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QUANTITATIVE ESTIMATION OF INSOLUBLE INORGANIC PHOSPHATE SOLUBILIZATION

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ABSTRACT
Phosphorus, the 'king pin' in Indian agriculture, occupies a unique position both in conventional as well as in alternative agriculture. This non-renewable source, is a second major plant nutrient. *Bacillus* sp. isolated from a rhizosphere soil sample of leguminous plant. The isolated bacterium was used to test for solubilization of insoluble inorganic phosphate sources i.e., tricalcium phosphate (TCP), rock phosphate (RP) aluminium phosphate ($AlPO_4$) and ferrous phosphate ($FePO_4$). The isolated *Bacillus* sp. solubilize maximum amount of phosphate from KH_2PO_4, on 3rd day, tricalcium phosphate (68.48 mg P_2O_5) on 9th day, rock phosphate (13.78 mg P_2O_5) on 12th day, aluminium phosphate (5.65 mg P_2O_5) on 9th day and ferrous phosphate (22.12 mg P_2O_5) on 9th day, after which phosphate solubilization activity was decreased. The precipitated inorganic phosphates are brought back into the medium by the action of mineral and organic acids produced by the bacterial isolate.

KEYWORDS: Phosphate solubilization, Insoluble phosphate source, Tricalcium Phosphate, Rock Phosphate, Aluminium Phosphate, Ferric Phosphate, *Bacillus* sp.

INTRODUCTION
Improving soil fertility is one of the most common practices in agricultural production. Phosphorus (P) is one of the most essential plant nutrients for maximizing crop productivity. This nutrient is limited in soils, which remain as a major challenge to agriculturists and land managers. Phosphorus is most conventionally divided into two categories; insoluble and readily soluble. Readily soluble or water soluble P, when applied in acid soils is rapidly fixed to unavailable forms and accounts for low phosphate use efficiency (Sarkar and Uppal, 1994). The insoluble P, which is not directly available to plants or microorganisms, usually comprises 95-99 percent of the total P (Hayman, 1975). The availability of soil P is influenced by a number of factors such as nature and content of clay, active sesquioxides, lime, pH and organic matter. Soil phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} or adsorbed to Fe-oxides and Al-oxides, Al-silicates and Ca-carbonates depending on the particular properties of a soil. In these forms, P is highly insoluble and unavailable to plants. In most tropical soils, phosphate is predominantly present in the form of inorganic phosphate (IP) compounds, which belong to two groups; those of calcium and those of iron and aluminium. The calcium is predominant under neutral to alkaline soil conditions, while iron and aluminium phosphates predominant under acidic conditions (Kalia, 1956 and Williams and Steinbergs, 1958). The objective of the present study was to analyze the solubilization of insoluble inorganic phosphate sources i.e., tricalcium phosphate (TCP), rock phosphate (RP), ferric phosphate (FP) and aluminium phosphate (AP) by *Bacillus* sp. isolated from a rhizosphere soil sample of leguminous plant.

MATERIALS AND METHODS
Testing the efficiency of *Bacillus* sp. in liquid PVK medium with different insoluble phosphate sources
Insoluble phosphates such as, tricalcium phosphate (TCP), rock phosphate (RP), ferric phosphate (FP) aluminium phosphate (AP) and a soluble form of dihydrogen potassium phosphate (KH_2PO_4) sources were used for the quantitative estimation of phosphate solubilization (PS). The PS activity of the *Bacillus* sp. estimated in PVK liquid medium. Hundred ml aliquots of PVK broth was transferred into 250 ml conical flasks and sterilized by autoclaving before the addition of phosphate source. Different insoluble phosphate sources were dried in hot air oven separately and added at the rate of 500mg / 100 ml. One ml of 24 hr bacterial suspension was added to each flask. All transfers of bacterial culture were carried out aseptically. Each treatment was replicated thrice and incubated at 28^0C in a shaker. At every three days interval up to 15 days of incubation period, the required amount of samples were withdrawn from each conical flask for estimation. The sub-samples were centrifuged and the supernatant was filtered. The filtrate was used to measure the pH and soluble P (Jackson, 1973).

