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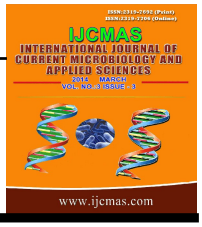

Original Research Article

Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil

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A B S T R A C T

Soil Microorganisms play a role in maintaining the ecological balance by active participation in Carbon, Nitrogen, Sulphur and Phosphorous cycles in nature. Phosphate Solubilizing Microbes plays an important role in plant nutrition through increase in phosphate uptake by plants and used as biofertilizers of agricultural crops. Phosphate is one of the most vital macronutrient required for the growth and development of plants. A large number of microorganisms present in the rhizosphere are known to solubilize and make available the insoluble phosphorus in the available form to the plants. A total of 37 Phosphate Solubilizing Microbial colonies were isolated on the Pikovskaya's agar medium, containing insoluble tri-calcium phosphate (TCP) from agricultural soil. The colonies showing clear halo zones around the microbial growth were considered as phosphate solubilization. Out of 37 microbial isolates 8 isolates showed highest Phosphate Solubilization Index (PSI) ranged from 1.13 - 3.0 were selected for further study as qualitative as well as quantitative activities. Among these 8 potent isolates, 3 strains showed maximum PSI of psm1, psm2 and psm6 in agar plates along with high soluble phosphate production of 0.37 mgL^{-1}, 0.30 mgL^{-1} and 0.28 mgL^{-1} in broth culture. These isolates psm1, psm2 and psm6 belongs to genus *Pseudomonas, Bacillus* and *Rhizobium* as identified by their morphological and biochemical properties respectively.

Keywords

Soil phosphorus; solubilisation; phosphate solubilizer; organic acids; *Bacillus; Pseudomonas; Aspergillus.*

Introduction

Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available for root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Ahmad Ali Khan *et al*., 2009). However, a greater part of soil phosphorous, approximately 95-99% is present in the form of insoluble phosphates and hence cannot be utilized

by the plants (Kannapiran and Sri Ramkumar, 2011). Phosphorus plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. It helps plants to survive winter rigors and also contributes to disease resistance in some plants (Amit Sagervanshi *et al*., 2012). P availability is low in soils because of its fixation as insoluble phosphates of iron, aluminium and calcium. Since deficiency of P is the most important chemical factor restricting plant growth, chemical phosphatic fertilizers are widely used to achieve optimum yields. Soluble forms of P fertilizer used are easily precipitated as insoluble forms, this leads to excessive and repeated application of P fertilizer to cropland (Sadia Alam *et al*., 2002).

Phosphorus is a plant macronutrient that plays a significant role in plant metabolism, ultimately reflected on crop yields. It is important for the functioning of key enzymes that regulate the metabolic pathways. It is estimated that about 98% of Indian soils contain insufficient amounts of available phosphorus, which is necessary to support maximum plant growth. The uptake of phosphorus by the plant is only a small fraction of what is actually added as phosphate fertilizer. Phosphorus deficiency is widespread and phosphorus fertilizers are required to maintain crop production. When it is added to the soil in the form of phosphatic fertilizer, only a small portion is utilized by plants (Padmavathi Tallapragada, 2010). Phosphate fertilizers can also be used to immobilize heavy metals in soil. Insoluble phosphate compounds can be solubilized by organic acids and phosphatase enzymes produced by plants and microorganisms (Jinhee Park *et al.,* 2010). Application of biological fertilizers such as biological phosphate fertilizers improves soil fertility (Abdol Amir Yousefi *et al.,*2011*).* Phosphorus can naturally be found in diverse forms in the soil solution. The roots take up several forms of phosphorus, out of which the greatest part is absorbed in the forms of H_2PO_4 and HPO_4 2- depending upon soil pH. The degree of fixation and precipitation of phosphorus in soil is highly dependent upon the soil conditions such as pH, moisture content, temperature and the minerals already present in the soil (Buddhi Charana Walpola, 2012).

Mechanism

Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively. Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms. Phosphate solubilization takes place through various microbial processes/mechanisms including organic acid production and proton extrusion. A wide range of microbial P solubilization mechanisms exist in nature and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi (Ahmad Ali Khan *et al.,* 2009). Phosphobacteria have been found to produce some organic acids such as monocarboxylic acid (acetic, formic), monocarboxylic hydroxy (lactic, glucenic, glycolic), monocarboxylic, ketoglucenic, decarboxylic (oxalic, succinic), dicarboxylic hydroxy (malic, maleic) and tricarboxylic hydroxy (citric) acids in order to solubilize inorganic phosphate compounds.

