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# Mineral phosphate solubilization by *Pseudomonas aeruginosa* isolates from chilli (*Capsicum annuum* L.) fields

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**Abstract**

Phosphorus (P) is the second imperative key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement. An adequate supply of P is obligatory for proper execution of various metabolic activities of plants. Identification of a potent phosphate solubilizing microorganism capable of transforming insoluble P into soluble and plant accessible forms is considered as the best eco-friendly option for providing inexpensive P to plants. In this study 12 phosphate solubilizing microorganisms were isolated from chilli rhizosphere by enrichment culture technique. P solubilisation efficiency was checked using vanadomolybdate phosphoric yellow colour method and two most efficient isolates, PS 2 and PS 3, were selected. Various parameters such as pH, temperature of incubation, carbon source, nitrogen source, NaCl concentration and incubation time were optimized for the selected isolates, *Pseudomonas aeruginosa* PS 2 (KR270346) and *Pseudomonas aeruginosa* PS 3 (KR270347). Maximum P solubilization rate was shown at pH 7, temperature 30°C and after 15 days of incubation. Glucose and ammonium sulphate were the best carbon and nitrogen sources for the selected isolates. Mineral phosphate solubilization was directly related to a pH drop in the culture medium. Production of gluconic acid by *Pseudomonas aeruginosa* during phosphate solubilization was confirmed by analyzing the culture medium by high performance liquid chromatography. The growth stimulation effect of selected isolates was confirmed by the vigour index determination and the treated rice seeds showed better germination percentage than control seeds.

**Keywords:** Gluconic acid, High performance liquid chromatography, Mineralization, Solubilization efficiency, Vigour index

## Introduction

Phosphorus is a macronutrient making up about 0.2% plant dry weight (Kuheli et al., 2003). Phosphorus increases biological activity of soil and maximizes crop yields. It plays a significant role in plant metabolism, ultimately reflected on plant yield (Karpagam and Nagalakshmi, 2014). P is important for the proper functioning of key enzymes that regulate the metabolic pathways. About 98% of Indian soils contain inadequate amounts of available phosphorus. Phosphorus deficiency may result in reduction of seedling growth, plant establishment and root development. Deficient plants appear stunted and dark green in colour, and exhibit delayed flowering and crop maturity (Khan et al., 2009). In nature phosphorus exists in a variety of organic and inorganic forms but majority of them are in unavailable fixed form and hence for improving agricultural productivity, application of phosphate fertilizers is necessary. But a large portion of inorganic phosphate applied to soil through these fertilizers is easily immobilized and are unavailable through precipitation reaction with cations like $Ca^{2+}$, $Fe^{2+}$ and $Al^{2+}$ present in soil (Gyaneshwar et al., 2002). Over application of phosphate fertilizers

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leads to over phosphatisation with less P availability to plants and results in adverse effect on plant growth and yield (Landweert et al., 2001). Thus the agriculture industry needs to find an ecofriendly alternative to chemical phosphate fertilizers.

Microbial solubilization of hardly soluble mineral phosphates is an important process in natural ecosystems and in agricultural soils. Though several phosphate solubilizing bacteria occur in soil, usually their numbers are not enough to compete with other bacteria commonly established in the rhizosphere. So the amount of P liberated by them is not enough for a substantial increase in *in situ* plant growth. As a result, inoculation of plants by a target microorganism at a much higher concentration is necessary to take the advantage of the property of P solubilization for plant yield enhancement (Rodriguez and Fraga, 1999). Seed or soil inoculation with phosphate solubilizing microbes helps to improve solubilization of fixed soil P and applied phosphates and results in higher crop yields (Sandeep et al., 2008). Several field studies using P-solubilizing microorganisms have been conducted in various parts of India in wheat, maize, paddy, peas, potatoes, soybean, cotton, tobacco and various other crops (Duby and Billore, 1992) with an increase in the P uptake and yield.

A wide range of bacteria belonging to *Pseudomonas* and *Bacillus* genera are known to be efficient phosphate solubilizers (Gulati et al., 2007). P solubilizing activity has been found to be highly dependent on the environmental conditions (Deepshikha et al., 2014). Each species or strain has characteristic cardinal temperature, pH and nutrient sources for maximum P solubilization. Several studies have reported the ability of different bacteria releasing phosphate from inorganic phosphate such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite and rock phosphate through solubilization and mineralization (Vassilev et al., 1996).

With emphasis on screening for potential PSB from the soil for agricultural purposes, the present experiment was designed to isolate and characterize environmental friendly and effective PSB isolates from chilli rhizosphere and optimize of parameters for effective phosphate mineralization.

