Tropical Ecology **57**(3): 465-474, 2016 ISSN 0564-3295

www.tropecol.com

Isolation, identification and characterization of phosphate solubilizing bacteria from different crop soils of Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu

N. TENZING BALIAH[1*], G. PANDIARAJAN[2] & B. MAKESH KUMAR[2]

[1]*Department of Plant Biology and Plant Biotechnology, Ayya Nadar Janaki Ammal College, Sivakasi 626 124*
[2]*Department of Plant Biology and Plant Biotechnology, G.Venkataswamy Naidu College, Kovilpatti 628 502*

Abstract: The use of phosphate solubilizing bacteria (PSB) as biofertilizers has concurrently increased phosphorous uptake in plants and improved yields in several crop species. A laboratory study was conducted to isolate, identify and characterize the phosphate solubilizing bacteria from different crop soils such as Okra, Chilli, tomato, Cotton and Egg plant. The population dynamic of PSB was higher in the rhizosphere soil of tomato followed by brinjal. Based on the solubilization zone production in the solid medium, two isolates from each crop plant were selected and used for studies. The selected PSB strains were screened *in vitro*. Among 10 PSB isolates, 6 strains were identified as *Bacillus megaterium*, 2 strains as *Pseudomonas putida* and CP2 and CTP2 as *P. fluorescence*. The selected strains differed in utilization of different carbon, nitrogen, amino acid and vitamin sources.

Key words: Characterization, crop soils, identification, isolation, phosphate solubilizing bacteria.

Handling Editor: Josef Gorres

Introduction

Phosphorus is one of the major plant nutrients required in optimum amount for proper plant growth. Phosphorus is known to involve many functions in the plant growth and metabolism. Several important cellular, metabolic and reproductive functions rely on sufficient phosphorus supply. Only about 25 per cent of the phosphorus applied to the soil is available for the crops and the rest become unavailable due to chemical fixation with aluminium and iron in acidic soils. Indian soils are characterized by poor and medium status with respect to available phosphorus (Baby 2002; Li *et al.* 2003; Ramanathan *et al.* 2004). Phosphorus ranks next to N in importance for living plants, however, in comparison with other nutrients; the concentration of phosphorus in the soil solution is generally low. Phosphorus in decomposing litter is subject to the same pattern of immobilization and uptake by micro-organisms as found for N (Bargali *et al.* 2015). In general, the application of phosphate solubilizing micro-organism greatly affects the biomass compared to control plants of *Dalbergia sissoo*. Seedlings exhibited maximum biomass production when

[*]*Corresponding Author*; e-mail: tens007@rediffmail.com

inoculated with *Penicillium chrysogenum* and *Aspergillus* sp. (Dash *et al.* 2013).

There are various types of soil microbes which can solubilize this fixed form of P and make it available to plants (Illmer *et al.* 1995; Kucey *et al.* 1989; Richardson 2001; Whitelaw *et al.* 1999). Such organisms are called phosphate solubilizers or Phosphate Solubilizing Microorganisms (PSMs). Phosphate solubilizing microorganisms include bacteria, fungi and actinomycetes. Several soil bacteria, particularly those belonging to the genera *Pseudomonas* and *Bacillus* possess the ability to convert the insoluble phosphate into soluble form by secreting organic acids resulting in improved phosphate availability to the plants (Fankem *et al.* 2006; Surange *et al.* 1997).

Most tropical soils are acidic, rich in iron and deficient insoluble forms of phosphorus (P), one of the essential elements in crop production (Baby 2002; Khan *et al.* 2010; Singh & Reddy 2011). In order to increase their fertility, chemical fertilizers containing soluble forms of P are applied on large scale. Yet, a great proportion of soluble P is rapidly precipitated into forms of low solubility, particularly Fe-P and Al-P complexes, which can be unavailable to plants (Gyaneshwar *et al.* 2002; Katznelson & Bose 1959; Parasanna *et al.* 2011). As an alternative strategy, phosphatic-bearing minerals, particularly Rock Phosphate (RP) are also used. RP, which usually contains some forms of the mineral apatite, can be applied directly to the soil with varying agronomic efficiencies depending on the type of soil and crop. The use of such a natural resource constitutes an economic, environmentally friendly, and efficient way of fertilizing crops in many tropical and subtropical countries (Kucey 1988; Yadav & Dadarwal 1997). Many soil microorganisms, particularly those colonizing the rhizosphere of plants, are able to mobilize insoluble inorganic phosphates from their mineral matrix to the soil solution and making them available to plant roots (Dave & Patel 2003; Dubey *et al.* 1997; Narayanasamy *et al.* 1981). Microbial inoculants are also found to be useful in enhancing growth of *Dalbergia sissoo* seedlings grown under stress conditions (Bisht *et al.* 2009).

