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SHORT COMMUNICATION

Phosphate Solubilising Fungi from Mangroves of Bhitarkanika, Orissa

NIBHA GUPTA*, SARITA JAYANTI DAS

Regional Plant Resource Centre, Bhubaneswar –751 015 Orissa, India

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Mangroves have evolved several adaptations to swampy and saline environments. It is situated at the inter-phase between marine and terrestrial environment, which is highly productive providing nutrients to surrounding micro biota. Similar adaptive characteristics in the form and function may occur with the associated microflora in such environments. Several free living and symbiotic microorganisms occurred in such saline habitats and some of them are reported for their beneficial activity in mangrove ecosystem like biomineralization of organic matter and bio-transformation of minerals. In view of this, 106 fungi isolated from rhizosphere and phyllosphere of mangrove plants grown in Bhitarkanika, Orissa were screened on plate culture containing Pikovaskaya medium for the phosphate solubilization. Selected fungi were evaluated for their phosphate solubilization potential under different cultural conditions. A total of 36 fungi were isolated that showed variable halo zone on medium containing tricalcium phosphate when grown under different pH and temperature. The highest zone was formed by *Aspergillus* PF8 (63 mm) and Aspergillus PF127 (46.5 mm). The observation on tricalcium phosphate solubilization activity of *Paecilomyces, Cladobotrytis, Helminthosporium* is rare. However, a detailed and elaborative studies are needed to confirm better mineral solubilization potential of these fungi.

Key words: mangrove, fungi, phosphate

Mangrove ecosystem is well studied as diversified habitat for plants, animals, and microbes. Several Nitrogen fixing, enzyme producers and mineral solubilisers are reported from mangrove rhizosphere (Kathiresan 2000; Vazquez 2000). Bhitakanika is second largest mangrove forest in India but not studied well for its microbial diversity and useful microorganisms especially phosphate solubilisers. Phosphorus is the second major plant nutrient and its availability to crops, rather than input, is more important as it is subjected to chemical fixation in soil with other metal cations depending on soil pH. Its availability through microbial solubilization in root zones or enhancement of absorption through other symbiotic organisms with crop roots, required to be explored. A large number of microorganisms including bacteria, fungi and actinomycetes are known to produce acidic metabolites which by change of soil pH or by direct chelation of metal cations, release fixed or or insoluble phosphorus in available form (Achal *et al*. 2007). 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 available to plants. Therefore, phosphorus biofertilisers 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 (Bucher 2007).

*Corresponding author. Phone: +91-0674-2557925, Fax: +91-0674-2550274, E-mail: nguc2003@yahoo.co.in

Several reports have been made on the occurrence of phosphate solubilising marine bacteria. Vazquez *et al*. (2000) reported the phosphate solubilizing microorganisms associated with the rhizosphere of mangroves in a semi arid coastal laggon. No study has been reported regarding the phosphate solubilising microbes of Bhitarkanika mangroves system. In the present study we screened the fungi obtained from mangrove area of Bhitarkanika for the phosphate solubilization potential at different pH and temperature.

26 plant samples of mangrove origin were collected from different locations and sites of Bhitarkanika. Leaf and root samples were used for the isolation of fungi. Leaves of individual plants were thoroughly washed and sterilized with 0.01% $HgCl_2$ for 30 sec and again rewashed with sterilized dist water. The leaves were cut into small pieces of 1 cm size and placed on plates of Pikovaskaya medium (7.2) added with 0.5% TCP (tricalcium phosphate) was used for the isolation and screening of phosphate solubilising fungi (Pikovskaya 1948; Kundu *et al*. 2000). Similarly, root pieces of 1 cm length were inoculated into the plates. All plates were incubated at 30 $^{o}C \pm 2$. After 7 days, discrete colonies showing clear zone around them were picked up, further purified and confirmed for phosphate solubilization. Selected fungal isolates were identified and maintained in agar slants at freezing temperature.

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. 30 and 37 ^{o}C for 7 days. The halo zone formed by the fungal colony was measured and expressed in terms of mm.

In the present investigation a good number of mineral phosphates solubilizing fungi were isolated from mangrove plants. Overall 36 fungi (14 fungi from phyllosphere and 22 fungi from rhizosphere) were found as solubilisers of tricalcium phosphate on solid Pikovaskaya medium of 7.2 pH (Souchie *et al*. 2005). These fungi were belonging to *Penicillium, Aspergillus, Fusrium, Helminthosporium, Cladobotrytis, Paecilomyces, Alternaria*. Phosphate solubilization by *Penicillium* and *Aspergillus* is reported very commonly where as findings of phosphate solubilization by other group of fungi presented is very rare (Gaur & Sacher 1980; Reyes *et al*. 2002; Reddy *et al*. 2002).

