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Solubilization of insoluble inorganic phosphate by soil inhabiting fungus *Penicillium citrinum* Thom

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ABSTRACT

Plant growth promoting fungus (PGPF) isolated from rhizosphere of sugarcane and identified as *Penicillium citrinum* Thom was tested for its phosphate solubilizing ability on four carbon sources *viz.* Glucose, Sucrose, Fructose and Mannitol, at varying range of pH in vitro condition. Among the carbon sources, in Glucose carbon source fungi was found to be solubilized maximum tricalcium phosphate (461µg/ml) at pH-8 while, minimum activity (421 µg/ml) as phosphate solubilization by the isolated fungal strain of *P. citrinum* Thom was found in Sucrose at pH-6.

Key Words: *Plant growth promoting fungus, Penicillium citrinum, Rhizosphere, Tricalcium phosphate*

INTRODUCTION

Plant growth promoting microorganisms have two major group that are plant growth promoting rhizobacteria (PGPR) and plant growth promoting fungi (PGPF). Both group of microorganism are equally important to enhance plant growth by means of mechanism of nutrition solubilization and their acquition to plants production of plant growth promoting substances and preventing the attack of pathogen. *Penicillium* sp. is also known as Plant Growth Promoting Fungi it has ability to solubilize fixed form of phosphorus and induced systematic resistance in plants (Rodriguez *et al* 1996).

Development of growth and activity of fungi is very much effected by source of carbon, nature and concentration of salt and pH of the soil (Johri *et al* 1999). Soils are often high in insoluble mineral and organic phosphates but deficient in available orthophosphate (Pi). Soil amendment with phosphatic fertilizer, produced via chemical processing of rock phosphate ore, is therefore an absolute requirement for crop production in order to feed the world's population. For over one hundered years, worker has recognized the ability of soil microorganism to solubilize Pi from insoluble (i.e. nutrionally unavailable) organic and mineral phosphates (Whitelaw 2000). Wide range of microbial biosolubilization mechanism exists, so that much of global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria & fungi. To increase the availability of phosphorus for plants, large amount of fertilizer are used on a regular basis. But after application of large proportion of fertilizer phosphorus is quickly transferred to the insoluble form. Therefore very little percentage of the applied phosphorus is used making continuous application necessary. It has been reported that many soil inhibiting fungi and bacteria can solubilize inorganic phosphates. Many soil fungi and bacteria are known to solubilize inorganic phosphates (Asea *et al* 1988, Illmer and Schinner 1992). Phosphate solubilizing microorganisms play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. Many

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bacterial, fungal, yeast and actinomycetes species capable of solubilizing sparingly soluble phosphorus in pure culture have been isolated and studied (Halder *et al* 1991, Abd-Alla 1994, Whitelaw 2000, Goldstein 1986). Application of PSMs in the field has been reported to increase crop yield. Species of Aspergillus and Penicillium are among fungal isolates identified to have phosphate solubilizing capabilities. Among the bacterial genera with this capability are Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Alcaligenes, 4 Serratia, Enterobacter, Acinetobacter, Flavobacterium and Erwinia (Rodriguez 1996).

Seed or soil inoculation with PSMs is known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yields (Jones *et al* 1999). PSMs are a low-cost solution that enriches the soil giving a thrust to economic development without disturbing ecological balance. Several mechanisms like lowering the pH by acid production, iron chelating and exchange reaction in growth environment have been reported to play a vital role in phosphate solubilization by PSMs, fungi perform better in acidic soil conditions. Alkaline soils rich in calcium phosphate complexes have a very strong buffering capacity (Ae *et al* 1990). Screening of phosphate solubilizing microbes using buffered media may lead to selection of more effective solubilizers (Gyaneshwar *et al* 1998). Many researchers have studied the effect of carbon sources of phosphate solubilization (Halder *et al* 1991, Narison *et al* 2000). Therefore the present investigation was under taken to find suitable carbon source, salt and pH for the solubilization of unavailable form of phosphorus in vitro condition by the fungus *P. citrinum* Thom.

