INTERNATIONAL JOURNAL OF SCIENCE AND NATURE
© 2004 - 2016 Society For Science and Nature(SFSN). All Rights Reserved
www.scienceandnature.org

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF PHOSPHATE SOLUBILIZING BACTERIA ISOLATED FROM ECONOMICALLY IMPORTANT TREE SPECIES

*Tensingh Baliah, N. & Jeeva, P.
Post Graduate Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, Tamilnadu, India.
*Corresponding author email: tens007@rediffmail.com

ABSTRACT
The present investigation is aimed at to isolation, identification and characterization of phosphate solubilizing bacteria from the economically important tree species such as lemon, jackfruit and mango. The population level of PSB was higher in the rhizosphere soils collected from lemon followed by jack fruit. Two isolates from each tree species, totally 6 PSB strains were isolated and used for further studies. The isolated strains such as JP1, JP2, LP1, MP1 and MP2 were identified as *Bacillus megaterium* and remain LP2 as *Pseudomonas putida*. The strains were characterized under *in vitro* by measuring the P solubilization zone in solid medium, determining pH change of the medium and estimating the phosphatase activity, organic acids production and available phosphorus. Among six strains, MP1 was found to be superior in forming halo zone formation in the solid medium followed by LP1; the maximum pH reduction noticed in MP1 followed by MP2; JP1 and JP2 good in organic acid production and JP1 able to produce more phosphatase followed by JP2. The isolated PSB strains were differed with respect to utilization of various carbon, nitrogen, aminoacid and vitamin sources.

KEY WORD: Isolation, identification, characterization, PSB, tree species.

INTRODUCTION

The world agriculture is dependent on chemical fertilizers as source plant nutrients to meet the increasing demand for food. The recent years, the environmentalists and agricultural scientists have realized that continued and unabated use of chemical fertilizers destroy the soil fertility, cause environmental pollution and imbalance in the soil microbial activity. Thus, increasing awareness is being created on the use of organic fertilizers including biofertilizers to sustain the soil fertility and plant productivity.

Biofertilizers offer a new technology to Indian agriculture holding a promise to balance many of the shortcomings of the conventional chemical based technology. It is a product that is likely to be commercially promising in the long run once information becomes available adequately to producers and farmers through experience and communication (Baby, 2004). Biofertilizers are carrier based preparations containing beneficial microorganisms in a viable state and improves the soil fertility and help plant growth. The commonly available biofertilizers are biological nitrogen fixers and phosphate solubilizers. They are environmental friendly, low cost agricultural input playing a significant role in improving nutrient availability to the crop plants. They make nutrients that are naturally abundant in soil or atmosphere usable for plants. The use of biofertilizers in combination with chemical fertilizers and organic manure offers a great opportunity to increase the crop productivity with less cost (Baby *et al*., 2006).

Phosphorus is the most important nutrient for plants and microorganisms. Phosphorus is known to involve in a functions in the plant growth and metabolism. The cellular machinery is difficult to be imaged without phosphorus being involved in its metabolic continuity and even perpetuation. Only about 25% of the phosphorus applied to the soils is available for the crops in the year of its application and remaining part is converted into insoluble unavailable forms. The soil is rich in phosphorus as it contains about 0.05 percent phosphorus but only one tenth of this is available to plants due to its poor solubility and chemical fixation in soil by aluminium, iron, magnesium *etc* (Wang *et al.*, 2009).

Microbial solubilization of inorganic phosphatic compounds is of great economic importance in plant nutrition. Phosphate solubilizing bacteria (PSB) play an important role in converting low grade insoluble inorganic phosphate sources like rock phosphate, bone meal, basic slag and the chemically fixed soil phosphorus into available form. Therefore, the use of phosphate solubilizing microbes in agricultural practice would not only offset the high cost of manufacturing phosphatic fertilizers but would also mobilize insoluble phosphorus in the fertilizers and soils to which they are applied. The mechanism of solubilization of insoluble phosphate is ability to secrete organic acids and phosphatase enzyme (Vessey, 2003; Mahantesh *et al.*, 2015). Apart from the solubilization of fixed form of P, PSB can also able to produce the plant growth promoting hormones like auxins, gibberellins and cytokinins. These hormones induce plant growth as well as dry matter production. Inoculation with PSB resulted in better growth and higher dry matter production in maize and is mainly attributed to solubilization of phosphate and the production of plant growth hormones.

