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Kumari Punam Pallavi
Krishi Vigyan Kendra, Harnaut, Nalanda, Bihar, India

Corresponding Author:
Kumari Punam Pallavi
Krishi Vigyan Kendra, Harnaut, Nalanda, Bihar, India

Effect of nitrogen sources on *phosphate solubilizing effieciency* of psychrotolerant bacterial strains

Kumari Punam Pallavi

Abstract

Tri-calcium phosphate, a source of insoluble phosphate and different sources of nitrogen and carbon were used to study the phosphate solubilizing capacity of *Pseudomonas lurida* (psychrotolerant strain).Tri-calcium phosphate solubilized in different carbon sources and incubation times, such as ambient (30°C) and 10°C, in the following order: glucose > maltose > galactose > sucrose > xylose. With all carbon sources and TCP presence, the pH shifts from being neutral to acidic. When TCP was solubilized with various nitrogen sources, ammonium sulphate caused the greatest amount of solubilization to occur. With glucose as the carbon source and ammonium sulphate as the nitrogen source, *Pseudomonas lurida* exhibited its highest level of phosphate solubilizing activity. In a liquid media, TCP solubilization was noticeably high.

Keywords: *Pseudomonas lurida*, insoluble phosphate source, tricalcium phosphate, NBRIP media

Introduction

Phosphorus (P) is an essential component of biological molecules and one of the key factors limiting biomass output because it only makes up a small portion of the total soil P. Growing demand for food worldwide and the use of P fertilizers in modern agriculture may result in excessive inorganic P inputs into intensively managed croplands, increasing P losses, and continuing eutrophication of surface waters.

The majority of the phosphorus in soil exists as insoluble phosphates, which the plants cannot use. Microbial activity, which in turn is regulated by a variety of factors such as plant species, soil type, and environmental factors, is principally responsible for mediating soil P conversions. Phosphate solubilizing microorganisms' (PSMs') ability to solubilize phosphate is also influenced by the presence of different carbon and nitrogen sources. PSM growth and activity are significantly influenced by the carbon source, the type and concentration of salt, and the pH of the soil (Yadav *et al*., 2010) [6]. While nitrogen source is crucial for the synthesis of inorganic acids, carbon source is crucial for the active proliferation of organisms and the production of organic acids. The Uttarakhand Himalayan soil, on the other hand, is acidic in character and contains little organic matter. The crops grown in this area have a low 'P' use efficiency because the applied water soluble 'P' fertilizers are quickly fixed to inaccessible forms. Therefore, research into some potentially psychrotolerant bacteria is necessary for sustainable agriculture in Uttarakhand's subtropical areas. The purpose of the current study was to investigate the ability of *Pseudomonas lurida* to saturate insoluble inorganic phosphate sources such tri-calcium phosphate at both ambient (30 °C) and subfreezing (10 °C) temperatures.

Materials and Methods

Collection of Organisms

Pseudomonas lurida bacteria were cultured on Nutrient agar slant for 3 days at 30 °C for further research after being obtained from a high altitude area (79.34 °E Latitude and 29.39°N Longitude) in Jageshwer district, Uttarakhand state.

Effect of various carbon and nitrogen sources on *Pseudomonas lurida's* ability to *solubilize* phosphate

(I) The preparation of a growth medium containing sources of carbon and nitrogen

In this experiment, two sets of flasks using NBRIP broth medium (Nautiyal, 1999) [4] and a single distinct phosphate source, such as TCP (Tri-calcium phosphate), were employed. By substituting four alternative carbon (C) sources-maltose, sucrose, xylose, and galactose-for glucose at a rate of 10 g/L, it was possible to see how each one affected the ability to solubilize phosphate. Ammonium sulfate is used as a nitrogen source to study the impact of various carbon sources on the phosphate solubilization (PS) process.

By substituting four distinct nitrogen sources-ammonium chloride, sodium nitrate, potassium nitrate, and urea-at a rate of 0.1 g/L for ammonium sulphate, the effects of the different nitrogen (N) sources on the solubilization of phosphate were assessed. To investigate how various carbon (C) sources affected PS, glucose was used as a carbon source. Distilled water was used to dissolve the carbon and nitrogen sources, which were then sterilized individually and supplied to the culture medium.

