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TCP and rock phosphate solubilization by mangrove fungi grown under different pH and temperature in liquid culture

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The objective of this work was to evaluate the efficiency of mangrove phyllosphere fungi in solubilization of tricalcium phosphate and rock phosphate in liquid culture. Total 14 fungi including *Aspergillus*, *Penicillium* and *Alternaria* were tested under different pH and temperature. Aspergillus PF 126 was solubilised and released 92.74 µg/ml P content where as *Aspergillus* PF 127 showed better efficiency of rock phosphate solubilization and produced 54.4 µg/ml P content into the liquid culture. *Penicillium* sp. was comparatively poor solubiliser of TCP and rock phosphate in liquid culture.

Key words: mangrove, fungi, phosphate, *Aspergillus*, *Penicillium*

Introduction

A large number of microorganisms including bacteria, fungi and actinomycetes are known to produce acidic metabolites which release fixed or insoluble phosphorus in available form (Silva and Vidor, 2001; Chung *et al.*, 2005). These organisms are very important with regard to savings of chemical fertilizer. The utilization efficiency of phosphate fertilizer by plant is only 20-25% due to chemical fixation in soil. Phosphate solubilising organisms dissolve the fixed mineral phosphate and make it available to plants (Zaidi and Khan, 2005). Therefore, phosphorus biofertilizers has gained importance in agriculture due to escalating cost of phosphatic fertilizers, environmental hazards posed by them and their dependence on nonrenewable energy resources for production. In present study, phosphate solubilising fungi were obtained and selected from different phyllosphere of mangroves growing in Bhitarkanika (Orissa). Since, these fungi were isolated from saline environment, the present work attains importance as no such report has been made earlier from the mangrove

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ecosystem of Bhitarkanika. However, some marine bacteria and fungi in India and abroad were reported as phosphate solubilisers (Vazquez *et al*., 2000; DeSouza *et al*., 2000). In the present studies these fungi were investigated for their phosphate solubilising potential using TCP and Rock phosphate in liquid culture (Wang *et al*., 2005).

Materials and methods

Medium for phosphate solubilising fungi

Pikovaskaya medium (7.2) added with 0.5% TCP (tricalcium phosphate) and /or rock phosphate was used for the present study (Pikovasakaya, 1948).

Source of fungi

Phosphate solubilising fungi were obtained from phyllosphere of mangrove plants previously collected from different locations and sites of Bhitarkanika. These plants were *Acanthus ilicifolius, Acrosticum sp, Aegiceras corniculatum, Aglaia cuculata, Avicennia officinalis, Brownloia tarsa., Bruigeira parviflora, Bruigeira gynnorrhiza, Caesalpinia cristae, Crinum, Dalbergia spinosa, Derris heterophyla, Exocoecaria agallocha, Heritiera fomes, Kalanchoe pinnata, Kendalia candel, Sonneratia apitala, Sonneratia caseolaris, Tamarix troupii.* Out of 56 phyllosphere fungi, 14 fungi were selected on the basis of their ability to form halozone around the growing colony in TCP added Pikovaskaya medium.

Effect of temperature and pH on phosphate solubilization

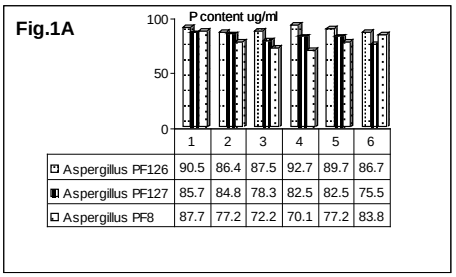
All selected fungi were grown in PDA agar of 6 pH to obtain pure culture for inoculation in further studies. Fresh culture of each fungus were cut into disc of 10 mm and inoculated into the Pikovaskaya of three different pH i.e. 4.5, 7.2 and 9.0 and incubated at two different temperature i.e. 30ºC and 37ºC for 10 days (Souchie *et al*., 2005; Bhargava and Raghupati, 1993). Three experimental sets were prepared (i) only media without phosphate (25 ml) (ii) media added with 0.5 g Tricalcium phosphate and, (iii) media added with 0.5g Rock phosphate (0.5 g), and inoculated with fresh culture of selected organisms in triplicate. These sets were incubated for 12 days. The total phosphate content available in 25 ml of culture filtrate was measured by UV-vis spectrophotometer at 420 nm. In each experimental set, final pH of culture filtrate was also measured.

