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Isolation and Characterization of Phosphate Solubilizing Fungi from the Soil Sample of MUTHUPET Mangroves

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ABSTRACT

Phosphorus replenishment, particularly in small holder agriculture, remains a challenge as it is mainly fertilizer dependent. While the user of soluble mineral phosphate fertilizers is the obvious best means to combat phosphate deficiency in soil, they were limited by high cost of fertilizers and availability at farmer's level. The thesis entitled "Isolation and characterization of phosphate solubilizing fungi from the soil sample of Muthupet mangroves".Physicochemical characteristics of soils such as moisture, electrical conductivity, pH, organic carbon, salinity, available nitrogen, available phosphorous and available potassium. Dilution plating of soil on Rose Bengal agar medium resulted in the occurrence of 10 species of fungi belonged to three genera viz., *Aspergillus* sp.*, Verticillium* sp. and *Nigrospora* sp.Total soil fungal population density was in the range from (19 to 16 x 10^{-2} CFU/g) and (22 to 17 x 10^{-2} CFU/g) in mangroves soil.Out of 10 species two only of fungi *Aspergillus niger* and *A. flavus* are solubilize phosphate.Phosphate solubilizing fungi at species under different temperature, pH and salinity showed appreciable growth in temperature 28°C, pH range 4 – 8 and salinity 2 to 10 percentage. The results indicate positive effect of co-application of rock phosphate with phosphate solubilizing fungi on plant growth.

Keywords : Mangrove, Rose bengal agar medium, phosphate solubilisation fungi (PSF), *Aspergillus niger* and *A. flavus.*

I. INTRODUCTION

Mangroves are the unique forests, representing intermediate vegetation between land and Sea that grow in oxygen deficient water logged soils. To survive in such harsh conditions mangrove have evolved a number of physiological and structural adaptations like Vivipary, Pneumatophores, Prop roots, Salt secretion, Ultra filtration etc. All mangrove species have mechanism to provide air to their root system from the atmosphere. Hence they can tolerate anaerobic conditions to some extent. Mangroves perform more vital for sustenance of both man and animal. However human dependency on mangrove resources has claimed heavily on its area and function (Krishnamurthy, and Prince Jayaseelan, 1984). As mangroves occur mostly in tropical regions where majority of the world population reside they have been gradually cleared through the years to meet the needs of the burgeoning population of late conversion in to shrimp farms aquaculture is emerging as a major threat to the future mangroves also. The mangrove ecosystem is at serious threats owing to anthropogenic pressure (Mohamed, 1996) Fungi are one of the important microbial components of the soil. Since 1860's, research have been carried out on the fungi of different soil types, such as soils of forest, driftwood, grasslands (Roy and Dwivedi, 1962) polar region, desert, marine and mangrove habitats and coastal sand belt 24 from various parts of the world. All these

studies revealed that the fungi might reside permanently, temporarily for a period in the soil. Their number and species composition in the soil habitat differs from place to place depending upon the physical, chemical and biological factors of the particular habitat (Ainsworth *et al.,* 1973).

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. A greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and cannot be utilized by the plants. To increase the availability of phosphorus for plants, large amounts of fertilizer are being applied to soil. But a large proportion of fertilizer phosphorus after application is quickly transformed to the insoluble form (Omar, 1998). Therefore, very little percentage of the applied phosphorus is available to plants, making continuous application necessary (AbdAlla, 1994). However, phosphorus deficiencies are wide spread on soil throughout the world and phosphorus fertilizers represent major cost for agricultural production. Many soil fungi and bacteria are known to solubilize inorganic phosphates (Illmer and Schinner, 1992).

