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Influence of Carbon and Nitrogen Sources in Solubilization of Hardly Soluble Mineral Phosphates by *Penicillium Oxalicum* CBPS-Tsa

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ABSTRACT : Phosphorus is one of the major plant growth limiting nutrients, despite being abundant in soils in both inorganic and organic forms. Phosphobioinoculants in the form of microorganisms can help in increasing the availability of accumulated phosphates for plant growth by solubilization. *Penicillium oxalicum* CBPS-Tsa, isolated from paddy rhizosphere, was studied for its phosphate solubilization. The influence of various carbon sources like glucose, sucrose, mannitol and sorbitol and nitrogen sources like arginine, sodium nitrate, potassium nitrate, ammonium chloride and ammonium sulphate were evaluated using liquid media with tricalcium phosphate (Ca-P), ferric phosphate (Fe-P) and aluminium phosphate (Al-P). Maximum soluble phosphate of 824 mg/L was found in the amendment of sucrose-sodium nitrate from 5 g/L of Ca-P. Mannitol, sorbitol, and ariginine were poor in phosphate solubilization. While sucrose was better carbon source in solubilization of Ca-P and Al-P, glucose fared better in solubilization of Fe-P. Though all the nitrogen sources enhanced P solubilization, nitrates were better than ammonium. In the amendments of ammonium chloride and ammonium sulphate, higher uptake of available phosphates by the fungus was found, and this resulted in depletion of available P in Fe-P amendment. Phosphate solubilization was accompanied by acidification of the media, and the highest pH decrease was observed in glucose amendment. Among the nitrogen sources, ammonium chloride favored greater pH decrease.

Key words: *Penicillium oxalicum* CBPS-Tsa, carbon, nitrogen, phosphate solubilization.

INTRODUCTION

Apart from soil moisture and nitrogen, phosphorus deficiency also limits crop growth on many soils[1]. Soil available microbes can solubilize rock phosphate by mechanism involving the production of organic or inorganic acids; chelation or exchange reactions and can serve as potential phospho-biofertilizers[2-7]. However, the roots through root exudation, including various sugar compounds, purines/nucleotides, amino acids, organic acids, vitamins, enzymes, and inorganic anions, may not only serve as a nutrient source to the soil microbial community but may also regulate their growth[8,9]. In addition, the chemical fertilizer application have the ability to create nitrogen, phosphorus and potash rich environments artificially in the fields, where both nitrogen and phosphates at higher quantities can bring deleterious effects to the plants. Hence, it is necessary to study the performance of various microorganisms in various amendments under *in vitro* conditions to develop them as successful phosphobiofertilizers.

A lot of studies carried out and reported the ability of *Penicillium* sp. to solubilize phosphates efficiently[5,10-18]. However, *P. oxalicum* CBPS-Tsa, a soil fungus isolated from the agricultural soils of Youngnam Province, Korea, has not been studied so far in detail. The present study was undertaken to identify the influence of various carbon and nitrogen sources as substrate in solubilization of hardly soluble mineral phosphates by *P. oxalicum* CBPS-Tsa.

MATERIALS AND METHODS

The laboratory isolate of *P. oxalicum* CBPS-Tsa was used in this study. The strain was inoculated onto PDA slants,

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grown for 7~10 days at 30℃ and maintained at 4℃. Stock cultures were maintained under these conditions for a maximum of three months and were sub cultured on fresh PDA slants frequently.

Unless otherwise stated Reyes basal medium was used throughout the study[17], and the cultures were maintained at 30℃ in a rotary shaker (120 rpm) and incubated in dark. The experiments were carried out in 100 mL Erlenmeyer flasks. Three forms of P, Ca-P, Fe-P and Al-P), were incorporated at 5 g/L concentration separately in each flask.

The basal medium was supplemented with different carbon sources, viz. glucose, sucrose, mannitol and sorbitol at 30 g/L concentration. Various nitrogen sources viz. arginine, ammonium chloride, ammonium sulphate, potassium nitrate and sodium nitrate were supplemented at 10 mM concentration. Each flask received a single inoculum disk (4 mm diameter) that was carved out from the freshly inoculated and actively growing *P. oxalicum* CBPS-Tsa colonies on PDA. All the flasks were incubated for seven days.