Phosphate solubilizing microorganisms are found in all soils but their number varies with soil climate as well as history (Gupta *et al.* 1986). Calcium phosphate dissolving microorganisms were found in larger number than microorganisms that dissolve other mineral phosphate compounds (Banik & Dey 1983; Dharmwal *et al.* 1989). Pikovskaya (1948) made a pioneering attempt in isolating an organism capable of actively solubilizing tricalcium phosphate and coined the name "Bacterium P". More recently, Reyes *et al.* (1999) formulated the basal medium and used for the isolation and enumeration of phosphate solubilizing microorganisms. Soil samples collected from sugarcane growing belt of north Bihar indicated that the population level of PSB ranged from 27 - 112 × 10^3 per gram soil. This large variation in their distribution in different soils might be due to the differences in organic carbon content (Yadav & Singh 1991).

This study aims to investigate and characterize various species of phosphorous solubilizing microorganisms found in soils under different crops in Virudhunagar District, Tamil Nadu.

Methodology

Study area

Tamil Nadu is situated in the Southern end of India, east of Kerala and north of Andhra Pradesh and Karnataka. Several folds or parts of Western Ghats separate the states of Tamil Nadu and Kerala. The area of investigation, Srivilliputtur is located in the southern side of Virudhunagar District, this area is boundary of Theni and Madurai in North, Tirunelveli in South and Kerala in Southwest. The study area covers totally 25 acres. The soil of the study is black soil.

Collection of soil and root samples

Soil and root samples were randomly collected from economically important plants such as Okra (BDP), Chilli, Tomato (TP), Cotton (CTP), Eggplant (BP) from Srivilliputtur Taluk, Virudhunagar District. The rhizosphere soils were used for the isolation and enumeration of PSB. The soil samples were air dried and used for the isolation and enumeration of Phosphate Solubilizing Bacteria (PSB).

Isolation and enumeration of PSB

Isolation and enumeration of PSB was carried out following dilution plate technique using hydroxy apatite medium (Katznelson & Bose 1959; Narsian *et al.* 1994). For the isolation of PSB, the soil samples were serially diluted up to 10^{-6} dilution, plated on Petridishes and incubated at 35 ± 2 ºC for seven days. At the end of incubation, PSB colonies were visually identified from the clear

zone around the bacterial colony. The colonies were sub cultured, purified and maintained in nutrient agar slants.

PSB was enumerated by observation of halo/solubilization zone around bacterial colonies surrounded by a turbid white back ground after 3-5 days of incubation. (Calculate the population of PSB per gram of soil on dry weight basis by counting the solubilization zone around the colonies). The PSB cultures were maintained on nutrient agar slants for further studies.

Identification of PSB

The isolated bacterial strains were identified using standard biochemical tests as listed in the Bergey's Manual of Determinative Bacteriology (Krieg & Dobereiner 1984).

Utilization of carbon, nitrogen, amino acid and vitamin sources

The utilization of carbon, nitrogen, amino acid and vitamin sources by PSB isolates were estimated in LB broth. Filter sterilized carbon, nitrogen, amino acid and vitamin sources are inoculated aseptically into the sterile medium at 1 per cent level. The PSB cultures were inoculated at the rate of 1.0 ml and incubated at room temperature. The growth was observed by the turbidity of the broth read at 560 nm.

Measurement of solubilization zone

The PSB strains were inoculated in solid medium and incubated for 7 days at 35 ± 2 °C. After the incubation period, the diameters of the solubilization zone produced around the colonies were measured.

Change in pH of the medium

PSB strains were isolated from the solubilization zone production, also known as the halo and were grown in LB broth and inoculated 1 ml to Pikovskaya's broth. After incubation period the pH was measured at different period of growth.

Estimation of organic acid production

The organic acid produced by PSB strain was estimated in terms of total titrable acidity of the culture filtrate (Sperber 1958). PSB strains were inoculated in the Pikovaskaya's broth and allowed to grow for 7 days. After incubation period the culture was centrifuged and removed the cell biomass. Two milliliter of culture filtrate was taken in a flask and added few drops of phenolphthalein and titrated against 0.01 N of sodium hydroxide. The volume of alkali consumed by culture filtrate was the total titrable acidity of the culture filtrate. The total titrable acidity was expressed by ml of 0.01 N NaOH consumed.

Organic acid produced by PSB in the culture was estimated following the method of Sperber (1958). PSB strains were inoculated in the Pikovaskaya's broth and allowed to grow for 7 days and after incubation period the culture was centrifuged. Two ml of culture filtrate was taken in a flask and titrated against 0.01 N of sodium hydroxide. The total titrable acidity was expressed by ml of 0.01 N NaOH consumed.

Estimation of phosphatase activity

Phosphatase activity was estimated by method described by Eivazi & Tabatabai (1977). From this, centrifuged the culture broth at 10,000 rpm for 10 minutes and the pellet was suspended in 5 ml of sterile distilled water. 1 ml of the sample was taken in a 50 ml conical flask and added 0.25 ml of toluene, 4 ml of modified universal buffer, 1 ml of p-nitrophenyl phosphate solution and incubated at room temperature for 1 h. After incubation, add 1 ml of 0.5 M of calcium chloride and 4 ml of 0.5 M sodium hydroxide and filtered the content through a filter paper. The absorbance was measured at 660 nm. The phosphatase activity was expressed µ moles of PNP released ml^{-1} of filtrate h^{-1}.