RESULTS
Phosphate solubilization in different insoluble phosphate sources by *Bacillus* sp.
The phosphate solubilizing bacteria *Bacillus* sp. examined for its PS activity and change in pH was measured with different insoluble phosphates along with soluble KH_2PO_4

and the results are given in Figs.1-5. In the figures the solubilized P is expressed as P_2O_5 / mg. The identified *Bacillus* sp. solubilized maximum amount of phosphorus (69.26 mg P_2O_5) from KH_2PO_4 on 3rd day. KH_2PO_4 is a soluble form of phosphate source. In case of insoluble form of phosphate source, *Bacillus* sp. solubilized maximum amount of phosphorus from TCP (68.48 mg P_2O_5) on 9th day, RP (13.78 mg P_2O_5) on 12th day, $AlPO_4$ (5.65 mg P_2O_5) on 9th day and $FePO_4$ (22.12 mg P_2O_5) on 9th day. Phosphate solubilization activity decreased after 9 (TCP, $FePO_4$, and $AlPO_4$) or 12 (RP) days of inoculation. A fall in pH was observed from the initial 7.0. There was a gradual reduction in pH from 3rd to 15th day. In case of $AlPO_4$ and $FePO_4$ the pH was greatly reduced to 2.8 (9th day) and 3.5 (12th day) respectively. The medium turned acidic during the incubation period. In case of KH_2PO_4 and RP the pH was reduced to 5.6 on 12th day. The isolate effectively converted the insoluble inorganic phosphate into soluble form.

FIGURE1. KH_2PO_4 Solubilization by *Bacillus* sp.

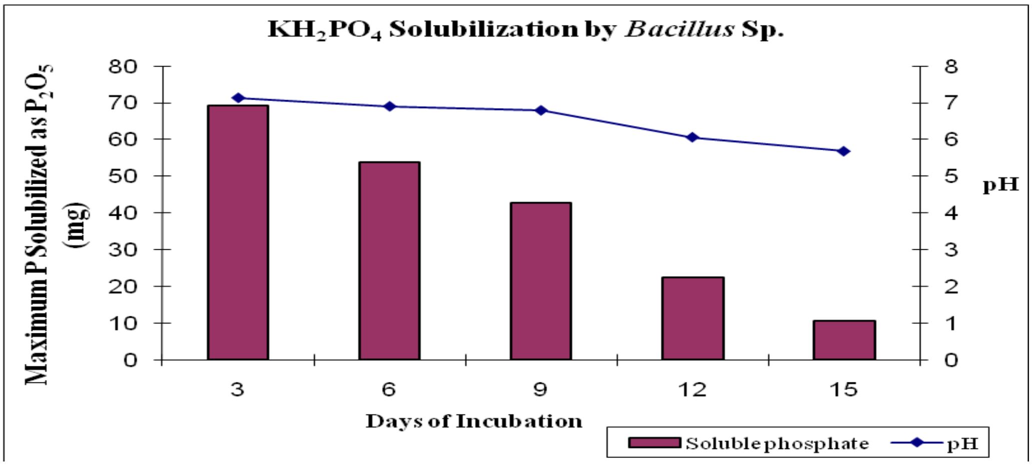


FIGURE 2. TCP Solubilization by *Bacillus* sp.

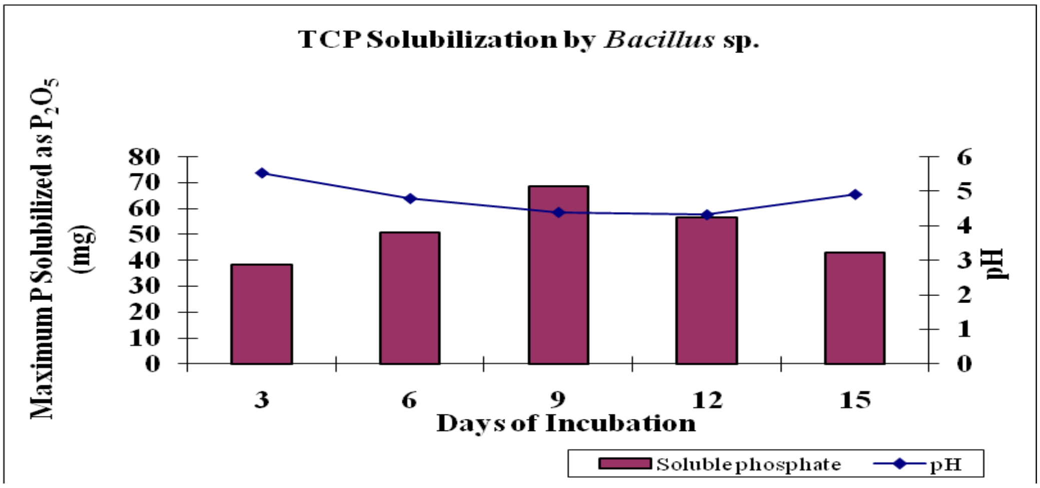


FIGURE 3. RP Solubilization by *Bacillus* sp.