After seven days incubation the cultures were centrifuged for 10 min (10,000 rpm at 4℃) and the supernatant was passed through a 0.45 M Millipore filter and was used to determine pH and phosphate. The pH of the broth was measured by directly immersing the glass electrode connected to a pH meter, into the filtrate. The P concentration was estimated by the chlorostannous reduced molydbophosphoric acid blue method[19].

The experiments were carried out in triplicate. Statistical interpretations of the various measurements were performed using Analysis of Variance (ANOVA) and comparison of means (LSD) following standard procedures[20].

RESULTS

Among the P sources tested, Ca-P was highly solubilized than Al-P and Fe-P. However, they revealed different patterns in solubilization. Among the three, Fe-P was poorly solubilized by the fungus in the presence of all the carbon sources. When Fe-P was used as substrate for P solubilization with various C and N sources, the fungus used almost 50% of soluble P present in the culture media. This is more evident in ammonium chloride and ammonium sulphate amendment, where the control flasks recorded more soluble P (data not shown) than the fungus inoculated flasks. The fungal biomass was also reduced considerably in Al-P and Fe-P than Ca-P in all the treatments (data not shown). Irrespective of the P source, some soluble P was detected in all the control flasks and this is especially alarming in Fe-P amended flasks.

Sucrose was found to be the best source for P solubilization followed by glucose. However, the difference in P solubilization between the two was marginal. The carbon source preference for *P. oxalicum* CBPS-Tsa in solubilization of Ca-P was in the order: sucrose > glucose > sorbitol > mannitol. For Al-P the order was sucrose > glucose > mannitol > sorbitol and for Fe-P it was glucose > sucrose > sorbitol > mannitol. Mannitol and sorbitol did not have any significant influence on P solubilization and growth of the fungus (Fig. 1). The highest and lowest values of P solubilization were observed in sucrose on Ca-P (562 mg/L) and mannitol on Fe-P (9.7 mg/L) respectively.

While nitrates facilitated better phosphate solubilization, ammonium and arginine decreased the activity. Phosphate solubilization by *P. oxalicum* CBPS-Tsa grown in various nitrogen sources facilitated Ca-P solubilization in the order: sodium nitrate > ammonium chloride > ammonium sulphate > potassium nitrate > arginine. Sodium nitrate was highest to solubilize P from Ca-P (824 mg/L) followed by ammonium chloride (779 mg/L) and potassium nitrate (762 mg/L). Solubilization of other difficult solubilizing P sources was lower than Ca-P. The order of preference for Al-P solubilization was sodium nitrate > potassium nitrate > ammonium sulphate > ammonium chloride > arginine. The rates of Fe-P solubilization obtained were good in sodium nitrate followed by potassium nitrate, arginine, ammonium chloride and ammonium sulphate in order. Among ammonium salts, ammonium chloride than ammonium sulphate in Ca-P and Al-P and ammonium sulphate than ammonium chloride in Fe-P amendments were better in P solubilization.

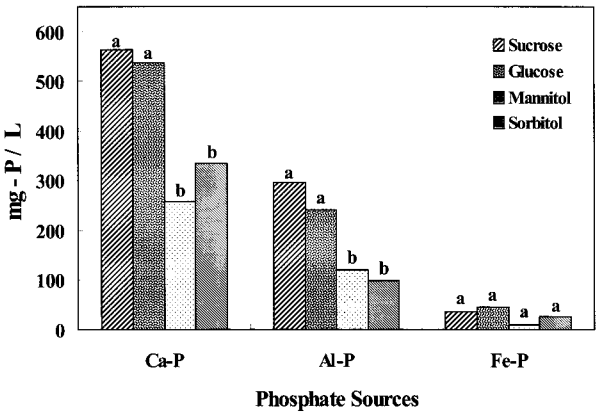

Fig. 1. Influence of carbon sources on solubilization of various mineral phosphates by *P. oxalicum* CBPS-Tsa after 7 days incubation. All values are means of three replicates. Among phosphate sources, significant differences according to LSD at $p<0.05$ level are indicated by different letters above the bars.

