RESEARCH ARTICLE

Studies on diversity and activity of *PSB* isolated from citrus field soil

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ABSTRACT

Phosphorous plays an important role in plant in many physiological activities such as cell division, photosynthesis and development of good root system and utilization of carbohydrate. Vidharbha is called as California of India for citrus as citrus is the main crop of this region of Maharashtra. The present study will focus on diversity and activity of PSB in citrus field as compare to cash crops. A total of 60 soil sample were collected from various villages of Katol tehsil (Vidharbha). These samples were subjected to isolation and identification of PSB. In present study total of 30 isolates each, was isolate obtained from normal crop and citrus field. The results showed that out of 30 isolates from various crop fields, 2 isolates (PSBV 14 and 22) showed highest phosphate solubilizing (28 mm). Whereas out of 30 strains from citrus field, 1 isolates (PSBC 16) showed highest zone of solubilization up to 25 mm. besides this strains PSBC22, 24 and 25 given 24 mm zone of phosphate solubilization. It was found that 17 isolated strains were given zone of Phosphate Solubilization more than 20mm, as compare to citrus field, in citrus field only 11 isolates given zone of Phosphate Solubilization in this range. Bacillus spp., Pseudomonas spp, and Enterobacter spp, were identified from the efficient phosphate solubilizers in both fields. This study concluded that the phosphate solubilization activity was higher in normal Crop field as compare to citrus field. The most efficient Strains of PSB (PSBV14), (PSBV22), (PSBV12) isolated from Normal Crop field. In Normal Crop field the crops rotation taken place which may leads the Phosphate Solubilization of Bound Phosphate.

Key words : Phosphate Solubilization, Citrus field, Diversity, PSB)

INTRODUCTION

Phosphorous is essential for growth and productivity of plant. It plays an important role in plant in many physiological activities such as cell division, photosynthesis and development of good root system and utilization of carbohydrate. Phosphorous deficiency result in the leaves turning brown accompanied by small leaves, weak stem and slow development. In most soil phosphors is available primarily as certain cation precipitate or poorly soluble organic compound or is bound to particle. The main inorganic phosphate in the soil is iron, aluminum and calcium phosphates (Baby , 2001).

Phosphorous is an essential nutrient for plant, but is often not available due to it fixation in soil Phosphate Solubilizing Bacteria (PSB) solubilized insoluble phosphate and make its available to the plant (Bhattacharya and Jain, 2000). Indian soil on an average contain 0.05% phosphorous that Constituent 0.2% of plant dry weight. Even applied phosphorous combine with metal ion PSB are required for its release (Bagyaraj and Varma, 1995).

Iron and aluminum phosphates are the major phosphate compounds in acidic soil. Whereas calcium phosphate in neutral to alkaline soil, phosphorus is the second vital nutrient next to nitrogen required for growth of microorganism and plants. But most of phosphors are not available to plants. Only 1-2% phosphors is supplied to above ground parts of the plants. Therefore, to meet out the phosphors demand of plant, exogenous sources of phosphorus is applied to plant as chemical fertilizers.

PSB secrete organic acids and enzyme that act on insoluble phosphate and convert into soluble form thus providing phosphorous to plant. PSB also produce amino acid, vitamin, and growth promoting substances (Bagyaraj and Varma, 1995, Schachtman *et al.,* 1998) which promote plants growth. Increased growth and yield of oats, coffee, tea, banana, mustard, maize, rice, sorghum, barley chickpea, soyabeans, groundnut, sugar beet cabbage,and tomato to the extent of 10-20 have been reported by using of PSB (Ponmungan and Gopy, 2006).

There are several phosphate Solubilizing microorganism (PSM) present soil, for example the species of *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Aspergillus, Penicillium, Fusarium* etc. They can utilize tri-calcium phosphate, apatite, rock phosphate, $FeSo_4$ sole phosphate source present in the medium. The indication of utilization is that they produce clearing zone around each colony.