Estimation of available P

The available phosphorus in the soil filtrate was estimated following the method of Olsen *et al.* (1954). Air dried soil (5 g) was extracted with extractant with a pinch of phosphate free activated charcoal and shake on a horizontal reciprocating shaker for 5 min and filtered through Whatman No.1 filter paper. One milliliter of culture/soil filtrate was pipetted out into a 25 ml standard flask and added 10 ml of distilled water and shake well. Then 5 ml of freshly prepared ascorbic acid and ammonium molybdate solution were added and made up to 25 ml. Absorbance was measured at 882 nm after half an hour. A standard curve was prepared using 0, 1, 2, 3, 4 and 5 ml of 5 ppm standard P solution.

Table 1. Code numbers and Population level of PSB in various crop plants.

Crop Plant/Sample	Population level ($\times 10^5$ g soil dry wt.)	Strains Code Number
Okra	1.06	BDP1
		BDP2
Chilli	1.04	CP1
		CP2
Tomato	6.38	TP1
		TP2
Cotton	0.12	CTP1
		CTP2
Egg plant	1.17	BP1
		BP2

Results

Isolation and population dynamics of PSB

The result indicated that the population level of PSB was higher in the rhizosphere soil of tomato. Based on the solubilization zone production, totally 10 PSB strains were selected and used for further studies (Table 1).

Based on the solubilization zone production in the solid medium, two isolates from each crop plant were selected and a total of 10 PSB strains were isolated. These 10 strains were used for further studies. The result indicated that the population level of PSB was higher in the rhizosphere soil collected from tomato and least in cotton (Table 1).

P solubilization, phosphatase activity and organic acid production by PSB

P solubilization by PSB was estimated by measuring the zone in the KNB solid medium (Plate 1). Under *in vitro*, the solubilization zone was maximum with CTP1 compared to other strains. All the PSB strains brought the pH down in the liquid culture medium. The maximum pH reduction was noticed in CTP2 with tricalcium phosphate (TCP) as phosphate source. The P solubilization potential of selected strains of PSB was tested *in vitro* by estimating available phosphorus in the culture medium. The results indicated a wide variation in the phosphate solubilization capacity of different strains in PSB. Among the 10 strains, CTP2 (46.0 ppm) released more phosphorus in the medium followed by TP1 (40.6) with TCP. The estimation of phosphatase activity indicated that the activity by PSB was highest with strain CTP2 and least with BP2. Meanwhile, the PSB strains TP1 (7.8, 0.1 N NaOH consumed) and TP2 (6.5, 0.1 N NaOH consumed) was good in organic acid production in the presence of TCP (Table 2).

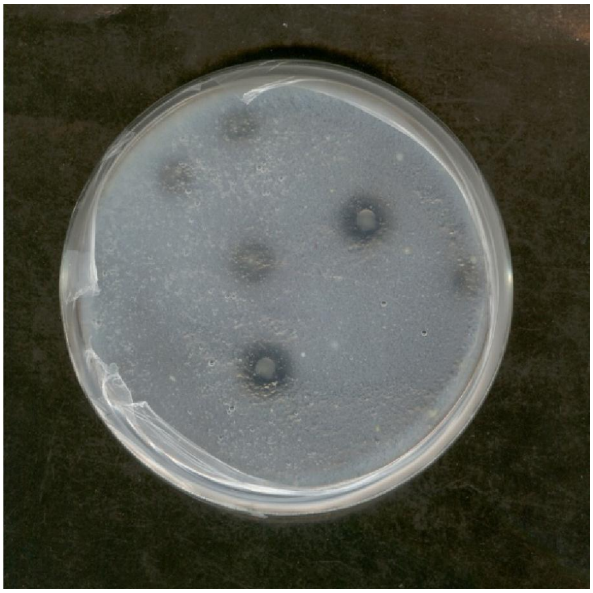

Plate 1: Phosphate solubilization by PSB.

Identification of PSB

Based on the biochemical tests, the PSB strains were identified up to species level. The results of various biochemical tests for ten PSB isolates were summarized in Table 3. Among ten PSB isolates, strains (i.e.) CP1, TP1, TP2, CTP1, BP1 and BP2 were identified as *Bacillus megaterium*, 2 strains as *Pseudomonas putida* and CP2, CTP2 as *Pseudomonas fluorescens*.

P. putida: Cells rod shaped, 0.5 - 0.8 × 1.0 - 4.0 µm in size. They were Gram negative, motile and their growth was strictly aerobic and spores were absent in all pseudomonads. *P. putida* were positive to oxidase, catalase, and arginine dihydrolase, acid from glucose and growth on citrate agar and negative to growth at 41 ºC, gelatin hydrolysis, starch hydrolysis and denitrification.