MATERIALS & METHODS

Collection of soil samples

Soil and root samples were collected from economically important tree species such as jackfruit, lemon and mango from Thaniparai, near Western Ghats, Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu, India. The soil samples were air dried under shade and used for the isolation and enumeration of PSB.

Isolation and enumeration of PSB

Isolation and enumeration of PSB was carried out following dilution plate technique using hydroxy apatite medium. For the isolation PSB, the soil samples were serially diluted up to 10^{-6} dilution and plated on petriplates and incubated at 35±2ºC for seven days. At the end of incubation period, PSB colonies were visually identified from the clear halo zone around the bacterial colonies. The colonies were sub cultured, purified and maintained in nutrient agar slants.

Identification of PSB

The isolated bacterial strains were identified up to genius level using standard biochemical tests as listed in the Bergey's Manual of Determinative Bacteriology (Krieg and Dobereiner, 1984).

Characterization of PSB

The isolated PSB strains were screened *in vitro* by measuring the P solubilization zone in solid medium, determining pH change of the medium and estimating the phosphatase activity, organic acids production and available phosphorus.

Measurement of halo zone

The PSB strains were inoculated in solid medium and incubated for 7 days. After incubation period, the diameter of the halo zone produced around the colonies was measured.

Change in pH of the medium

Selected PSB strains were grown in LB broth and inoculated 1ml to Pikovskaya's broth. After incubation period the pH was measured.

Organic acid production

The organic acid produced by PSB strain was estimated in terms of total titrable acidity of the culture filtrate (Sperber, 1958). 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.

Phosphatase activity

The PSB isolates were grown in Pikovaskaya's broth where TCP was replaced with organic source (p-glycerophosphate) (Eivazi and Tabatabai, 1977). The colour intensity was measured at 660 nm and calculated the p-nitrophenol content by reference to a calibration graph plotted from the results obtained with standard p-nitrophenol. The phosphatase activity was expressed µ moles of PNP released/ml of filtrate/hour.

Available Phosphorus

The available phosphorus in the culture filtrate was estimated following the method of Olsen *et al.* (1954). A standard curve was prepared using standard P solution for quantify the available phosphorus.

Utilization of carbon, nitrogen, aminoacid and vitamin sources

The utilization of different carbon, nitrogen, aminoacid and vitamin sources by PSB isolates were estimated in LB broth. Filter sterilized sources were inoculated aseptically into the sterile medium at 1 % 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.

RESULTS

Population dynamics of PSB

Soil samples were air dried and subjected to the isolation of PSB using hydroxy apatite medium. The result showed that the population level of PSB was higher in the soil collected from lemon followed by jack fruit. Based on the solubilization zone formed around the bacterial colonies in the hydroxy apatite solid medium, two isolates from each plant were selected and totally 6 PSB strains were isolated. These 6 strains were regularly sub cultured and used for further studies (Table 1).

TABLE 1: Population level of PSB in crop plants

S. No.	Crop Plants	Population level ($\times 10^5$ /g soil dry weight)	Code No.
1	Jack fruit	2.02	JP1 JP2
2	Lemon	2.42	LP1 LP2
3	Mango	1.35	MP1 MP2

Identification of PSB strains

Based on the biochemical and morphological tests, PSB were identified up to species level. Among six PSB isolates, five strains (JP1, JP2, LP1, MP1 and MP2) were identified as *Bacillus megaterium* and remain LP2 as *Pseudomonas putida* (Table 2). *Bacillus megaterium* cells were Gram positive and motile in nature. Growth in glucose agar was mucoid. They were positive to catalase, acid from glucose, hydrolysis of casein, gelatin and starch hydrolysis, citrate utilization and nitrate reduction. *B. megaterium* were negative to anaerobic growth, gas from glucose, VP test and indole production.

Pseudomonas putida were Gram negative and motile in nature. The bacterial cell growth was strictly aerobic. *P. putida* were positive to oxidase, catalase, arginine dehydrolase, acid from glucose and growth on citrate agar medium. Further, they were negative to growth at 41ºC, gelatin hydrolysis, starch hydrolysis and denitrification.

