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INFLUENCE OF DIFFERENT CARBON AND NITROGEN SOURCES ON INSOLUBLE INORGANIC PHOSPHATE SOLUBILIZATION BY *BACILLUS SUBTILIS*

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ABSTRACT
Phosphate solublization activity of *Bacillus subtilis* in different carbon and nitrogen sources with insoluble form of phosphate sources like tricalcium phosphate and rock phosphate were studied. TCP solubilization with different carbon sources irrespective of incubation period can be related in the following order: glucose, fructose, arabinose, mannose, sucrose, lactose, and sorbitol. The pH drifted from neutral to acidic with all carbon sources in presence of TCP. All the sugars with rock phosphate did not have any marked influence on phosphate solubilization activity. Regarding TCP and RP solubilization with different nitrogen sources maximum solubilization was recorded in the presence of ammonium sulphate. *Bacillus subtilis* showed maximum PS with glucose and ammonium sulphate. RP solubilization in liquid medium was very slow.

KEY WORDS: Phosphate solubilization, Insoluble phosphate source, Tricalcium Phosphate, Rock Phosphate, *Bacillus* sp.

INTRODUCTION

Phosphorus (P) is one of the major nutrients, second only to nitrogen in requirement for plants. A greater part of soil phosphorus is in the form of insoluble phosphates and cannot be utilized by the plants. Soil P transformations are primarily mediated by microbial activity, which in turn is influenced by a combination of factors including plant species, soil type and environmental factors [1]. Carbon and nitrogen sources greatly influence phosphate solubilization process. In the presence of various carbon and nitrogen sources microorganisms have diverse levels of phosphate solubilization activity. Carbon source is an important parameter for active proliferation of organisms and production of organic acids and nitrogen source is important for the production of inorganic acids. Solubilization of insoluble phosphates depends upon a multitude of factors including decrease in pH, microorganism and the insoluble phosphate used [2 & 3]. The response of microorganisms to the surroundings is not always consistent, because soil environment and plant factors influence the activity of PSM [4].The objective of the present study was to examine phosphate solublization activity of *Bacillus subtilis* in different carbon and nitrogen sources with insoluble inorganic form of phosphate sources like tricalcium phosphate and rock phosphate.

MATERIALS AND METHODS

Collection of Organisms

The bacterial culture *Bacillus subtilis* was collected from the Department of Biology, Gandhigram Rural Institute, Gandhigram, Dindigul and grown on Nutrient agar slant for 3 days at 30^0C for further study.

Influence of different carbon and nitrogen sources on phosphate solubilization by *Bacillus subtilis*.

Preparation of growth medium with carbon and nitrogen sources

Three sets of flasks containing Pikovskaya's (PVK) broth medium with two different phosphate source such as TCP and RP were used in this experiment. The effect of different carbon (C) sources on phosphate solubilizing activity was observed by replacing glucose with five different carbon sources viz., fructose, lactose, arabinose, sucrose and galactose at the rate of 0.5 g/l. For the effect of different C sources on phosphate solubilization (PS), ammonium sulphate was a nitrogen source.

The effect of different nitrogen (N) sources on phosphate solubilization was evaluated by replacing ammonium sulphate with five different nitrogen sources viz., ammonium nitrate, sodium nitrate, calcium nitrate, potassium nitrate and urea at the rate of 0.5 g/l. Glucose was a carbon source to study the effect of different nitrogen (N) sources on PS. Carbon and nitrogen sources were dissolved in distilled water, sterilized separately and added to the culture medium prior to inoculation.

Estimation of Phosphate solubilization activity

One ml of 24 hrs bacterial culture was inoculated in 100 ml of PVK medium containing different C and N sources separately and incubated at room temperature. At every three days interval up to 15 days of incubation period, the required amount of sample was withdrawn from each conical flask for estimation of PS activity. The sub-samples were centrifuged and the supernatant was filtered. The filtrate was used to measure the pH and soluble P was estimated by Chlorostannous reduced molybdophosphoric acid blue method [5].