They secret organic acid such as acetic acid, lactic acid, succinic acid, propionic acid, formic acid etc. Consequently bound form phosphates are solubilized and charge molecule of phosphorus is absorbed by the plant. Therefore, the PSM save 30-50 kg/ha of supper phosphate and increase crop yield up to 200-500 kg/ha. Phosphorus was probably discovered around 1669 by a German Alchemist, H. Brandt in Hamburg. He derived the word phosphorus from its property in a Greek terminology "phos" meaning "light"and" phorus" meaning "bringing". It exist in two allotropic forms yellow (white) and red (brown) froms (Goeland Pathode, 2004).

In small quantity as aluminum andiron phosphate and largely in the form of rock phosphate (Ca_3 $(Po_4)2$ it is also found in soil as insoluble phosphates, soluble phosphates, organic phosphate and residual phosphates (Goeland Pathode, 2004, Dardarwal, 1992). Assimilation of phosphate from organic compound by plants and microorganism take place through the enzyme "phosphate" which is present in wide variety of soil microorganism plant can absorb phosphate only in soluble from. The transformation of insoluble phosphate into soluble form is carried out by a number of microbes present in the soil. "A large fraction of soil microbes can dissolve insoluble inorganic phosphate present in the soil and make them available to the plants" (Bhattacharya and Jain, 2000; Richardson, 2001).

The medium used to estimate the population density of phosphate solubilizers show a clear zone around the colonies indicating phosphate solubilization. Phosphorus the "Master key" element in crop production is next only to nitrogen as a major plant nutrient due to chemical fixation of phosphate it remain largely unavailable to growing plant. In alkaline soil, the predominal form of fixed phosphate is tri calcium phosphate carried out by majority of bacteria and fungi (Gaur, 1990).

Bromfield (1959) found dissolution of rock phosphate by fungi mainly by the secretion of organic acids. Yin (1988) observed the solubilization of hydroxyapatite and rock phosphate by a variety of Gram negative and positive bacteria, fungi and Actinomycetes under liquid medium conditions. Gaur (1974) found that TCP and hydroxyapatite were easily Solubilizers than RP by different groups of bacteria normally, TCP is solubilized with an equal case by fungi and bacteria but fungi solubilized RP in more amount than bacteria.

Vidharbha is called as California of India for citrus as citrus is the main crop of this region of Maharashtra in

Vidharbha around 100,000 hectare of land is under cultivation of citrus that is lemon, orange, sweet lemon. In our country 48% of citrus is produce in Vidharbha such a large area under cultivation lion's share of around 50% in country productivity. Citrus of Vidharbha attract academic interest and motivate to research.

The present study was focused on the assessment of PSB diversity and its Solubilizing activity in citrus field as compare to cash crops. This study will help in improving phosphate intake of Citrus crop which will lead to increase productivity, The Study also aimed to assess the efficiency of Phosphate Solubilization of isolates in various normal crop field and citrus field.

MATERIALS AND METHODS

Isolation of Phosphate Solubilizing Bacteria (PSB)

Collection of soil sample

Soil sample were collected from different villages of Katol, tehsil for the isolation of phosphate Solubilizing Bacteria the samples were air dried under shade and used for isolation and identification of organisms. A total of 60 soil sample were collected from various villages of Katol tehsil. These samples were used for isolation and identification of PSB. The samples were processed to analyze within same day. The samples were collected by using standard procedure. Mix the sample and fill the sample bag make sure that all the cores are thoroughly mixed together. Soil samples were filled plastic bag about 1/2 full (approximately 1 cup) with the mixed sample.

Preparation of soil suspension

1 gm of air dried soil sample was taken in l00 ml sterilized distilled water and shaken for 5 minutes stand the tube for settling the soil and upper supernatant was discarded these procedure was followed by 4 times lastly 4 th tubes supernatant was collect out for inoculation of the plate

Sterilization of Medium and Inoculation

Media, Petri dishes, were kept in an autoclave for sterilization at 121 ^{0}c for 20 min at 15 lb pressure after sterilization, plating of these media and inoculation the soil suspension by point inoculation and incubate at 37^{0}c or 2 days. At the end of incubation, PSB colonies were identified by the formation of clear zone of phosphate Solubilization around the bacterial colony (De, freitas *et al,* 1997).