TABLE 2: Identification of PSB

S. No.	PSB Strains	Identified Phosphate Solubilizing Bacteria
1	JP1	*Bacillus megaterium*
2	JP2	*Bacillus megaterium*
3	LP1	*Bacillus megaterium*
4	LP2	*Pseudomonas pudita*
5	MP1	*Bacillus megaterium*
6	MP2	*Bacillus megaterium*

Characterization of PSB

Phosphate solubilization zone was estimated by measuring solubilized zone around the bacteria. Among these six strains, MP1 was found to be superior in forming halo zone followed by LP1**.** All the PSB strains brought down the pH in the liquid medium and the maximum pH reduction was noticed in MP1 followed by MP2 with tricalcium phosphate (TCP) as phosphate source. Among 6 PSB strains, the strains JP1 (6.26 0.1 N NaOH consumed) and JP2 (5.31 0.1N NaOH consumed) were good in organic acid production in the presence of TCP as phosphate source. The result further revealed that the strain JP1 was able to produce more phosphatase followed by JP2. There was a wide variation in the phosphate solubilization capacity of different strains in PSB. Among six PSB strains, JP2 (35.08 ppm) released more phosphorus in the medium followed by LP1 (34.76 ppm) with TCP (Table 3).

TABLE 3: Characterization of PSB

S. No.	PSB Strains	Solubilization zone (mm)	pH reduction	Organic acid (0.1NaOH consumed)	Phosphatase activity (µ mole/ml/hr)	Available P (ppm)
1	JP1	3	4.10	6.26	26.8	34.04
2	JP2	2	4.28	5.31	24.3	35.08
3	LP1	4	4.35	4.39	21.2	34.76
4	LP2	3	4.21	4.55	19.9	33.72
5	MP1	5	4.56	4.69	20.7	31.60
6	MP2	3	4.39	4.53	21.8	31.48

TABLE 4: Utilization of carbon source by PSB strains

S. No.	PSB Strain	Carbon source				
		Glucose	Lactose	Maltose	Sucrose	Fructose
1	JP1	+++ (1.456)	++ (0.859)	++ (1.095)	++ (0.875)	+++ (1.134)
2	JP2	+++ (1.521)	++ (0.954)	+++ (1.178)	++ (0.945)	+++ (1.276)
3	LP1	++ (1.024)	++ (1.075)	++ (1.065)	++ (0.926)	+++ (1.167)
4	LP2	+++ (1.542)	++ (0.683)	++ (0.924)	++ (1.032)	+++ (1.255)
5	MP1	+++ (1.482)	++ (1.028)	++ (1.031)	++ (1.033)	+++ (1.239)
6	MP2	+++ (1.381)	+++ (1.153)	++ (1.263)	++ (1.021)	++ (1.173)

Values in parentheses indicate OD value

(+)-0.0-0.5 (Poor Growth); (++) - 0.51-1.0 (Moderate Growth); (+++) -1.1-1.5 (Best Growth)

TABLE 5: Utilization of nitrogen source by PSB strains

S. No.	PSB Strain	Nitrogen source				
		Ammonium nitrate	Potassium nitrate	Ammonium chloride	Urea	Ammonium sulphate
1	JP1	+++ (1.173)	++ (0.958)	++ (0.623)	++ (1.098)	++ (1.025)
2	JP2	+++ (1.169)	++ (1.013)	++ (0.958)	+++ (1.120)	++ (0.932)
3	LP1	+++ (1.174)	++ (0.531)	+++ (1.256)	++ (0.732)	++ (1.052)
4	LP2	++ (1.086)	++ (0.932)	+ (0.432)	++ (0.925)	++ (1.077)
5	MP1	++ (0.953)	++ (1.032)	++ (0.757)	++ (0.532)	++ (0.950)
6	MP2	++ (0.965)	+++ (1.141)	++ (1.032)	++ (0.832)	+++ (1.060)

Utilization of nitrogen source

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

Utilization of Aminoacid source

All the PSB strains were utilized various aminoacids source, but the utilization amino acid by PSB strains varied with strain to strain. All the PSB strains were moderately utilized the leucine. The result also indicated that the strains LP1 and LP2 were poorly utilized the cytosine (Table 6).

Utilization of vitamin source

Utilization of vitamins by PSB strains varied with strain to strain. The result indicated that the most of the strains preferred ascorbic acid, myoinosital as vitamin source. Thiamine and biotin were moderately utilized while nicotinic acid was found to be poor source (Table 7).