## Materials and Methods

*Isolation of phosphate solubilizing bacteria*

Soil samples of chilli *(Capsicum annuum* L.*)* rhizosphere were collected from different parts of Kerala, India using standard protocols (Son et al., 2006) during 2012-2013. Care was taken to select representative samples from different geographical areas.

Pikovskaya's medium (Pikovskaya, 1948) was used for the enrichment, isolation, screening and maintenance of phosphate solubilizing bacteria. Enriched soil samples were serially diluted and were spread-plated on modified Pikovskaya's (PVK) agar, and the bacterial colonies producing a distinct tri-calcium phosphate (TCP) solubilisation zone were grown in pure culture and maintained in 30% glycerol at -20°C.

The phosphate solubilization ability of the isolates was checked by the method described by Srivastav et al., (2004). National Botanical Research Institute's Phosphate (NBRIP) medium with bromophenol blue (BPB) as indicator (Mehta and Nautiyal, 2001) was also used for screening of potential isolates and selection was done on the basis of dye decolourisation.

Nutrient broth was used to study the growth pattern of the selected isolates. Phosphate solubilizing *Pseudomonas striata* obtained from the division of microbiology, Indian Agricultural Research Institute (IARI), New Delhi, served as control in this study.

*Measurement of phosphate solubilization potential*

Each bacterial strain was cultured on nutrient broth overnight and 2 µl of each inoculum [Optical Density (OD) at 600 nm = 1] was spotted on to

NBRIP medium for the measurement of Solubilization Index (SI). Each strain was inoculated in triplicate and after two days of incubation at 28º C, solubilization Index (SI) was measured by the following formula: SI = solubilization halo diameter/colony diameter. Decrease of pH of the medium was also measured.

For quantification of soluble phosphate produced by each strain 1% bacterial culture (OD = 1) was added to 10 ml of Pikovskaya's (PVK) medium (pH 7) containing tricalcium phosphate as sole phosphate source. After 72 h of incubation at 28º C cell free supernatant (CFS) was obtained by centrifugation at 10000 rpm for 20 min and amount of soluble phosphate was measured following the vanadomolybdate phosphoric yellow colour method (Jackson, 1973).

*Identification of selected bacteria*

The selected isolates were subjected to cultural, morphological, biochemical and physiological characterization as mentioned in Bergey's manual of determinative bacteriology (Holt et al., 1994).

The molecular identification of PSB was done on the basis of 16S rRNA gene sequencing. The genomic DNA of PSB isolates was extracted by the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA). The primers 8F: 5'-AGAGTTTGATCMTGG-3' and 1492R: 5'-ACCTTGTTACGACTT-3' were used for amplification of 16S rRNA gene (Weisburg et al., 1991). The total PCR reaction mixture was 50.0 µl comprising 200 µM dNTPs, 50 µM of each primer, 1X PCR buffer, 3 U *Taq* polymerase, and 100 ng genomic DNA. The thermo cycling conditions involved an initial denaturation at 94°C for 4 min, followed by 30 cycles at 94° C for 1 min, 52° C for 1 min, and 72° C for 2 min and final extension at 72° C for 8 min. The 16S rRNA gene was purified by gel electrophoresis, ligated to pGEM-T vector (Promega, Madison) and transformed in *E. coli* JM109. The sequences of the insert were determined using a Big-Dye Terminator Cycle Sequencer and an ABI Prism 310 Genetic Analyzer (Applied Biosystems, CA). Each nucleic acid sequences was edited manually to correct falsely identified bases and trimmed to remove unreadable sequence at the 3' and 5' ends (considering peak and quality values for each base) using the sequence analysis tools. The edited sequences (16S rDNA) were then used for similarity searches using BLAST (Basic Local Alignment Search Tool) programme in the NCBI GenBank (www.ncbi.nlm.nih.gov) DNA database for identifying the bacterial strains.

*Standardization of conditions for efficient TCP solubilization by selected isolates*

The conditions for maximum phosphate solubilization by selected bacterial isolates were optimized by varying the cultural conditions like pH (6, 6.5, 7, 7.25 and 7.5), temperatures (25, 27.5, 30, 32.5 and 35° C), carbon source (glucose, fructose, mannitol), nitrogen source (ammonium sulphate, urea, potassium nitrate,) and incubation period (1-30 days). The effect of different concentrations of sodium chloride (0.1, 0.2, 0.3, 0.4 and 0.5 g%) on TCP solubilization by the selected isolates were also tested. During optimization process one of the conditions was varied in each experiment keeping the other variables constant.