A fall in the pH of the culture medium was invariably recorded due to the inoculation of flasks with the fungus. The results are presented in Fig. 2 and 4. From the initial value of 6.5, the pH dropped below 2.0 in the amendment of ammonium chloride and sucrose. Though, in general, the pH drop was recorded in all the treatments the P solubilization and pH drop were not comparable. This is evident in the case of nitrate amendments, which produced more soluble P did not reduce the pH (2.37) than ammonium chloride could (1.91). However, a significant negative correlation was observed between medium pH and P solubilization. The pH of the medium in the control flasks was not altered much. However, in Fe-P the drop in pH was alarming where it varied between 2.8~3.9.

When all the amendments are considered, the sucrose -sodium nitrate amendment was found to be the best in influencing maximum P solubilization.

DISCUSSION

Earlier studies indicate increase in plant growth and yield by fungal inoculation[13,21-24]. Microbes are influenced by various plant root exudates viz. amino acids, organic acids, sugars, etc and by the application of various chemical fertilizers in the agricultural environments. Among these, sugars and organic acids occupy a major portion than others[8,9,25]. In addition, all the agricultural crops receive heavy dose of chemical fertilizers, among which nitrogenous fertilizers score high. Hence, it becomes necessary to study these influencing factors on candidate bioinoculant in *in vitro* conditions for its ability to solubilize phosphates.

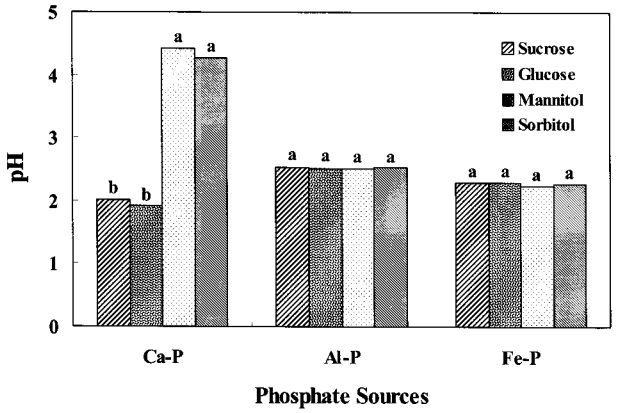

Fig. 2. Influence of carbon sources on the final pH in the mineral phosphate solubilization media inoculated with *P. oxalicum* CBPS-Tsa after 7 days incubation. All values are means of three replicates. Among phosphate sources, significant differences according to LSD at $p<0.05$ level are indicated by different letters above the bars.

Nutritional status of the culture media, especially nitrogen and phosphates are reported to affect phosphate solubilization by *P. rugulosum*[18]. In the present study, the nutrition in the form of carbon, nitrogen and phosphates were found to influence the P solubilization according to the source. Sucrose and sodium nitrate highly influenced the P solubilization by the fungus *P. oxalicum* CBPS-Tsa. The high P solubilization observed in Ca-P than Al-P and Fe-P could be attributed to the nature of the sources, which Ca-P is highly soluble and Fe-P and Al-P are poorly soluble[26].

In general, an increase of soluble P in the presence nitrates and inhibition in the presence arginine amendment was observed. This was attributed to the tendency of nitrates to increase P solubilization at the end of incubation period[27]. Since, our result was a one-time analysis, it is difficult to confirm the above and further studies are needed, especially for *P. oxalicum* CBPS-Tsa. Our observations on the superiority of nitrates over ammonium are well supported by an earlier study[18] and was in disagreement with the findings where ammonium was reported to be the best source for P solubilization[27].

The fungus poorly solubilized Fe-P and it consumed soluble P in the culture media. This was evident from the results where, at the end of incubation, the control recorded more soluble P than the fungus inoculated plates. This phenomena draws support from earlier reports that suggested the same phenomena in *P. rugulosum* and *P. radicum*[18,27]. In the ammonium sulphate and ammonium chloride amendments, the control recorded more soluble P than the flasks inoculated with fungus. However, the fungal biomass recorded was very low (data not presented), contradicting

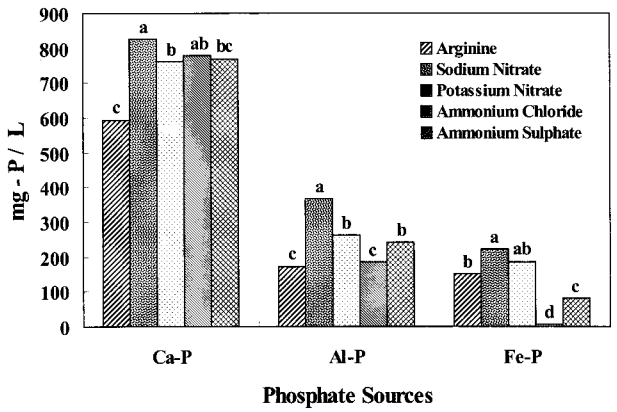

Fig. 3. Influence of nitrogen sources on solubilization of various mineral phosphates by *P. oxalicum* CBPS-Tsa after 7 days incubation. All values are means of three replicates. Among phosphate sources, significant differences according to LSD at $p<0.05$ level are indicated by different letters above the bars.

