JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2008. Vol. **2**(1), p. 219-222

A Study on Phosphate Solubilizing Microorganisms of an Orchard Ecosystem in Tamilnadu

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(Received: 18 February 2008; accepted: 12 April 2008)

The study showed the presence of five fungi such as *Aspergillus niger* strain 1, *A.niger* strain2, *A.niger* strain 3, *A.flavus and A.fumigatus* and six types of bacteria such as *Escherichia coli, Micrococcus spp, Erwinia spp, Alcaligens spp, Serratia spp* and *Citrobacter spp.* All the fungal and bacterial isolates were further tested for their efficiency in solubilizing phosphate (i.e tricalcium phosphate). The fungal isolate *A. niger* strain 1 and the bacterial isolate *Micrococcus spp* were found to be efficient in phosphate solubilization.

Keywords: Phosphate solubilizing microorganisms, fungi, bacteria, orchard ecosystem & tricalcium phosphate.

Phosphorus is one of the major nutrients, second only to nitrogen in requirements for plants. A greater part of soil phosphorus, approximately 95.99 percent is present in the form of insoluble phosphates and cannot be utilized by the plants (Vassieleva *et al.,* 1998). 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). Many soil fungi and bacteria are known to solubilize inorganic phosphates (Asea *et al.,* 1988; Illmar and Schinner, 1992; Singal *et al.,* 1994). Soil microorganisms play a significant role in mobilizing phosphate for plants (Halder *et al.,* 1991). Microbial solubilization of hardly soluble mineral phosphates in soil is an important process in natural ecosystems and in agricultural soils (Mikanova & Novakova, 2002). The present study was aimed to screen and characterize the phosphate solubilizing microorganisms from the decayed residues of on orchard located in the Gandhigram Rural University Campus, TamilNadu.

MATERIAL AND METHODS

Decayed orchard residue was collected from the university orchard, Gandhigram Rural University, Gandhigram, Tamil Nadu. The sample was serially diluted from 10^{-1} to 10^{-8} using sterile distilled water. Phosphate solubilizing bacteria and fungi were isolated from this residue using Pikovskaya's medium (Pikovskaya, 1948). The medium contained 10.0g glucose, 0.5g $(NH_4)_2$, SO_4, 0.1g $MgSO_4$. $7H_2O$, 0.5g yeast extract, 0.2g Kcl, 2.0mg Fe SO_4. H_2O, 5.0g Ca_3 $(PO_4)_5$ and 20g agar in 1000 ml distilled and adjusted to pH 7.0.

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Enumeration of Phosphate Solubilizing Bacteria (PSB)

0.1 ml of the sample from the decomposed orchard residue of 10^{-5} and 10^{-6} dilutions were plated on Pikovskaya (PVK) medium and incubated at 37°C for 10days. The predominant, morphologically distinct phosphate solubilizing bacteria (PSB) were isolated on the basis of the formation of clear zone around the colonies in the Pikovskaya medium contain tricalcium phosphate [Ca_3 $(PO_4)_5$].

The predominant PSB were selected, purified by repeated culturing and maintained on nutrient agar slants at 4°C. All these PSB were identified by their colony characteristics, gram's staining, motility study and various biochemical tests such as methyl red reaction, indole production, Vogas – proskaur test, citrate utilization, catalase test, oxidase test, nitrate reduction, H_2S production and casein hydrolysis.

Enumeration of Phosphate Solubilizing Fungi (PSF)

0.1 ml of the sample from the decayed orchard residue of 10^{-3} and 10^{-4} dilutions were plated on Pikovskaya (PVK) medium supplemented with streptomycin as inhibitor for the bacterial growth and incubated at room temperature (i.e., 28°C - 30°C) for 6 days. The predominant, morphologically distinct PSF colonies were isolated on the basis of the formation of clear zone around the colonies in the Pikovskaya medium with tricalcium phosphate [Ca_3 $(PO_4)_5$].

All the predominant PSB were selected, purified by repeated culturing and maintained on potatoes dextrose agar (PDA) slants at 4°C. All these PSF were identified by their colony characteristics, spore characteristics and microscopic observations.

Solubilizing activity of PSB and PSF on tricalcium phosphate

All the PSB and PSF were tested to solubilize insoluble phosphates (i.e tricalcium phosphate). About 0.5 percent (w/v) insoluble phosphate (i.e tricalcium phosphate) was added in Pikovskaya agar medium, sterilized and poured on petriplates. Six strains of PSB and five strains of PSF were inoculated and incubated at 37°C for 10 days for PSB and at room temperature (i.e. 28C°-30C°) for 6 days for PSF. At every 24 hours,

Table 1. Identification characteristics of bacterial isolates

Isolate No.	Colony morphology	Gram stain	Shape	Motility	Indole production	Methyl red reaction	Vogas praskaur reaction	Citrate utilization	Oxidase activity	Urease activity	Casein hydro-lysis	H_2S produ-ction	Identifi-cation result
1	White irregular	-	Rod	+	+	+	-	-		-	+	-	*E. coli*
2	Light yellow	+	Cocci	-	+	+	-	-		-	-	-	*Micrococcus spp.*
3	Rhizoid	-	Rod	+	+	-	+	-		-	+	-	*Erwinia spp.*
4	White irregular	-	Cocco-bacilli	-	+	-	-	-		-	-	-	*Alcaligens spp.*
5	Pink	-	Rod	+	-	+	+	+		-	+	-	*Serratia spp.*
6	White irregular	-	Rod	-	+	+	-	-		-	-	-	*Citrobacter spp.*