Identification

The organisms were identified on the basis of standard procedure including gram staining and IMViC test (Indole, MR, VP, Citrate test) for identification of Phosphate Solubilization bacteria selective medium were used.

Analysis of Phosphate Solubilizing Activity

The phosphate Solubilizing activities of isolated strains were observed by zone of phosphate solubilization on Pikovskaya's agar medium.

RESULTS AND DISCUSSION

In present study a total of 60 isolates were isolated from general crop and citrus crop field. The results showed that out of 30 isolates from various crop fields, 2 isolates (PSBV 14 and 22) showed highest phosphate solubilizing (28 mm). Whereas out of 30 strains from citrus field, 1 isolates (PSBC 16) showed highest zone of solubilization up to 25 mm. besides this strains PSBC22, 24 and 25 given 24 mm zone of phosphate solubilization. It was found that 17 isolated strains were given zone of Phosphate Solubilization more than 20mm, as compare to citrus field, in citrus field only 11 isolates given zone of Phosphate Solubilization in this range.

In 2005 Chen *et al* studied on phosphate solubilizing bacteria from subtropical soil and their tri-calcium phosphate solubilizing abilities are they reported that, the ability of a few soil microorganism to convert insoluble forms of phosphorus to an accessible form is an important trait in a plant growth Promoting bacteria for increasing plant yields. The use of phosphate solubilizing bacteria as inoculants increases the phosphorous uptake by plants. In this study, isolation, screening and characterization of 36 strains of phosphate Solubilizing bacteria from central Taiwan were carried out.

In various crop fields, 18 isolates (PSBV) were showed highest phosphate Solubilization activity whereas 12 isolaets solubilized the phosphate moderately (Fig. 1).

In 2006 Ponmurugan and Gopi studied the distribution pattern and screening of phosphate solubilizing

Bacteria isolated from different food and forage crops are they found that the distribution pattern and population density of phosphate solubilizing bacteria (PSB)was assessed in cultivated soils. PSB isolates were assessed for phosphate Solubilizing capacity, production of growth regulators, phosphatase activity, PH changes and titrable acidity. The population levels of PSB were highest in the rhizosphere soil of groundnut and lowest in the rhizosphere of Ragi, Sorghum and Maize.

It could be observed from the data that the distribution pattern of PSB in the Rhizosphere soils showed that the Population levels decreased with the distance of soil sampling from the plants. A wide variation the capacity of solubilize phosphorous by the PSB isolates was observed. Further, all the isolates were able to secrete phytohormones like gibberelic acid (GA3) and Indole acetic acid (IAA) and acid phosphatase under in vitro condition.

In present study from both field the common phosphate solubilizers *Bacillus spp*. *Pseudomonas, Enterobacter, Erwinia* spp. Were isolated. It was found that the *Bacillus* spp*.* given highest zone of solubilization whereas *Pseudomonas, Enterobacter, Erwinia* show low phosphate solubilization activity in both the field.

Vazquez *et al.* (1999) Studied on phosphate-Solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon and they found that the Phosphate-Solubilizing potential of the rhizosphere microbial community in mangroves was demonstrated when culture media supplemented with insoluble, tri-basic calcium phosphate and incubated with roots of black (*Avicennia germinans* L.) and White mangrove became transparent after a few days of incubation. He was isolated phosphate-Solubilizing bacterial strains from the Rhizosphere of both species of mangroves. *Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus atrophaeus, Paenibacillus maceran,, Pseudomonas stutzeri.*

In present study total of 30 isolate were isolated from citrus field. The results analysis were showed that isolates PSBC 16 isolate showed highest phosphate solubilization zone i.e. 25mm. where isolate no. PSBC3, PSBC5, PSBC9 and PSBC 26, showed low phosphate solubilization zone (Fig. 2).

When the Phosphate solubilization activity of Normal crop fields isolates were analyze and compared with citrus field isolates. It was found that 17 isolates gives zone of Phosphate Solubilization more than 20mm, 28 isolates give zone of Phosphate Solubilization more than 10mm and 3 isolates gives zone of Phosphate Solubilization more than 25mm.

