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EFFECT OF DIFFERENT CARBON AND NITROGEN SOURCES ON SOLUBILIZATION OF INSOLUBLE INORGANIC PHOSPHATE BY PSYCHROTOLERANT BACTERIAL STRAINS

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ABSTRACT
Most agricultural soils contain large reserves of phosphorus (P), a considerable part of which accumulates as a consequence of regular applications of P fertilizers. However, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants. Phosphate solubilizing microorganisms convert insoluble phosphates into soluble forms generally through the process of acidification, chelation and exchange reactions. Thus, such microorganisms may not only compensate for higher cost of manufacturing fertilizers in industry but also mobilizes the fertilizers added to soil. In the present study *Pseudomonas lurida* isolated from agriculture soil, having potential to solubilize insoluble inorganic phosphates at low temperature was characterized. Phosphate solubilization was related to pH decrease caused by growth of bacteria in medium containing glucose as carbon source. Strains show diverse levels of phosphate solubilization activity in liquid broth culture in presence of various carbon and nitrogen sources. TCP (Tri-calcium phosphate) solubilization with different carbon sources irrespective of incubation period can be related in the following order: glucose, maltose, sucrose, galactose, xylose both at ambient as well as 10°C. The pH changes from neutral to acidic with all carbon sources in presence of TCP. Regarding TCP, solubilization with different nitrogen sources maximum solubilization was recorded in the presence of ammonium sulphate. *Pseudomonas lurida* showed maximum phosphate solubilizing with glucose as carbon source and ammonium sulphate as nitrogen source. TCP solubilization in liquid medium was significantly high.

INTRODUCTION

Phosphorus (P) is an essential nutrient for biological growth. Absence of this element in the soil could limit the plant development (Prejambada *et al.,* 2009; Victoria *et al.,* 2009). A greater part of soil phosphorus is in the form of insoluble phosphates and cannot be utilized by the plants. To increase the availability of phosphorus for plants, large amount of fertilizer are being applied to soil. But a large proportion of phosphatic fertilizer applied is quickly transformed to the insoluble forms which decrease the efficiency of fertilizers. So, the need of those microbes arose which have the capacity to solubilize phosphorus. These microorganisms are called Phosphate solubilizing micro organisms (PSMs).

Phosphate-solubilizing microbes can transform the insoluble phosphorus to soluble forms HPO_4^{2-} and $H_2PO_4^-$ by acidification, chelation, exchange reactions, and polymeric substances formation (Delvasto *et al.,* 2006). The phosphate solubilizing activity of Phosphate solubilizing microorganisms (PSMs) is affected by the presence of various carbon and nitrogen sources. Development of growth and activity of PSMs is very much affected by nature and concentration of salt and pH of soil (Yadav *et al.,* 2010). Carbon source is an important parameter for active proliferation of organisms and production of organic acids whereas nitrogen source is important for the production of inorganic acids. Many researchers have studied the effect of various carbon sources on phosphate solubilization (Narsian and Patel, 2000).

However, the soils of Uttarakhand Himalayas are acidic in nature, low in moisture content and organic matter. The applied water soluble 'P' fertilizers are rapidly fixed to unavailable forms which accounts for the low 'P' use efficiency of the crops grown in this region (Pal, 1998). Thus, the exploration of some potential psychrotolerant microorganisms is a need for sustainable agriculture in the sub-tropical regions of Uttarakhand. Since these psychrotolerant microorganisms showed more efficient phosphate solubilization when different carbon and nitrogen sources has been taken.

The present phosphate solublization activity of *Pseudomonas lurida* at different carbon and nitrogen sources.

MATERIALS AND METHODS

Collection of organisms

The bacterial culture *Pseudomonas lurida were* collected from high altitude location (79.34°E Latitude and 29.39°N Longitude) in Jageshwer district, of Uttarakhand state and grown on Nutrient agar slant for 3 days at 30°C for further study.

Influence of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas lurida*.

Preparation of growth medium with carbon and nitrogen sources

Two sets of flasks containing NBRIP broth medium (Nautiyal., 1999) with one different phosphate source such as TCP were used in this experiment. The effect of different carbon (C) sources on phosphate solubilizing activity was observed by replacing glucose with four different carbon sources viz., maltose, sucrose, xylose and galactose at the rate of 10 g/L. For the effect of different Carbon 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 four different nitrogen sources viz., ammonium chloride, sodium nitrate, potassium nitrate and urea at the rate of 0.1 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.