*Analysis of organic acid production*

Production of organic acids by the selected isolates were analyzed by HPLC (Shimadzu LC 20 AP). The isolates were grown in King's B broth for 14 days and the resultant culture filtrate was filtered through 0.22 µm nylon filter. The organic acids were separated using Enable C18 column (4.6×250 mm) set at the following parameters, solvent methanol: deionized water (20:80); flow rate 0.8 ml $min^{-1}$; pressure-300 kgf $cm^{-2}$; UVdetector – 210 nm and injection volume 20 µl. The organic acids were identified by comparing the retention time of standard acids like oxalic, gluconic, acetic and succinic acids (Patel et al., 2007).

*Effect of isolates on growth promotion*

Effect of the selected phosphate solubilizing

bacterial isolates on seed germination and seedling vigor of rice seeds (*Oryza sativa)* were assayed by employing the procedure described by Chandrasekhara et al., (2007) with minor modifications. Three replicates of 20 seeds were used per treatment. Rice seeds of the variety Ponmani were selected and surface sterilized using 2% sodium hypochlorite solution for 3 minutes followed by serial washing with sterile distilled water five times. The rice seeds were then aseptically transferred to 100 ml of 4 days old Pikovskaya's broth in 250 ml Erlenmeyer Flasks inoculated with one ml ($2x10^8$ cfu $ml^{-1}$) of each selected bacterial cultures and incubated for 24 hours in a rotary shaker at 120 rpm at 28 ± 2°C. After incubation, broth in each flask was discarded and randomly selected 20 rice seeds treated with bacterial isolates were placed aseptically in Petriplates lined with sterile moistened filter paper. Seeds treated with sterile Pikovskaya's broth were kept as control. All the plates were set for germination under dark at 28 ± 2°C for 7 days. After incubation, length of roots and shoots of individual seeds were measured in the test and control plates and vigour index (VI) was calculated using the following formula (Abdul-Baki and Anderson, 1973): VI= (Mean Root lenght+Mean Shoot Lenght) (Germination [%])

*Statistical analysis*

The statistical analysis for all the parameters was performed using analysis of variance. The means were compared by Duncan's Multiple Range Test using the statistical package SAS version 8.3 (SAS Institute Inc., Cary, NC, USA). The differences among the LS MEANS were analyzed by constructing diffograms (Hsu, 1996).

## Results and Discussion

*Isolation and screening of phosphate solubilizing bacteria*

32 soil samples from chilli fields were collected from different districts of Kerala, India. All soil samples showed acidic pH. 17 phosphate solubilizing bacteria were isolated and all the isolates were subjected to *in vitro* tricalcium phosphate solubilization. The isolates showed

*Table 1.* Screening of phosphate solubilizing bacteria from chilli rhizosphere

| Isolate | Final pH of the medium | Amount of P solubilized (mg 100 $ml^{-1}$) | Phosphate solubilization efficiency [SE%=Z-C/C×100] |
|---|---|---|---|
| PS 1 | 4.5 | 17.92 ± 0.1 | 148.33 ± 2.88 |
| PS 2 | 3.4 | 56.11 ± 1.05 | 300 ± 2.88 |
| PS 3 | 3.13 | 69.1 ± 0.1 | 420 ± 2.30 |
| PS 4 | 4.71 | 30.95 ± 0.02 | 213.33 ± 2.88 |
| PS 5 | 4.11 | 39.04 ± 0.4 | 238.33 ± 12.58 |
| PS 6 | 5.17 | 6.52 ± 0.48 | 135 ± 2.3 |
| PS 7 | 4.39 | 44.32 ± 0.02 | 281.66 ± 2.88 |
| PS 8 | 5.09 | 34.97 ± 0.01 | 206.66 ± 5.77 |
| PS 9 | 4.7 | 32.45 ± 0.02 | 153.66 ± 3.21 |
| PS 10 | 4.22 | 22.22 ± 0.69 | 185 ± 5.0 |
| PS 11 | 4.05 | 30.11 ± 0.02 | 115.33 ± 2.88 |
| PS 12 | 4.51 | 21.92 ± 0.01 | 110.66 ± 10.06 |
| PS 13 | 4.34 | 32.09 ± 0.4 | 125 ± 2.88 |
| PS 14 | 4.19 | 13.98 ± 0.02 | 121.66 ± 9.07 |
| PS 15 | 4.250 | 27.22 ± 0.02 | 120.33 ± 2.88 |
| PS 16 | 4.09 | 23.29 ± 0.50 | 110.66 ± 10.06 |
| PS 17 | 5.03 | 19.22 ± 0.01 | 135 ± 13.22 |
| Std | 4.73 | 50.66 ± 0.1 | 230 ± 2.88 |