A diverse group of soil microflora was reported to be involved in solubilizing insoluble phosphorous complexes enabling plants to easily absorb phosphorous. Several fungal and bacterial species, popularly called as PSMs assist plants in mobilization of insoluble forms of phosphate. PSMs include different groups of microorganisms, which not only assimilate phosphorus from insoluble forms of phosphates, but they also cause a large portion of soluble phosphates to be released in quantities in excess of their requirements. Species of *Aspergillus* and *Penicillium* are among fungal isolates identified to have phosphate solubilizing capabilities. Among then bacterial genera with this capability are *Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Alcaligenes, Serratia, Enterobacter, Acinetobacter, Flavobacterium and Erwinia.*

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. Among the bacterial genera with this capacity are Pseudomonas, *Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium and Erwinia.* There are considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizosphere.

Effect of PSMs on growth, yield and phosphorus economy

Inoculation of plants with PSMs generally results in improved plant growth and yield, in particular, under glasshouse conditions (Khan *et al.,* 2010; Zaidi *et al.,* 2009). More importantly, investigations conducted under field level using wheat and maize plants have revealed that PSMs could drastically reduce the usage of chemical or organic fertilizers (Singh and Reddy, 2011). As reported by Vessey and Heisinger (2001), enhancement of plant phosphorus nutrition might be due to stimulation of root growth or elongation of root hairs by specific microorganisms, thus no direct increase in the availability of soil phosphorus is always expected. PSMs have been isolated from soil of various plants such as walnut (XuanYu, 2011), rice (Chaiharn and Lumyong, 2009), mustard (Chandra *et al.,* 2007), oil palm (Fankem et al., 2006), soybean (Son *et al.,* 2006), aubergine and chili (Ponmurugan and Gopi, 2006), and maize (Alam *et al.,*2002). Better crop performance was reported to beachieved from several horticultural plants and vegetables, which were successfully inoculated with PSB (Young etal., 2003). Phosphorus use efficiency in agricultural lands could effectively be improved through the inoculation ofrelevant PSMs, which is in fact, an integrated and sustainable mean of nutrient management of crop production systems.

Apart from phosphate solubilizing abilities, some of these microorganisms can benefit plant growth by several different mechanisms such as enhancing nitrogen fixation, plant hormone production etc. Although phosphorus PSMs are abundant in many of the soils, isolation, identification and selection of PSMs have not as yet been successfully commercialized, thus application is still found to be limited. Investigations on the subject are often designed to confirm a specific response of PSMs to a particular environment, thus large scale application infield level is still limited.

Materials and Methods

Collection of sample

The soil samples were collected from depth of 6-15cm from the agricultural land. Soil samples were collected from rhizosphere of tomato plant. Collected soil samples were stored in polythene bags aseptically and maintained at the laboratory for further study.

Isolation of Strains

For isolation of Phosphate Solubilizing Microbes, 1g rhizosphere soil was suspended in 100ml of distilled water. An aliquot (100µl) from decimal dilutions was inoculated on Picovskaya's medium by pour plate technique and incubated at 30^{o}C. Colonies showing phosphate solubilizing zone around them were considered as PSM. Single colonies appearing on Picovskaya's agar plates were transferred in liquid broth of Picovskaya's and on agar slants for further study.

Identification of Microbes

For identification of fungi, a drop of lactophenol cotton blue placed on glass slide and observed under microscope. The isolated bacteria was identified by morphological characteristic in which gram staining, shapes, IMViC test and motility including catalase, oxidase test, sucrose, lactose fermentation, starch hydrolysis, Gelatin hydrolysis and Nitrate reduction.

Analysis of Phosphate Solubilizing Activity

From the isolates, larger halo zone producing strains were selected for further study. The qualitative as well as quantitative analyses of phosphate solubilizing activity of the selected isolates were conducted by plate screening method and broth culture method.

Qualitative method

All the suspected colonies were screened for phosphate solubilization on Pikovskayas medium. Isolates showing phosphate solubilizing ability were spot inoculated at the centre Pikovskaya's plate and incubated at 37^{0}C.Diameter of clearance zone was measured successively after 24 hours, up to 7 days. The Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter .i.e. clearance zone including bacterial growth and the colony diameter.

$$PSI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

All the observations were recorded in triplicate. Strains developing clear zones around their colonies could easily identify as PSM.

