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Research article

Variation in rock phosphate solubilization by three isolates of *Aspergillus niger* van Tieghem grown in liquid media supplemented with different carbon and nitrogen sources

Hruda Ranjan Sahoo and Nibha Gupta*

Division of Plant Pathology and Microbiology, Regional Plant Resource Centre, Bhubaneswar, Odisha, India
***Corresponding Author:** nguc2003@yahoo.co.in [Accepted: 25 April 2016]

Abstract: In the present study, we worked out the phosphate solubilization potential of three different isolates of *Aspergillus niger* from various sources such as leaf (L), root (R) and soil (S) under different carbon and nitrogen supplementation in liquid culture. The fungal cultures were inoculated in Czapek Dox Medium containing different carbon and nitrogen sources and supplemented with Moroccan rock phosphate. The fungal strains exhibited good potential favoring the solubilization of rock phosphate in laboratory conditions. On carbon sources modification, *Aspergillus niger* (L) and (S) showed highest P solubilization activity 27.6% and 29.6% respectively in presence of glucose whereas *Aspergillus niger* (R) showed highest P solubilization of 27.8% in presence of inositol. Similarly on modification of nitrogen sources, *Aspergillus niger* (L) and (S) showed maximum solubilization of 35.25% and 40.8% respectively, but *Aspergillus niger* (R) showed maximum solubilization of 37% in presence of amino acid L-phenylalanine in culture broth. Further pot experiment with different soil composition and host plants may exhibit its exploitable potential.

Keywords: Rock phosphate - Phosphate solubilization - Carbon sources - Nitrogen sources.

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INTRODUCTION

Phosphorus is an important nutritional element required indirectly for plant growth and development by incorporating itself in several biomolecules such as phospholipids, nucleic acids and nucleotides (Ahemad *et al.* 2009). Besides, cellular organization, phosphorus plays key role in many physiological and biochemical role and ultimately leads to crop growth and yield (Bagyaraj *et al.* 2000). However it is deficient in soil as it is fixed resulting into low availability and demands more input of chemical fertilizer. Under such poor soil conditions, phosphate solubilizing microbes can be helpful in mineralization of fixed phosphorus (Whitelaw 2000). A wide range of fungi especially *Aspergillus* and *Penicillium* are reported to solubilize insoluble form of phosphorus depending upon their ability to produce and release organic acids to their surrounding environment (Seshadri *et al.* 2004, Wakelin *et al.* 2004). The organism metabolic activity is dependent upon the cultural, nutritional and environmental factors (Nahas 1996, Kundu *et al.* 2002). Mostly carbon sources are the important factor behind the microbial metabolism and proliferation (Yadav *et al.* 2010, Khan *et al.* 2013). Different nitrogenous components in the growth media has also impact upon the microbial activity (Habte & Osorio 2012). Keeping this view, a study was planned to evaluate the efficiency of three different isolates of *Aspergillus niger* van Tieghem for phosphate solubilization under different nutritional conditions added with rock phosphate.

MATERIALS AND METHODS

The Fungal cultures of *Aspergillus niger* van Tieghem Leaf, Root and Soil isolated from leaf, root and soil, were grown on Sabouraud Dextrose Agar slant for 3 days at 30°C for further study. The plate solubilization index of the three isolates was calculated by the formula given by Gaur *et al.* (1990) and Premono *et al.* (1996). Triplicate sets of Czapek Dox broth medium with rock phosphate as phosphate sources and separately with

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different carbon and nitrogen sources were used and inoculated with these fungal isolates and incubated at 28±2ºC for 10 days. P content was estimated by vanadophosphomolybdate method and represented in the form of % solubilised (Jackson 1958).