RESULTS

Influence of different C sources on phosphate solubilization

Phosphate solubilization activity of *Bacillus subtilis* influenced by different carbon sources was studied with TCP and RP is shown in Fig.1. *Bacillus subtilis* utilized a variety of carbon compounds as energy source but the phosphate solubilization activity varied with different carbon sources and the type of phosphate source**.** Though all the test sugars supported phosphate solubilization activity, glucose (62.40 mg P_2O_5) influenced highest activity on 3rd day followed by fructose (46.28 mg P_2O_5) on 3rd day and arabinose (46.08 mg P_2O_5) on 9th day. On the basis of maximum TCP solubilization, the different carbon sources irrespective of incubation period can be related in the following order: glucose > fructose > arabinose > mannose > sucrose > lactose > sorbitol. In the conical flask supplemented with of RP as a phosphate source, all the sugars did not have any marked influence on phosphate solubilization activity. Among the carbon sources tested, glucose (13.11 mg P_2O_5) found to be the best on 9th day followed by mannose (5.71 mg P_2O_5) on 3rd day and arabinose (5.39 mg P_2O_5) on 12th day for RP solubilization, which was in the order of glucose > mannose > arabinose > fructose > sucrose > lactose > sorbitol. The *Bacillus subtilis* showed greater phosphate solubilization activity with RP in presence of glucose than with other monosaccharides and disaccharides. The pH drifted from neutral to acidic with all carbon sources in the presence of TCP recording lowest of 4.01 after 15 days of incubation and RP recording lowest of 3.54 after 3 days of incubation. Lowest pH range was observed when glucose was used as a carbon source with TCP and RP.

Influence of different N sources on phosphate solubilization

The effect of five different nitrogen sources on phosphate solubilization activity of the *Bacillus subtilis* using TCP and RP is shown in Fig.2. Maximum TCP and RP solubilization was recorded in the presence of ammonium sulphate. Though all the tested nitrogen sources supported phosphate solubilization activity, ammonium sulphate recorded highest activity (43.27 mg P_2O_5) followed by ammonium nitrate (42.83 mg P_2O_5) and potassium nitrate (41.46 mg P_2O_5). TCP solubilization activity influenced by nitrogenous compounds can be arranged in the following order: ammonium sulphate > ammonium nitrate > potassium nitrate > calcium nitrate > sodium nitrate > urea. Maximum RP solubilization was found in the presence of ammonium sulphate (20.11 mg P_2O_5) followed by ammonium nitrate (7.60 mg P_2O_5) and sodium nitrate (4.97 mg P_2O_5). RP solubilization as influenced by nitrogenous compounds can be arranged in the following order: ammonium sulphate > ammonium nitrate > sodium nitrate > calcium nitrate > potassium nitrate > urea. The phosphate solubilization activity was lesser in the presence of organic nitrogen source (urea). All nitrogen sources showed highest activity of TCP solubilization on sixth day but urea showed maximum solubilization on 12th day. The drift in pH was directly related to phosphate solubilization activity i.e., when phosphate solubilization activity was more, the fall in pH was more.

DISCUSSION

Effect of different carbon and nitrogen sources on phosphate solubilization

Carbon

Microorganisms showed diverse levels of PS activity in the presence of various carbon and nitrogen sources. Microorganisms utilized a variety of carbon compounds as energy source, but the amount of PS varied with different substrates. In the present study, the *Bacillus subtilis* showed maximum PS with glucose in TCP containing PVK media whereas RP solubilization in liquid medium was very slow. Maximum PS was observed after 12 days of incubation for RP. Bacteria were able to solubilize very little phosphate in the absence of sucrose [6]. Glucose was found to be best carbon source followed by sucrose and galactose for phosphate solubilization by *Pseudomonas striata* [7]. Fructose proved to be the best carbon source for *Rhodotorula minuta* NCIM 3359 and *Saccaromyces cerevisiae* ATCC 9896 cultures [8]. Increasing glucose concentration from 0.5 to 2.0 % in PVK medium progressively enhanced solubilizing effect of *Bacillus megaterium*, but with RP such enhancement was not observed beyond 0.5 percent [9]. In the presence of TCP and all sugars, viz., arabinose, fructose, galactose, sorbitol, mannitol, xylose, sucrose, maltose and lactose, *Enterobacter aerogenes* showed positive effect on phosphate solubilization (PS) activity except xylose but in the presence of RP all sugars showed negative effect on PS activity [10]. Phosphorus release enhanced with increasing concentrations of glucose, which can be attributed to greater availability of the energy source for the growth of organism and acid production [9]. All the monosaccharides proved superior than disaccharides, polysaccharides and sugar alcohols for RP solubilization, while all the monosaccharides and two disaccharides sucrose and maltose, proved best for TCP solubilization [11].