Quantitative method

Pikovskaya's broth medium (100 ml) with Tricalcium phosphate (0.3g/100ml) was prepared and sterilized; 1ml of each isolates was inoculated into the broth medium. Then the inoculated sample were incubated for 5 days on rotatory shaker 37^{0}C after incubation, culture broth was centrifuged at 10,000rpm for 30min. Uninoculated broth served as control. The available Phosphorous was determined using colorimetrically at 410nm with standard KH_2PO_4.

Optimization of physiological conditions (Temperature and pH)

The phosphate solubilization efficiency (PSE) of the isolates was studied on

Pikovskaya agar and (pH 7.0) and incubation temperature 25ºC,30ºC,35ºC, 40ºC and 45ºC and Pikovskaya agar adjusted at different pH values 5,6,7,8, and 9,with incubation temperature 28ºC.

Effect of PSB on growing Tomato (*Solanum lycopersicum*)

The following treatments with tomato seeds as follows,

1. Control soil
2. Control soil + Zinc phosphate
3. Control soil + psm1
4. Control soil + psm2
5. Control soil + psm6

All the treated seeds were sown in sterilized soil within the polythene bag. After 10 days of sowing, the growth of tomato plant was noted.

Results and Discussion

Isolation and Identification of PSM

The collected soil samples were evaluated for Phosphate solubilizing microbe in Pikovskaya's agar medium. A total of 37 Phosphate Solubilizing Microbial colonies were isolated on the Pikovskaya's agar medium, containing insoluble tri-calcium phosphate (TCP) from agricultural soil. Out of 37 microbial isolates 6 isolates showed highest Phosphate Solubilization Index (PSI) ranged from 1.13 - 2.23 were selected for further studies. The colonies showing clear halo zones around the microbial growth were considered as phosphate solubilization were shown in the photo 1.

The bacterial isolates were further characterized by a series of biochemical reaction and identified as *Azotobacter sp., Bacillus sp., Pseudomonas sp*., and *Rhizobium*. The fungal isolates were identified as *Aspergillus* by LPCB mount. The morphological and biochemical characteristics of these isolates were shown in the Table 1.

Analysis of phosphate solubilizing activity

From the isolates, larger halo zone producing strains were selected for further study. The qualitative as well as quantitative analyses of phosphate solubilizing activity of the selected isolates were conducted by plate screening method and broth culture method.

Qualitative method

All the selected isolates were found to be potent phosphate solubilizers showing clear zone around their colonies. Out of 37 microbial isolates 6 isolates showed highest Phosphate Solubilization Index (PSI) ranged from 1.13 - 2.23 were selected for further studies .Among these 6 potent isolates, 3 strains showed maximum PSI of psm1, psm2 and psm6 in agar plates. The PSI measurement was shown in the table 2.

Quantitative method

Pikovskaya's broth medium (100 ml) with Tricalcium phosphate (0.3g/100ml) was prepared and sterilized; 1ml of each isolates was inoculated into the broth medium. It was observed that psm1 (*Pseudomonas sp*.), psm2 (*Bacillus sp*.) and psm6 (*Rhizobium s*p.) showed highest percent P solubilization. The results were showed in the Table 3 and figure 1.

Optimization of physiological conditions

The phosphate solubilization efficiency of these isolates were analysis using various

parameters such as pH (5-9), temperature (30ºC - 45ºC). The results were shown in the table 4, figure 2 and table 5, figure 3.

Effect of PSB on growing Tomato (*Solanum lycopersicum*)

In this study comparative analysis of vegetative and reproductive plant growth patterns of tomato from germination of seeds to maturation of plant in polythene bag experiment were done. Each containing control soil, control soil with Zinc Phosphate and psm1, psm2 and psm6 with control soil. Crop development data were collected on an average interval of 10-23 days from the sowing of seeds. Several parameters related to vegetative as well as reproductive plant growth patterns i.e., date of sowing, date of germination, number of plants germinated, number of leaves, length of leaves, colour of leaves, length of plants etc. were recorded for comparative evaluation in different combinations. Initially the best growth was recorded for the plant growing in control with strain psm1 and psm2, followed by control with zinc phosphate which was shown in the table 6.