RESULTS AND DISCUSSION

Phosphate solubilizing potential of three isolates of *Aspergillus niger* in solid state and liquid culture conditions is depicted in table 1. Solubilisation index evaluated through the plate test with PK medium added with TCP showed good activity of these isolates of *Aspergillus niger* and ranged as 1.27 to 1.85. All the three *Aspergillus niger* showed 23.7% to 25.83% whereas difference in solubilization of rock phosphate in liquid culture have been observed among these fungal isolates. *Aspergillus niger* isolated from root showed highest solubilization (36.2%) followed by other two isolated from soil (31.3%) and leaf (22.7%). Though fungi contribute more to the soil biomass being the important constituents of soil microflora depending on the conditions like soil depth and nutrient conditions, *Aspergillus niger* sourced from leaf showed better solubilization efficiency in plate test.

Table 1. Phosphate solubilising potential of three isolates of *Aspergillus niger* in solid state and liquid culture conditions.

	P solubilized	***Aspergillus niger* (L)**	***Aspergillus niger* (R)**	***Aspergillus niger* (S)**
Plate test	Solubilization Efficiency (%)	87.5	50	27
(solid medium)	Solubilization Index	1.875	1.5	1.27
Broth culture	TCP Solubilization (%)	25.83±6.54	23.9±2.27	23.7±4.3
(liquid medium)	RP solubilization (%)	22.7±0.46	36.2±5.6	31.3±1.99

Carbon and nitrogen sources greatly influence phosphate solubilization process. In the presence of various carbon and nitrogen sources, micro-organisms have diverse levels of phosphate solubilization activity. Rock phosphate solubilization under carbon and nitrogen supplementation in media separately has shown in table 2–3. It is clearly evident that carbon sources affect the P solubilization capacity of fungi indicating that micro-organisms utilizes different carbon sources as energy sources and most of the test sugars support phosphate solubilization activity. *Aspergillus niger* (R) was able to solubilize rock phosphate more in different carbon source used except lactose and sorbose (Table 2). The pH measured after 10 day incubation period of fungal culture under such circumstances varies from 4.46 to 6.28 (Fig. 1). Similarly *Aspergillus niger* (S) performed good for rock phosphate solubilization in liquid culture and exhibited solubilised P % ranged 24.3–29.6 except for lactose. Presence of glucose and maltose in culture confirmed to be better carbon sources for this organism as far as phosphate solubilization is concerned. Though, all carbon sources tested in the present experiment showed good support for the metabolic activity pertaining to phosphate solubilization, lactose did not contribute well in this regard. Data measured on the drift of pH after 10 days of incubation period of 10 days are presented in figure 2. It is observed that carbon metabolism in presence of sugar fructose is more as compared to other sugars used as pH of the final culture was drifted to 3.9 (Fig. 3). *Aspergillus niger* (L) did not show much solubilization efficiency in presence of different carbon sources used except glucose (27.6 %). Though the fungi showed decrease in pH in presence of fructose (3.9), it could not affect the solubilization as *Aspergillus niger* (L) showed 14.9±5.6% phosphate solubilization only.

Table 2. Effect of Carbon sources on phosphate solubilization activity (% P solubilized) of different isolates of *Aspergillus niger*.

Carbon sources	***Aspergillus niger* (L)**	***Aspergillus niger* (R)**	***Aspergillus niger* (S)**
Fructose	14.9±5.6	22.9±2.1	25.2±1.45
Glucose	27.6±2.5	26±1.8	29.6±1.16
Inositol	3.4±0.49	27.8±1.15	24.3±4.16
Lactose	0.8±0	0.9±0.45	3.1±0.95
Maltose	0.75±0.07	22.2±2.25	28.9±0.71
Mannose	11.6±1.8	21.7±1.16	26.5±1.52
Raffinose	8.8±0	21.9±0.51	26.2±1.8
Sorbose	3±0.42	13.9±1.46	24.4±4.7
Sucrose	3.4±0.49	24.5±0.7	27.3±2.48

All the fungi showed diverse levels of RP solubilization activity in presence of different carbon sources. Glucose being the simplest sugar is favorable for growth of organism and acid production thereby enhances solubilized P release in the medium. All the 3 fungi showed good P solubilization activity in presence of glucose. The pH drift and P solubilization potential of organisms was estimated after 10 days of incubation.