P. fluorescens: Cells rod shaped and 0.5 - 1.0 × 1.5 - 5.0 µm in size. They were Gram negative, motile and growth was strictly aerobic and not producing spores. On King's B medium they produced fluorescein, a water soluble pigment. Cells were positive to oxidase, catalase, lipase activity, arginine dihydrolase, gelatin hydrolysis, acid from glucose, urease and negative to growth at 41 ºC and starch hydrolysis.

Table 2. Characteristics of PSB strains. Significant differences are indicated by different letters.

PSB strains Code No.	Solubilization zone (mm)	pH* reduction	Available P (ppm)	Phosphatase activity (μ moles ml^{-1} hr^{-1})	Organic acid (0.1N NAOH consumed)
BDP1	3^{bc}	4.21^{b}	32.5^{e}	28.5^{e}	4.5^{fg}
BDP2	2^{d}	4.12^{c}	32.4^{e}	28.8^{de}	5.5^{d}
CP1	2^{d}	4.56^{a}	22.3^{g}	29.3^{cd}	4.0^{g}
CP2	2^{cd}	4.47^{a}	30.2^{f}	26.5^{f}	4.2^{fg}
TP1	3^{b}	4.04^{cd}	40.6^{b}	32.3^{b}	7.8^{a}
TP2	3^{b}	4.09^{c}	39.5^{c}	29.5^{c}	6.5^{c}
CTP1	6^{a}	4.10^{cd}	35.4^{d}	29.2^{c}	4.0^{g}
CTP2	2^{cd}	3.8^{d}	46.0^{a}	34.7^{a}	4.5^{b}
BP1	2^{d}	4.31^{b}	29.9^{f}	29.5^{c}	4.8^{ef}
BP2	2^{cd}	4.57^{a}	35.8^{d}	25.3^{g}	4.7^{e}
CD P = 0.05 %	0.985	0.173	1.140	0.370	0.277

***** Initial pH of the medium 7.0.

Table 3. Utilization of Carbon sources by PSB strains. (+) indicates poor growth, (++) indicates moderate growth, (+++) indicates better growth.

Strains	Carbon Sources				
	Glucose	Lactose	Maltose	Sucrose	Fructose
BDP1	0.148	0.455	1.113	0.842	0.481
	(+)	(+)	(+++)	(++)	(+)
BDP2	0.193	0.492	1.144	0.583	0.763
	(+)	(+)	(+++)	(++)	(++)
CP1	0.831	1.042	1.142	0.137	0.936
	(++)	(+++)	(+++)	(+)	(++)
CP2	0.688	0.956	0.834	0.964	0.861
	(++)	(++)	(++)	(++)	(++)
TP1	0.751	1.047	0.701	0.436	0.876
	(++)	(+++)	(++)	(+)	(++)
TP2	0.860	0.963	0.976	0.712	0.997
	(++)	(++)	(++)	(++)	(++)
CTP1	0.864	1.010	1.011	0.936	0.637
	(++)	(+++)	(+++)	(++)	(++)
CTP2	0.678	0.845	0.946	0.538	0.521
	(++)	(++)	(++)	(++)	(++)
BP1	0.948	0.792	0.976	0.437	1.137
	(++)	(++)	(++)	(+)	(+++)
BP2	0.876	0.731	1.122	0.689	1.063
	(++)	(++)	(+++)	(++)	(+++)

B. megaterium: Rod shaped, cells 0.5 - 1.5 × 1.5 - 6.0 μm in size. Growth was strictly aerobic and produced endospores. Cells were Gram positive and motile in nature. Growth in glucose agar was mucoid. They were positive to catalase, acid from glucose, casein hydrolysis, gelatin hydrolysis, starch hydrolysis, citrate utilization nitrate reduction and growth at pH 6 - 8 on nutrient broth and negative to anaerobic growth, gas from glucose, VP test, indole production and growth with lysozyme.

Table 4. Utilization of Amino acids sources by PSB strains. Here, (+) indicates poor growth, (++) indicates moderate growth, (+++) indicates better growth.

Strain	Amino Acid Sources				
	Tyrosine	Tryptophan	Valine	Methionine	Phenylalanine
BDP1	1.021	1.024	0.612	0.744	0.325
	(++)	(+)	(+)	(+)	(+)
BDP2	0.836	0.847	(0.421)	0.85	0.612
	(+)	(+)	(+)	(+)	(+)
CP1	1.124	0.954	0.423	0.752	0.478
	(++)	(+)	(+)	(+)	(+)
CP2	1.652	1.256	0.654	1.425	0.586
	(+++)	(++)	(+)	(++)	(+)
TP1	1.952	1.125	1.045	1.325	0.654
	(+++)	(++)	(+)	(++)	(+)
TP2	1.233	1.452	(0.652)	1.044	0.254
	(++)	(++)	(+)	(++)	(+)
CTP1	1.842	1.652	0.523	1.56	0.651
	(+++)	(+++)	(+)	(+++)	(+)
CTP2	1.452	1.256	0.541	1.542	0.521
	(++)	(++)	(+)	(+++)	(+)
BP1	1.852	1.325	0.898	1.325	0.754
	(+++)	(++)	(+)	(++)	(+)
BP2	1.654	1.235	1.01	1.253	0.568
	(+++)	(++)	(+)	(++)	(+)

Table 5. Utilization of Nitrogen sources by PSB strains. Here, (+) indicates poor growth, (++) indicates moderate growth, (+++) indicates best growth.