* Values are mean ± SD of three replications

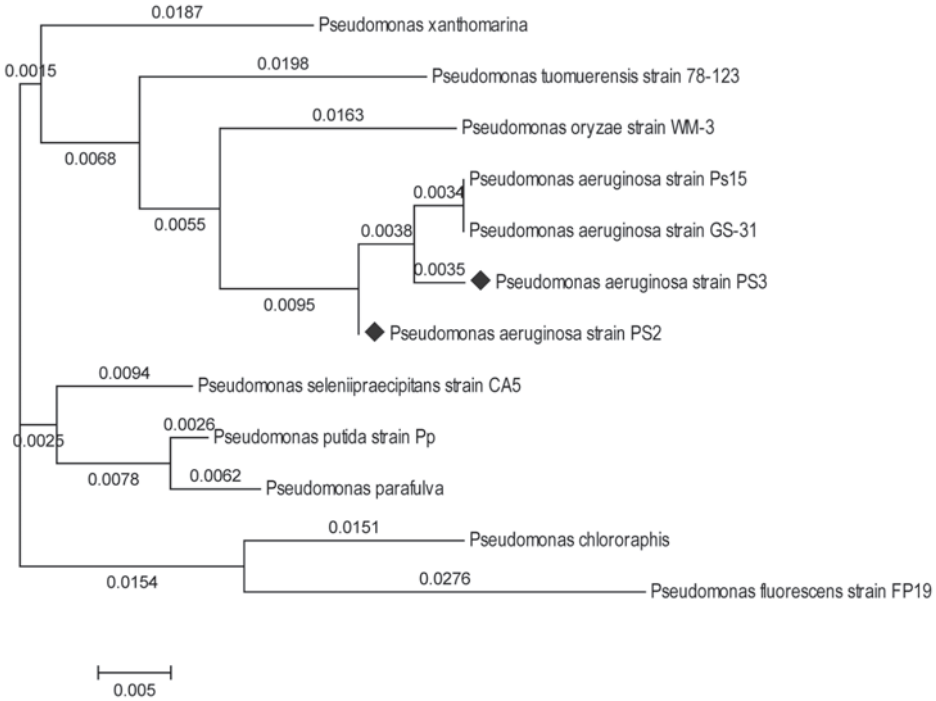



*Figure 1.* Phylogenetic tree of selected isolates *Pseudomonas aeruginosa* PS 2 and *Pseudomonas aeruginosa* PS 3

variation in their P solubilizing activity and the results are given in Table 1. P solubilization ranged between 6.52 and 69.1 mg 100 ml$^{-1}$. There was noted reduction in pH of the culture medium in all the inoculated flasks. Zone of clearance produced in the Pikovskaya's agar and dye decolonization in the NBRIP-BPB medium (Mehta and Nautiyal, 2001) were also taken as criteria for selection of the efficient isolates. Among the isolates PS 2 showed solubilization efficiency (SE%) 300, pH reduction of 3.4 and solubilized 56.11 mg ml$^{-1}$ P whereas PS 3 showing SE% 420, pH reduction of 3.13 and P solubilization of 69.1mg ml$^{-1}$. From the total 17 isolates, based on the primary and secondary screening, two isolates which showed promising results were used for further studies**.** There are several reports on the isolation of phosphate solubilizing bacteria from the rhizosphere of different plants such as rice (Stephen et al., 2015), tomato (Karpagam and Nagalakshmi, 2014), and chilli (Moumita et al., 2011).

The two selected isolates were identified as *Pseudomonas aeruginosa* based on biochemical and molecular characterization. Molecular identification was carried out by 16SrDNA sequencing and the resultant sequence was deposited in the NCBI gene bank database and designation KR270346 and KR270347 was obtained. Stephen and Jisha (2011) identified phosphate solubilizing *Burkholderia* sp. based on 16S rDNA sequencing. Phylogenetic tree was constructed using the neighbour-joining method using the software MEGA 4. Similar method was used by Qurban et al. (2014) for the identification of phosphate solubilizing PSB7 (*Burkholderia thailandensis*), PSB21 (*Burkholderia seminalis*), and PSB17 (*Sphingomonas pituitosa*) (Figure 1).