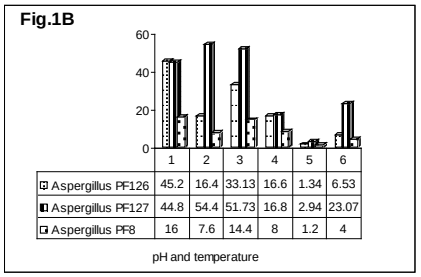
Results

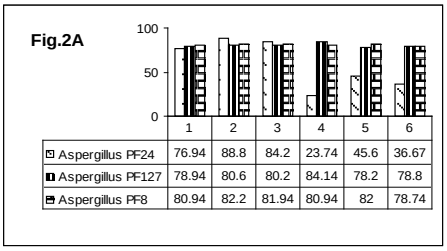
All fungi tested (10 *Aspergillus* sp., 3 *Penicillium* sp. and 1 *Alternaria* sp.) were found to be solubilser of TCP in liquid medium (Figs. 1-5). All the *Apsergillus* sp. was found to be better solubilisers than *Penicillium* sp. The P content released into the medium from TCP was 92.74 µg/ml by *Aspergillus* PF126 (Fig. 1A). It was followed by *Aspergillus* PF24 and PF8 that produced 88.8 µg/ml and 87.74 µg/ml P content into the medium (Fig. 2A.). These *Aspergilli* performed well in both temperature (30ºC and 37ºC) and three pH (4.5, 7.2 and 9.0). Other *Aspergillus* sp. poorly exhibited phosphate solubilization (Fig. 3A). Similarly, *Penicillium* PF110 performed well in all pH and temperature tested. However, it showed maximum solubilization of phosphates at 37ºC in 4.5 pH and released 88.14 µg/ml P content (Fig. 4A). One and only isolate of *Alternaria* had shown TCP solubilization in liquid culture and released P content in the range of 31.6 to 35.94 µg/ml. However, these fungi did not show preference for 9 pH and 37ºC.

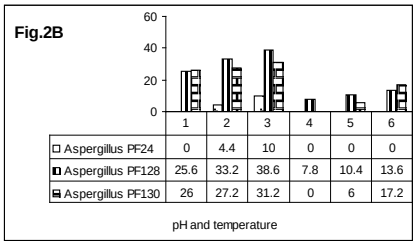
Rock phosphate is low efficiency P fertilizer that is directly applied to soil and can be solubilized by phosphate solubilising microorganisms (Wang *et al*., 2005; Duponnois *et al*., 2005; Dwivedi *et al*., 2004). In present study, all *Aspergillus* sp. except *Aspergillus* PF58, *Penicillium* PF34 and PF110, *Alternaria* sp. were able to solubilize rock phosphate in liquid culture (Fig. 1B-5B). Three *Aspergilli* i.e. PF126, PF127 and PF 8 have shown rock phosphate solubilization in all temperature and pH in varied capacity. The highest rock phosphate solubilization and release of phosphorus was observed by *Aspergillus* PF 127 (54.4 µg/ml) and *Aspergillus* PF 126 (45.2 µg/ml). These two fungi were found to be best among 14 fungi evaluated in this experiment.