We obtained 3 *Penicillium* sp., 10 *Aspergillus* sp., and one *Alternaria* sp. from phyllosphere of mangrove plants as phosphate solubiliser. All phyllosphere fungi exhibited best zone formation at 7.2 pH. All the three *Penicillium* sp. could not solubilize phosphate in medium of 4.5 at 30 and 37 °C temperature (Table 1) where as all the *Aspergilli* exhibited better response towards phosphate solubilization in 4.5 pH. *Aspergillus* PF8 and PF130 and *Alternaria* PF50 performed well in both the pH and temperature. However, other phyllosphere fungi showed preference to specific medium and/or pH. In all, the highest zone forming fungi was *Aspergillus* PF8 (63 mm) and PF127 (46.5 mm) at 7.2 pH at 37 °C.

Studies on rhizosphere of mangrove plants showed presence of 22 fungi as phosphate solubiliser (PS). Among them *Aspergillus* (2), *Penicillium* (10), *Fusrarium* (5), *Helminthosporium*, *Cladobotrytis*, *Paecilomyces* were obtained. In contrast to phyllosphere, rhizosphere exhibited occurrence of more potent fungi. The population of *Aspergilli* may be poor at rhizosphere site, their performance with reference to phosphate solubilization is superior than *Penicillium* sp. (Table 2). The occurrence of *Helminthosporium, Cladobotrytis,* and *Paecilimyces* as phosphate solubiliser is rare observation (Ha que & Dave

Table 1. Halo zone formation by mangrove phyllosphere fungi grown under different pH and temperature (mm)

Organism code	30 °C (pH)			37 °C (pH)		
	4.5	7.2	9	4.5	7.2	9
Alternaria sp. PF50	27 (2.83)*	28 (5.66)	28.5 (0.71)	16.5 (3.54)	27.5 (6.36)	12 (0)
Aspegillus sp. PF97	18.5 (0.71)	26 (8.49)	21.5 (2.12)	0 (0)	30.5 (0.71)	22 (1.41)
Aspergillus sp. PF126	0 (0)	41 (5.66)	32.5 (2.12)	31 (0)	44.5 (2.12)	34.5 (0.71)
Aspergillus sp. PF127	30 (1.41)	34.5 (3.54)	36.5 (4.95)	0 (0)	46.5 (2.12)	41 (1.41)
Aspergillus sp. PF24	23 (1.41)	28 (1.41)	26 (2.83)	24 (0)	31.5 (2.12)	31 (1.41)
Aspergillus sp. PF8	38.5 (2.12)	47.5 (23.33)	47 (2.83)	37.5 (0.71)	63 (0.00)	48 (1.41)
Aspergillus sp. PF73	0 (0)	24.5 (0.71)	0 (0)	0 (0)	25 (2.83)	0 (0)
Aspergillus sp. PF84	0 (0)	31.5 (3.54)	0 (0)	0 (0)	0 (0)	0 (0)
Aspergillus sp. PF128	32.5 (3.54)	24 (0)	26 (0)	31 (1.41)	0 (0)	0 (0)
Aspergillus sp. PF130	0 (0)	32.5 (0.71)	26 (2.83)	0 (0)	27.5 (0.71)	29 (1.41)
Aspergillus sp. PF58	19 (1.41)	29.5 (0.71)	0 (0)	21.5 (2.12)	29 (1.41)	0 (0)
Penicillium sp. PF55	0 (0)	34 (5.66)	20.5 (0.71)	0 (0)	33 (4.24)	16 (1.41)
Penicillium sp. PF110	0 (0)	0 (0)	20 (0)	0 (0)	12 (2.83)	13 (4.24)
Penicillium sp. PF34	0 (0)	32.5 (4.95)	22 (0)	0 (0)	35 (4.24)	16 (2.83)

*Means of three replications and value of standard deviation in parentheses.