Phosphate solubilizing microorganisms (PSMs) play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers. Microorganisms are involved in a range of process that effect the transformation of soil phosphorus (P) and thus are integral component of the soil 'P' cycle. Many 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; Goldstein, 1986). Application of PSMs in the field has been reported to increase crop yield. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSMs (Goldstein, 1986). Among PSMs, fungi perform better in acidic soil conditions (Ahmad and Jha, 1968). Species of *Aspergillus, Penicillium* and yeast have been widely reported solubilizing various forms of inorganic phosphates (Whitelaw, 2000).

Approximately 95– 99% of soil phosphorous is present in the form of insoluble phosphates and cannot be utilized by the plants (Vassileva *et al.,* 2001). A greater portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized after application therefore; it becomes unavailable to plant Thus, the insoluble and fixed form of phosphorous is released in order to increase the soil phosphorous availability (Arpana *et al.,* 2002). Seed or soil inoculation with phosphate solubilizing bacteria is known to improve solubilization of fixed soil phosphorus and applied phosphates resulting in higher crop yields (Yadav, and Dadarwal, 1997).

Several soil fungi, particularly those belonging to the genera *Penicillium* and *Aspergillus* possess ability to bring insoluble soil phosphates into soluble forms by secreting weak organic acids such as formic, acetic, propionic, lactic, glucolic, fumaric and succinic. Therefore, very few amount of work has done in the similar kind of research, since the present work have been designed the following objectives such as to isolate and identified the phosphate solubilizing fungi. Then to determined the phosphate solubilizing efficiency of isolated fungi finally to established the effect of the pH and temperature on the phosphate solubilization efficiency of selected species.

II. METHODS AND MATERIAL

Collection of samples

The soil samples were collected from Manakattu and Sellimunai mangroves of Muthupet, Thiruvarur District, Tamil Nadu, India.

Analysis of physico –chemical characteristics of the soil

The physico chemical properties were analyzed with standard procedures by Jackson, (1973).Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkey and Black, (1934), available nitrogen was estimated by alkaline permanganate method as described by Subbiah and Asija, (1956) and available phosphorus by Brayl method as described by Bray and Kutz, (1945).

Available micronutrients such as Zn and Cu were determined in the diethylenetriaminepenta acetic extract of soil using Perkin- Elmer model 2280 Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978).

Isolation of phosphate solubilizing fungi

Phosphate solubilizing fungi (PSF) were isolated from each sample by serial dilution and spread plate method (Gaur, *et al.,* 1973). The collected soil samples were serially diluted using sterile water blanks and plated on Rose Bengal Agar Medium. The Rose Bengal agar medium with following composition was used (g/L): glucose, 10; Peptone, 2 ; KH_2PO_4,0.5 ; $MgSO_4$,0.5; Rose Bengal dye,a pinch; Agar,16; The pH was adjusted at 5 (2 g of agar was added in addition, for solid medium), and density of the medium was 1.001 g. All media and glassware used were sterilized in autoclave before use.The plates were incubated at 28^0C for 3-5 days. After incubation the phosphate solubilizing microorganisms were selected based on the zone of clearing around the colonies. The isolated phosphate solubilizing fungi were purified by repeated culturing and maintained on Potato Dextrose Agar slants at 4°C.

Identification of PSF

Lacto phenol cotton blue is a strain commonly used for making semi-permanent microscopic preparations of fungi. Place a drop of Lacto phenol cotton blue on a clean slide. Transfer a small tuft of the fungus with the sterilized inoculation needle with spores and spore bearing structurer in to the drop. Gentle tease the sample using the two mounted needles. Mix gently the strain with the mold structure place a cover glass over the preparation and taking care to avoid trapping air bubbler in the stain. The slides were observed under bright field microscope with oil immersion objective. Morphological features of fungi were photographed using Nikon microscope. All the fungi were identified with help of the standard manual of Gillman, 1957.

Optimization of media and growth conditions for phosphate solubilization

Phosphate solubilizing ability of fungal strains was tested in types of media. Flask were inoculated with 8% spore suspension and incubated on shake at 28°C for 6 days.