Estimation of phosphate solubilization activity

One ml of 24 hrs old bacterial culture was inoculated in 50ml of NBRIP medium containing different C and N sources separately and incubated at ambient as well as 10°C temperature. At 1st, 3rd, 7th and 10th day of incubation period, the required amount of sample was withdrawn from each conical flask for estimation of PS activity. The subsamples were centrifuged at 4000 rpm 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 (Jackson, 1973).

RESULTS

Influence of different carbon sources on phosphate solubilization

Phosphate solubilization activity of *Pseudomonas lurida* influenced by different carbon a source was studied with TCP is shown in Fig.1. *Pseudomonas lurida* utilized a variety of carbon compounds as energy source but the phosphate solubilization activity varied with different carbon sources and incubation temperature. Though, all the test sugars supported phosphate solubilization activity, glucose (3853.10 ppm P_2O_5) influenced highest activity on 10th day at 10°C followed by maltose on 7th day and galactose (1008.60 ppm P_2O_5) at 10°C. On the basis of maximum TCP solubilization, the different carbon sources irrespective of incubation period can be related in the following order: glucose > maltose > galactose > sucrose > xylose. Among the carbon sources tested, glucose (2550 ppm P_2O_5) found to be the best on 10th day followed by maltose (2140.10 ppm P_2O_5) on 7th day and galactose (990.50 ppm P_2O_5) on 7th day at 30°C for TCP solubilization, which was in the order of glucose >maltose > galactose >sucrose > xylose. The *pseudomonas lurida* showed greater phosphate solubilization activity with TCP 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 3.70 after 7th days of incubation at 10°C and recording lowest of 3.75 after 7th days of incubation at ambient temperature (30°C). Lowest pH range was observed when glucose was used as a carbon source with TCP and incubation temperature both at ambient and 10°C.

Influence of different Nitrogen sources on phosphate solubilization

The effect of five different nitrogen sources on phosphate solubilization activity of the *pseudomonas lurida* using TCP is shown in Fig. 2. Maximum TCP solubilization was recorded in the presence of ammonium sulphate. Though all the tested nitrogen sources supported phosphate solubilization activity, ammonium sulphate recorded highest activity on 3rd day of incubation (3839.19 ppm P_2O_5) followed by sodium nitrate (1100.20 ppm P_2O_5) and potassium nitrate (1010.05 ppm P_2O_5) on 3rd day at 10°C of incubation temperature. TCP solubilization activity influenced by nitrogenous compounds can be arranged in the following order: ammonium sulphate > Sodium nitrate > Potassium nitrate > ammonium chloride > urea. Maximum TCP solubilization at 30°C of incubation was found in presence of ammonium sulphate (2550 ppm P_2O_5) on 10th day followed by sodium nitrate (700.20 ppm P_2O_5) and potassium nitrate (695.10 ppm P_2O_5). TCP solubilization as influenced by nitrogenous compounds at 30°C of incubation can be arranged in the following order: ammonium sulphate > sodium nitrate > potassium nitrate> ammonium chloride. The phosphate solubilization activity was lesser in the presence of organic nitrogen source (ammonium chloride). All nitrogen sources showed highest activity of TCP solubilization on 3rd day. Here, phosphate solubilization seems to be link with decrease in pH of the medium but this was not strictly proportional to the amount of phosphate solubilized.

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. Solubilization of 'P' increased when there is a sufficient amount of energy available to the organisms to result in the formation of organic acids. Significant differences were observed among the carbon sources and isolates in relation to the solubilization of phosphates in the culture medium, reduction in pH of the culture filtrate. Glucose produced the greatest increase in total soluble phosphate. The effect of different carbon sources (glucose, galactose, fructose) has been determined on the production of enzyme (Qureshi *et al.*, 2010). In the present study, the *Pseudomonas lurida* showed maximum PS at 10°C with glucose and ammonium sulphate in TCP containing NBRIP media whereas at 30°C incubation temperature was very slow. Maximum PS was observed after 7th days of incubation . Glucose was found to be best carbon source followed by sucrose and galactose for phosphate solubilization by *Pseudomonas striata* (Gaur, 1990). Narsian and Patel (2000), reported maximum P solubilization by