In the present study, the collected soil samples were evaluated for P solubilizing in PKV plates. Phosphate solubilizing microbes are detected by the formation of clear halos around their colonies. The halo is produced due to solubilization of insoluble phosphates, which in turn is mediated via the production of organic acid in the surrounding medium (Gaur,1990). A total of 37 Phosphate Solubilizing Microbial colonies were isolated on the Pikovskaya's agar medium, containing insoluble tri-calcium phosphate (TCP) from agricultural soil. Out of 37 microbial isolates 6 isolates showed highest Phosphate Solubilization Index (PSI) ranged from 1.13 - 2.23 were selected for further studies. Among these 6 potent isolates, 3 strains showed maximum PSI of psm1, psm2 and psm6 in agar plates as 2.23, 2.15 and 2.11.These isolates were identified as genus *Pseudomonas* sp., *Bacillus* sp., and *Rhizobium.* These bacteria were well known identified as phosphate solubilizer by Hilda Rodríguez and Reynaldo Fraga, 1999.

The solubilization levels varied with different isolates, all the potent isolates were capable of solubilizing tricalcium phosphate in broth medium. It was observed that isolate psm1 (0.864 U/ml) and psm2 (0.786 U/ml) showed highest percent P solubilization when compared to other isolates. Tripti *et al.*, 2012, showed that isolates S2 (*Pseudomonas sp.)* and S30 (*Bacillus sp.*) showed highest P Solubilization. Our findings are very well supported by Gupta *et al.*, 2002, that *Pseudomonas* sp. is a potent phosphorus solubilizer.

To analyze whether bacteria can grow in a range of pH 5 to 9 and to test their ability to change the pH value of the medium, all the potent strains were inoculated separately. Optical density of culture medium revealed that rapid growth at pH 5 and 7.These changes were well documented by Rodriguez and Fraga, 1999 and Md.T.Islam *et al.*, 2007. Nautiyala *et al*., (1999) reported that the strains isolated from alkaline soil have the potential to solubilize phosphates at high

Table.1 Morphological and Biochemical characteristics of the isolates

S.No	Characteristics	Psm1 P*	Psm2 B*	Psm6 R*	Psm4 B*	Psm5 A*
1	Gram reaction	G-ve	G +ve	G -ve	G +ve	G -ve
2	Shape	Rods	Rods	Rods	Rods	Rods
3	Pigments	+/-	-	+/-	-	+/-
4	H_2S production	+	-	+	-	-
5	Indole	-	-	+	-	+
6	Methyl red	-	-	+	-	+
7	Vogues Proskauer	-	+	+	+	+
8	Citrate utilization	+	+	-	+	+
9	Nitrate reduction	+	+	+	+	+
10	Starch hydrolysis	+	+	+	+	+
11	Gelatin Hydrolysis	-	+	-	+	-
12	Lactose fermentation	-	+	+	+	+
13	Sucrose fermentation	+	+	+	+	+
14	Mannitol fermentation	-	+	+	+	+

*****P=*Pseudomonas,* B=*Bacillus,* R=*Rhizobium,* A=*Azotobacter*

Table.2 Phosphate Solubilizing Activities of Six most P solubilizing activities

S.No	Isolates of PSM	Colony measurement(cm)	Zone measurement(cm)	Solubilization Index(SI)*
1	Psm1(*Pseudomonas sp*)	1.3	1.6	2.23
2	Psm2(*Bacillus sp.*)	2.0	2.3	2.15
3	Psm3(*Aspergillus*)	0.6	0.6	2.0
4	Psm4(*Bacillus sp.*)	1.2	1.3	2.08
5	Psm5(*Azotobacter)*	1.2	1.3	2.08
6	Psm6(*Rhizobium*)	1.9	2.1	2.11

***SI**= (Colony diameter + Halo zone)/colony diameter

Table.3 Phosphate Solubilizing Activities of Six most P solubilizing activities

S.No	Isolates of PSM	Soluble P concentration (mg/l)
1	Psm1 (*Pseudomonas sp*)	0.864
2	Psm2 (*Bacillus sp.*)	0.786
3	Psm3 (*Aspergillus*)	0.561
4	Psm4 (*Bacillus sp.*)	0.612
5	Psm5 (*Azotobacter)*	0.593
6	Psm6 (*Rhizobium*)	0.686