Acid production is commonly observed during P solubilization (Scervino *et al.* 2011). Hence, P solubilization takes place due to release of organic acids by fungi resulting in reduction of pH (Yadav & Singh 1991, El-Komy 2005). The pH change from neutral to acidic condition is found in presence of most of the sugars at the end of 10 days. However, no correlation could be established between P solubilization in liquid broth and acidic pH recorded for the same. Similar observations were also noticed in experiments conducted by Wani *et al.* (1979).

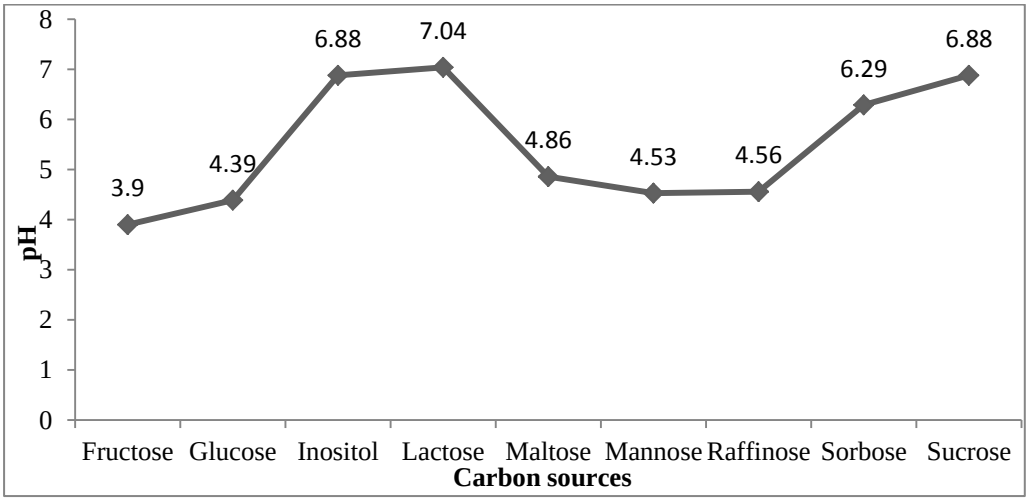

Figure 1. Effect of carbon sources on pH drift during P solubilisation in *Aspergillus niger* (L).

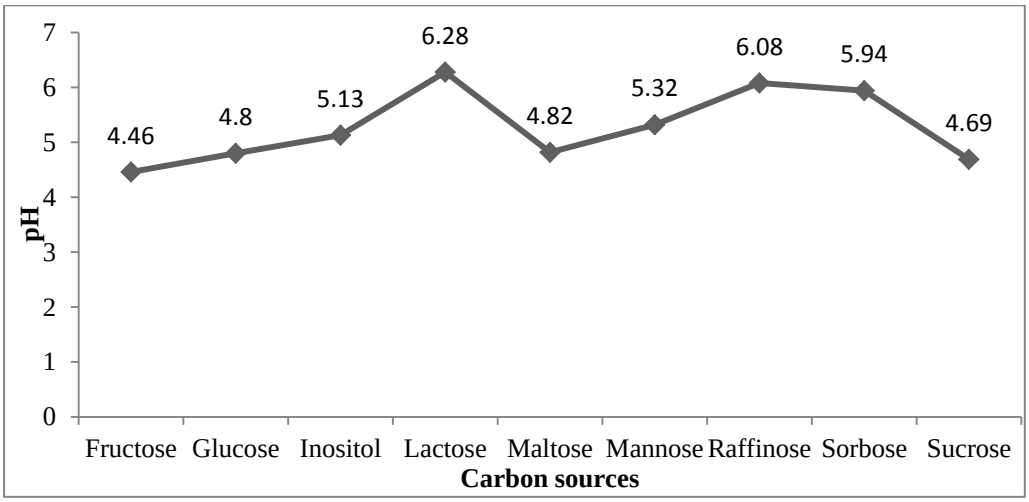

Figure 2. Effect of carbon sources on pH drift during P solubilisation in *Aspergillus niger* (R).

