Effects of Inorganic Soluble Phosphate on Phosphate Solubilization and Acid Phosphatase Activity of the Bacterial Strain *Pantoea agglomerans*, Isolated from Soil

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The present study assessed the effect of application of inorganic soluble phosphate (P) on phosphatase enzyme activity of phosphate solubilizing bacterial strain identified as *Pantoea agglomerans*. The strain was employed in phosphatase enzyme induction studies using NBRIP medium which contained $Ca_3(PO4)_2$ as a source of inorganic insoluble P. The medium was amended with four levels of inorganic soluble P (0, 0.5, 1.0 and 1.5 g/l KH_2PO_4 viz., P_0, P_1, P_2 and P_3). As revealed by the results, phosphatase activity and phosphate solubilization were decreased by inorganic soluble P (KH_2PO_4) in the NBRIP medium inoculated with phosphate solubilizing bacterial strain. The highest phosphatase activity (118.85 U/ml) and phosphate solubilization (642 µg/ml) were recorded when medium contained only inorganic insoluble P and the lowest phosphatase activity (95.3 U/ml) and phosphate solubilization (210 µg/ml) were recorded at the highest level of inorganic soluble P (1.5 g/l). Therefore it can be concluded that presence of soluble P represses the P solubilization activity of *P. agglomerans* under *in vitro* conditions

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Introduction

Phosphorus is the second important macro nutrient required by plants. Compared to other essential macronutrients (with exception of nitrogen), it is one of the less-abundant (0.1% of total) elements in the lithosphere (Jones and Oburger, 2011) and often regarded as a limiting nutrient in agricultural soils. After application of inorganic phosphorus to soil as chemical fertilizer, a considerable amount of phosphorus is rapidly transformed into less available forms by forming a complex with Al^{3+} or Fe^{2+} in acidic soils or with Ca^{2+} in calcareous soils (Toro, 2007) and thus become unavailable to plants. It has been observed by many investigators that a high proportion of P solubilizing microorganisms (PSMs) especially bacteria, fungi and actinomycetes reside in the rhizosphere of plants and play an important role in solubilization of bound phosphates, making them available to plants (Sujatha *et al.*, 2004).

Microbialy mediated phosphorus management has gained increasing attention as a cost effective way of soil phosphorus nutrition. This process not only compensates high cost of phosphatic fertilizer usage but also minimizes environmental contamination. Phosphate solubilizing microorganisms (PSM) are able to carryout solubilization and mineralization of inorganic and organic soil phosphorus, respectively, into the bio-available form for plant uptake. PSM facilitates solubilization of inorganic phosphorus by production of low molecular weight organic acids and the mineralization of organic phosphorus by synthesis of a variety of different extra cellular enzymes (Khan *et al.*, 2009). Since organic phosphorus constitutes 4-90% of the total soil phosphorus, mineralization of soil organic phosphorus plays an imperative role in phosphorus management. Among the variety of enzymes released by PSM, phosphomonoesterases (phosphatases) are the most abundant enzyme that can be divided into acid and alkaline phosphomonoesterases. Both phosphomonoesters can be produced by PSM depending upon the external conditions (Jorquera *et al.*, 2008). The relationship between PSM introduced into soil, phosphatase activity and the subsequent mineralization of organic phosphorus still remains poorly understood (Chen *et al.*, 2003). Therefore, developing inoculants with high phosphatase activity would be of great practical importance in organic phosphorus solubilization especially in phosphorus deficient soils. The present work was aimed at studying the effect of application of inorganic soluble phosphorus source on phosphatase activity of an isolated phosphate solubilizing bacterial strain.

Materials and Methods

Isolation of phosphate solubilizing bacterial strain

Phosphate solubilizing bacterial (PSB) strain used for the experiment was isolated from tomato growing rhizosphere soil samples collected from green houses at Chungchugnam-do province, Gongju-Gun area in South Korea. Serial dilutions of field moist soils were inoculated using National Botanical Research

Institute's Phosphate growth medium (NBRIP) agar plates containing 10 g glucose, 5g $Ca_3(PO_4)_2$ -TCP, 5g $MgCl_2.6H_2O$, 0.25g $MgSO_4.7H_2O$, 0.2g KCl, 0.1g $(NH_4)_2SO_4$ in 1L distilled water (Nautiyal, 1999). The colonies with clear halos were considered to be phosphate solubilizing colonies. The partial sequencing of 16S rRNA for the bacterial strain was done with the help of DNA sequencing service, SOLGENT, Daejeon, South Korea. The bacterial strain identified as *Pantoea agglomerans* was selected for the experiment and the pure cultures were maintained in a glycerol suspension (30% v/v) at -80°C until use.