TABLE 6: Utilization of aminoacid source by PSB strains

S. No.	PSB Strain	Vitamin source				
		Nicotinic acid	Thiamine	Ascorbic acid	Myoinositol	Biotin
1	JP1	++	+	+++	+++	++
		(0.890)	(0.140)	(1.352)	(1.260)	(0.953)
2	JP2	++	++	+++	++	++
		(0.886)	(0.954)	(1.198)	(1.042)	(1.056)
3	LP1	++	++	++	+++	++
		(0.902)	(1.045)	(1.042)	(1.259)	(0.518)
4	LP2	++	++	+++	++	++
		(0.860)	(1.065)	(1.133)	(1.065)	(0.852)
5	MP1	++	++	+++	+++	+++
		(0.954)	(1.029)	(1.282)	(1.349)	(1.185)
6	MP2	++	+++	+++	+++	+++
		(0.834)	(1.257)	(1.274)	(1.304)	(0.964)

TABLE 7: Utilization of vitamin source by PSB strains

S. No.	PSB Strain	Aminoacids				
		Leucine	Threionine	Isoleucine	Thiamine	Cytosine
1	JP1	++	++	+	++	+++
		(0.864)	(1.089)	(0.432)	(0.943)	(1.175)
2	JP2	++	++	++	++	++
		(1.058)	(1.042)	(0.953)	(1.042)	(0.963)
3	LP1	+++	++	+++	++	+
		(1.185)	(0.531)	(1.164)	(0.842)	(0.328)
4	LP2	++	+++	++	++	++
		(0.962)	(1.186)	(0.953)	(1.073)	(0.573)
5	MP1	++	++	++	++	++
		(0.514)	(0.931)	(1.053)	(0.852)	(0.952)
6	MP2	++	++	+++	+++	++
		(0.963)	(1.073)	(1.185)	(1.142)	(1.057)

DISCUSSION

Isolation and population dynamics of PSB

PSB strains were capable of forming a halo zone around the colony on the solid medium containing in soluble phosphate compounds, was identified as a phosphate solubilizers. The PSM was 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. 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 (Illmer and Schinner, 1992; Reena *et al.*, 2013).

PSB have been found in almost all soils tested, although the number varies with soil, climatic and cropping history (Kucey, 1983). Soil samples collected from sugarcane growing belt of north Bihar indicated that the population level of phosphate solubilizing bacterial range from 27-112 x $10^{-3}g^{-1}$. This large variation in their distribution in different soils may be due to the differences in organic carbon content (Yadav and Singh, 1991).

Identification of PSB strains

The most efficient phosphate dissolving bacterial isolates belong to the genera *Bacillus* and *Pseudomonas*, though species of *Brevibacterium*, *Corynebacterium*, *Micrococcus*, *Sarcina* and *Achromobacter* have also been reported to be active in solubilization of insoluble

phosphates. Among fungi, the most efficient phosphate solubilizers belong to the genera *Aspergillus* and *Penicillium,* though species belonging to the genera *Cephalosporium* and *Alternaria have* also been known to solubilize insoluble phosphates. Among cyanobacteria, *Anabaena, Nostoc, Calothrix* and *Tolypothrix* were efficient phosphate solubilizers (Gupta *et al.,* 1998). Frietas and Germida (1990) isolated the phosphate solubilizing microorganisms such as *Pseudomonas aeruginosa, P. cepacia, P. fluorescens.*and *P. Putida* from the rhizosphere of wheat and *Bacillus licheniformis, B. mycoides, B. megaterium* from rhizosphere of paddy (Perez *et al.,* 2007; Gandhi *et al.,* 2014).

CHARACTERIZATION OF PSB

pH reduction by PSB

The pH of the culture filtrate turned to acidic was indicated that production of organic acids by PSB, which facilitate the solubilization phosphate (Sperber, 1958; Gaur and Sachar, 1980). The pH lowered down due to the liberation of the organic acids in liquid media (Nahas, *et al.,* 1996). The maximum decline in pH was recorded with *Bacillus megaterium* from 6.0 to 4.2 and *B. 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 and Patel, 1999). The minimum pH of 3.2 was shown by isolate PKN 3 after 7th day. The bacterial isolates, PKS 4, PBS 4, PKS 3 and PKU 5 significantly decreased the pH of media up to 3.3, 3.6, 3.8, 3.6 and 3.4 respectively after 7th day of growth (Fatima *et al.,* 2015).