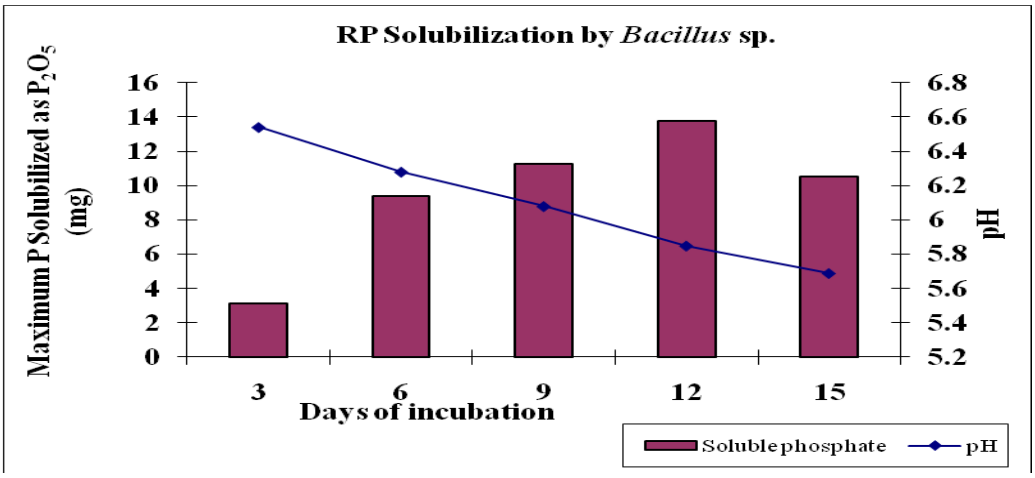


FIGURE 4. $AlPO_4$ Solubilization by *Bacillus* sp.

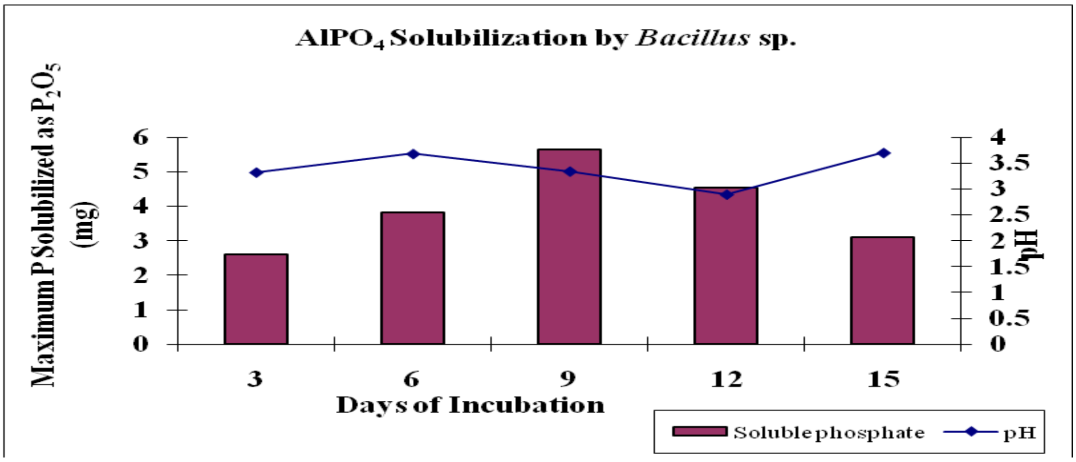
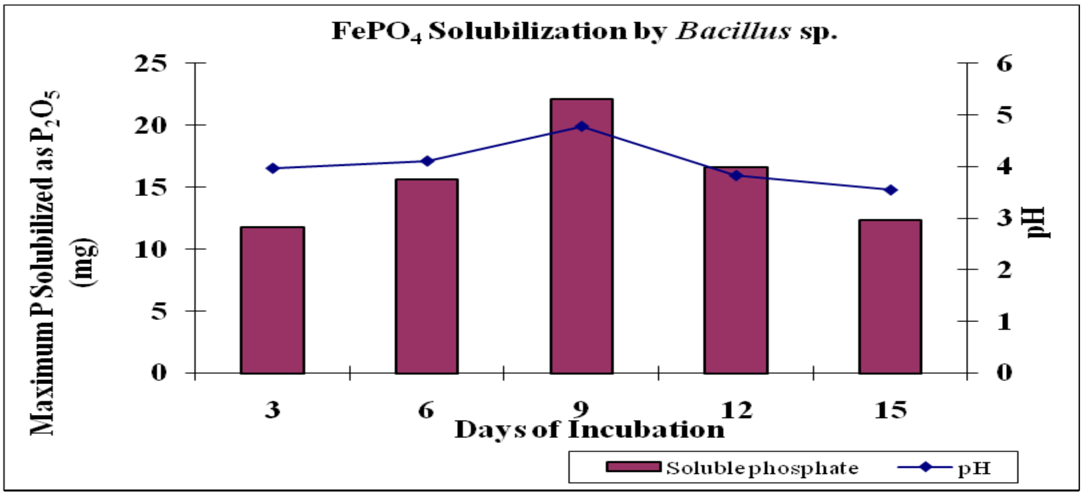


FIGURE 5. $FePO_4$ Solubilization by *Bacillus* sp.