As compare to citrus field, 11 isolates give zone of Phosphate Solubilization more than 20mm, and 25 isolates give zone of Phosphate Solubilization more than 10mm and only one isolate give zone of Phosphate Solubilization more than 25mm (Fig. 1 & 2).

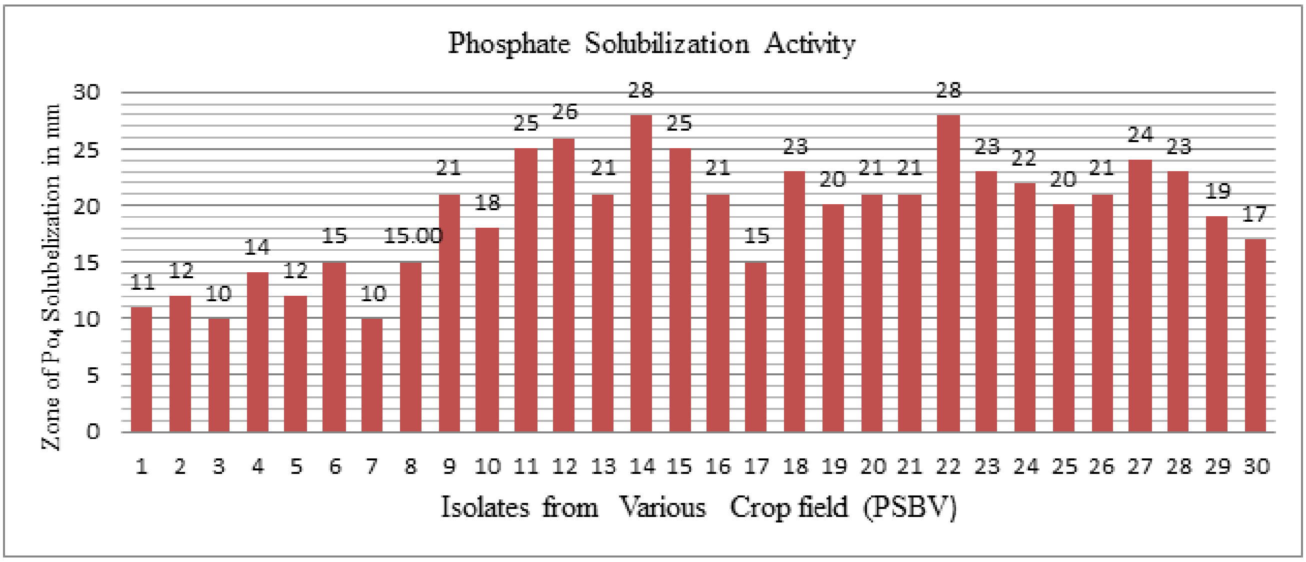

Fig . 1: Phosphate Solubilization Activity of normal crops fields

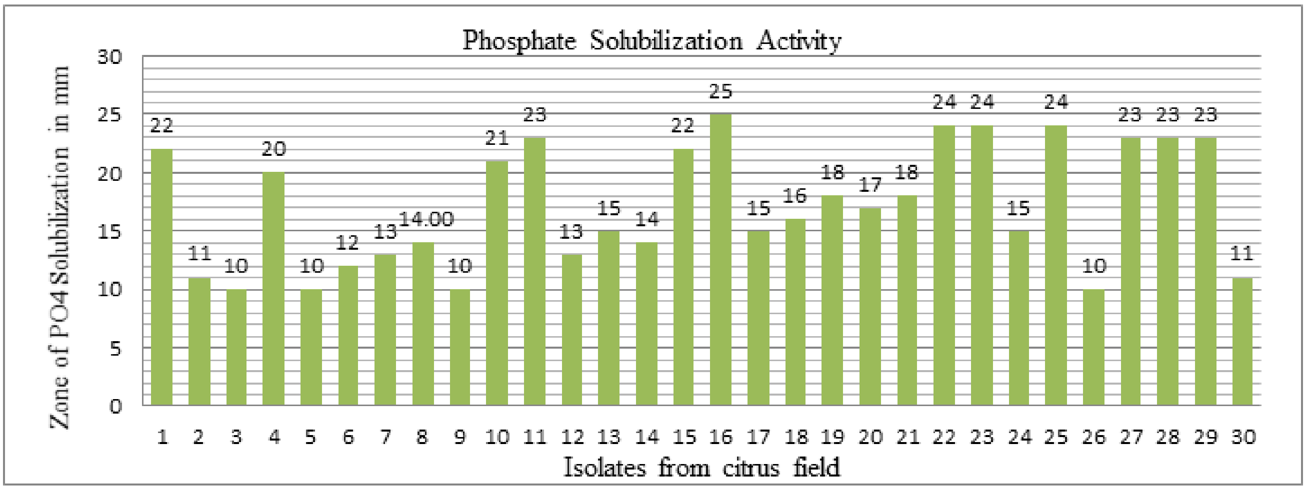