Strain	Nitrogen Sources				
	Ammonium Nitrate	Potassium Nitrate	Ammonium Chloride	Urea	Ammonium Sulphate
BD1	1.596	1.067	1.544	1.206	0.926
	(+++)	(++)	(+++)	(++)	(+)
BDP2	1.596	1.016	1.344	1.138	0.868
	(+++)	(++)	(++)	(++)	(+)
CP1	1.110	1.146	1.499	1.073	0.786
	(++)	(++)	(++)	(++)	(+)
CP2	0.984	1.212	1.513	1.098	0.746
	(+)	(++)	(+++)	(++)	(+)
TP1	1.256	0.956	1.496	1.231	0.868
	(++)	(+)	(++)	(++)	(+)
TP2	1.461	0.971	1.453	1.234	0.906
	(++)	(+)	(++)	(++)	(+)
CTP1	1.461	1.064	1.596	1.196	0.944
	(++)	(++)	(+++)	(++)	(+)
CTP2	1.111	0.938	1.579	1.176	0.976
	(++)	(+)	(+++)	(++)	(+)
BP1	0.914	0.716	1.366	1.178	0.878
	(+)	(+)	(++)	(++)	(+)
BP2	1.213	1.116	1.442	1.264	1.262
	(++)	(++)	(++)	(++)	(++)

Table 6. Utilization of Vitamin sources by PSB strains. Here, (+) indicates poor growth, (++) indicates moderate growth, (+++) indicates better growth.

Strain	Vitamin Sources			
	Nicotinic Acid	Thiamine	Pyridoxine	Myoinositol
BDP1	1.342 (++)	0.933 (+)	1.142 (++)	1.499 (++)
BDP2	1.354 (++)	0.838 (+)	1.069 (++)	1.469 (++)
CP1	1.453 (++)	0.898 (+)	0.914 (+)	1.456 (++)
CP2	1.554 (+++)	0.906 (+)	0.908 (++)	1.497 (++)
TP1	1.187 (++)	1.070 (++)	0.993 (++)	1.474 (++)
TP2	1.123 (++)	0.874 (+)	0.861 (+)	1.481 (++)
CTP1	1.489 (++)	0.792 (+)	0.806 (+)	1.498 (++)
CTP2	1.374 (++)	0.843 (+)	0.946 (+)	1.468 (++)
BP1	1.048 (++)	0.784 (+)	0.711 (+)	1.390 (++)
BP2	1.283 (++)	1.112 (++)	1.116 (++)	1.489 (++)

Biology of PSB

Utilization of carbon source

All PSB strains utilized for various carbon sources. The preferred carbon source varied from strain to strain. Most of the strains preferred maltose and lactose as carbon source, but fructose was moderately utilized while sucrose was found to be a poor source (Table 3).

Utilization of amino acids

All the amino acids supported the growth of PSB strains. Amino acids like tyrosine, tryptophan and methionine were the preferred sources by PSB compared to others (Table 4).

Utilization of nitrogen source

All the PSB strains were utilized for various nitrogen sources. The result revealed that the most of the strains preferred ammonium chloride, as nitrogen source, but ammonium nitrate, potassium nitrate and urea were moderately utilized while, Ammonium sulphate was found to be a poor source (Table 5).

Utilization of vitamin source

Utilization of vitamins by PSB varied from strain to strain. The result indicated that the most of the strains preferred nicotinic acid, as vitamin source, whereas pyridoxine and myoinositol were moderately utilized. Thiamin was found to be as a poor source (Table 6).

Discussion

Ten PSB strains were selected based on the P solubilization zone. Gaind (1987) reported that the PSB strains were isolated using the Pikovskaya's medium based on the formation of halo zone around these microorganisms. These findings were also supported by Ahamad & Jha (1967) that the phosphobacteria were identified by noting the solubilizing zone formed around the bacterial colony.