Figure.1 Phosphate Solubilizing Activities of Six most P solubilizing activities

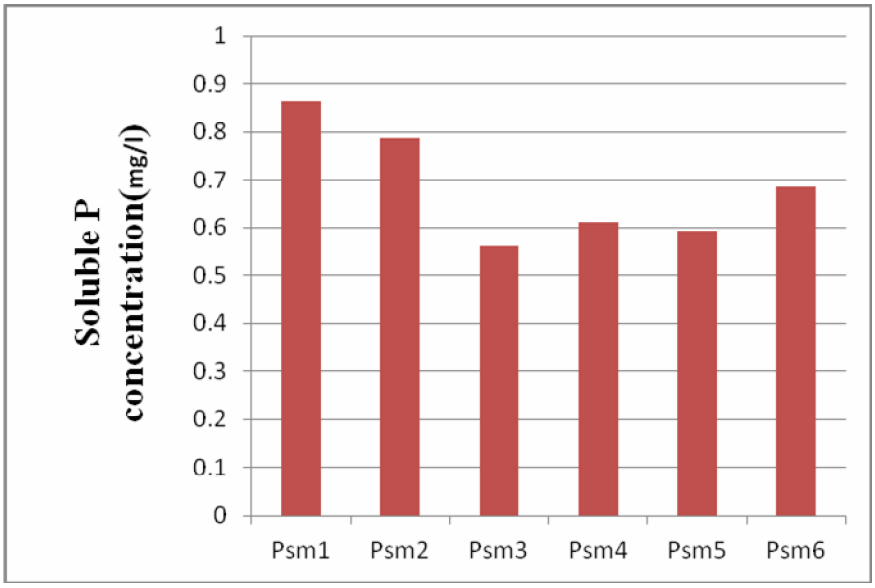

Table.4 Optimization of pH on Phosphate solubilisation

S.No	pH Range	Phosphate solubilization(mg/l) (Mean of triplicate values)		
		Psm1	**Psm2**	**Psm6**
1	5	0.20	0.19	0.21
2	6	0.25	0.20	0.26
3	7	0.38	0.30	0.29
4	8	0.34	0.25	0.24
5	9	0.28	0.23	0.22

Figure.2 Optimization of pH on Phosphate solubilisation

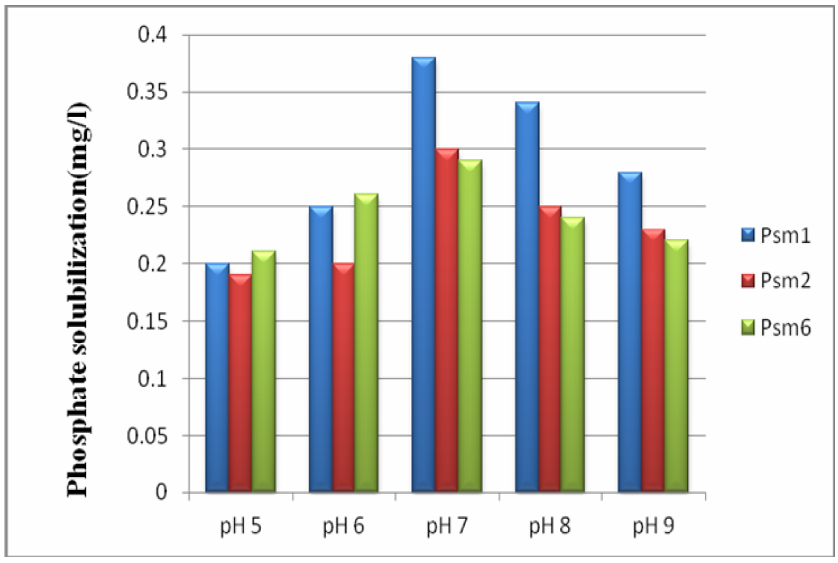

Table.5 Optimization of Temperature on Phosphate solubilization

S.No	Temperature (ºC)	Phosphate solubilization(mg/l) (Mean of triplicate values)		
		Psm1	Psm2	Psm6
1	25	0.25	0.20	0.22
2	30	0.26	0.25	0.23
3	35	0.33	0.30	0.26
4	40	0.36	0.33	0.28
5	45	0.31	0.28	0.24