*Pseudomonas* sp. represents one of the most abundant genera of the root microbiome (Zamioudis et al., 2013). *Pseudomonas aeruginosa* has been frequently isolated as endophytes from different crop plants and most of such strains have been reported to show inhibitory activity against various

*Table 2*. Optimization of cultural conditions for efficient P solubilisation by the selected isolates

| Treatments | | Phosphorus released (mg 100 ml$^{-1}$) | | |
|---|---|---|---|---|
| | | *Pseudomonas aeruginosa* PS 2 | *Pseudomonas aeruginosa* PS 3 | *Pseudomonas striata* (Std) |
| pH | 6 | 22.30$^{i}$ | 21.18$^{i}$ | 19.89$^{i}$ |
| | 6.5 | 38.83$^{g}$ | 40.55$^{g}$ | 40.25$^{g}$ |
| | 7 | 68.47$^{a}$ | 64.58$^{b}$ | 50.99$^{e}$ |
| | 7.25 | 66.27$^{ab}$ | 57.68$^{d}$ | 46.62$^{f}$ |
| | 7.5 | 61.56$^{c}$ | 50.23$^{e}$ | 34.49$^{efg}$ |
| Temperature | 25 | 32.17$^{h}$ | 37.94$^{ef}$ | 36.36$^{fgh}$ |
| | 27.5 | 46.53$^{d}$ | 40.24$^{e}$ | 33.45$^{gh}$ |
| | 30 | 68.08$^{a}$ | 65.27$^{ab}$ | 53.18$^{c}$ |
| | 32.5 | 63.69$^{b}$ | 56.95$^{c}$ | 48.58$^{d}$ |
| | 35 | 46.80$^{d}$ | 54.77$^{c}$ | 36.45$^{efg}$ |
| Carbon sources | Glucose | 67.81$^{a}$ | 56.77$^{b}$ | 51.87$^{b}$ |
| | Fructose | 52.42$^{b}$ | 43.87$^{c}$ | 46.99$^{c}$ |
| | Mannitol | 36.03$^{d}$ | 28.48$^{e}$ | 25.86$^{e}$ |
| Nitrogen sources | Ammonium sulphate | 69.34$^{a}$ | 62.30$^{b}$ | 53.94$^{c}$ |
| | Potassium nitrate | 44.39$^{d}$ | 10.21$^{g}$ | 23.70$^{f}$ |
| | Urea | 32.09$^{e}$ | 61.68$^{b}$ | 45.37$^{d}$ |
| Salt concentration (g%) | 0.1 | 34.24$^{g}$ | 36.91$^{g}$ | 50.46$^{ef}$ |
| | 0.2 | 57.03$^{bcd}$ | 45.02$^{f}$ | 45.17$^{f}$ |
| | 0.3 | 68.13$^{a}$ | 62.62$^{ab}$ | 52.22$^{de}$ |
| | 0.4 | 55.98$^{cd}$ | 60.57$^{bc}$ | 23.08$^{g}$ |
| | 0.5 | 54.04$^{de}$ | 52.06$^{de}$ | 18.64$^{g}$ |

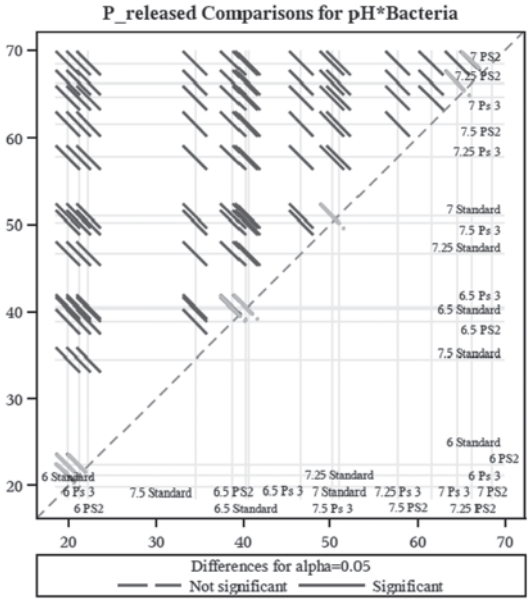
*The mean values with a common letter in the superscript within each treatment do not differ significantly at 5% level of significance.

phytopathogens (Thomas and Sekhar 2016). Sasirekha and Srividya (2016) reported the isolation of *Pseudomonas aeruginosa* from chilli rhizosphere. About 140 strains of *Pseudomonas*, comprising of 55 strains of *P. aeruginosa*, 26 of *P. cepacia*¸ 37 of *P. fluorescens* and 22 strains of *P. putida* were isolated from potato rhizosphere at Dehradun, India (Deshwal et al., 2013). Nineteen endophytic *P. aeruginosa* strains were isolated from red fruit of chilli by Sudhir et al. (2014). *P. aeruginosa* 7NSK2 isolated from maize rhizosphere acts as biocontrol agent against *R. solanacearum* causing bacterial wilt in eucalyptus (Ran et al., 2005).