Treatments and Experimental procedure

The selected *Pantoea agglomerans* strain was used for the phosphatase enzyme induction studies using Ca_3PO_4 containing NBRIP medium supplemented with four levels of inorganic soluble P (0, 0.5, 1.0 and 1.5 g/l KH_2PO_4viz., P_0, P_1, P_2 and P_3) in triplicates. They were inoculated with 1ml culture having final concentration 10^8 CFU/ml. Aliquot (10 ml) of treatments were taken 1, 2 and 3 days after inoculation and centrifuged at 5000 rpm for 10 min. The clear supernatant was used in determining the acid phosphatase activity and amount of phosphorous released into the medium. The phosphorus availability was determined using phospho-molybdate blue color method (Murphy and Riley, 1962).

Assay of acid phosphatase activity

Acid phosphatase activity was determined using a modified assay of Juma and Tabatabai (1988). Aliquot of centrifuged culture supernatant (1 ml) was incubated at 37°C with 1 ml of 25 mM p-nitrophenyl phosphate (pNPP) and 4 ml of modified universal buffer (pH adjusted to 6.5). After 1hr, the reaction was terminated by adding 1ml of 0.5 M $CaCl_2$ and 4ml of 0.5 M NaOH. The assay mixtures were filtered and spectrophotometer readings were taken at 410 nm to quantify the intensity of yellow color (Tabatabai, 1982). The amount of released p-nitrophenol (pNP) was quantified using the pNP standard and expressed in terms of units (U). One unit (1 U) of phosphatase activity is the amount of enzyme required to release 1 µg pNP in ml of culture filtrate under assay conditions (1U= 1 µg pNP/ml) (Prasanna *et al.*, 2011).

Results and Discussion

Phosphatase activity and phosphate solubilization in different treatments with different time intervals are presented in Table 1 and 2 respectively. Phosphatase activity was decreased by inorganic soluble (KH_2PO_4) phosphates in the NBRIP medium inoculated with phosphate solubilizing bacterial strain. The phosphatase activity reached to the maximum at 48 hours after the inoculation of the strain followed by significant decrease when incubation progressed. The decrease in phosphatase activity after reaching a maximum at 48 hours might be due to the repression of phosphatase by available phosphorus released through the dissolution of $Ca_3(PO_4)_2$ in the NBRIP medium (Kapri and Tewari, 2010).

Table 1: Acid phosphatase enzyme activity (U/ml^{-1}) of culture filtrates at different time intervals

Treatment	Phosphatase enzyme activity(U/ml)		
	24 hours	48 hours	72 hours
P_0	$112.8^{a}\pm1.18$	$118.85^{a}\pm1.73$	$109.2^{a}\pm2.83$
P_1	$100.7^{b}\pm2.52$	$105.7^{b}\pm1.54$	$103.1^{b}\pm3.11$
P_2	$98.5^{bc}\pm1.78$	$100.9^{c}\pm2.31$	$96.6^{c}\pm3.24$
P_3	$95.3^{c}\pm2.08$	$95.9^{d}\pm2.11$	$95.3^{c}\pm2.73$

Values are given as means ± SD for triplicate samples. Within each column, means followed by same letter(s) are not

Table 2: Soluble phosphorous (µg/ml) in culture filtrates at different time intervals

Treatment	Soluble phosphorous (µg/ml)		
	24 hours	48 hours	72 hours
P_0	$254.71^{a}\pm1.53$	$642.25^{a}\pm2.23$	$640.38^{a}\pm2.52$
P_1	$240.75^{b}\pm1.22$	$630.24^{b}\pm1.54$	$630.21^{b}\pm3.75$
P_2	$232.24^{c}\pm1.57$	$622.32^{c}\pm1.35$	$620.21^{c}\pm2.38$
P_3	$210.85^{d}\pm2.85$	$608.54^{d}\pm1.15$	$604.35^{d}\pm3.03$

Values are given as means ± SD for triplicate samples. Within each column, means followed by same letter(s) are not significantly different at P=0.05 level according to DMRT

Phosphatase activity and phosphate solubilization in the NBRIP medium inoculated with *Pantoea agglomerans* decreased significantly when the application of inorganic soluble phosphate. The lowest phosphatase activity and phosphate solubilization were recorded at the highest rate of inorganic soluble phosphate (1.5g/l). This is obviously due to the presence of readily available source of P which represses phosphatase enzyme activity and phosphate solubilization. Similar to our findings, negative correlation between inorganic soluble phosphate source with phosphatase activity and phosphate solubilization has been observed by Wyss *et al.* (1999) and Anil and Lakshmi (2010). The treatment of P_3 which contains 1.5g/L KH_2PO_4 showed the lowest phosphatase activity and phosphate solubilization, which clearly proves the need of an insoluble phosphate source [$(Ca_3(PO_4)_2$] in the medium for the induction of phosphatase enzyme.

Conclusion

Phosphatase activity and phosphate solubilization in the NBRIP medium inoculated with *Pantoea agglomerans* remarkably decreased with the application of inorganic soluble phosphate source under *in vitro* conditions. Therefore composition of insoluble and soluble P in the medium affects the induction and activity of Acid Phosphatase enzyme activity, where increased abundance of soluble phosphates represses the enzyme activity.

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