Figure.3 Optimization of temperature on Phosphate solubilization

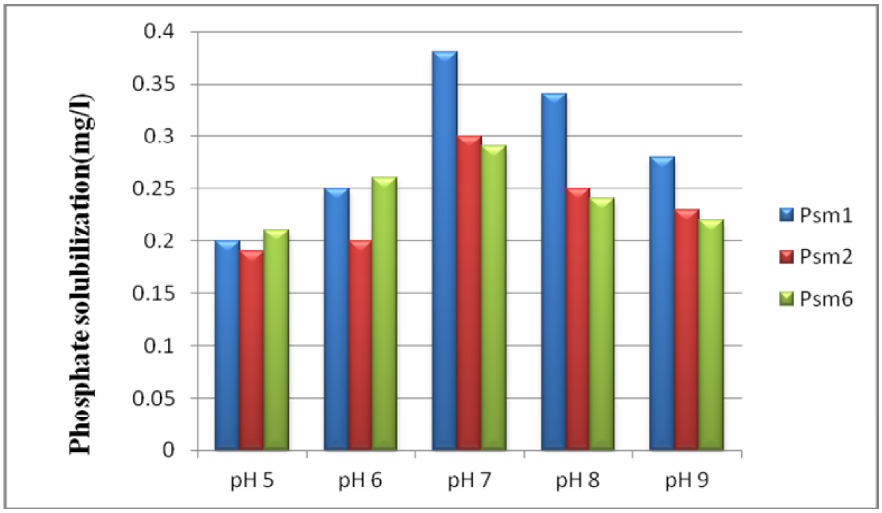

Table.6 Effect of selected microbial isolates on Tomato

S.No	Nature of soil	Date of sowing	Date of Germination	No. of plant germinated	No. of leaves/plant	Leaves length (cm)	Colour of leaves	Plant Length (cm)	Inflores cence	No. of fruits
1	Control	15.02.2013	25.02.2013	4	4	2.4	Green	10	-	-
2	Control + Zinc phosphate	15.02.2013	25.02.2013	4	4	2.0	Green	8.5	-	-
3	Control+ Psm1	15.02.2013	25.02.2013	4	4	2.0	Deep green	8.0	-	-
4	Control+ Psm2	15.02.2013	25.02.2013	3	3	1.7	Deep green	6.5	-	-
5	Control+ Psm6	15.02.2013	25.02.2013	3	3	1.5	Deep green	5.4	-	-

salt, high pH and high temperature concentration. The phosphate solubilization of all potent strain were optimized for temperature (30ºC - 45ºC), in which growth seen in between 35ºC and 40° C in which our findings were similar to Uma Maheswar and Sathiyavani, (2012).

In this study comparative analysis of vegetative and reproductive plant growth patterns of tomato from germination of seeds to maturation of plant in plastic bag experiment were done. Each containing control soil, control soil with Zinc Phosphate and psm1, psm2 and psm6 with control soil. In this study, these combinations were used for the evaluation of comparative growth patterns of plants. Initially the best growth was recorded for the plant growing in control with strain psm1 and psm2, followed by control with zinc phosphate.

Many phosphate solubilizing bacteria are reported as plant growth promoter (Hafeez 2004; Katiyar and Goel., 2003; Hilda Rodríguez and Fraga, 1999). It is well established that introduction of plant growth promoting bacteria (PGPB) in soil improves the plant growth. PGPB promote plant growth through the production of plant growth hormones (Patten and Glick, 2002; Bottini *et al.,* 2004). Present study was conducted to screen the potential PGPRs and their use as a biofertilizer. psm1 , psm2 and psm6 were selected for pot scale trial because of their positive effect on the germination of tomato, their efficient phosphate solubilization abilities on plate assay as well as their release of free P in liquid culture and their auxin production abilities to enhance the growth of three different crops.

All three PGPRs, improves plant growth by various mechanisms which could not be mutually exclusive. It is likely that phosphate solubilizing strains might have helped in plant root proliferation and production of plant growth regulators by the bacteria at root interface which resulted in better water absorption and nutrients such as P by host plant (Barea *et al.,* 2005; Gupta *et al.,* 2002). The above experiments were carried out in complete absence of any synthetic plant growth regulators. Increase in shoot and root length of the tested plants may be attributed to the production of growth promoting substances.