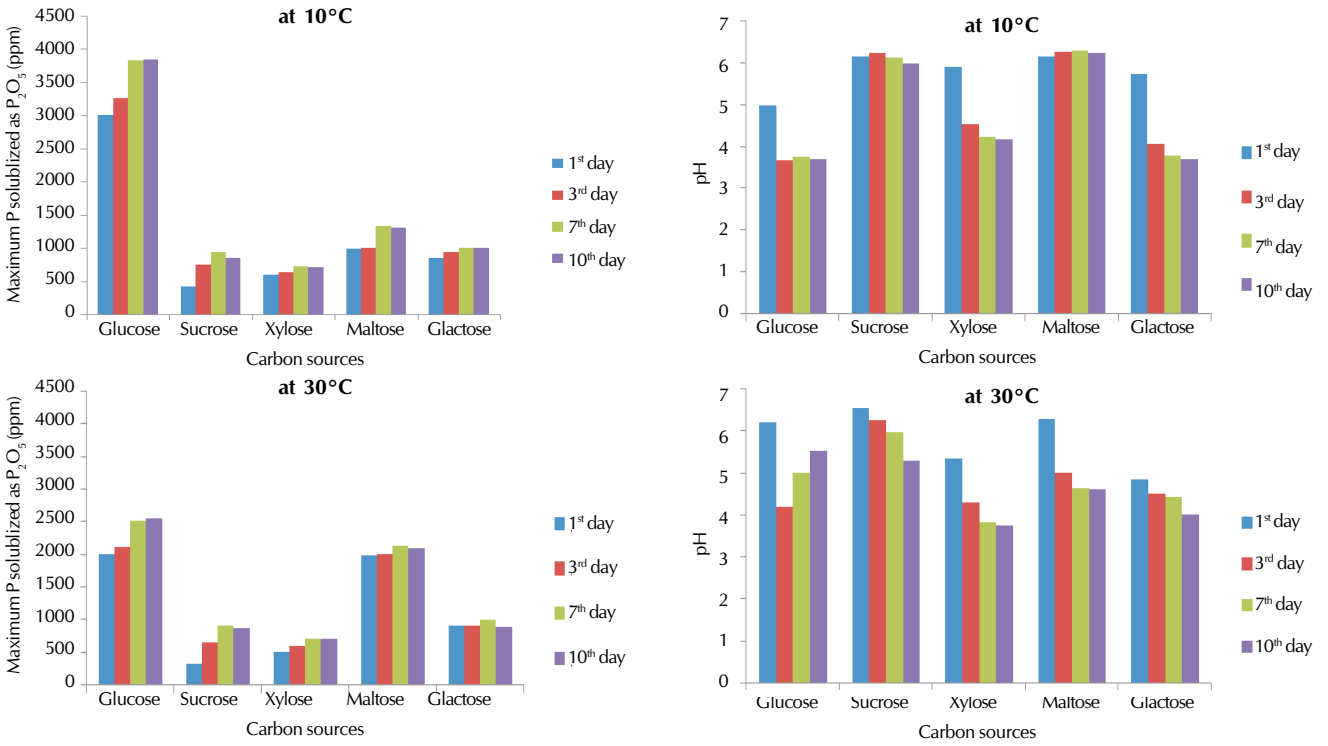

Figure 1: Effect of different Carbon souces on TCP solubilization by *Pseudomonas lurida* at ambient as well as 10°C