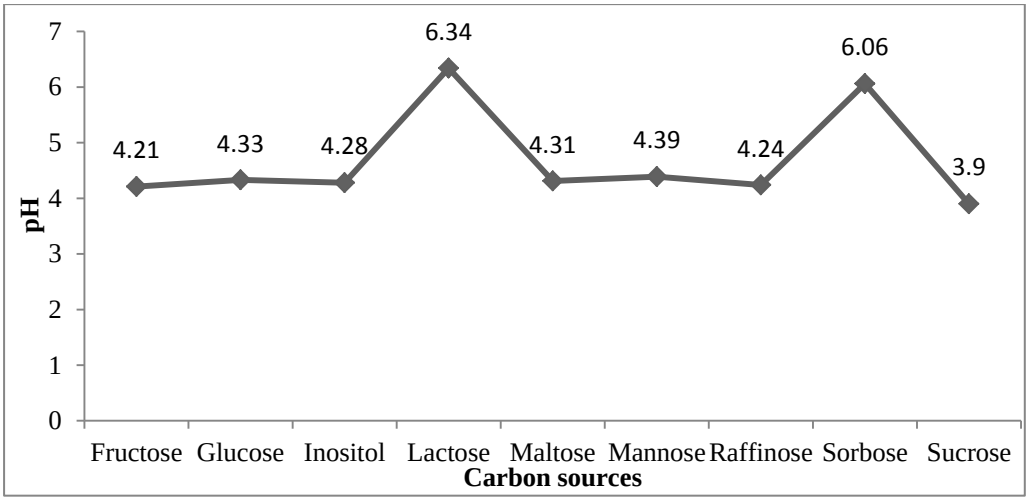

Figure 3. Effect of carbon sources on pH drift during P solubilisation in *Aspergillus niger* (S).

Table 3. Effect of nitrogen sources on phosphate solubilisation activity (% P solubilized) of different isolates of *Aspergillus niger*.

Nitrogen sources	*Aspergillus niger* (L)	*Aspergillus niger* (R)	*Aspergillus niger* (S)
Ammonium chloride	23.4±4.81	29.4±1.41	39.3±1.1
Ammonium Sulphate	27.45±3.75	34.9±0.7	39.7±1.35
L-Glutamine	30.8±9.05	22.4±1.6	38.7±0.36
L-Phenylalanine	29.8±6.4	37±2.3	39±0.75
L-Threonine	35.25±0.07	22.7±2.76	40.8±1.67
L-valine	25.7±11	34.1±3.6	35.2±0.51
Potassium nitrate	32.6±2.12	34.9±2	30.2±3.1
Urea	24.85±7.7	6.8±4.1	22.8±1.46
Sodium nitrate	34.2±0.14	32.75±1.34	28.9±1.3

All the tested *Aspergillus niger* L, R, S influenced RP solubilization process in presence of different nitrogen sources (salts/amino acids). The results obtained during experiment have been presented in table 3. All the three fungi have shown good solubilization potential in presence of different nitrogen sources in combination of glucose as basal carbon source. *Aspergillus niger* (L) solubilised rock phosphate at 35.25 and 34.2% in presence of L-threonine and sodium nitrate respectively whereas *Aspergillus niger* (R) preferred L-phenylalanine and could be able to solubilize 37.2% rock phosphate in liquid culture conditions. *Aspergillus niger* (S) showed good P solubilization activity in presence of most of the nitrogen sources which demonstrates that this organism is well adapted and can grow and function in different Cultural conditions. However, no significant changes in the pH of the culture filtrate was observed as an effect of different nitrogen sources (Fig. 4, 5). The decline in the pH of the culture filtrate of *Aspergillus niger* (S) has been observed and presented in figure 6. Highest drift in pH to highly acidic range *i.e.* 2–3 was measured in the presence of L-phenylalanine followed by potassium nitrate (3.3) and sodium nitrate (3.45). However, it is known that nitrates were more efficient nitrogen source for P solubilization activity due to presence of assimilatory enzymes for nitrate reduction in organisms (Dave & Patel 2003). Moreover inorganic nitrogen sources proved to be better source for P solubilization activity as compared to organic ones (Selvi *et al.* 2012).