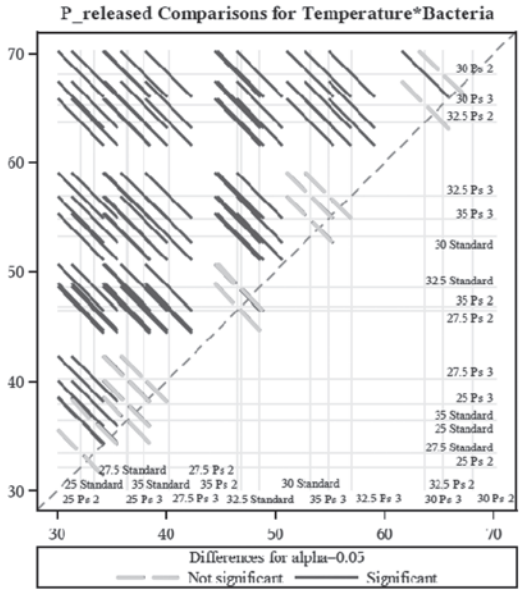
*Standardization of conditions for efficient TCP solubilization*

The impact of different carbon and nitrogen sources, pH, temperature and salt concentration on the P solubilization activity of the isolates was studied (Table 2 & Figure 2). The carbon sources of the media used were glucose, fructose and mannitol, and the isolates showed maximum P solubilization rate when glucose was used as the carbon source. In the case of nitrogen sources ammonium sulphate was proved to be the best one. Maximum P solubilization rate was shown when pH 7 and temperature of 30°C were maintained in the culture medium. Among the different salt concentrations analyzed, 0.3% proved to be the best one. Maximum activity was shown after 15 days of incubation; after that the rate of P solubilization declined. This is in accordance with the findings of Stephen and Jisha (2011) who reported optimum pH, temperature, and salt concentration for phosphate solubilizing *Burkholderia* sp. as 7, 32.5º C, and 0.5 to 1% respectively.

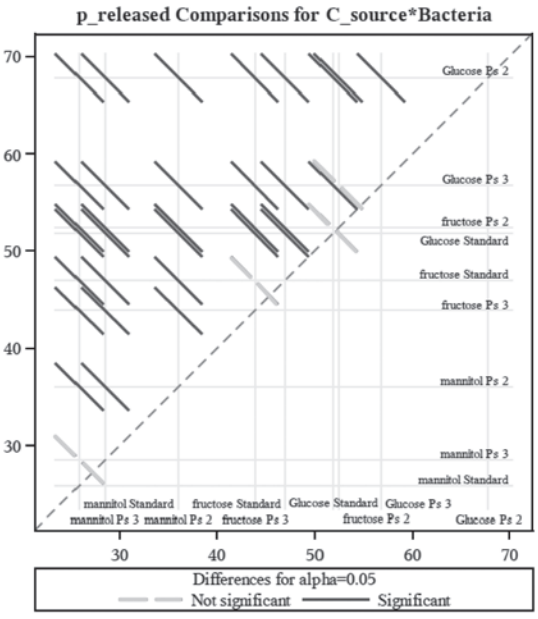
All microbial activities are sensitive to environmental temperature. Each isolate has a



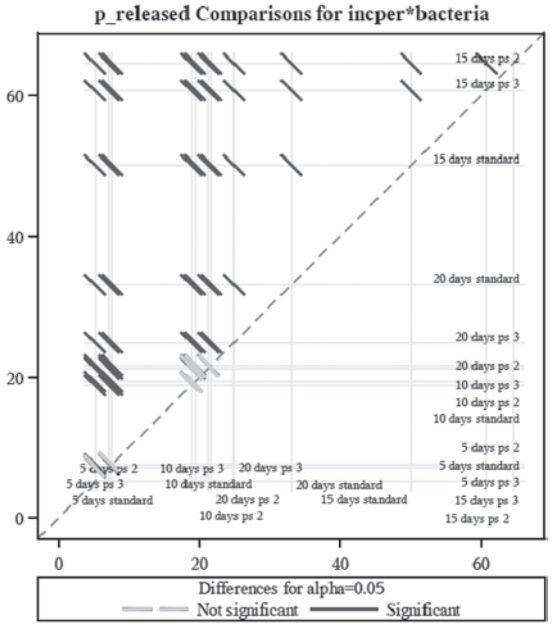

a). pH




b) Temperature




c) Carbon source




d) Incubation period (days)