Table 2. Halo zone formation by mangrove phyllosphere fungi grown under different pH and temperature (mm)

Organism code	30 °C (pH)			37 °C (pH)		
	4.5	7.2	9	4.5	7.2	9
Aspergillus sp. RF4	26.5 (2.12)	32.5 (0.71)	21.5 (0.71)	18.5 (0.71)	31 (4.24)	24 (1.41)
Aspergillus sp. RF9	0 (0)	31.5 (6.36)	29 (0)	0 (0)	39 (0)	30.5 (3.54)
Cladobotrys sp. RF10	0 (0)	35 (2.83)	34 (0)	0 (0)	18 (2.83)	14 (0)
Fusarium sp. RF23	0 (0)	24 (1.41)	28.5 (2.12)	0 (0)	27.5 (3.54)	25.5 (0.71)
Fusarium sp. RF31	0 (0)	21.5 (2.12)	0 (0)	0 (0)	21.5 (0.71)	0 (0)
Fusarium sp. RF32	31 (1.41)	0 (0)	0 (0)	36 (0)	33 (0)	31.5 (2.12)
Fusarium sp. RF35	0 (0)	21.5 (2.12)	0 (0)	0 (0)	23.5 (0.71)	0 (0)
Fusarium sp. RF6	0 (0)	38 (0)	39 (0)	0 (0)	0 (0)	16.5 (0.71)
Fusarium sp. RF7	0 (0)	28.5 (3.54)	0 (0)	0 (0)	14.5 (4.95)	0 (0)
Fusarium sp. RF8	0 (0)	24 (1.41)	0 (0)	0 (0)	14 (0)	13 (0)
Helminthosporium sp. RF3	0 (0)	35 (0)	0 (0)	0 (0)	16 (0)	0 (0)
Paecilomyces sp. RF29	0 (0)	24.5 (0.71)	0 (0)	11.5 (0.71)	19.5 (2.12)	14 (1.41)
Paecilomyces sp. RF21	24 (1.41)	24.5 (0.71)	0 (0)	22 (0)	24.5 (0.71)	21 (0)
Penicillium sp. RF11	31.5 (0.71)	24 (2.83)	0 (0)	31 (1.41)	28 (0)	0 (0)
Penicillium sp. RF18	24 (4.24)	17.5 (0.71)	18 (1.41)	0 (0)	0 (0)	0 (0)
Penicillium sp. RF19	0 (0)	23.5 (0.71)	26 (0)	0 (0)	17.5 (2.12)	0 (0)
Penicillium sp. RF25	0 (0)	36 (0)	27 (0)	30 (0)	30 (4.24)	20.5 (4.95)
Penicillium sp. RF26	27.5 (2.12)	28.5 (2.12)	22.5 (3.54)	20.5 (2.12)	26 (1.41)	20.5 (0.71)
Penicillium sp. RF27	0 (0)	30 (2.83)	24.5 (2.12)	19 (1.41)	26.5 (0.71)	19 (1.41)
Penicillium sp. RF38	0 (0)	24.5 (3.54)	31 (1.41)	24 (1.41)	26.5 (2.12)	0 (0)
Penicillium sp. RF2	0 (0)	22.5 (2.12)	18 (1.41)	0 (0)	21 (1.41)	18 (1.41)
Penicillum sp. RF14	0 (0)	24 (2.83)	0 (0)	0 (0)	0 (0)	0 (0)

*Means of three replications and value of standard deviation in parentheses.

2005). This zone of mangrove plant was also discriminated with the leaf part due to the presence of more number of PS *Penicillia* and *Fusarium*. All rhizospheric *Penicilllia* did not prefer 4.5 pH at 30 °C for phosphate solubilization except *Penicillium* RF11 and RF26. Similarly, Fusarium sp. did not show preference either for 4.5 pH or 9.0 pH except *Fusarium* RF32 and *Fusarium* RF6. Overall, highest phosphate solubilization in the form of zone formation was exhibited by *Aspergillus* RF9 and *Fusarium* sp. RF6 i.e. 39 mm.

In conclusion, fungi obtained from phyllopshere of mangroves plants of Bhitarkanika were found to be best as compared to the rhizosphereic fungi. However, Aspergillus sp. exhibited best phosphate solubilization whatever their source may be (Turan *et al*. 2006). Poor occurrence of phosphate solubilizing fungi in the mangrove phyllosphere showed the host specific interaction where as highly populated rhizosphere with distinctive fungi indicates the nutritionally rich environment (Dave & Patel 2003; Wang *et al*. 2005).

This finding is very well corroborated with the several reports available on richness of mangrove microbial diversity due the support they extend through production of large quantities of vegetative matter (Ananda & Sridhar 2004). The present study extends preliminary but important observations towards the development of phosphatic biofertiliser required for saline and alkaline soils (Narsha & Patel 1997; Harris *et al*. 2006).

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