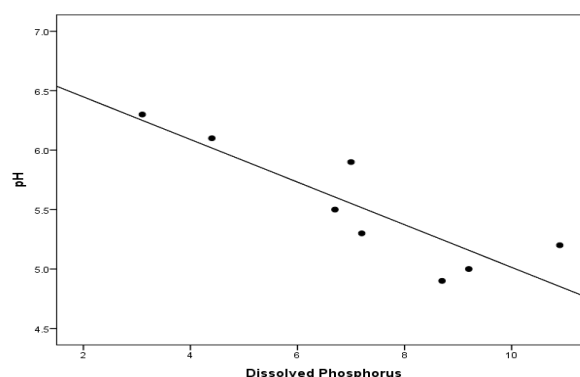


Fig. 6. Regression of dissolved phosphorus ( $\text{mg L}^{-1}$ ) by final pH of the medium by composite inoculation of *B. megaterium* MTCC 8755 with *P. fluorescens* PSMI treatment ( $Y = -0.18x + 6.81$ ;  $R^2$  linear = 0.760). The regression coefficient is significant at  $P < 0.05$ .

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