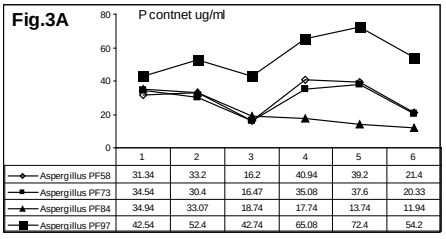
Almost all fungal isolates used in this study were producers of acid into the medium. The decline in the final pH of culture filtrate was observed (Table 1). In control experimental sets (without phosphate) pH were decreased towards acidic condition. Similarly, in test experimental sets pH were also declined as compared to initial pH. All *Aspergillus* sp. produced more acidic metabolite than *Penicillium* sp. as evident from the results mentioned in Table 1 that initial pH of the medium was reduced up to 3.92 and 4.03 in *Aspergillus* PF8 and PF 127 respectively. However, addition of rock phosphate did not affects much pH of the medium rather marginal increase in pH could be observed.

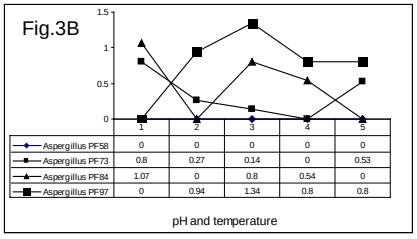


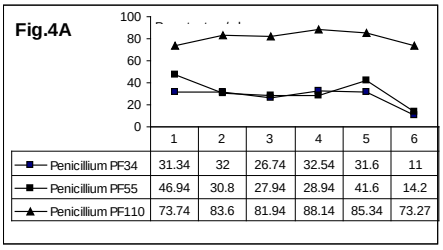


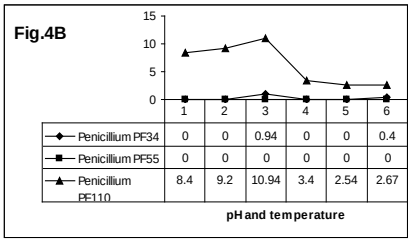


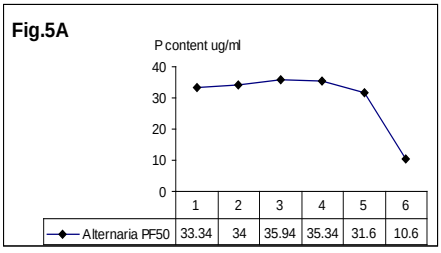


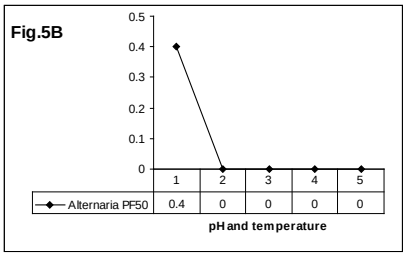












Figs. 1-5. (A-B) 1 -30 ºC, 4.5 pH, 2 - 30 ºC, 7.2 pH, 3 – 30 ºC, 9.0 pH, 4 - 37 ºC, 4.5 pH, 5. - 37 ºC, 7.2 pH, 6 - 37 ºC., 9.0 pH, Fig. A - TCP solubilisation, Fig. B - Rock phosphate solubilisation.

Table 1. Changes in pH of culture filtrates of phosphate solubilising fungi.