Control experiment revealed that deficiency of available phosphate retard plant growth in various parameters such as root and shoot length of rye as compared to test. From these results we conclude that inoculation of tomato with efficient PGPRs significantly enhance plant growth in small scale experiment. The pronounced plant growth by PGPRs observed in the present study can be attributed to the production of IAA, IBA and solubilization of phosphate. Such findings are in agreement with many authors who reported phytohormones production by *Pseudomonas* (Glick, 1995).

In conclusion, it is attractive to speculate that coordination of above mentioned mechanisms may act to stimulate plant growth. Because of their efficient phosphate solubilization and auxin production ability, it is justifiable to propose here that these bacterial strains have plant growth potentials and could be exploited as biofertilizers or bioinoculant. Greater attention should be paid to studies and application of new combination of phosphate solubilising bacteria and other PGPR for improved results.

References

Abdol Amir Yousefi, Kazem Khavazi, Abdol Amir Moezi, Farhad Rejali and HabibAllahNadian.2011. hosphate Solubilizing Bacteria and Arbuscular Mycorrhiza Fungi Impacts on Inorganic Phosphorus Fractions and Wheat Growth. *World Applied Sciences Journal*. 15 9: 1310-1318.

Aftab Afzal and Asghari Bano.2008. Rhizhobium and Phosphate Solubilizing Bacteria improve the yield and phosphours uptake in Wheat *Triticum aestivum*. *International Journal of Agriculture and Biology.*101:85-88.

Aftab Afzal, M, Ashraf, Saeed, A Asad and Farooq, M .2005.Effect of phosphate solubilizing microorganism on phosphorus uptake, yield traits of wheat *Triticum aestivum L..International Journal of Agriculture and Biology*.72:207-209

Ahmad Ali Khan, Ghulam Jilani, Mohammad Saleem Akhtar, Syed Muhammad Saqlan Naqvi, Mohammad Rasheed.2009. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Journal of Agricultural Biological science.* 11:48-58.

Alvaro Peix, Rau´ Rivas, Ignacio Santa-Regina, Pedro F. Mateos, Eustoquio Martı´nez-Molina, Claudino Rodrı´guez-Barrueco and Encarna Vela´zquez.2004.*Pseudomonas lutea* sp. nov., a novel phosphate-solubilizing bacterium isolated from the rhizosphere of grasses. *International Journal of Systematic and Evolutionary Microbiology*. 54: 847–850.

Amardip Singh, Poonam and Ghosh, A.K.2012. Screening and Assessment of Phosphate Solubilising Microbes as Potential Biofertilizer, isolated from Ecosystem of Bihar. *Asian Journal of Experimental Biological Science* .32:397-406.

Amit Sagervanshi, Priyanka Kumari, Anju Nagee and Ashwani Kumar.2012. Isolation and Characterization Of Phosphate Solublizing Bacteria from Anand Agriculture Soil.*International Journal of Life Sciences and Pharma Research. 23:*256-266.

Barea, J., M. J. Pozo, R. Azcon and C. Azcon-Aguilar.2005. Microbial co-operation in the rhizosphere *J. Exp. Bot*. 56: 1761 - 1778.

Bottini, R., F. Cassán and P. Piccoli. 2004. Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Appl. Microbiol. Biotechnol*. 65:497-503.

Buddhi Charana Walpola and Min-Ho Yoon.2012. Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: A review.*African Journal of Microbiology Research.* 637: 6600-6605.

Federica Tamburini, Verena Pfahler, Else K. Bünemann, Kathi Guelland, Stefano M. Bernasconi and Emmanuel Frossard.2012.Oxygen Isotopes Unravel the Role of Microorganisms in Phosphate Cycling in Soils. *Environmental Science and Technology*.46:5956-5962.

Fengling Zhu, Lingyun Qu, Xuguang Hong, and Xiuqin Sun. 2011.Isolation and characterization of a phosphate-solubilizing Halophilic Bacterium Kushneria sp.YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. *Evidence-based Complementary Alternative Medicine*.1-6.

Gaur, A.C. 1990. Phosphate solubilizing microorganisms as biofertilizers. *Omega Scientific Publishers, New Delhi, India*.5761: 114.

Glick, B.R., 1995. The enhancement of plant growth by free living bacteria. *Can. J. Microbiol.* 41:109–117.

Gupta,A., J.M.Meyer and R.Goel.2002.Development of Heavy

metal- resistant Mutants of phosphate Solubilizing *Pseudomonas* sp. NBRI 4014 and their characterization. *Current Microbiology*.45:323-327.