Organic acid production

The production of organic acids by PSB seems to the main reason for solubilization of P and some metabolites like CO_2, H_2S and alkalinity or HNO_3 produced by organotrophs or autotrophs are also implicated to a little extent in P solubilization (Illmer and Schinner, 1995). Almost all PSM produced different amount and types of organic acids and among them di, tri-carboxylic and aliphatic acids are more effective than monobasic and aromatic acids (Illmer and Schinner, 1992). Within the various organic acids, hydroxyl acids are more efficient in phosphate solubilization because of their chelating property (Banik and Dey, 1983). 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 aquatic environment (Al-ghazalli *et al*., 1986). Organisms like *B. subtilis, B. megaterium,* and *Pseudomonas aeruginosa*were produced large quantity of phosphatase in the culture media, which mineralized organic phosphate. Acid and neutral pH influenced the production of acid phosphatase, whereas the alkaline phosphatase was produced in alkaline pH (Dick and Tabatabai, 1984).

Liberation of Phosphorus

It was suggested that the phosphate solubilizing activity in liquid medium ranged from 11% to 72% of total phosphate solubilization which was much more than phosphate solubilization observed (13%-58%) for *Pseudomonas* spp . The rate of phosphate solubilization was more with sorghum isolate followed by pearl millet, maize, cowpea and cotton (Kundu *et al*., 2002). *B. megaterium, B. polymyxa* and *Pseudomonas stutzeri* strains isolated from the soil samples were found to efficient in solubilizing of insoluble phosphate. The organisms obtained from rhizosphere of crop plants were solubilized more of tricalcium phosphate as compared to those obtained from non-rhizosphere soils (Dave and Patel, 1999).

The phosphate solubilizing activity in liquid medium ranged from 11% to 72% of total phosphate solubilization which was much more than phosphate solubilization observed (13%-58%) for *Pseudomonas* spp. *Penicillium* sp and *Pseudomonas* sp. were isolated from forest soil, solubilized 46.7 µ g/ml and 51.70 µg/ml of phosphate respectively from inorganic phosphate. The factors like nutrition, aeration and temperature were greatly influenced the phosphate solubilization (Illmer and Schinner, 1992).

Biology of PSB strains

All PSB strains were utilized various carbon source and their preferential carbon source varied from strain to strain. *Pseudomonas fluorescence* was utilized variety of carbon compounds as energy source. Among all the carbon sources tested, glucose was found best followed by galactose for both tricalcium phosphate and rock phosphate solubilization. Galactose was followed by sucrose for TCP and mannose for RP solubilization. All the monosaccharides were proved that superior to disaccharides and polysaccharides for rock phosphate solubilization, while all the monosaccharide and two disaccharides, sucrose and maltose, proved best for TCP solubilization (Dave and Patel, 2003; Singh *et al.,* 2015).

The form of available carbon sources were greatly affected the phosphate solubilization under *in vitro* and *in situ* in soil and was more active in presence of hexoses and pentoses or dissacharides (Patil *et al*., 2002). Phosphorus release was enhanced with increasing concentration of glucose and this was attributed to greater availability of the energy source for the growth of organisms and acid production. The maximum solubilization occurred at 2% than 1.5, 1.0 and 0.5% (Yadav and Singh, 1991). Gyaneshwar *et al.* (1998) found that *Enterobacter asburiae* solubilized phosphate when grown in presence of ammonia/nitrate as nitrogen source.

CONCLUSION

It is concluded that the population dynamics of PSB was varied with tree species with majority of *Bacillus megaterium.* All the PSB isolates were able to solubilize the insoluble tricalcium phosphate (TCP) and the solubilizing capacity was varied with strains. PSB strains were able to produce organic acids and phosphatase enzyme and the production ability varied with strain to strain. These variations reflected in the phosphate solubilization. Further, the isolated PSB strains were differed in their utilization of various sources such as carbon, nitrogen, aminoacids and vitamins. The selection of elite PSB strain depends on the *in vitro* studies. The use of these PSB as bioinoculants/biofertilizers will increase the available P in soil, helps to minimize the phosphatic fertilizer application, reduces ecological pollution and enhance the soil fertility and plant growth and development for the sustainable agriculture.