The present study revealed that the population level of PSB was varied in different rhizosphere soil. This is mainly due to the abiotic factors of the soils. This was supported by Kucey (1983) that the PSB have been found in almost all soils tested, although the number varies with soil, climatic and cropping history. This large variation in the distribution of PSB in different soils may be due to the differences in organic carbon content of the soil (Yadav & Singh 1991). The soil physical, chemical and biological properties were significantly changed after the flood. There was an increase of soil moisture content, pH, dehydrogenase activity and

C/N ratio. At the same time, soil microbial load and microbial diversity declined after flooding in different land use systems (Balasubramanian *et al*. 2015). Screening of PSB clearly indicated that there was wide variation the PSB strains in solubilization zone formation, pH change, P solubilization in the liquid medium, phosphatase activity and organic acid production. These characters were determined the efficient strains of PSB. The phosphate solubilizing microorganisms were preliminarily screened by measuring the radii of the clear zones around their colonies and further selection was based on the ability of strains of release P into the culture medium (Illmer & Schinner 1992). The clear or halo zone was formed due to the solubilization of insoluble phosphates by acidification of association of either proton extrusion or organic acid secretion (Darmwall *et al*. 1989). Bacteria, fungi and actinomycetes are active in solubilizing insoluble inorganic phosphate with high efficiency (Banik & Day 1983; Narsian *et al*. 1994; Narsian & Patel 1995; Kapoor *et al*. 1989; Sperber 1958). Among phosphate solubilizing microorganisms the most efficient ones belong to the genera *Pseudomonas* and *Bacillus* (Dave & Patel 1999). Frietas & Germide (1990) isolated the phosphate solubilizing microorganisms such as *Pseudomonas aeruginosa*, *P. cepacia, P. fluorescence* and *P. putida* from the rhizosphere of wheat and *Bacillus licheniformis, B. mycoides, B. megaterium* from the rhizosphere of rice (Watanabe & Hayano 1993).

The pH of the culture medium turned to acidic was indicated that production of organic acids by PSB, which facilitate the solubilization of phosphate (Sperber 1958; Gaur & Sachar 1980). The maximum decline in pH was recorded with *B. megaterium* from 6.0 to 4.2 and *Bacillus cerus* from 6.6 to 5.6. A fall in pH accompanied phosphate solubilization due to the production of organic acids, but no correlation could be established between acidic pH and quantity of P_2O_5 liberated (Dave & Patel 1999). Medium pH tended to decrease in all cases of growth, however, no constant relationship was found between amounts of P released and the drop in pH. The lack of relationship between pH drop and P solubilizied may be due to the liming effect of rock P and the production of other metabolites by the microbes (Kucey 1983).

Another important criterion for phosphate solubilization by PSB is the action of phosphatase enzyme. The liberation of phosphorus from organic phosphate compounds was mainly due to the action enzyme esterase type. PSM produced the phosphatase enzyme which solubilized the phosphate in the aquatic environment (Al-ghazalli *et al*. 1986). Organisms like *Bacillus subtilis, B. megaterium*, and *Pseudomonas aeruginosa* were produced large quantity of phosphatase in the culture media, which mineralized organic phosphate. Phosphatases were of the microbial origin, which played a significant role in the solubilization of phosphate. These enzymes were classified into acid and alkaline phosphatase (Eivazi & Tabatabai 1977; Gould *et al*. 1979). Acid phosphatase promoted the hydrolysis of the synthetic organophosphatic substrates like p-nitrophenyl phosphate (Pradel & Boquet 1988).

The PSB strains were identified upto species level. Gaur *et al*. (1973) studied the bacterial cultures morphological, cultural and physiological and biochemical characteristics using the manual of microbiological methods and identified the organism *Bacillus* sp., using Bergey's manual of Determinative Bacteriology.

Frietas & Germida (1990) isolated the phosphate solubilizing microorganisms such as *Pseudomonas aeruginosa*, *Pseudomonas cepacia, P. fluorescence* and *P. putida* from the rhizosphere of wheat and *Bacillus licheniformis, Bacillus-mycoides, B. megaterium* from rhizosphere of paddy (Watanabe & Hayano 1993).

PSB strains were utilized the carbon, nitrogen, amino acids and vitamin sources but preferential is varied from strain to strain. This will greatly affect the P solubilization by PSB. The form of available carbon sources greatly affected the growth as well as the phosphate solubilization and was more active in presence of hexoses and pentoses or dissacharides (Patil *et al*. 2001). The nitrogen sources supported the growth and P solubilization of *Pseudomonas fluorescence* (Dave & Patel 2003). Likewise, all the amino acids and vitamins supported the growth of PSB strains but the preference differed from strains to strains.

Conclusions

This study has revealed that, the phosphate solubilizing efficacy of the isolated species could be used to solublize higher amount of phosphates found in the soils of Southern Tamil Nadu, especially in the dry areas of Virudhunagar, Ramanathapuram, Sivagangai and Thoothukudi Districts and make it a fertile one.

References

Ahmad, N. & K. K. Jha. 1967. Solubilization of rock phosphate by microorganisms isolated from Bihar soils. *Journal of General and Applied Microbiology* **14:** 89-95.

Al-ghazalli, R., S. H. Mustafa, K. Mohammad & D. Algazawl. 1986. Some observations on "P" solubilization by aerobic microorganisms isolated from sediments of Al Khairriver Baghdad (Iraq). *Journal of Biosciences Research* **47:** 157-172.

Baby, U. I. 2002. Biofertilizers in tea. *Planters' Chronicle* **98**: 395-396.