Fig . 2 Phosphate Solubilization Activity of citrus fields

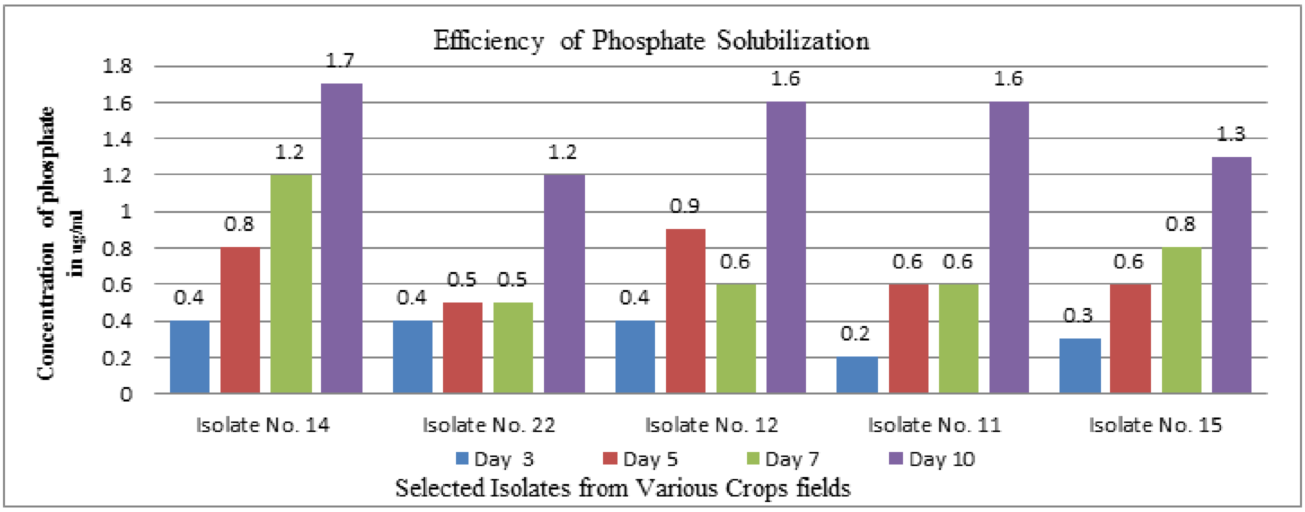

Fig No:-3:- Efficiency of Phosphate solubilization by isolated strains. PSBC 11 showed the solubilization of phosphate day 3, day 5, day 7, and day 10 are 0.4 µg/ml ,0.5 µg/ml , 0.7 µg/ml and 1.5 µg/ml. PSBC 23 showed the solubilization of phosphate in day 3, day5, day 7, and day 10 are 0.2µg/ml, 0.4µg/ml, 0.8µg/ml 1.5 µg/ml. respectively (Fig 3).