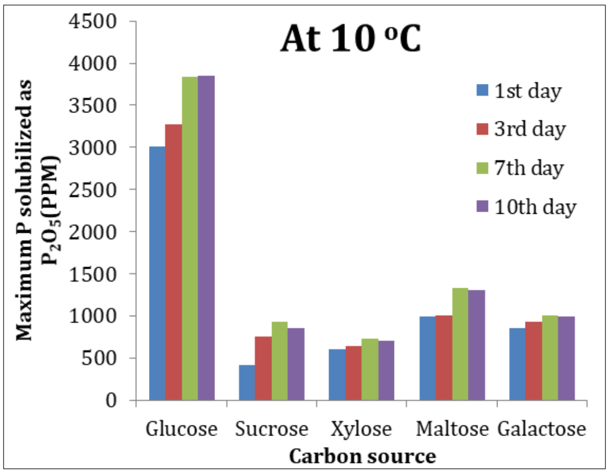
Activity estimation for phosphate solubilization

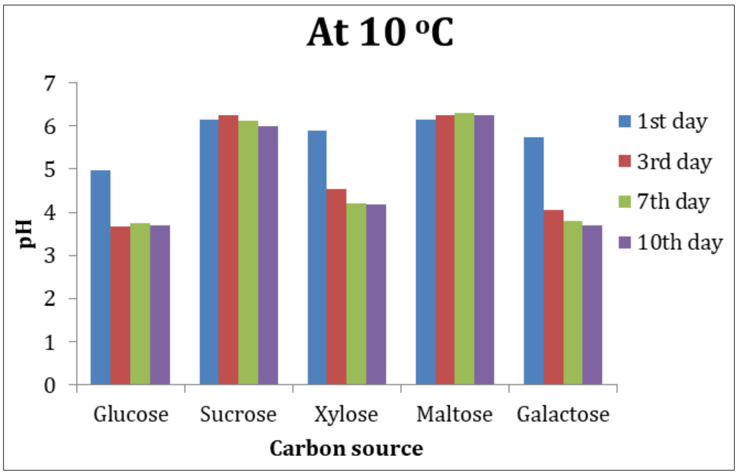
50 ml of NBRIP medium with various C and N sources separately was inoculated with one ml of a 24-hour-old bacterial culture and incubated at both room temperature and 10 ºC. The necessary amount of sample was removed from each conical flask on the first, third, seventh, and tenth days of the incubation period in order to estimate PS activity. At a speed of 4000 rpm, the subsamples were centrifuged, and the supernatant was filtered. By using the filtrate to assess pH and the chlorostannous reduced molybdophosphoric acid blue technique to estimate soluble P, respectively (Jackson, 1973) [1].

Results

Effects of various carbon sources on the solubilization of phosphate

The influence of various carbon sources on Pseudomonas lurida's, phosphate solubility activity was investigated using TCP, as seen in Fig. 1. A variety of carbon compounds were used by *Pseudomonas lurida* as an energy source, however the phosphate solubilization activity varied depending on the carbon source and incubation temperature. The maximum activity was impacted by glucose (3956.10ppm P_2O_5) on day 10 at 10 ºC, followed by maltose on day 7 and galactose (1085.50ppm P_2O_5) at 10^{O}C, even though all the test sugars supported phosphate solubilization activity. Regardless of incubation time, the various carbon sources can be connected in the following sequence based on maximum TCP solubilization: glucose > maltose > galactose > sucrose > xylose. In terms of TCP solubilization, glucose (2685 ppm P_2O_5) performed the best among the investigated carbon sources on day 10, followed by maltose (2235 ppm P_2O_5) on day 7, and galactose (1101 ppm P_2O_5) on day 7. These results were in the following order: glucose > maltose > galactose > sucrose > xylose. In comparison to other monosaccharides and disaccharides, the *pseudomonas lurida* exhibited enhanced phosphate solubilization activity with TCP in the presence of glucose. With all carbon sources in the presence of TCP, the pH moved from neutral to acidic, reaching a minimum of 3.65 after 7 days of incubation at 10 ºC and 3.78 after 7 days at ambient temperature (30 ºC). When glucose was employed as a carbon source with TCP and incubation temperatures at ambient and 10 ºC, the lowest pH range was seen.