*Figure 2.* Diffogram on the impact of different factors on the TCP solubilization by PSB

minimum, optimum and maximum temperature. Deepshikha et al., (2014) reported pH 7, temperature of 28° C and incubation time of 72 hrs as optimum for phosphate solubilizing fluorescent *Pseudomonas* isolated from normal and replant sites of apple and pear. Jena and Chandi (2013) also reported 72 hrs as best for maximum P solubilization by *Pseudomonas* isolates. In the study carried out by Kumar and Kumar (2013), *Pseudomonas* strains produced optimum P solubilization in presence of

0 to 1.25% NaCl. Ashish et al., (2014) showed pH 7 as optimum for phosphate solubilization by *Bacillus* spp NPSBS 3.2.2 obtained from the cotton plant rhizosphere. Zhu et al., (2011) and Sahu et al., (2007) also reported that pH 7 was the most suitable for phosphate solubilization activity by isolates *Kushneria* sp. and *Streptomyces gallus* PS3 respectively.

In the work carried out by Ashish et al. (2014) sucrose proved to be the best C source followed by dextrose and starch, and least activity was in the presence of mannitol. Similarly in the present study, lowest level of P solubilization was obtained with mannitol as C source. Srividya et al., (2009) stated that the isolate DASA 68056 had the highest phosphate solubilization efficiency when sucrose was used as carbon source. Ashish et al., (2014) and Kumari and Gupta (2013) testified ammonium sulphate as the best nitrogen source.

*High Performance Liquid Chromatography (HPLC) analysis of organic acid production by the isolates*

HPLC analysis showed the presence of organic acids in the culture media of both the isolates PS 2 and PS 3 (Figure 3). Culture supernatant of Pseudomonas aeruginosa PS 2 showed a major peak at retention time RT: 4.145 and Pseudomonas aeruginosa PS 3 at retention time RT: 4.524. The results were compared with the retention time of the standard gluconic acid (RT: 4.651). From the result it is clear that both the isolates produce gluconic acid which is a major mechanism of phosphate solubilization. The minor peaks present in the HPLC profile may be due to the presence of minor organic acids or impurities.

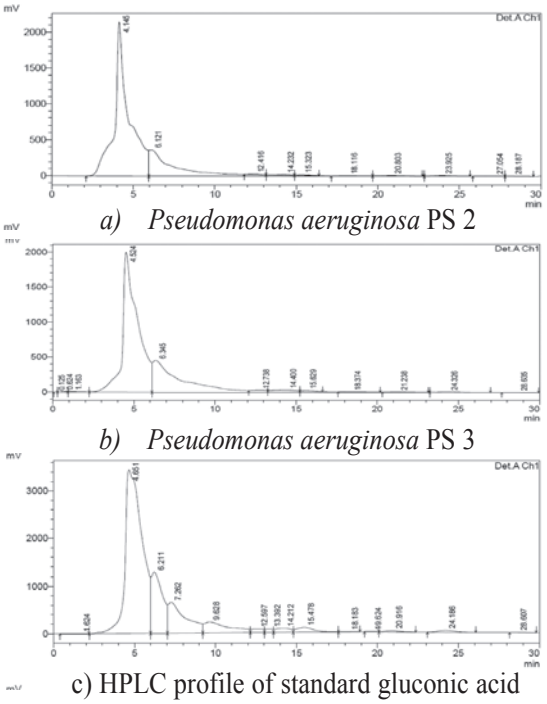



*Figure 3*. Organic acid production profile of selected isolates

It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids (Jisha and Stephan, 2011) which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Sagoe et al., 1998). However, P-solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture. Organic acid production results in acidification of the microbial cell and its surroundings. As a result P may be released from a mineral phosphate by proton substitution for $Ca^{2+}$ (Goldstein, 1994).

*Table 3*. Vigour index of rice seeds treated with selected phosphate solubilising microorganisms

| | Root length (cm) | Shoot Length (cm) | Root Dry weight (g) | Shoot dry weight (g) | Germination percentage (%) | Vigor index (VI) |
|---|---|---|---|---|---|---|
| PS2 | 21.28±0.4 | 14.34 ± 0.05 | 0.091 ± 0.05 | 1.44±0.24 | 90.00 | 1590.00 |
| PS3 | 16.25 ± 0.18 | 9.57± 0.54 | 0.085±0.18 | 0.97 ± 0.4 | 76.67 | 990.83 |
| Control | 11.4±0.4 | 7.69 ± 0.4 | 0.0 54 ± 0. 4 | 0.84± 0.01 | 85.00 | 646.23 |

* Values are mean ± SD of three replications

Gluconic acid is one of the prominent organic acids responsible for P solubilization and is produced by direct oxidation of glucose, and the resultant pH change and reduction potential are thought to be responsible for the dissolution of tricalcium phosphate in the culture medium (Chen et al., 2006). Stephen and Jisha (2011) also reported gluconic acid production by phosphate solubilizing *Burkholderia* spp.