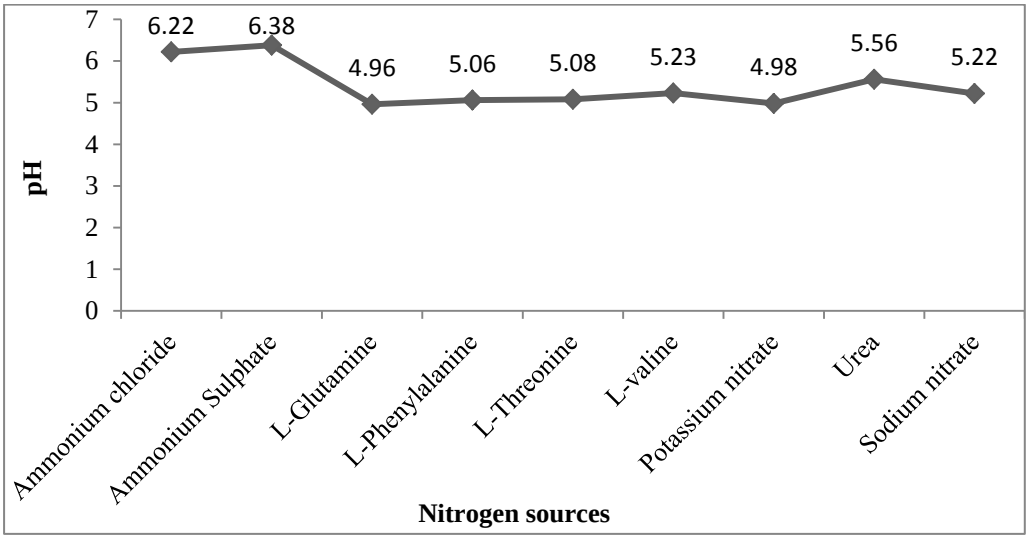

Figure 4. Effect of nitrogen sources on pH drift during P solubilisation in *Aspergillus niger* (L).

The role of *Aspergillus niger* towards solubilization of phosphate sources is clearly evident from the present study. Though many fungi obtained from the different sources other soil have been observed as potent candidate to be exploited for the bioinoculant development, the role of soil fungi stand unbeatable as fungi present in the soil convert unavailable forms of phosphorus to available phosphorus for plant to absorb by employing different strategies. Soil P transformations are mediated by microbial activity and influenced by various factors such as plant species, soil type and environmental factors (Chen *et al.* 2004). In the present study, the fungal strain exhibited good potential favoring the solubilization of rock phosphate in laboratory conditions with supplementation of different carbon and nitrogen sources but further pot experiment with different soil composition and host plants may exhibit its exploitable potential.