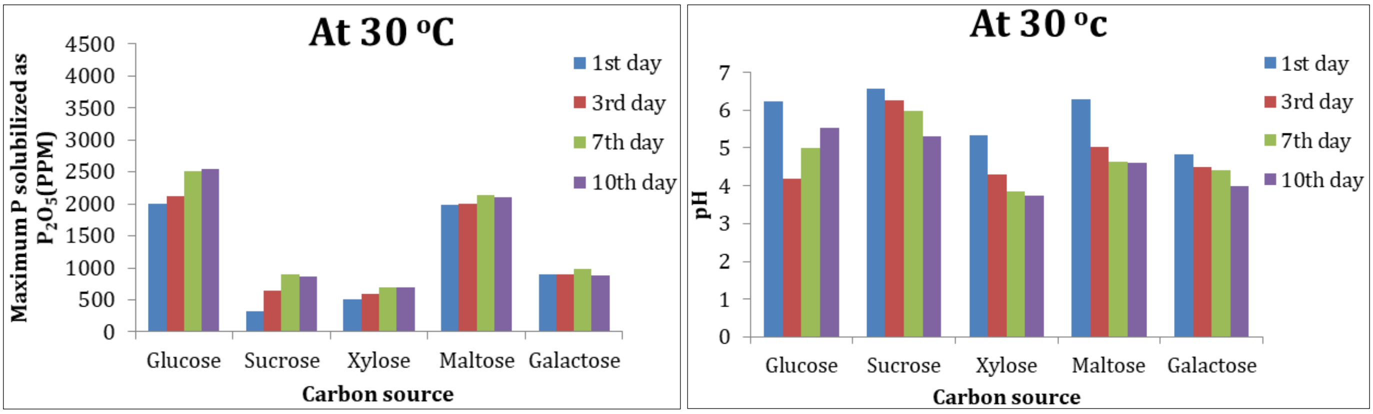


Fig 1: Effect of different Carbon souces on TCP solubilization by Pseudomonas lurida at ambient as well as 10 ºC

Effect of various nitrogen sources on the solubilization of phosphate

Figure 2 depicts how five different nitrogen sources affected the *pseudomonas lurida's* ability to solubilize phosphate using TCP. Ammonium sulphate caused the greatest amount of TCP to dissolve. However, ammonium sulphate recorded the highest activity on the third day of incubation (3845.19 ppm P_2O_5), followed by sodium nitrate (1095.20 ppm P_2O_5) and potassium nitrate (1015.08 ppm P_2O_5) on the third day at 10 ºC of incubation temperature. All of the tested nitrogen sources supported phosphate solubilization activity. The order of nitrogenous sources that affect TCP solubilization activity is: ammonium sulphate > sodium nitrate > potassium nitrate > ammonium chloride > urea. On the tenth day of incubation, ammonium sulphate (2585 ppm P_2O_5), sodium nitrate (715.20 ppm P_2O_5), and potassium nitrate (699.25 ppm P_2O_5) were found to have the highest levels of TCP solubilization at 30ºC. The order of nitrogenous sources influencing TCP solubilization at 30 ºC of incubation is as follows: ammonium sulphate > sodium nitrate > potassium nitrate > ammonium chloride. On the third day, TCP solubilization demonstrated the highest activity across all nitrogen sources. At this point phosphate solubilization seemed to be associated with a drop in the medium's pH, but this relationship was not quite linear with the amount of phosphate solubilized.