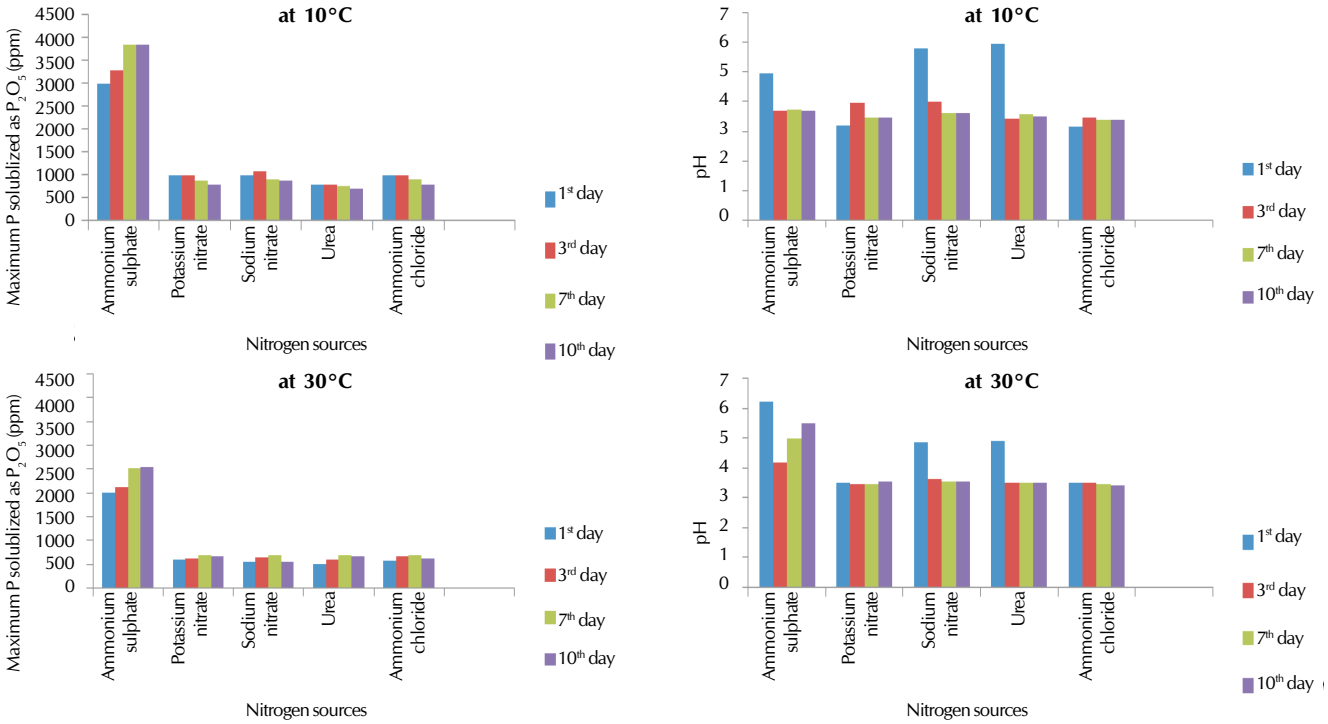

Figure 2: Effect of different Nitrogen souces on TCP solubilization by *Pseudomonas lurida* at ambient as well as 10°C

Aspergillus aculeatus with arabinose and glucose. while all the monosaccharides and two disaccharides sucrose and maltose, proved best for TCP solubilization (Dave and Patel, 2003).

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 sodium nitrate (Gaur, 1990). All the test nitrogen sources viz., ammonium chloride, potassium nitrate, sodium nitrate, and urea proved inferior to the ammonium sulphate in context to solubilization of TCP and RP with *Enterobacter aerogenes* (Thakker *et al.,*1993). A number of bacteria had been reported of being able to solubilize phosphate only in the presence of ammonium as the nitrogen source (Illmer and Schinner, 1992; Lapeyrie *et al.,* 1991). The nitrogen source in salt form seems to be important, as it was necessary for better solubilization of rock phosphate (Asea, 1988). Previous reports on phosphorus solubilizing microorganisms (Lapeyrie *et al.,* 1991) have attributed to the differences in phosphate solubilization (when ammonium and nitrate were used) due to the use of different mechanisms for the generation of acidity in the culture.

On analyzing the comparative profile of 'P' solubilized by the strains using different media, it was observed that after 7th day of incubation i.e. on 10th day at 10°C and 30°C, a considerable drop in the values were observed. So, keeping in view that the potential of the strains to solubilize 'P' was best on 7th day, the values of 'P' solubilized and drop in pH both at 10°C and 30°C, using NBRIP broth as media were analysed. 'P' released by the organisms was associated with reduction in pH of the medium (Yadav and Singh., 1991). Acidic pH of the medium enhanced phosphate solubilizing activity. The ability of the given strains to solubilize mineral phosphate in glucose as a carbon source was increased at a high level up to 7 days. These results are consistent with the earlier report where P-solubilizing ability increased with increasing concentration of glucose in *Pseudomonas* sp. (Banik, 1983) and *P. agglomerans R-42* (Son *et al.*, 2006). Phosphate solubilization by the *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum* spp. were accompanied with pH reduction of the culture medium (El-Komy., 2005).

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