MATERIALS AND METHODS

Isolation and identification of fungus: Rhizospheric soil was collected from healthy sugarcane plant raised at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, (India) from 15-25 cm depth from the rhizosphere soil of Sugarcane (*Colletotrichum folcatum)*. Pikovskayas agar medium (PKV) was used for the isolation, cultivation and maintenance of phosphate solubilizing bacteria (Gaur 1990). The fungal colonies producing distinct zones of TCP solubilization were raised into pure cultures, maintainted on Potato Dextrose Agar slants at 4ºC and identify on the basis of cultural and microscopic features followed by the method of Subramanian (Subramanian 1971, Barnett and Hunter 1972). The phosphate solubilizing fungus identified as *Penicillium citrinum* Thom. At Agarkhar Research Institute, Pune, Maharastra, on the basis of phenotypic characters was selected for further studies on ability to solubilize TCP at different carbon source at varying range of pH in vitro condition.

Media and Growth condition: Phosphorus solubilizing ability of fungal strain was tested in four different carbon sources on modified PVK broth with 0.5% TCP. Effect of different carbon source on phosphate solubilization was done with addition of 1% respective sugars like glucose, fructose, sucrose & mannitol. Further we examined the phosphate solubilizing ability of fungus at different pH 6, 7 & 8 on modified Pikovskaya’s broth with 0.5% TCP. Flasks were inoculated with 5% v/v, spore suspension and incubate on a orbital shaking incubator at 30 ºC for 7, 14 & 21 days.

Estimation of Phosphorus: Cultures were harvested after different growth periods in order to record to change in pH and concentration of Phosphorus released in the medium. After centrifugation at 10,000 rpm for 30 min. The pH of culture medium was measured with a pH meter equipped with a glass electrode.

Dissolved phosphate concentration in the culture filtrate was determined by Vanado-molybdate method as described in APHA 1995. It was expressed in terms of µg/ml of phosphorus in culture medium.

Table 1 Effect of solubilization of Phosphorus by the fungus *Penicillium citrinum* Thom in different carbon sources at pH-6 after 7, 14 and 21 days, respectively.

Week of Incubation							
pH-6							
		7 days		14 days		21 days	
Carbon Sources		**pH**	**P-solubilization** (µg/ml)	**pH**	**P-solubilization** (µg/ml)	**pH**	**P-solubilization** (µg/ml)
Glucose	R1	5.20	192.00	5.1	201.00	4.8	196.00
	R2	5.30	191.00	5.0	200.00	5.0	198.00
	R3	5.20	192.00	5.1	201.00	4.8	196.00
Mean		5.23	191.60	5.06	200.60	4.86	196.6
Sucrose	R1	5.42	188.00	5.40	191.00	5.3	421
	R2	5.44	187.00	5.39	192.00	5.0	418
	R3	5.45	188.00	5.40	192.00	5.2	420
Mean		5.43	187.66	5.39	191.66	5.16	419.6
Maltose	R1	6.3	310	5.9	378	5.1	438
	R2	6.2	309	5.7	376	5.0	437
	R3	6.3	310	5.7	376	5.0	437
Mean		6.26	309.6	5.76	376.6	5.03	437.3
Fructose	R1	6.1	321	5.7	388	5.0	451
	R2	6.2	322	5.7	388	5.4	455
	R3	6.2	322	5.6	387	5.5	456
Mean		6.16	321.6	5.66	387.6	5.3	454

Week of Incubation							
pH-7							
		7 days		14 days		21 days	
Carbon Sources		**pH**	**P-solubilization** (µg/ml)	**pH**	**P-solubilization** (µg/ml)	**pH**	**P-solubilization** (µg/ml)
Glucose	R1	5.9	324	5.1	394	4.8	461
	R2	5.8	323	5.2	395	4.7	460
	R3	5.9	324	5.3	396	4.7	460
Mean		5.86	323	5.2	395	4.73	460.3
Sucrose	R1	6.4	295	5.9	367	5.3	421
	R2	6.3	294	5.8	366	5.2	420
	R3	6.4	295	5.7	365	5.2	420
Mean		6.36	294.6	5.8	366	5.25	420.3
Maltose	R1	6.3	310	5.9	378	5.1	438
	R2	6.3	310	5.8	376	5.0	437
	R3	6.1	308	5.9	378	5.0	437
Mean		6.23	309.3	5.86	377.3	5.03	437.3
Fructose	R1	6.1	321	5.7	388	5.0	451
	R2	6.1	321	5.6	387	5.1	452
	R3	6.2	322	5.6	387	5.0	451
Mean		6.13	321.3	5.63	387.3	5.03	451.3