Nitrogen

The efficiency of five different nitrogen sources on PS activity was studied. Inorganic nitrogen sources proved better than organic one. Nitrates were more efficient than rest of the compounds, which could be due to the presence of assimilatory enzymes for nitrate reduction in organisms. The effect of inorganic and organic nitrogen sources on PS activity of *Schwanntomyces occidentalis* with RP and found ammonium sulphate exhibiting maximum activity followed by ammonium nitrate [7]. All the test nitrogen sources viz., ammonium nitrate, postassium nitrate, calcium nitrate, sodium nitrate, urea and asparagines proved inferior to the ammonium sulphate in context to solubilization of TCP and RP with *Enterobacter aerogenes* [10]. All the nitrates were efficiently used by *Pseudomonas fluorescens* and showed a peak value of TCP solubilization on 5th day [11]. Maximum RP solubilization was found in the presence of ammonium sulphate followed by potassium nitrate, ammonium nitrate, asparagines, sodium nitrate, urea and calcium nitrate. Ammonium sulphate was the best nitrogen source. Some strains, showed a well-developed ability for phosphate solubilization, lost their abilities after several cycles of

inoculation and cultivation [12]. On the other hand, an increase in the ability of some strains to solubilize inorganic phosphates was observed, which might mean that they have adapted to P deficient conditions.

pH

Acid production during phosphate solubilization appears to be an event of common occurrence and is dependent upon the type of phosphate. The pH changed from neutral to acidic condition with all carbon sources were recorded with both TCP and RP (Fig.1).

FIGURE 1. Effect of different carbon sources on TCP and RP solubilization by *Bacillus* sp. (error bars indicate ± SD)