Balasubramanian, D., K. Arunachalam & A. Arunachalam. 2015. Soil microbial and biochemical properties as affected by floods in different land use systems of Burachapori Wildlife Sanctuary, northeast India. *Tropical Ecology* **56**: 289-302.

Banik, S. & B. K. Dey. 1983. Phosphate - solubilizing potentiality of the microorganisms capable of utilizing aluminium phosphate as a sole phosphate source. *Central Microbiology* **138**: 17-23.

Bargali, S. S., Kiran Shukla, Lalji Singh, Lekha Ghosh & M. L. Lakhera. 2015. Leaf litter decomposition and nutrient dynamics in four tree species of dry deciduous forest. *Tropical Ecology* **56**: 191-200.

Bisht, R., S. Chuturvedi, R. Srivastava, A. K. Sharma & B. N. Johri. 2009. Effect of arbuscular mycorrhizal fungi, *Pseudomonas fluorescens* and *Rhizobium leguminosarum* on the growth and nutrient status of *Dalbergia sisoo* Roxb. *Tropical Ecology* **50**: 231-242.

Dash, S., A. K. Mohapatra & N. Gupta. 2013. Growth response of *Dalbergia sissoo* Roxb. to mineral solubilizing bacteria and fungi in nursery conditions. *Tropical Ecology* **54**: 109-115**.**

Dave, A. & H. H. Patel. 1999. Inorganic phosphate solubilizing soil *Pseudomonas*. *Indian Journal of Microbiology* **39**: 161-164.

Dave, A. & H. H. Patel. 2003. Impact of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas fluorescens*. *Indian Journal of Microbiology* **43**: 33-36.

Dharmwal, N. S., R. B. Singh & R. Rai. 1989. Isolation of phosphate solubilizers from different sources. *Current Science* **58**: 570-571.

Dubey, S. K., V. R. Balasundaram, L. M. Pant, N. Jayasheela, B. R. Kawale & B. Mishra. 1997. Effect of phosphate dissolving bacteria applied with rock phosphate on nodulation and yield of rain fed soybean under different agriculture climatic conditions. *Journal of Indian Society for Soil Science* **45**: 503-505.

Dubey, S. K., R. S. Sharma & S. K. Vishwakarma. 1997. Integrated nutrient management for sustainable productivity of important cropping system in Madhya Pradesh. *Indian Journal of Agronomy* **42**: 13-17.

Eivazi, F. & M. A. Tabatabai. 1977. Phosphatases in soils. *Soil Biology and Biochemistry* **9**: 167-172.

Fankem, H., D. Nwaga, A. Deubel, L. Dieng, W. Merbach & F. X. Etoa. 2006. Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. *African Journal of Biotechnology* **5**: 2450-2460.

Frietias, J. R. & J. J. Germide. 1990. PGPR for winter wheat. *Canadian Journal of Microbiology* **36**: 265-273.

Gaind, S. 1987. Studies on thermo tolerant phosphate solubilizing microorganisms. Ph.D. Thesis, P.G. School, IARI, New Delhi.

Gaur, A. C. & S. Sachar. 1980. Effect of rock phosphate and glyucose concentration on phosphate solubilization by *Asporgillus gwanori*. *Current Science* **49**: 533-554.

Gaur, A. C., M. Madan & K. P. Ostwal. 1973. Solubilization of P compounds by native micro flora of rock phosphate. *Indian Journal of Experimental Biology* **11:** 427- 429.

Gould, W. D., D. C. Coleman & A. J. Rubink. 1979. Effect of bacteria and amoebae on rhizosphere phosphatase activity. *Applied and Environmental Microbiology* **37:** 943-946.

Gupta, R. D., K. Bhardwaj, B. C. Marwah & B. R. Tripathi. 1986. Occurrence of phosphate dissol-ving bacteria in some soils of north - west Himalayas under varying bio - sequences and climo-sequence. *Journal of Indian Society for Soil Science* **34**: 498-504.

Gyaneshwar, P., G. Naresh Kumar, L. J. Parekh & P. S. Poole. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil* **245:** 83-93.

Illmer, P. & F. Schinner. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biology and Biochemistry* **24:** 389-395.

Illmer, P., A. Barbato & F. Schinner. 1995. Solubilization of hardly-soluble AlPO4 with P- solubilizing microorganisms. *Soil Biology and Biochemistry* **27:** 265-270.

Katzenelson, H. & B. Bose. 1959. Metabolic activity and phosphate dissolving ability of bacterial isolates from wheat roots rhizosphere and non-rhizosphere soil. *Canadian Journal of Microbiology* **5:** 79-85.

Kapoor, K. K., M. M. Mishra & K. Kukreja. 1989. Phosphate solubilization by soil microorganisms. *Indian Journal of Microbiology* **219**: 119-127.

Khan, M. S., A. Zaidi, M. Ahemad, M. Oves & P. A. Wani. 2010. Plant growth promotion by phosphate solubilizing fungi: Current perspective. *Archieves in Agronomy and Soil Science* **56:** 73-98.