*Growth promotion of paddy seeds*

Induction of seedling vigour by beneficial microorganisms has been considered as an easy way of screening then for seed or soil application. Results of the growth promotion study are given in table 3 and plate 1. The selected isolates induce germination of paddy seeds. Among the isolates PS 2 was found to be the best isolate and seeds treated with this isolate showed 90% germination. The vigor index calculation also supports the results and recorded a vigor index of 159.00 of seeds treated with the isolate PS 2. High seedling vigor plays a major role in plant growth promotion and biocontrol activities in indigenous rhizospheric bacterial isolates (Chandrasekhara et al., 2007).

It is well established that phosphatic fertilizers are essential inputs for harnessing the yield potential of high yielding varieties in intensive and multiple cropping systems. Hence it is worthwhile to isolate and screen efficient phosphate solubilizing bacteria and to work out the optimum factors for maximum solubilization of insoluble inorganic phosphates. Here seventeen phosphate solubilizing bacteria were isolated from chilli fields. Among them two isolates showed promising results on phosphate solubilization in solid and liquid medium. Conclusively, PSM are likely to serve as an efficient bio-fertilizer especially in areas deficient in P to increase the overall performance of crops.

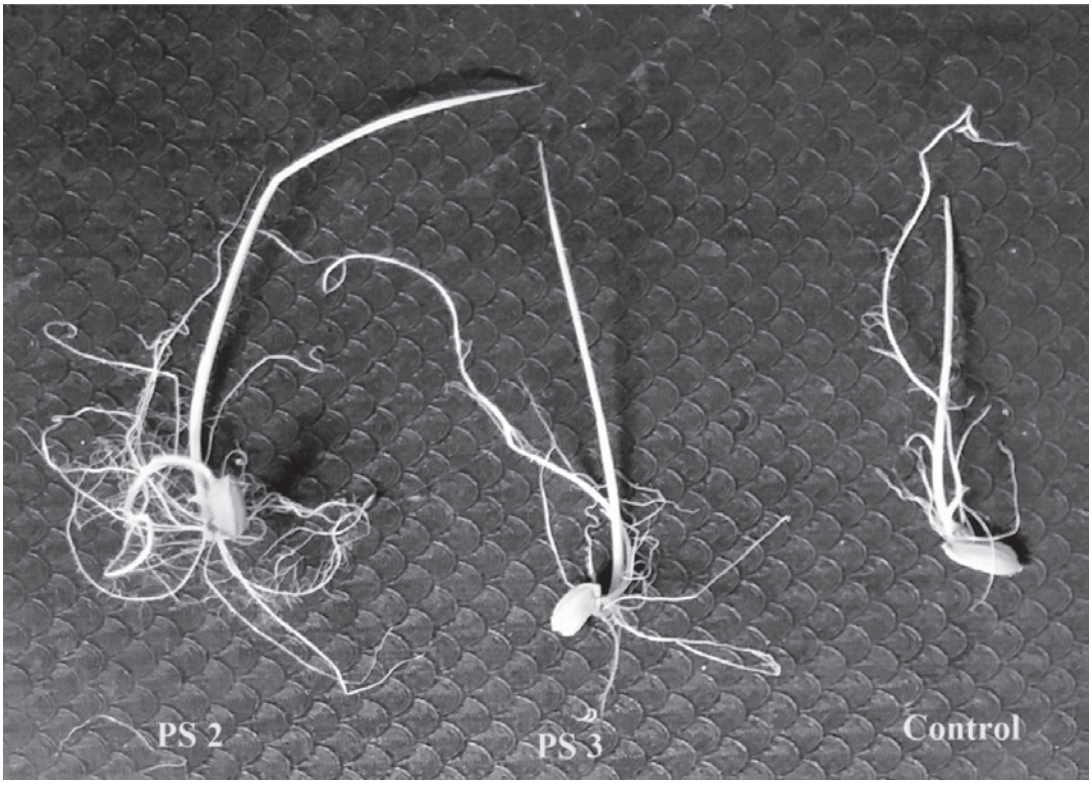



*Plate 1.* Germination of paddy seeds treated with selected phosphate solubilising bacteria

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