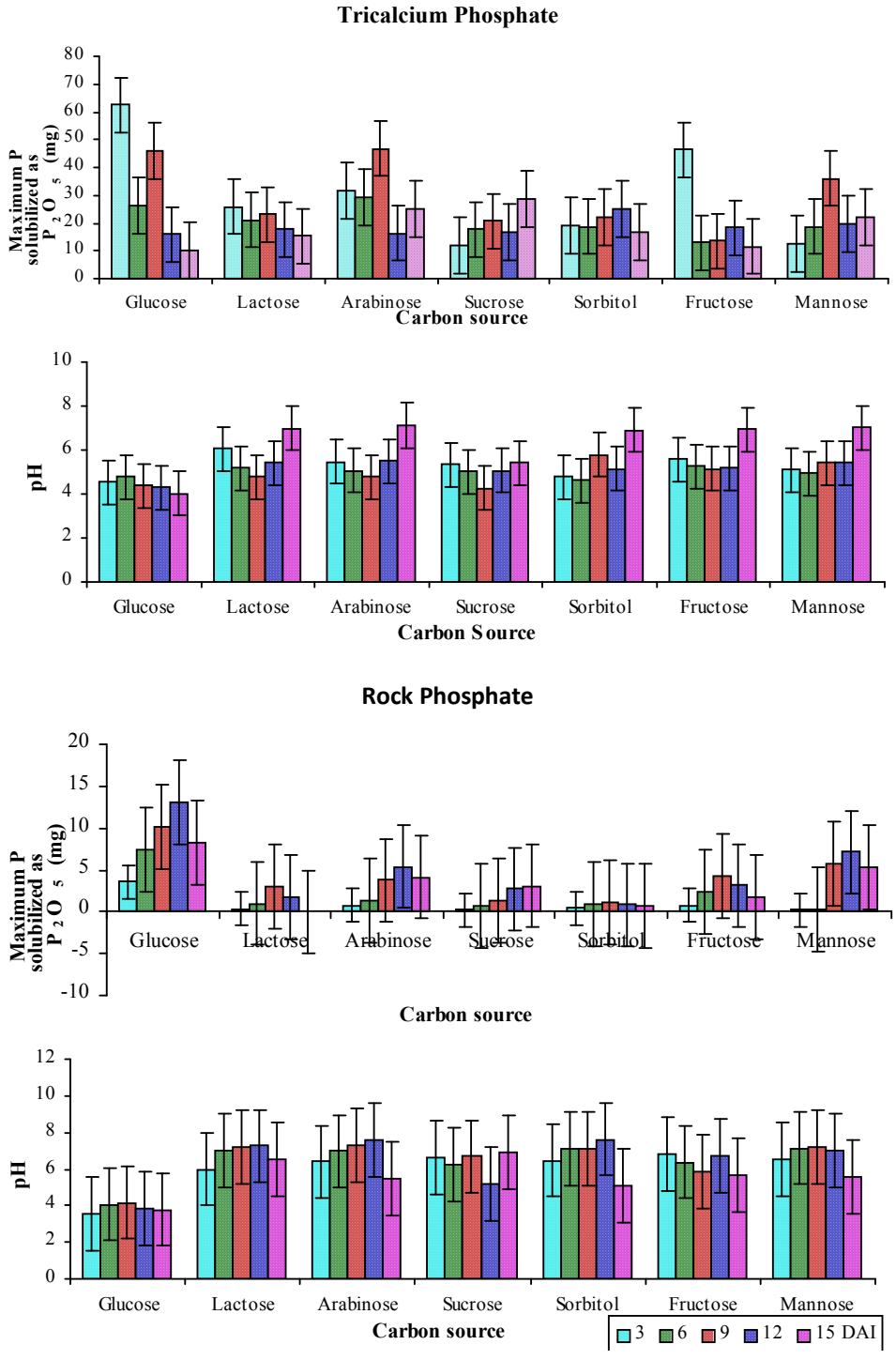
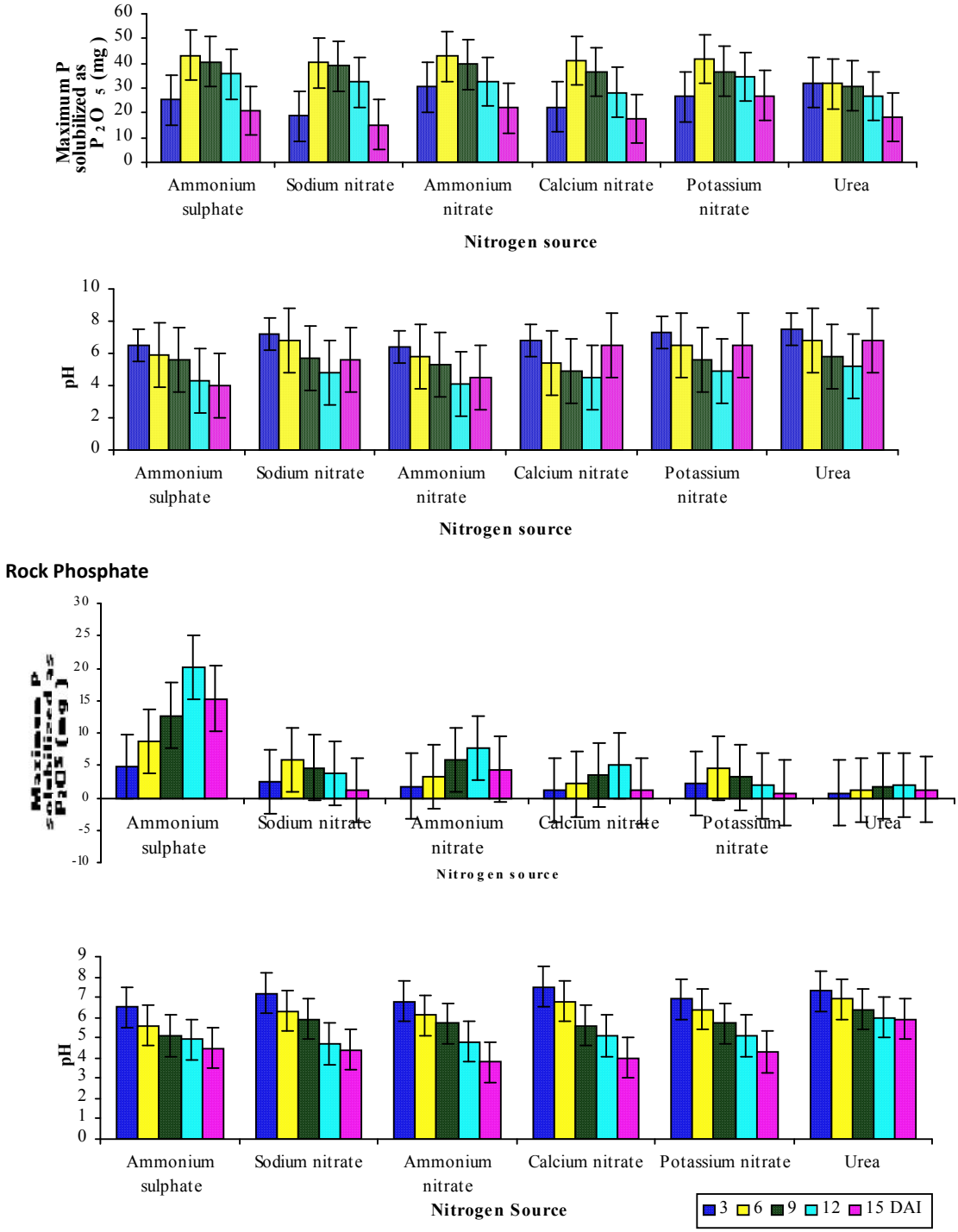