(+) Positive (-) Negative

the halo and colony diameter were measured and expressed as solubilization efficiency (SE) according to Nguyen *et.al.,* (1992).

where,

$$SE = \frac{\text{Solubilization diameter (S). 100}}{\text{Growth diameter}}$$

RESULTS AND DISCUSSION

Six predominant phosphate solubilizing bacteria (PSB) and five species of predominant Phosphate Solubilizing Fungi (PSF) were isolated from the decayed residue of an orchrd by plating on Pikovskaya medium amended with 0.5 percent (w/v) tricalcium phosphate on the basis of formation of the clear zone around the colonies. These bacterial isolates were identified as *Escherichia coli, Micrococcus spp., Erwinia spp., Alcaligens spp., Serratia spp.* and *Citrobacter spp.* (Table 1) and the fungal colonies were identified as *Aspergillus niger*-1, *A.niger* 2, *A.niger* 3, *A.flavus* and *A.fumigaatus* using colony characteristics, spore characteristics and microscopic observations (Table 2).

Several investigations had already been made by others on isolation of phosphate solubilizing bacteria and fungi from agricultural soil. Pradhan and Sukla (2000) isolated two fungal strains i.e., *Aspergillus spp.* and *Penicillium spp.* and found them to carry the potential to solubilize insoluble inorganic phosphate i.e tricalcium phosphate. Peix *et.al.,* (2003) isolated a novel bacteria that actively solubilized phosphate in *invitro* condition and it was identified as *Pseudomonas rhizosphaerae* sp. nov.

In the present study, phosphate solubilization activity of the isolated bacterial cultures were determined by observing the halo and colony diameter and expressed as solubilization efficiency (Fig.1& 2). Among the isolates, the bacteria Micrococcus spp. and the fungi, *A.niger* showed better solubilization efficiency with a percentage of 100 and 62.5 respectively in the Pikovskaya medium amended with tricalcium phosphate. However In Pikovskaya medium enriched with rock phosphate all the isolates showed growth but they did not produce any clearance zone. The bacterial strain *Micrococcus spp* and the fungal strain, *A.niger* 2 showed good growth with a growth diameter of 1.4 and 5.7 cm respectively.

Some of the related studies carried out by Chen *et. al.,* (2005) sharred that nearly six species of *Serratia* can solubilize tricalcium phosphate.

Pradhan and Sukla (2005) shared that *Aspergillus* spp *and Penicillium* spp can solubilize tricalcium phosphate both in the solid and liquid medium.

Table 2. Identification characteristics of fungal isolates

Fungal isolate	Macroscopic characters (Colony morphology)	Microscopic characters (Lacto phenol cotton blue staining features)	Name of the isolates
1.	Cottony colony with grayish black spores.	Septate hyphae with erect conidiophore bearing chain of spherical conidiospores.	*Aspergillus niger 1*
2.	Cottony colony with grayish black spores.	Septate hyphae with erect conidiophore bearing chain of spherical conidiospores.	*A. niger 2*
3.	Cottony colony with grayish black spores.	Septate hyphae with erect conidiophore conidiophore bearing chain of spherical conidiospores.	*A. niger 3*
4.	Granualar flat yellow at first becoming bright to dark yellow	Septate hyphae, hyaline, conidial heads are radiate	*A. flavus*
5.	Blue-green surface pigmentation with cottony mycelium	Conidiospores are short with conical shaped terminal vesicle.	*A. fumigatus*

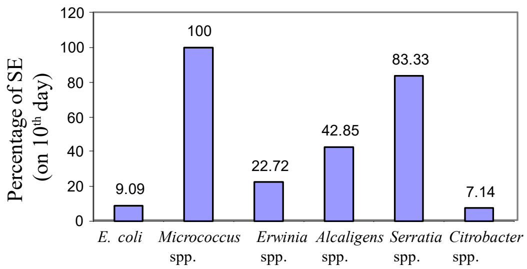

Fig. 1. Solubilizing efficiency (SE) of PSB in PVK medium with tricalcium phosphate

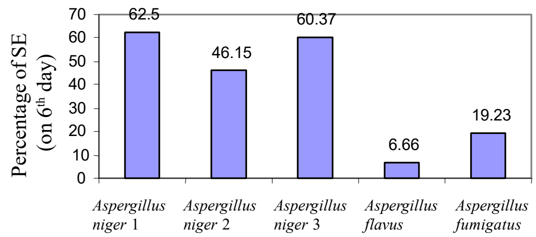

Fig. 2. Solubilizing efficiency (SE) of PSB in PVK medium with tricalcium phosphate (0.5%)

CONCLUSION

The results that all the six PSB (*E.coli, Micrococcus* spp*., Erwinia* spp*., Alcaligens* spp*., Serratia spp.* and *Citrobacter* spp.) and the five PSF (*A.niger* 1*, A.niger* 2*, A.niger* 3*, A.flavus* and *A. fumigatus*) carry the ability to solubilize insoluble phosphate (i.e. tricalcium phosphate). Among all the isolates, the bacteria, *Micrococcus spp*. and the fungi, *A. niger* 1 were found to be the efficient strains with higher percentage of solubilization efficiency (SE). Hence these strains could be used as phospho biofertilizer in agricultural fields to improve the availability of soluble phosphate for crop growth and productivity.

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