		Week of Incubation				
		pH-8				
	7 days		14 days		21 days	
Carbon Sources	pH	P-solubilization (µg/ml)	pH	P-solubilization (µg/ml)	pH	P-solubilization (µg/ml)
Glucose R1	7.2	324.00	6.81	394.00	6.50	461
R2	5.80	325.00	5.30	394.00	4.60	461
R3	5.80	323.00	5.10	393.00	4.80	461
Mean	5.83	324.00	5.20	393.60	4.76	461
Sucrose R1	6.4	295.00	5.90	367.00	5.3	421
R2	6.3	294.00	5.80	366.00	5.2	420
R3	6.3	295.00	5.90	366.00	5.3	421
Mean	6.3	294.60	5.86	366.33	5.3	421
Maltose R1	6.3	310	55.9	378	5.1	438
R2	6.2	310.00	6.0	380	5.1	438
R3	6.2	309.00	5.9	378	5.2	439
Mean	6.2	309.33	5.93	378.66	5.13	438.33
Fructose R1	6.1	321	5.7	388	5.0	451
R2	6.2	320	5.6	387	5.1	452
R3	6.3	323	5.8	389	5.0	451
Mean	6.2	321.33	5.7	388	5.03	451.33

RESULTS AND DISCUSSION

Microorganisms: Fungi isolated from the different soils of agriculture research farm, Institute of Agriculture Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh (India). Only this fungus *P. citrinum* Thom show significant zone of phosphate solubilization. A clear halo zone was formed around the colonies after 5 days of incubation on solidified PVK medium. Supplemented with tri-calcium phosphate, indicating phosphate solubilizing ability of the fungal isolate. It was selected for further studies. *P. citrinum* Thom. Shows greenish pigmentation with a rough surface.

Effect of different pH on solubilization of phosphorus with four different Carbon source: After confirming the phosphorus solubilizing ability on solid media, the phosphorus solubilization in liquid media was carried out in modified PVK broth using four different carbon source like Glucose, Fructose, Sucrose & Mannitol, at pH 6.0, 7.0 & 8.0 for 7, 14, & 21 days incubation to find out which media formulation was best for new isolated *P. citrinum* Thom. Species in three replicate. Modified PVK broth containing Glucose as a carbon source show maximum phosphorus solubilization at a rate of 324, 394, & 461 µg of P/ml at pH 8.0 of cultured filtrate with resulting final pH of 5.9, 5.1, & 4.8 respectively after incubation for 7, 14, & 21 days respectively. Low level of phosphorus solubilization was observed in modified PVK broth containing sucrose as a carbon source shows 295, 367, & 225 µg of P/ml after 7, 14 , & 21 days of incubation respectively. There are drop in pH if we used modified PVK broth 6.50, 5.90 & 5.70 after 7, 14 & 21 days of incubation respectively (Table 1). Based on the above result we conclude that if Glucose is used as a carbon source in PVK broth at pH 8.0 then phosphorus solubilization efficiencies shown by this new isolates is more than any other carbon source. So Glucose is a prime carbon source at pH 8 for our new isolates. Phosphate solubilization activity of *P. citrinum* Thom. species in the presence of three different in pH 6, 7, & 8 modified PVK broth at This strain demonstrated diverse level of phosphate solubilization activity in the presence of four different type of carbon source at different pH. Production of acid was greatly effected by the nature of pH of the media. (Table 2). The solubilizing ability of microorganism is related to its organic acid production; however, the nature of acid is also important (Vassileva *et al* 1998). Nahas 1996 and

Kucey *et al* (1988) showed that the solubilization of insoluble phosphate depends upon a multitude of factor including decrease in pH microorganisms and the insoluble phosphates used. Many studied have shown that the production of soluble phosphate is not necessarily correlated with study. Bardyia and Gaur (1974) suggested that the nature of organic acid produced is more important than the total acidity.

However, further study is needed to confirm the mechanism involved in phosphate solubilization by the fungal isolate in different carbon source as well as different pH. The results thus report the isolation of fungal sp. With the capability to solubilize different carbon source on modified PVK broth and with different pH in fact that the phosphate solubilization ability was enhanced maximally in the presence of glucose in PKV media at pH 8, the strain *P. citrinum* Thom. Can thus be of great benefit in the maintaining the available phosphates level for crops in saline alkaline soils. A large fraction of land arid and semiarid regions is affected by salinity in India along about 7.5 million of hectares of land are saline or alkaline. The fungal strain *Penicillium citrinum* Thom. Can thus utilize in land reclamation of the salinity regions along with boilogical nitrogen fixers.

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