Krieg, N. R. & J. Do¨bereiner. 1984. Genus *Azospirillum*. pp. 94-104. *In*: N. R. Krieg & J. G. Holt (eds.) *Bergey's Manual of Systematic Bacteriology*. Vol. 1. The Williams & Wilkins Co., Baltimore.

Kucey, R. M. N. 1983. Phosphate solubilizing bacteria and fungi in various cultivated and fungi in various cultivated and virgin Alberta soils. *Canadian Journal of Soil Science* **63:** 671-678.

Kucey, R. M. N. 1988. Effect of *Penicillium bilaji* on the solubility and uptake of P and micronutrient from soil and wheat. *Canadian Journal of Soil Science* **68**: 261-270.

Kucey, R. M. N., H. H. Jenzen & M. Leggett. 1989. Microbial mediated increases in plant available phosphorus. *Advances in Agron*omy **42:** 199-228.

Li, S. T., J. I. Zhou, H. Y. Uang, X. Q. Chen & C. W. Du. 2003. Characteristics of fixation and release of phosphorus in three soils. *Acta Pedologzca Sznica* **40**: 908-914.

Narayanasamy, G., S. K. Ghosh & M. C. Sarkar. 1981. Chemical and mineralogical composition of P-rock deposits occurring in India. *Fertilizer News* **26**: 3-9.

Narsian, V., J. Thakkar & H. H. Patel. 1994. Isolation and screening of phosphate solubilizing fungi. *Indian Journal of Microbiology* **34**: 113-118.

Narsian, V. & H. H. Patel. 1995. Inorganic phosphate solubilization by some yeast. *Indian Journal of Microbiology* **35**: 127-132.

Olsen, S. R., C. V. Cole, F. S. Watanabe & L. Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate U. S. D. A. Circular, U. S. Govt. Printing Office, Washington D.C.

Patil, M. G., R. S. Sayyed, A. B. Chaudhari & S. B. Chincholkar. 2001. Phosphate solubilizing microbes: A potential bio-inoculants for efficient use of phosphate fertilizers. pp. 107-117. *In*: S. M. Reddy, S. Ram Reddy, M. A. Singarachary & S. Girisham (eds.) *Proceeding of National Symposium Bioino culants for Sustainable Agriculture and Forestry*. Scientific Publishers (India), Jodhpur.

Pikovskaya, R. I. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiologiya* **17**: 362-370.

Pradel, E. & P. L. Boquet. 1988. Acid phosphatase of *Escherichia coli,* molecular cloning and analysis of *agp*, the structural gene for periplasmic acid glucose phosphatase. *Journal of Bacteriology* **170**: 4916-4923.

Parasanna, A., V. Deepa, P. Balakirashna Murthy, M. Deecaraman, R. Sridhar & P. Dhandapani, 2011. Insoluble phosphate solubilization by bacterial strains isolated form rice rhizosphere soils from southern India. *International Journal of Soil Science* **6:** 134-141.

Ramanathan, S., K. Natarajan & P. Stalin. 2004. Effect of foliar nutrition on grain yield of rice fallow black gram. *Madras Agriculture* **91:** 160-163.

Reyes, I., R. Brnir, S. Simard & H. Antoun. 1999. Characteristic of phosphate solubilisation by an isolate of a tropical *Penicillium regulusum* and UV-induced mutants. *FEMS Microbiology Ecology* **23**: 291-295.

Richardson, A. E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorous by plants. *Australian Journal of Plant Physiology* **28:** 897-906.

Singh, H. & M. S. Reddy. 2011. Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. *European Journal of Soil Biology* **47:** 30-34.

Sperber, J. I. 1958. Release of phosphate from soil minerals by hydrogen sulphate. *Nature* **181**: 934-936.

Surange, S., A. G. Wollum, N. Kumar & C. S. Nautiyal. 1997. Characterizations of *Rhizobium* from the root nodules of leguminous trees growing in alkaline soils. *Canadian Journal of Microbiology* **43**: 891-894.

Watanabe, K. & K. Hayano. 1993. Distribution and identification of proteolytic bacteria *Bacillus* sp. in paddy field soil under rice cultivation. *Canadian Journal of Microbiology* **39**: 674-680.

Whitelaw, M. A., T. J. Harden & K. R. Helyar. 1999. Phosphate solubilisation in solution culture by the soil fungus *Penicillium radicum*. *Soil Biology and Biochemistry* **31:** 655-656.

Yadav, K. & T. Singh. 1991. Phosphorus solubilization by microbial isolate from Caci fluvent. *Journal of Indian Society for Sciences* **39**: 89-93.

Yadav, K. S. & K. R. Dadarwal. 1997. Phosphate solubilization and mobilization through soil microorganisms. pp. 293-308. *In*: K. R. Dadarwal (ed.) *Biotechnological Approa-ches in Soil Microorganisms for Sustainable Crop Production*. Scientific Publications, Jodhpur.

(Received on 06.10.2012 and accepted after revisions, on 28.10.2014)