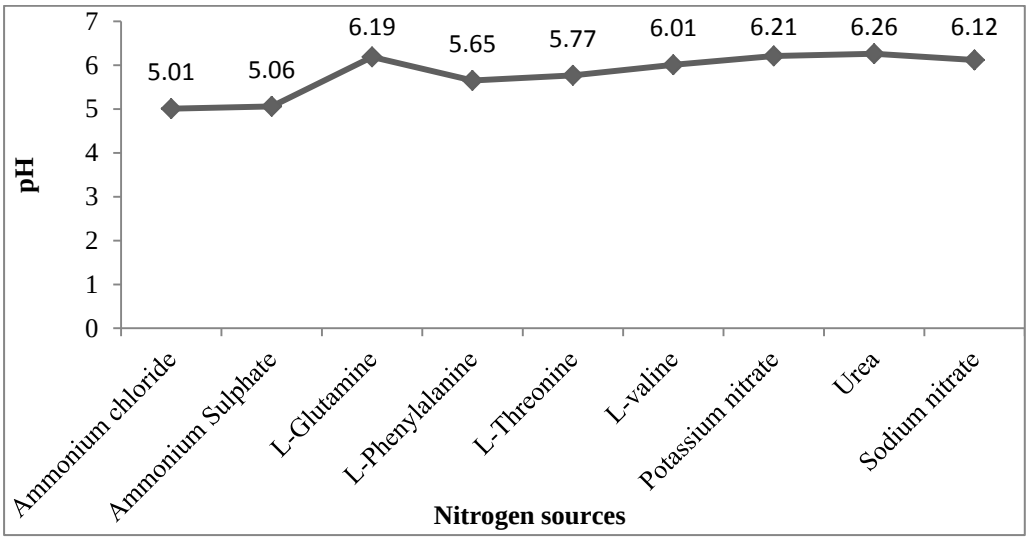

Figure 5. Effect of nitrogen sources on pH drift during P solubilisation in *Aspergillus niger* (R).

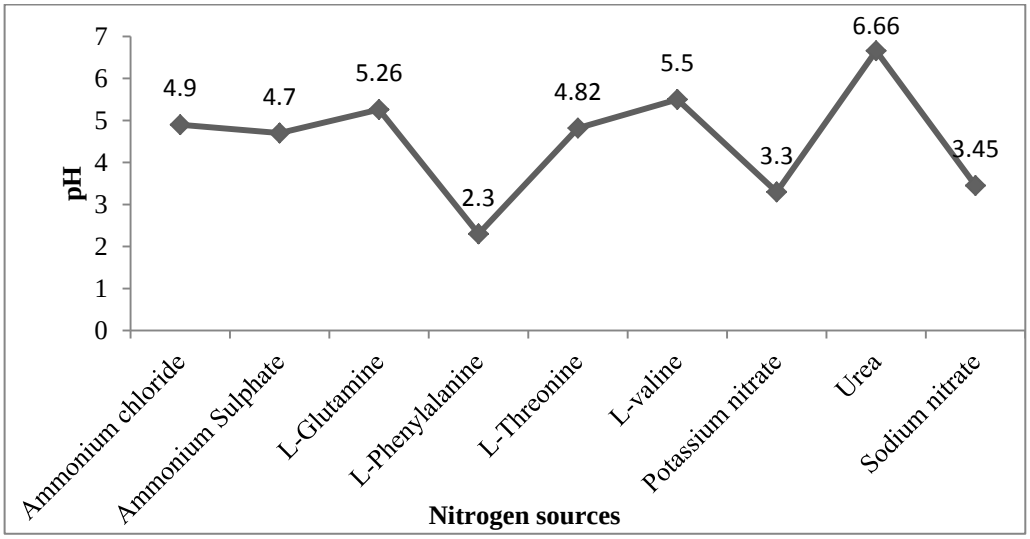

Figure 6. Effect of nitrogen sources on pH drift during P solubilisation in *Aspergillus niger* (S).

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REFERENCES

Ahemad M, Zaidi A, Khan MS & Oves M (2009) Biological importance of phosphorus and phosphate solubilising micro-organisms- an overview, In: Khan MS & Zaidi A (eds) *Phosphate solubilizing microbes for crop improvement*. *Nova*, New York, pp. 1–4.

Bagyaraj DJ, Krishnaraj PU & Khanuja SPS (2000) Mineral phosphate solubilization: agronomic implication, mechanism and molecular genetics. *Proceedings of the Indian National Science Academy* 66: 69–82.