DISCUSSION

Effect of different insoluble phosphate sources and incubation time on phosphate solubilization by *Bacillus* sp.

Periodical estimates of P in media revealed the potential of the isolate in releasing P from insoluble phosphate sources. In the present study, the *Bacillus* sp. solubilizes the insoluble phosphate sources, such as TCP, RP, $FePO_4$, and $AlPO_4$ which were slow initially, gradually increased in the middle period of incubation and declined later. The better performance of *Bacillus* sp. to release the available P in PVK medium indicates the role played by the different insoluble phosphate sources to trigger microbial action. With regard to the incubation period, the solubilization ability of the organism was high till 9 days of incubation. The *Bacillus* sp. solubilizes more amount of phosphate during 9 days of incubation except in case when KH_2PO_4 is used, which was readily available to the organism. In case of insoluble phosphate source the available P increased in the middle and declined later. The isolated *Bacillus* sp. solubilize maximum amount of phosphate from KH_2PO_4 on 3rd day, TCP, $AlPO_4$ and $FePO_4$ on 9th day and RP on 12th day, after that PS activity was decreased (Figs.1-5).

Gaind and Gaur (1989) reported that the drop in solubilization after a maximum value might be attributed to deficiency in nutrients in the culture medium. The decrease in soluble P at later periods of incubation may be either due to decreased solubilizing activity or increased P absorption and refixation of solubilized P with metal ions present in the broth. These results are in confirmation with the results observed by Gaur (1990) in *Pseudomonas striata*. The P concentration in solution did not follow a sigmoid curve type, but with some fluctuations, which may be due to cell lysis and P precipitation brought about by organic metabolites (Illmer and Schinner, 1992). Fluctuation in the bacterial activity is in accordance with the findings of Narsian *et al.* (1994) and Patel and Dave (2000). Decrease in P concentration during initial stages can be attributed to the utilization of existing P for growth and development of the organism. In the later stage the bacteria would have started acting on the substrate for want of nutrients, thus releasing P from insoluble sources. The cells after the initial shock would have utilized the available free P for metabolism and later acclimatized to the given environment or due to substrate stress (Seshadri *et al.,* 2000). Length of incubation period is one of the factors, which influences microbial activities. These results are in conformity with the findings of Goswami and Sen (1962), who observed maximum solubilization of phosphate occurring within two weeks period under controlled conditions. Similar results have been recorded by Chhonkar and Subba Rao (1967) and Mehta and Bhide (1970). Incubation up to 9 days may be effective in increasing PS. Degree of PS varied with the organism and phosphate compound (Leyval and Berthelin, 1985; Sujatha *et al.*, 2004).

Except TCP, other insoluble phosphate sources solubilization was very slow; this is similar to earlier findings. The poor solubilization of rock phosphates may be due to the complex mineral composition and other non-phosphorus content of the rocks (Bardiya and Gaur, 1972). The solubilization was reduced to a great extent in case of the RP presumably because of the strong apatite bond (Gupta and Biswas, 1994). RP solubilization depended on the nature of RP and the organism. The relative efficiency of the organisms for RP solubilization could be due to the nature and quantity of organic acids secreted into the medium (Narsian *et al.,* 1994). In an experiment conducted by Gaur (1990) where ferric phosphate was incorporated as the insoluble phosphate source, a marked blackening of the particles by numerous organisms was observed. The hydrogen sulphide presumably reduced ferric phosphate to black ferrous sulphide with the release of phosphate.

In studies conducted by Kang *et al*. (2001) on all the inorganic phosphates, aluminium PS resulted in a very slight decline of pH from 6.5 to 6.0, after 10 days of incubation. $AlPO_4$ was thus solubilized to the maximum at pH 6.0. In case of TCP there was an initial decrease in pH after 6 days incubation followed by a decline up to 5.7. A fall in pH accompanied PS was observed in this study during the various incubation periods. Rise in the pH observed later may be due to the use of organic acids by the organism. The results showed that the change in pH is dependent upon the type of phosphate source. Several potential mechanisms reported for PS that includes modification of pH by secretion of organic acids and protons or cation dissociation (Illmer and Schinner, 1992). Bardiya and Gaur (1974) suggested that the nature of organic acids produced is more important than the total acidity. Leyval and Berthelin (1985) observed that the production of acids during PS depends upon the microorganism and also on the type of insoluble phosphate used as the substrate. The precipitated inorganic phosphates are brought back into the medium by the action of mineral and organic acids produced by the bacterial isolate.

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