ACKNOWLEDGMENT

The authors are thankful to the College Management and the Principal of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu, India for providing necessary help and laboratory facilities to carry out the present work.

REFERENCES

Afzal, A. and Asghari, B. (2008) *Rhizobium* and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). *Int. J. Agric. Biol.* **10,** 85–88.

Akhtar, M.S. and Siddiqui, Z.A. (2009) Effects of phosphate solubilizing microorganisms and *Rhizobium* sp. on the growth, nodulation, yield and root-rot disease complex of chickpea under field condition. *Afr. J. Biotechnol*. **8,** 3489-3496.

Al-ghazalli, R., Mustafa, S.H., Mohammad, H.A and Al-gazawl, D.(1986) Some observations on "P" solubilization by aerobic microorganisms isolated from sediments of Al Khairriver Baghdad (Iraq). *J. Bio. Sci. Res.* **47,** 157 –172.

Aslantas, R., Cakmakci, R. & Sahin, F. (2007) Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Sci. Hortic*.**111,** 371-377.

Baby, U.I. (2006) Use of biofertilizers in tea gardens. *Planters' Chronicle.* **102**(2), 4-6

Baby, U.I. (2004) Sustainable productivity. *Contemporary Tea Time.***10**, 51-52.

Banik, S. and Dey, B.K. (1983) Phosphate – solubilizing potentiality of the microorganism capable of utilizing aluminium phosphate as a sole phosphate source. *Central Microbiol.***138,** 17-23.

Dave, A. and Patel, H.H. (1999) Inorganic phosphate solubilizing soil Pseudomonas. *Ind. J. Microbiol.* **39,** 161-164.

Dave, A. and Patel, H.H. (2003) Impact of different carbon and nitrogen source on phosphate solubilization by *Pseudomonas fluorescens. Ind. J. Microbiol.* **43**(1), 33-36.

Dick, W. and Tabatabai, M.A. (1984) Kinetic parameters of phosphatases in soils and organic waste materials.*Soil Sci.* **137**, 7-15.

Eivazi, F. and , M.A. Tabatabai (1977) Phosphatases in soils. *Soil Biol. Biochem.* **9,** 167-172.

Fatima, F., Pathak, N.and Verma, S.R. (2015) Isolation and characterization of potential phosphate solubilizing bacteria from the rhizoplane of Kukrail forest, Lucknow. *Int. J. Adv. Engi. Tech. Manage. Appl. Sci.* **2,** 1-15.

Fernandez, L.A, Zalba, P., Gomez, M.A. and Sagardoy M.A. (2007) Phosphate solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. *Biol. Ferti. Soil* **43,** 805-809.

Frietias, J.R. and Germide, J.J. (1990) PGPR for winter wheat. *Can. J. Microbiol*.**36,** 265-273.

Gandhi, A., Muralidharan, G. and Sudhaka, E. (2014) Isolation and identification of elite phosphate solubilizing bacteria from soil under paddy cultivation. *Int. Lett. Nat. Sci*. **11(1)**, 62-68.

Gaur, A.C. and Sachar, S. (1980). Effect of rock phosphate and glucose concentration on phosphate solubilization by *Asporgilluesgwanori*. *Curr. Sci*. **49,** 533-554.

Gulati, A., Rahi, P. and Vyas, P. (2007) Characterization of phosphate solubilizing florescent *Pseudomonas* from the rhizosphere of sea buckthorn growing in the cold desert of Himalayas.*Curr. Microbiol.***56,** 73–79.

Gupta, R., Singh, P. and Pandhar, M.S. (1998) Role of phosphate solubilizing microorganism in P- economic and crop yield. **In:** Soil Plant Microbes Interaction in Relation to Integrated Nutrient management (Ed. B.D. Kaushik), pp. 95- 101, Venus Printers and Publishers, New Delhi.

Gyaneshwar, P., Kumar, C.N and Parekh, L.J. (1998) Biochemical and genetic characterization of mineral P solubilizing *Enterobacter asburiae*. **In:** Biofertilizers and Bipesticides. A.M. Deshmukh (ed.), pp. 112 – 118, Technosci Publication, Jaipur.