Growth in different temperature

The fungi were inoculated individually in the plates containing modified Rose Bengal Agar medium and grown under 15, 28 and 37°C, in an incubator, for period of a week. The growth and phosphate solubizing efficiency were estimated.

Growth in different pH

pH ranging from 4 to 8 at an interval of 1pH was altered in the Rose Bengal Agar medium by adding 1N HCl (to reduce the pH). The plates were incubated at room temperature (28 ± 2°C) for a period of 7 days. Their growth and solubilization zone was measured using mm scale. From this, solubilization efficiency was worked out as described earlier.

Growth in different salinity

Modified Rose Bengal agar medium with different salinity ranging from 2 to 10%. Intervals was prepared using addition of salt (NaCl). The fungi were inoculated individually in the plates containing different salinity medium incubated, their growth and

phosphate solubilizing efficiency were estimated after 7 days of incubation as described earlier.

III. RESULTS AND DISCUSSION

Phosphorus deficiencies are wide spread on soil throughout the world and one of the limiting factors for crop productivity. Phosphorus fertilizers represent major cost for agricultural production. Many bacteria, fungi and a few actinomycetes are potential solubilizers of bound phosphates in soil thus playing an important role making it available to plants in the soluble form (Perez, *et al.,* 2007; Fasim, *et al.,* 2002).

Physicochemical characterization of soil

Phosphorus is known to play an important physiological and biochemical role in crop plants. Therefore, it regulates the crop growth and yield to the greater extent. A recent estimate revealed that 49.3% of cultivated lands are deficient in available phosphorus. The added fertilizer phosphorus gets fixed in soil and it is not available to the crops. Therefore, primary approach in agronomic management of phosphorus is to scavenge the native fixed phosphorus and also to overcome the fixation of applied p-fertilizer.

The analysis of macronutrients and micronutrients from mangrove soil ,were reported (Table 1&2). Parameters such as moisture, temperature, pH, electrical conductivity (EC), organic carbon, organic matter, available nitrogen, available phosphates and available potassium were taken with phosphorous and available potassium was taken into consideration for the present investigation as these parameters are reported to influence the Fungal population (Swart, 1958; Ramanathan,1997). These parameters are responsible for population dynamics of microorganisms in the mangrove environment, which coincided with those in other mangrove along the east coast (Shanmukhappa and Neelakantan 1987; Rangaro,*et al.*, 1988). Muthepet mangroves exhibit the tropical characteristics features of the mangroves.

Table 1. Physicochemical properties of the soil sample

S. No	Sampling Places	Texture	Ph	Bulk Density (g/cm³)	Water holding capacity (%)	Electrical conductivity	Organic carbon	Available Nutrients (mg/g)		
								N	P	K
1	Manakattu	Sandy Clay	7.60	1.270	28	0.610	0.60	1.48	0.190	1.60
2	Sellimunai	Sandy	7.5	1.230	25	0.590	0.58	1.45	0.155	1.55

