JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2009. Vol. **3**(2), p. 709-713

Phosphate Solubilizing Efficiency of Some Fungi Isolated from Soils of Aurangabad, M.S.

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(Received: 20 March 2009; accepted: 27 April 2009)

In the present study random sampling of agricultural soil was carried out. Total 94 fungal species were isolated and identified. All the fungal strains were screened for their phosphate solubilizing activity. 81 species showed distinct activity. 10 isolates with maximum activity were selected for further studies. These 10 isolates were identified as, *Aspergillius sp.* 1 & 2, *Cephalotrichum microsporum, Cladosporium cladosporioides, Cladosporium oxysporum, Curvularia, penniseti, Gliocladium catanulatum, one* unidentified species of *Gliocladium*, *Penicillium crysogenum* and *Penicillium oxalicum.*. The fungi could express a better P solubilizing activity in liquid medium rather than on solid medium.

Key words: Fungal sp., Agricultural soil, Phosphate.

Phosphorus (P) is an essential nutrient for plant growth and development. Despite its worldwide distribution in nature, P is deficient in most soils & its content is about 0.05% of which only 0.1% is available for plant. (G. Richa et al, 2007). Compared with other major nutrients, P is by far the least mobile & least available to plants in most soil conditions (I.EI-Azouni, 2008) Although P is abundant in soil in both organic and inorganic forms it is frequently the prime limiting factor for plant growth. P is added in the form of phosphoric fertilizers, part of which is utilized by plants and the remainder is converted into fixed forms. (Khan et al, 2007). To avoid P-deficiency phosphate-solubilizing micro-organisms (PSM) could play an important role in supplying phosphate to plants in a more eco-friendly manner.

Many soil micro-organisms are known to solubilize insoluble forms of inorganic phosphatic compounds. (Thomas 1985,) The PSM include different types of microorganisms that convert phosphatic compounds into soluble forms. In vitro studies with PSM from soil in earlier investigation have indicated that fungi (PSF) are

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more efficient in solubilization of inorganic phosphates as compared to bacteria (PSB). Efficient PSF could be used as inoculants to improve the utilization of phosphates by the crop plants from soil (Wahegaonkar & Bharaswadkar, 2006). Many investigators have observed that a high proportion of PSM are concentrated in the rhizosphere of plants. (Mohammed S. Khan et al 2007). The principal mechanism of mineral phosphate solubilization is the production of organic acids and acid phosphatases synthesized by soil micro organisms. Production of organic acids results in acidification of the microbial cell and its surroundings.

Many researchers have quantitatively investigated the ability of the PSM to solubilize insoluble P in pure liquid smedium culture. Scientists have also shown that microbial solubilization of soil phosphate in liquid culture medium was probably due to the organic acids like oxalic acid; citric acid, lactic acid etc have been determined by various chromatographic techniques (Maliha *et al* 2004, Mattey, 1992).

In India, it is estimated that about 260 million tons of phosphate rock deposits are available and this material can be used as cheap source of phosphate fertilizer for crop production. (Fai, 2002)

The present study was primarily undertaken to isolate highly phosphate solubilizing filamentous fungi and quantify their ability to use insoluble inorganic phosphates and develop them for their probable use as future biofertilizers.

MATERIAL AND METHODS

Isolation and identification of fungi:

Fungal strains were isolated from different soil samples collected from some agricultural fields around the city of Aurangabad, MS, India.

Serial dilution of soil solution on was carried out on Czapek - Dox solid medium. Distinct colonies were selected, purified and maintained on Czapek - Dox slants, at 4°C. The isolates were identified using illustrations given by Barnett (1998), M.B. Ellis, (1993). Gilman (2001) etc.

Isolations were also done by enrichment technique (Dubey, Maheshwari, 2002) in Pikovskaya's (PKVK) (Pikovskaya, 1948) medium for PSM.

Primary screening of isolates:

Preliminary screening of phosphate solubilization was dome by depth of clearing zone method evolved by us. Butts of PKVK agar medium were prepared by sterilizing and cooling the medium in test tubes. Each fungus was inoculated in the test tubes and incubated for 7 days at 28°C $\pm$ 2°C. Depth of clear zone in an otherwise opaque PKVK medium was measured as the phosphate solubilizing activity of the fungus.