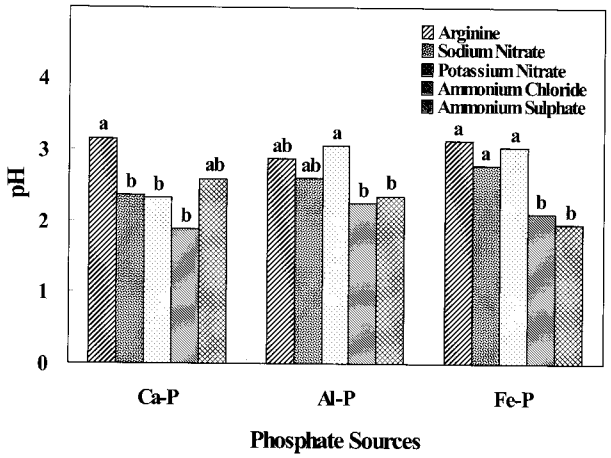

Fig. 4. Influence of nitrogen sources on solubilization of various mineral phosphates inoculated with *P. oxalicum* CBPS-Tsa after 7 days incubation. All values are means of three replicates. Among phosphate sources, significant differences according to LSD at $p<0.05$ level are indicated by different letters above the bars.

the earlier report on the high amount of biomass[27]. Hence, a thorough examination into the nutritional preference of genus *Penicillium* that possess P solubilization potential is necessary at species level to get a clear picture in this regard.

As found in other studies, a decrease in the pH of medium was observed in all the amendments viz. phosphates, carbon and nitrogen sources. Earlier studies indicate an increase in P solubilization against a pH decrease[15,16,19,26-32]. While, the lowest pH recorded was in the presence of glucose, the highest pH was recorded in mannitol. Among nitrogen sources, the lowest was in the presence of ammonium chloride and the highest was in arginine. Illmer and Schinner[16] attributed the high amount of soluble P in culture media, at the end of incubation, to the cell lysis and P liberation. Some other reports attribute the property to the kind of organism and insoluble phosphates used[33], which is in agreement with our results. From our results, we further hypothesize that the ability depends not only on the organism and P source but also depend on the carbon and nitrogen available for the fungus to act. This is further checked in *P. oxalicum* CBPS-Tsa. When analyzed statistically, a correlation between pH and soluble phosphate level was observed in the present study. However, except a few cases (perfect positive values in sorbitol and Fe-P and perfect negative values in potassium nitrate and Ca-P) the results are not significant (Table 1). Our reports are in consonance with the findings of all the above reports[12,31,34].

Table 1. Correlations(r) between final pH and soluble phosphate

	Phosphate sources		
	Ca-P	Al-P	Fe-P
Carbon sources			
Sucrose	-0.085	0.909	0.567
Glucose	0.622	0.809	0.931
Mannitol	-0.976	-0.744	0.899
Sorbitol	-0.982	-0.043	0.992*
Nitrogen Sources			
Arginine	0.291	-0.746	-0.912
Sodium nitrate	0.929	0.956	-0.574
Potassium nitrate	-0.999*	-0.564	-0.968
Ammonium chloride	-0.909	-0.911	0.713
Ammonium sulphate	0.938	-0.866	0.635

Thus, our results reveal the ability of *P. oxalicum* CBPS-Tsa to solubilize Ca-P, Al-P and Fe-P. These *in vitro* results indicate that their inoculation would enhance the available soil P in the rhizosphere. However, the requirement sucrose and sodium nitrate, in *in vitro* conditions, for the fungus to solubilize phosphates indicates their requirement in the rhizosphere for the action. Considering the above qualities of the selected fungus, it can be recommended for use in the chemical nitrate fertilizer applied soils.

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