Chen CR, Condron LM, Davis MR & Sherlock RR (2004) Effects of plant species on microbial biomass phosphorus phosphatase activity in a range of grassland soils. *Biology and Fertility of Soils* 40: 313–322.

Dave A & Patel HH (2003) Impact of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas fluorescens*. *Indian Journal of Microbiology* 43(1): 33–36.

El-Komy MAH (2005) Coimmobilization of *Azospirillum lipoferum*, *Bacillus megaterium* for Successful Phosphorus, Nitrogen Nutrition of Wheat Plants. *Food Technology and Biotechnology* 43 (1): 19–27.

Gaur A., Rana J, Jalali B & Chand H (1990) Role of VA mycorrhizae, phosphate solubilizing bacteria and their interactions on growth and up-take of nutrients by wheat crops. In: The National Conference on Mycorrhizae (1990: Hisar, India). Proceeding. Hisar, India. *Trends in Mycorrhizal Research* 105–106.

Habte M & Osorio NW (2012) Effect of nitrogen form on the effectiveness of a P-solubilizing fungus to dissolve rock phosphate. *Journal of Biofertiliser and Biopesticides* 3: 1–4.

Jackson ML (1958) *Soil Chemical Analysis*. Prentice Hall Inc. Englowoodcliff, New Jersey, USA.

Khan MS, Ahemad E, Zaidi A & Oves M (2013) Functional Aspect of phosphate solubilizing bacteria: Importance in crop production, In: Maheswari DK (ed) *Bacteria in Agrobiology: crop productivity*. Springer, Berlin. pp. 237–265.

Kundu BSR., Gera N, Sharma A & Bhatia R (2002) Host specificity of phosphate solubilizing bacteria. *Indian Journal of Microbiology* 42: 19–21.

Nahas E (1996) Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World Journal of Microbiology and Biotechnology* 12: 567–572.

Premono EM, Moawad MA & Vleck PLG (1996) Effect of phosphate solubilizing *Pseudmonas putida* on the growth of maize and its survival in the rhizosphere, *Indonasian Journal of Crop Science* 11: 13–23.

Scervino JM, Papinutti VL, Godoy MS, Rodriguez MA, Monica ID, Recchi M, Pettinari MJ & Godeas AM (2011) Medium pH, carbon and nitrogen concentrations modulate the phosphate solubilization efficiency of *Penicillium purpurogenum* through organic acid production. *Journal of Applied Microbiology* 110(5): 1215–1223.

Selvi KB & Ravindran AD (2012) Influence of different carbon and nitrogen sources on insoluble inorganic phosphate solubilization by *Bacillus subtilis*. *International Journal of Advanced Biological Research* 2(3): 441–445.

Seshadri S, Ignacimuthu S & Lakshminarasimhan C (2004) Effect of nitrogen and carbon sources on the inorganic phosphate solubilization by different *Aspergillus niger* strains. *Chemical Engineering Communications* 191: 1043–1052.

Wakelin SA, Warren RA, Harvey PR & Ryder MH (2004) Phosphate solubilization by *Penicillium spp.* closely associated with wheat roots. *Biology and Fertility of Soils* 40: 36–43.

Wani PV, More BB & Patil PL (1979) Physiological studies on the activity of phosphorus solubilizing microorganisms. *Indian Journal of Microbiology* 19(1): 23–25.

Whitelaw MA (2000) Growth promotion of plants inoculated with phosphate solubilizing fungi. *Advances in Agronomy* 69: 99–151.

Yadav J, Verma JP, Yadav SK & Tiwari KN (2010) Effect of salt concentration and pH on soil inhabiting fungus *Penicillium citrinum* Thom. For solubilisation of tricalcium phosphate. *Microbiology Journal* 72: 625–630.

Yadav K & Singh T (1991) Phosphorus solubilization by microbial isolate from a calcifluvent. *Journal of Indian Society of Soil Science* 39(7): 89–93.