Fungi	pH of culture filtrate											
	Tri Calcium phosphate						Rock phosphate					
	30°C			37°C			30°C			37°C		
	4.5	7.2	9	4.5	7.2	9	4.5	7.2	9	4.5	7.2	9
Aspergillus PF 126	4.87	4.9	4.74	4.66	4.82	4.56	5.42	5.59	5.2	5.64	5.7	5.62
Aspergillus PF 127	4.03	4.73	4.91	4.79	4.66	4.95	4.43	5.24	4.99	5.45	5.72	4.78
Aspergillus PF 8	4.8	4.54	4.94	3.92	4	4.88	5.42	5.74	5.61	5.59	5.82	5.9
Aspergillus PF 24	4.85	4.51	4.62	5.13	5.39	4.28	5.44	5.12	5.29	6.57	6.86	6.42
Aspergillus PF 128	4.79	4.82	4.92	4.76	4.76	4.7	5.27	5.44	5.32	5.74	5.85	4.78
Aspergillus PF 130	4.68	5.09	4.74	5.02	4.9	4.77	5.51	5.31	5.31	5.9	5.82	5.53
Aspergillus PF 58	5.09	5.03	5.46	4.95	5.18	5.45	6.4	6.36	5.94	6.03	5.72	5.91
Aspergillus PF 73	5.37	5.69	5.78	5.52	5.5	5.68	6.23	6.61	6.41	6.29	6.35	6.42
Aspergillus PF 84	5.24	5.24	5.5	5.74	5.83	5.65	7.25	7.08	7.05	7.14	7.08	6.71
Aspergillus PF 97	5.43	5.41	5.73	4.62	4.34	4.38	5.5	5.47	5.27	5.74	4.78	5.33
Penicillium PF 34	5.41	5.48	5.76	5.4	5.43	5.88	6.63	6.9	7.09	6.75	6.99	7.16
Penicillium PF 55	5.56	5.46	5.94	6.48	5.42	5.88	7.28	7.08	7.08	7.21	7.33	7.22
Penicillium PF 110	5.11	4.73	4.32	4.65	4.88	4.43	5.46	5.34	5.01	5.69	5.26	5.06
Alternaria PF 50	5.27	5.33	5.4	5.24	5.38	5.48	6.56	6.29	6.37	5.7	5.87	6.16

*****Data are mean of three replications.

Discussion

In the present investigation a good number of mineral phosphates solubilizing fungi were obtained from mangrove plants. Phosphate solubilization by *Penicillium* and *Aspergillus* is reported very commonly where as findings of phosphate solubilization by *Alternaria* presented is very rare (Gaur and Sachar, 1980; Reyes *et al*., 2002; Reddy *et al*., 2002). However, very poor solubilization of rock phosphate have shown by this fungi. It is well known that phosphate solubilising microorganisms in soil solubilize insoluble phosphates mainly by secreting acids into the medium (Dave and Patel, 2003; Chung *et al*., 2005). This observation is also corroborated with Villegas and Fortin (2001). A large number of microorganisms are known to produce acidic metabolites which by change of pH and by chelation of metal ions release fixed or insoluble phosphorus in available form (Yadav and Dadarwal, 1997). The decline in pH of the medium indicates the same mechanism used by the inoculated fungi (Narsian and Patel, 1997).

Tricalcium phosphate solubilizing abilities of microbes is reported well (Rudresh *et al.*, 2005). All the fungi tested could be able to solubilise TCP in liquid culture state. However, degree of Phosphate solubilization varied with the type of organisms involved (Sujatha *et al*., 2004; Srivastav *et al*., 2004).

Aspergillus Pf 126 and PF127 were found to be superior to other fungi in solubilization of both tricalcium phosphate and rock phosphate but not in equal efficiency. Several reports are mentioned regarding the effects of carbon and nitrogen sources on phosphate solubilization capacity and its enhancements (Yu *et al*., 2005; Silva and Vidor, 2001).The findings of 14 fungi as rock phosphate solubiliser including *Aspergillus* and *Penicillium* sp. suggested the further improvement of strains with respect to nutritional components (Narsian and Patel, 2000).

In conclusion, some phyllopshere fungi of mangrove plants of Bhitarkanika were found to be phosphate solubilisers. However, *Aspergillus* sp. exhibited best phosphate solubilization in any source (Silva and Vidor, 2001). Occurrence of phosphate solubilizing fungi in the mangrove phyllosphere showed the host specific interaction (Ananda and Sridhar, 2004). This finding is very well corroborated with the several reports available on richness of mangrove microbial diversity due to the support they extended through production of large quantities of vegetative matter. Though occurrence of phosphate solubilising microbes in marine system is documented (Seshadri *et al*., 2002; DeSouza *et al*., 2000), reports on phosphate solubilisers from mangrove origin is meager (Vazquez *et al.,* 2000).The present study extends preliminary but important observations towards the development of phosphatic biofertiliser required for saline and alkaline soils.

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