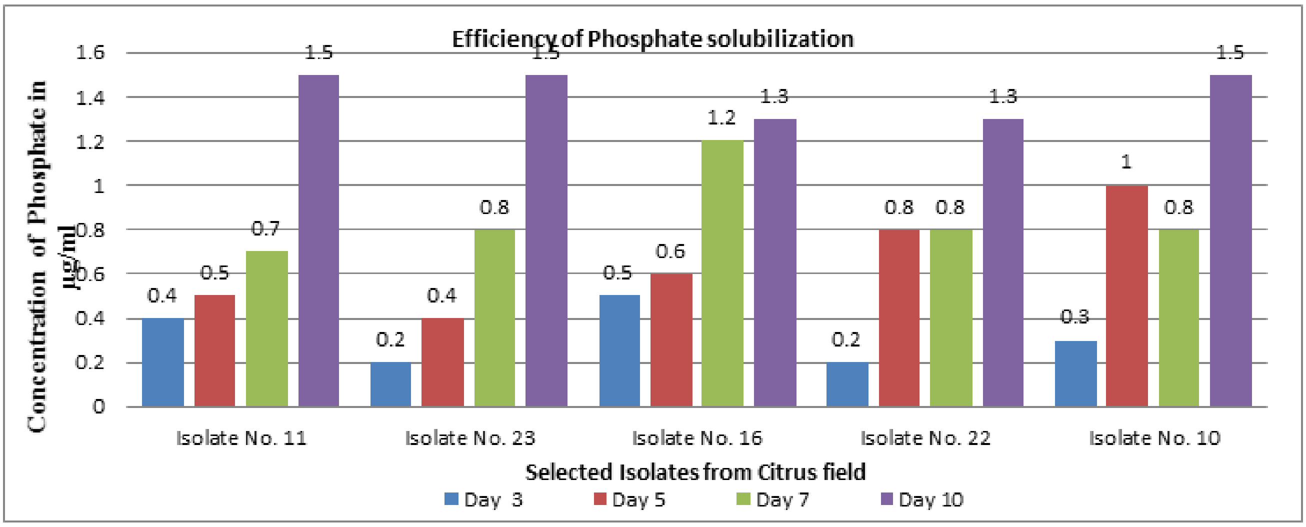

Fig No. 4 Phosphate Solubilizing Efficiency of Isolates from Citrus Field.

The efficiency of Phosphate solubilization was checked by using colorimetric method. The results and data obtained from various crop field (PSBV) and Citrus Crop filed (PSBC) were compared.

The Results showed that out of 30 isolate, only five isolates (PSBV) were subjected to estimate solubilization of phosphate and found the efficiency of phosphate solubilization at within 3,5,7,and10 days (Fig. 3). PSBV 14, PSBV 22, PSBV 12, PSBV 11, PSBV 15 showed highest solubilization at the end of 10 dayPSBV14 showed amount of phosphate in day 3, day5 day 7and day 10 are 0.4µg/ml, 0.8µg/ml, 1.2µg/ml,1.7ug/ml respectively. PSBV 22 showed the solubilization of phosphate within day 3, day 5, day7, and day 10 are 0.4µg/ml, 0.5µg/ml 0.5µg/ml, and 1.2µg/ml respectively. PSBV 12 showed solubilization of Phosphate in 3, day 5, day7, day10, was 0.4ug/ml, 0.9µg/ml, 0.6µg/ml, 1.6µg/ml respectively.

When isolates from citrus field (PSBC) were subjected for analyzing the colorimetric efficiency it was found that out of 30, PSBC 11, PSBC 23, PSBC16, PSBC 2 and PSBC10were showed highest zone of phosphate solubilization.

PSBC16 showed the solubilization of phosphate in day 3, day 5, day 7 and day 10 are 0.5 µg/ml 0.6µg/ml 1.2 µg/ml 1.3 µg/ml respectively.

PSBC 22 showed the solubilization of Phosphate in day 3, day 5, day7. Day10, are 0.2µg/ml,0.8 µg/ml, 0.8µg/ml, 1.3 µg/ml (Fig. 4).