Hameeda, B., Harini, G., Rupela, O.P.,Wani, S.P. and G. Reddy (2008) Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macro fauna. *Microbiol. Res*. **163,** 234-242.

Illmer, P. & Schinner, F. (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biol. Biochem*. **24,** 389-395.

Illmer, P. & Schinner, F. (1995) Solubilization of inorganic calcium phosphates, solubilization mechanisms. *Soil Biol. Biochem*. **27,** 257 – 263.

Krieg, N.E. and Dobereiner, J. (1984) Genus *Azospirillum*. In: *Bergey's Manual of Systematic Bacteriology*. Vol I. (eds.) N.R. Krieg and J.G. Holt. Williams and Wilkins, Baltimore, London. pp. 94-104.

Kucey, R.M.N. (1983) Phosphate solubilizing bacteria and fungi in various cultivated and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil Sci.* **63,** 674-678.

Kundu, B.S., Geva, R., Sharma, N., Bhatia, A. and R. Sharma (2002) Host specificity of phosphate solubilizing bacteria.*Ind. J. Microbiol.***42,** 19-21.

Mahantesh, S.P., Patil, C.S. and Himanshu (2015) Isolation and characterization of potent phosphate solubilizing bacteria. *ISOI J. Microbiol. Biotechnol. Food Sci.* **1**, 23-28.

Nahas, E., Banzatto, D.A. and Assis, L.C. (1990) Fluorapatite solubilization by *Aspergillus niger* in vinasse medium. *Soil Biol. Biochem*. **22**, 1097–1101.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L. (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. pp.939.

Patil, M.G., Sayyed, R.Z., Chaudhari, A.B. and S.B. Chincholkar (2002) Phosphate solubilizing microbes: A potential bioinoculants for efficient use of phosphate fertilizers. In: *Bioinoculants for Sustainable Agriculture and Forestry*. Reddy SM, Reddy SR, Grisham S. (Ed.), pp. 107-118, Scientific Publisher, Jodhpur.

Perez, E., Sulbaran, M., Ball, M.M. and L. A. Yarzabal (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. *Soil Biol. Biochem.* **39,** 2905–2914.

Reena, T., Dhanya, H., Deepthi, M.S. and D. Pravitha (2013) Isolation of Phosphate Solubilizing Bacteria and Fungi from rhizospheres soil from banana plants and its effect on the growth of *Amaranthus cruentus* L. *IOSR J Pharmacy Biol. Sci.* **5,** 6-11.

Sahin, F., Cakmakci, R. and F. Kantar (2004) Sugar beet and barely yields in relation to inoculation with N2-fixing and phosphate solubilizing bacteria. *Plant Soil* **265,** 123-129.

Sapak, Z., Meon, S. and Z.A.M. Ahmad (2008) Effect of endophytic bacteria on growth and suppression of *Ganoderma*infection in oil Palm. *Int. J. Agric. Biol.* **10,** 127–132.

Singh, A., Budholiya, N. and S. Kr. Singh (2015) Isolation and identification of phosphate solubilizing bacteria by 16s rRNA sequencing and evaluation of pesticide tolerance capacity. *World J. Pharm. Pharmaceut. Sci.* **4**, 964-978.

Sperber, J.I. (1958) The incidence of apatite – solubilizing organisms in the rhizosphere and soil. *Aust. J. Agric. Res.* **9,** 778 – 781.

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

Taurian, T., Anzuay, M.S., Angelini, J.G., Tonelli, M.L., Luduena, L., Pena, D., Inanez, F. and A. Fabra (2010) Phosphate-solubilizing peanut associated bacteria, screening for plant growth-promoting activities. *Plant Soil* **329,** 421-431.

Vessey, J.K. (2003) Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* **255,** 571-586.

Wang, X., Wang, Y., Tian, J., Lim, B.L., Yan, X. and H. Liao (2009) Over expressing AtPAP15 enhances phosphorus efficiency in soybean. *Plant Physiol*. **151,** 233–240.

Xuan Yu, A., Xu Liu, D., TianHui Zhu, Z., GuangHai Liu, R.T. and G. Cui Mao (2011) Isolation and characterization of phosphate solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biol. Fert. Soil.* **47,** 437-446.

Yadav, K. and Singh, T. (1991) Phosphorus solubilization by microbial isolate from cacifluvent. *J. Ind. Soc. Soil Sci.* **39,** 89-93.