Table – 2

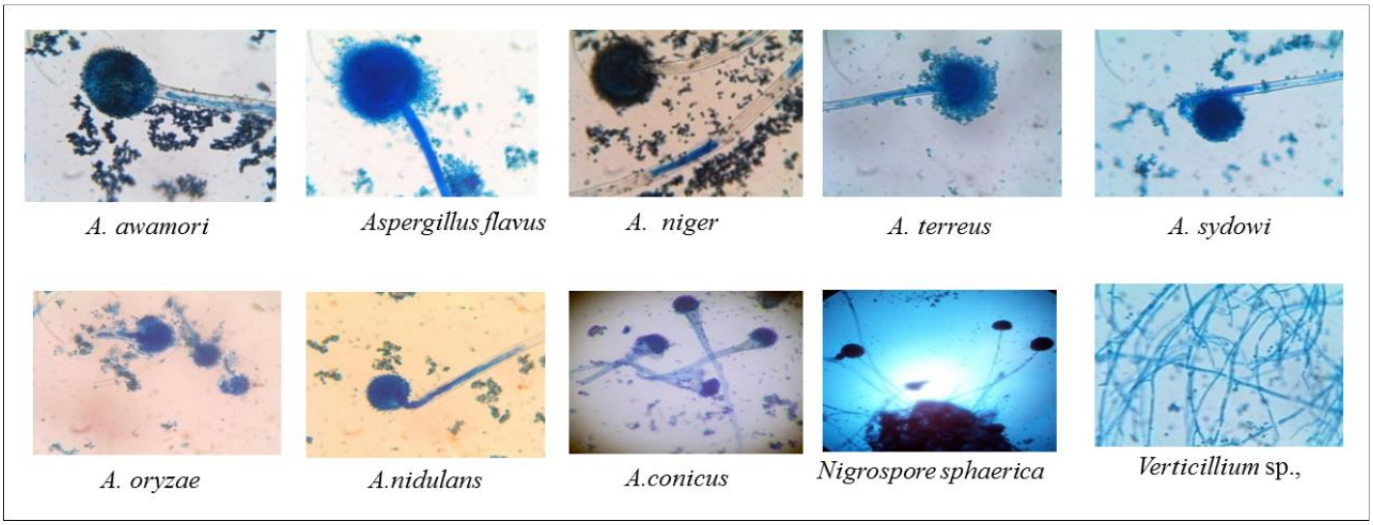
S.No	Sampling sites	Salinity Units	Macro nutrients (%)			Available Micro nutrients (%)				
			N	P	K	Zn	Cu	Fe	Mn	B
1	Manakattu	4.8	1.48	1.60	2.40	2.40	1.60	5.5	2.70	0.550
2	Sellimunai	5.0	1.45	0.185	1.55	2.55	1.50	5.4	2.45	0.510

Density of fungal growth in mangroves soil

The dilution plating of the soils collected from the mangroves showed the fungal population in the range from 19 to 16 CFU/ml isolated. The fungal strains were identified as *Aspergillus flavus, A. niger, A. terreus, A. sydowi, A. awamori, A. oryzae, A. nidulans, A. conicus, Nigrospora sphaerica* and *Verticillium* sp., sterile mycelium based upon their colony morphology, spore characteristics and microscopic studies. The isolated fungal species are belong to one division Deutromycetes (Fig.:1).

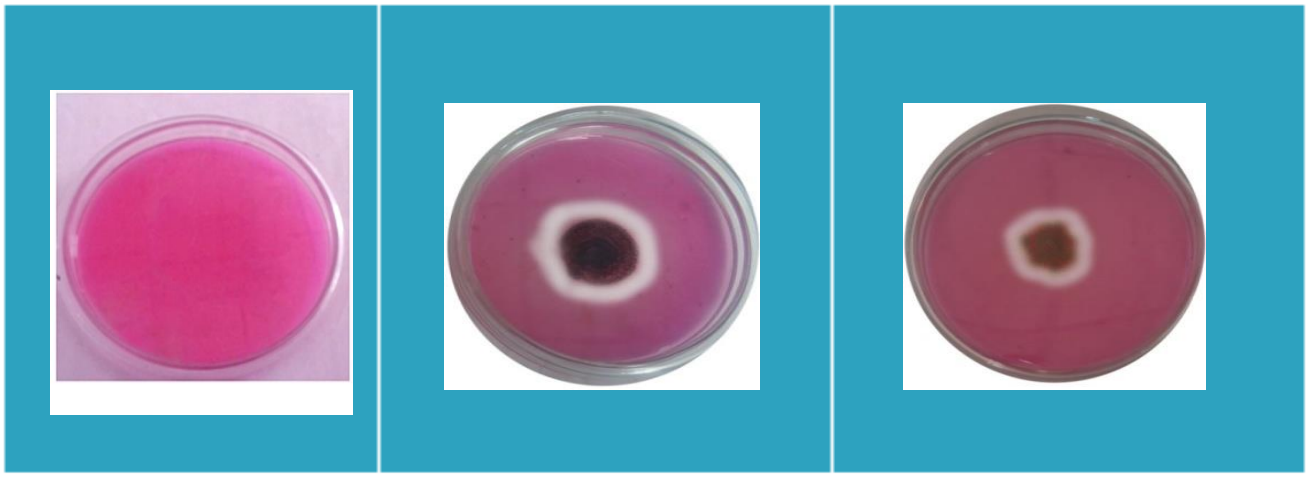
In the previous study, Identification of fungal isolates was done by observing colony characteristics on PDA plates. On the basis of growth pattern the isolates were identified as *Aspergillus* and *Penicillium* sp. This was confirmed by microscopic analysis of colony using lacto phenol blue stain. *Aspergillus* gave black dense felt like mycelial growth front side of PDA plate with dirty white color on back side (Sane and Mehta, 2015).