Rudresh *et al* 2004 was found that, Improvement of Phosphate Solubilizers an alternative approach for the use of phosphate solubilizing bacteria as microbial inoculation is the use of mixed inoculation of PGPR strains comprising phosphate solubilizing Bacteria. The effect of a combined inoculation of Rhizobium, a phosphate solubilizing *Bacillus megaterium sub sps. phosphaticum strains-PB* and a biocontrol fungus, *Trichoderma sps*. On growth, nutrient uptake and yield of chickpea were studied under glass house and field condition combined inoculation of these three organism showed increased germination, nutrient uptake plant height, number of branches, nodulation, pea yield and total biomans of chickpea compared to either individual inoculation or an uninoculated control .

Balamurugan *et al* in 2010, carried out the study on isolation characterization are they found that phosphate solubilizing bacteria of tea garden soil were isolated, screened in vitro and studied its bio ecology. Among the 25 isolates, the five strains were shown higher phosphates enzymes activity compared to other strains. (PSB 12,PSB 25 and PSB 37) and among these three strains PSB 37 was found to be superior in forming halo zone of phosphate solubilization followed by PSB 25. All strains brought down the pH of culture medium. The biochemical characterization of the isolates PSB7, PSB12, PSB20, PSB22, PSB25, PSB26, PSB37 were closer to Pseudomonas species. The selected PSB strains preferred temperature at 28^0c to 35^0c for its better growth.

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REFERENCES

Baby UI, Tensingh Balaiah N, Ponmurugan P and Premkumar R (2001) Population dynamics of nitrogen fixing and Phosphate Solubilizing bacteria in tea soil. uPASI Tea found. *Inst. Newslett*, 10;4-4

Bagyaraj DJ.and Varma A. (1995) Mineral Phosphate Solubilization; Agronomic implication Mechanism and Molecular genetics. *Adv. Microbial Ecol*. 14;119-142.

Balamurugan A, Princy T, Vidhya R, Pallavi Nepolean P Jayanthi R and. Premkumar R (2010) Isolation and Characterization of Phosphate Solubilizing bacteria in tea". *J. Biosci, Res,*1(4);285-293.

Bhattacharya P. and Jain RK (2000) Phosphorous Solubilizing biofertilizers in the Whirl pool of rock Phosphate- challenges apportunities fertile. New, 45;45-52

Chen YP,Rekha PD, Arun AB, Shen FT, Lai WA,Young CC (2006) Phosphate Solubilizing bacteria from Subtropical Soil and their tricalcium Phosphate Solubilizing abilities. *Applird Soil Ecology* 34;33-41.

Dardarwal,K.P1992., Plant growth promoting Rhizobacteria as biofertilizer, plantandsoil.255;571-586.

Gaur, A.C. and Sachar, S.1990., Effect rock Phosphate and Glucose Concentration on Phosphate Solubilization by Aspergillus awamori. *Curr. Sci.*49;533-554.

Goel P.K.,G.K. Pathade 2004., Biotechnological application in environment andagriculture ABD Publication 57-117

Ponmurgan P.and C.Gopi,2006., Distribution pattern and Screening of Phosphate Solubilizing Bacteria isolated from different food and forage crops. *Journal of Agronomy*, 5;600-604.

Richardson AE, Hadobas PA and Hayes JE (2001) Extracellular Secretion of Aspergillus phytase from Arabidopsis roots enable plants to obtain Phosphorus from phytate. *Plant J.*,641-649.

Rudresh DL, Shivaprakash MK, Prasad RD (2004), Effect of Combined application of Rhizobium, Phosphate Solubilizing Bacterium and Trichoderma spp. On growth nutrient uptake and yield of Chickpea. *Applied Soil Ecology* 28;139-146.

Suh JS, Lee SK, Kim KS and Seong KY (1995) Solubilization of Insoluble Phosphate by Aspergillus niger, Pseudomonas putida, Penicillium sps. Isolated from Korean Soils. *JK SSf*, 28;3 278-386.

Vazquez, P.and Holguin G, Puente ME, Lopezcortes A, Bashan Y (2000) Phosphate Solubilizing Microorganism associated with the Rhizosphere of Mangroves in a Semiarid Coastal lagoon. *Biol Fertile Soils*, 30;460-468.

Yin R (1988) Phosphate Solubilizing microbes in non-irrigated Soils in China. *Soils*,20;243-246.