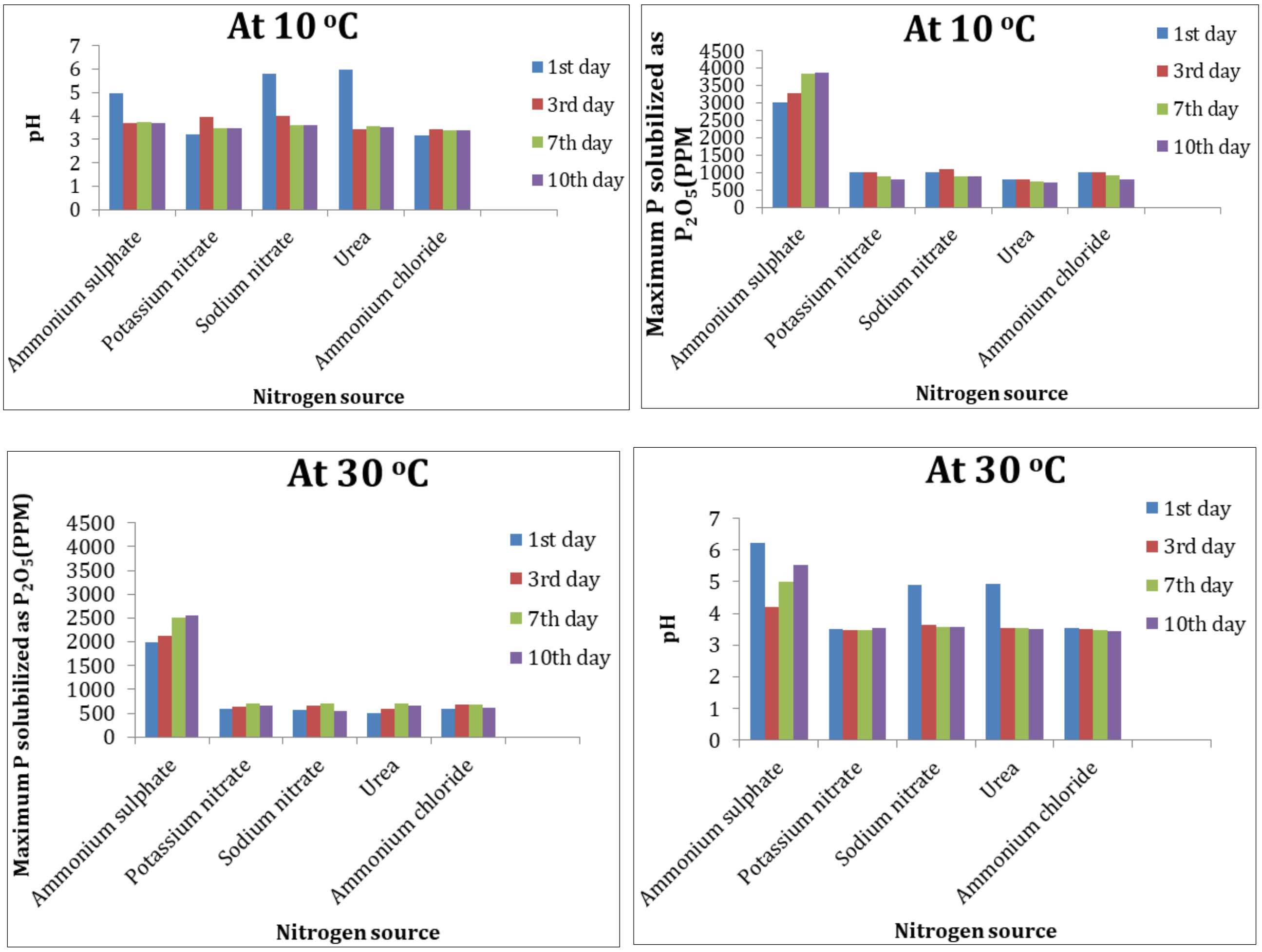


Fig 2: Effect of different Nitrogen sources on TCP solubilization by Pseudomonas lurida at ambient as well as 10 ºC

Discussion

Impact of various sources of carbon and nitrogen on the solubilization of phosphate

a. **Carbon:** When different carbon and nitrogen sources were present, different amounts of PS activity were displayed by microorganisms. Numerous carbon compounds were used by microorganisms as energy sources, however the amount of PS differed with different substrates. When the organisms have access to enough energy to cause the creation of organic acids, the solubilization of "P" increases. Regarding the solubilization of phosphates in the culture medium and the lowering of the pH of the culture filtrate, significant differences were seen between the carbon sources and isolates. The highest increase in total soluble phosphate was caused by glucose. According to Qureshi *et al*. (2010), the impact of several carbon sources (glucose, galactose, and fructose) on enzyme synthesis has been identified. At 10 ºC with glucose and ammonium sulphate in TCP-containing NBRIP media, *Pseudomonas lurida* displayed maximum PS in the current investigation, but incubation was very slow at 30 ºC. The peak PS was noticed after seven days of incubation. For *Pseudomonas striata* to solubilize phosphate, glucose was shown to be the optimum carbon source, followed by sucrose and galactose.

b. **Nitrogen:** Five distinct nitrogen sources' effectiveness on PS activity was investigated. Organic nitrogen sources did not perform as well as inorganic ones. The fact that nitrates were more effective than the other substances may be because organisms have assimilatory enzymes for nitrate reduction. When TCP and RP were solubilized with *K. pneumoniae S6C1*, all of the test nitrogen sources-ammonium chloride, potassium nitrate, sodium nitrate, and urea-proved to be less effective than ammonium sulphate (Kshetri *et al*., 2017) [2]. Previous studies on the microorganisms that solubilize phosphorus have linked variations in phosphate solubilization (when ammonium and nitrate were used) to the utilization of various methods for producing acidity in the culture.

Analysis of the comparative profile of "P" solubilized by the strains in various mediums revealed that the values significantly decreased after the seventh day of incubation, or on the tenth day at 10 ºC and 30 ºC. Since the strains' ability to solubilize P was at its peak on day 7, the values of "P" solubilized and pH drop at both 10 ºC and 30 ºC, using NBRIP broth as the media, were analyzed. The organisms' release of P was linked to a drop in the medium's pH (Gonzalez *et al*., 2022). The acidic pH medium promoted phosphate-solubilizing action. Up to 7 days, the provided strains' capacity to solubilize mineral phosphate in glucose as a carbon source grew significantly. These findings are in line with a previous study found that *Enterobacter hormachei's* P-solubilizing capacity increased when glucose content increased (Pallavi, 2018) [3].

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