Fig -1. Microphotograph of isolated fungal species (400x)



Phosphate solubilizing assay by different isolates

Out of all fungi isolated from the soil only two fungi showed significant zone of phosphate solubilisation. A clear halo zone was formed around the colonies after 7 days of incubation on blue colored PVK medium incorporated with 5% senegal RP indicating phosphate-solubilizing ability of the fungal isolates. Maximum (2.1) was shown by *Aspergillus* followed by *A .flavus* (1.3) (Fig -1). This study is corroborated by Yu (2005), Silva and Vidor (2002) investigated the solubilization of rock phosphate in liquid culture by *Aspergillus niger* and *Penicillium oxalicum*. Several reports have mentioned the effect of carbon and nitrogen sources on phosphate solublization capacity and its enhancements.



Control *Aspergillus niger* *A.flavus*

Fig -2 Phosphate solubilization assay

Growth rate of phosphate solubilizing fungi under different pH

The two isolates were tested with various pH in 4, 5, 6, 7 and 8. Where as grow pH - 8 in only *A. niger* compare to others (Table-3).

Table-3 Growth rate of phosphate solubilizing fungi under different pH

S.No	Microorganisms	PH				
		4	5	6	7	8
1.	*Aspergillus niger*	+	++	++	+	+++
2.	*A.flavus*	-	-	+	+	++

(-) – No growth; (+) – Slight growth; (++) – Moderate growth; (+++) – High growth

Growth rate of phosphate solubilizing fungi under different temperature

Phosphate solubilization was also seen at 30°C and it was found that after 35°C there was growth retardation and fall in phosphate solublization. Different temperature have been reported by earlier workers for solublization, most of them have found 25°C to 28°C to be optimum temperature (Sayer and Gadd.,1998) In the present study two fungal isolates were seen at 28°C which is the optimum temperature for the growth and phosphate solublization. (Table-5)

Table-4 Growth rate of phosphate solubilizing fungi under different temperature

S.No	Microorganisms	Temperature (°C)	
		15	28
1.	*Aspergillus niger*	++	+++
2.	*A. flavus*	+	++

(-) – No growth; (+) – Slight growth; (++) – Moderate growth; (+++) – High growth

Growth rate of phosphate solubilizing fungi under different salinity

Slat (NaCl) at all the concentration (2, 4, 6, 8 and 10%) were found to influence all the isolates positively. *Aspergillus niger* are tolerate to grow at all the concentration of NaCl percentage (Table-5).

Table-5. Growth rate of phosphate solubilizing fungi under different salinity

S.No	Microorganisms	NaCl (%)				
		2	4	6	8	10
1.	*Aspergillus niger*	++	+	+++	+	++
2.	*A.flavus*	-	-	+	+++	++

(-) – No growth; (+) – Slight growth; (++) – Moderate growth; (+++) – High growth

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