Estimation of solubilization Efficiency:

Sterilized PKVK medium was poured into sterile Petri plates. After solidification of the medium, a pin point inoculation of fungal strains was made on the plates under aseptic conditions. The plates were incubated at 28°C $\pm$ 2 °C for 7 days. The colony diameter and the diameter of the halo zone formed around the growing colony showing phosphate solubilization was measured after 7 days. Solubilization efficiency (Nguyen, 1992) was calculated as:

$$SE = \frac{\text{Total diameter (colony + halo zone)}}{\text{s Colony diameter}} \times 100$$

Phosphate solubilizing activity in PKVK broth:

50 ml aliquots of PKVK broth (pH 7.0) in conical flasks were inoculated with equal amounts of inoculums of each fungal strain. The flasks were incubated on a shaker at 28°C $\pm$ 2°C for 7 days. The contents were filtered through pre dried & pre washed Whatman filter paper No 42. The mycelial mat was dried and weighed to measure the vegetative growth of the organism. The culture filtrate was used to measure the phosphate solubilizing activity by Barton's reagent method (Dubey, Maheshwari, 2002). The pH of the culture filtrate was noted using Hanna make (2003) pH meter.

RESULTS AND DISCUSSION

Isolation identification & screening of fungi: (PLATE 1)

Total 94 fungal species were isolated, identified and maintained of Czapek-Dox agar

slants. All the fungal strains were screened for their phosphate solubilizing activity by measuring the depth of clearing zones in PKVK solid medium. 81 species showed distinct zone of clearance. 10 isolates with maximum depth of clear zone were selected for further studies. These 10 isolates were identified as, *Aspergillius sp. 1 & 2, Cephalotrichum microsporum, Cladosporium cladosporioides, Cladosporium oxysporum, Curvularia, penniseti, Gliocladium catanulatum ,* one unidentified species of *Gliocladium*, *Penicillium crysogenum,*and *Penicillium oxalicum.*

Phosphate solubilization efficiency on solid PKVK medium: (Table: 1, PLATE 1)

The fungi under study showed vegetative growth between 0.7 and 4.8 cms. The diameter of clear zone was recorded between 1.5 cm (*Aspergillus* sp.1) and 9.2 cm (*Aspergillus* sp.2). Solubilization efficiency was high in both species of *Cladosporium* (485.7 & 314.28) & *Cephalotrichum microspoium* (320.0). Similar to the earlier reports (Aad Alla et al 2001) species of *Aspergillus* & *Penicillium* were fairly good in the solubilization efficiency.

Phosphate solubilization in PKVK liquid medium: (Table: 1)

The fungi were studied after 7 days incubation to determine the final change in pH of the growth medium and phosphate solubilizing activity.

A significant drop in the pH of the medium was observed in all fungal culture media. The drop was the maximum in *Aspergillus* sp. 2 (2.8). Similar results were observed by Iman M.EI-Azouni (2008). The change in pH in the other organisms ranged from 3.5 to 5.2 average drop being 4.21. Drop in the pH of the medium was due to the release of organic acids by the fungus (Maliha et al.,2002, Mattey, 1992) Presence of organic acids in the medium perhaps supports the enzymatic digestion of the insoluble phosphate in the medium.

The phosphate solubilizing activity of the fungal isolates when tested on PKVK liquid medium indicated that all the strains released high amounts of phosphates. (998 ug to 3065 ug P/ ml). Highest activity was expressed by the two species of *Gliocladium*, 3065 ug & 2582 ug respectively, followed by *Aspergillus & Penicillium* species.

When the activity in broth was correlated with the vegetative growth of the test organisms it was observed that the two species of *Gliocladium, Aspergillus* sp.2 and *Curvularia penniseti* were the most ideal organisms to be used as phosphate solubilizing organisms in broth cultures. The mycelial dry weight of these fungi was comparatively very less and the activity was very high.

Statistical analysis

Kar, Pearsons correlation coefficient was studied to test whether there is a linear dependence of mycelial dry weight on solubilization of P. One

Table 1. Phosphate solubilizing activity of selected fungal strains

S. No	Name of Fungi	Colony Diameter (cm)	Zone of Clearance on 5th day (cm)	Solubili-zation Efficiency	pH on 7th Day	P ug/ml on 7th Day	Dry Weight of Mycelium (mg)
1	*Curvularia pennseti*	2.2	6.2	281.81	5.2	1232.16	0.0233
2	*Cephaoitrichum microsporum*	1.0	3.2	320.0	4.5	1282.16	0.3066
3	*Aspergillus* sp 1	0.9	1.5	166.66	4.1	1632.16	0.0766
4	*Aspergillus.*sp 2	4.8	9.2	191.66	2.8	1432.16	0.2866
5	*Cladosporium cladsoporioides*	0.7	3.4	485.7	4.9	1498.83	0.2733
6	*Cladosporium oxysporum*	0.7	2.2	314.28	3.5	1065.5	0.2800
7	*Gliocladium catanulatum*	2.1	3.7	176.19	4.5	2582.16	0.210
8	*Gliocladium*	2.8	4.2	150.0	4.5	3065.5	0.040
9	*Penicillium crysogenum*	3.0	4.2	140.0	3.9	998.83	0.266
10	*Penicillium oxalicum*	1.5	4.1	273.33	4.2	1448.83	0.4633

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