FIGURE 2. Effect of different N sources on TCP and RP solubilization by Bacillus sp. (error bars indicate ± SD)



The fluctuation in pH during TCP solubilization in the presence of nitrogenous compounds ranged from 4.0 to 7.5 and for RP is ranged from 3.8-7.5 (Fig. 2). *Bacillus* sp. and *Aspergillus carbonum* lowered the pH of the medium to 5.0 and 2.7 respectively, when compared to control pH of 6.8 [13]. No correlation could be established between the final acidity produced by the microorganisms and P solubilization in the liquid medium [14]. P released by the organisms was associated with reduction in pH of the medium [9]. Acidic pH of the medium enhanced phosphate-solubilizing activity [10]. *Aspergillus japonicus* and *A. foetidus* were found to solubilize five types of Indian rock phosphates at pH 8 and 9. Solubilization was higher in the presence of pyrite than in controls lacking either pyrite or fungal inoculums. Both the *Aspergillus* sp. was found to be good pyrite solubilizers and could grow over a wide range of pH. Solubilization of rock phosphates was found to be the result of organic acid release and

pyrite oxidation [15]. $AlPO_4$ solubilization was accompanied by a distinct pH decrease to about pH 2 [16]. Solubilization of RP was associated with steep decline in pH from 6.5 to 2.5 within two days of incubation [17]. The pH remained at 2.5 for 20 days indicating the production of strong acids. $AlPO_4$ solubilization resulted in a very slight decline from 6.5 to 6.0 after 10 days of incubation period. Phosphate solubilization of *Penicillum oxalicum* CBPs-3F-Tsa was associated with the decrease of the pH values from 6.5 to 2.35, 2.74 and 2.54 for TCP, $AlPO_4$ and $FePO_4$ respectively [18]. During phosphate solubilization *A. niger* P39 had a stronger ability to acidify the culture media than *P. oxalicum* P66 after 20 days of incubation [19]. Phosphate solubilization by the *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum* spp. were accompanied with pH reduction of the culture medium [20]. Maximum pH reduction was 2.8, 1.2 and 0.5 units for *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum lipoferum* strain 137, respectively.

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