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

Hafeez, F. Y., M. E. Safdar, A. U. Chaudhry and K. A. Malik 2004. Rhizobial inoculation improves seedling emergence, nutrient uptake and growth of cotton. *Australian Journal of Experimental Agriculture* .446: 617 – 622.

Hassan Shokri Vahed, Parisa Shahinrokhsar and Fatemeh Heydarnezhad.2012. improving growth and yield of rice *Oryza Sativa* L. in the presence of phosphorus fertilizer .*International Journal of Agriculture and Crop Sciences.* 4 17:1228-1232.

Hilda Rodríguez and Reynaldo Fraga.1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*. 17 1999:319–339.

Humaria Yasmin and Asghari Bano.2011.Isolation and Characterization of Phosphate Solubilizing Bacteria from Rhizospheric soil of weeds of Khewra Salt Range and Attock. *Pakistan .J .Bot.,* 433:1663-1668.

Ivanova, R Bojinova,D Nedialkova, K.2006. Rock Phosphate Solubilization by Soil Bacteria. *Journal of the University of Chemical Technology and Metallurgy*. 413: 297-302

Jinhee Park, Nanthi Bolan, Mallavarapu Megharaj, and Ravi Naidu . 2010. Isolation of Phosphate-Solubilizing Bacteria and their Effects Characterization of on Lead Immobilization. *Pedologist*. 67-75.

Katiyar V and R. Goel, 2003. Solubilization of inorganic phosphate and plant growth promotion by cold tolerant mutants of *Pseudomonas fluorescens*. *Microbiol Res* .158:163–168.

Kannapiran, E and Sri Ramkumar, V. 2011. Isolation of phosphate Solubilizing bacteria from sediments of Thondi coast, Palk Strait, Southeast coast of India. *Annals of Biological Research*.25:157-163.

Khosro Mohammadi and Yousef Sohrabi2012. Bacterial Biofertilizers For Susstainable Crop production: A Review.ARPN Journal of Agricultural and biological Science.7:307-316.

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

Leticia Fernández , Betina Agaras , Pablo Zalba ,Luis G. Wall , Claudio Valverde.2011. *Pseudomonas* spp. isolates with high phosphate-mobilizing potential and root colonization properties from agricultural bulk soils under no-till management. *Biol Fertil Soils*. 48:763–773.

Md. Tofazzal Islama, Abhinandan Deoraa, Yasuyuki Hashidokoa, Atiqur Rahmana, Toshiaki Itoa, and Satoshi Taharaa .2007.Isolation and Identification of Potential Phosphate Solubilizing Bacteria from the Rhizoplane of *Oryza sativa* L. cv. BR29 of Bangladesh. *Z. Naturforsch*. 62c, 103-110.

Mohammad Ali Malboobi , Mandana Behbahani , Hamid Madani , Parviz Owlia Ali Deljou , Bagher Yakhchali , Masoud Moradi and Hassan Hassanabadi.2009. Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. *World J Microbiol Biotechnol.*

Padmavathi Tallapragaada, Usha Seshachala 2010. Phosphate-Solubilizing microbes and their occurrence in the rhizospheres of Piper betel in

Karnataka, India. *Turk. Journal of Biology*. 362012:25-35.

Panhwar,Q.A.,Radziah,O,Zaharah,A.R.,Sariah,M.,Mohd.Razi,L.2010.Role of phosphate solubilizing bacteria on rock phosphate solubility and growth of aerobic rice. *Journal of Environmental Biology*.32:607-612.

Patten, C. L. and B. R. Glick 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl. Environ. Microbiol.* 68:3795-3801.

Ponmurugan, P and Gopi, C.2006. In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria.*African Journal of Biotechnology*. 5 4: 348-350.

Pradhan, N and Sukla, L B.2005 .Solublization of inorganic phosphates by fungi isolated from agriculture soil. *African Journal of Biotechnology.* 510:850-854.

Qurban Ali Panhwar, Radziah Othman, Zaharah Abdul Rahman, Sariah Meon and Mohd Razhi Ismail. 2012. Isolation and Characterization of Phosphate- Solubilizing bacteria from aerobic rice. *African Journal of Biotechnology*. 1111:2711-2719.

Sadia Alam, Samina Khalil, Najma Ayub and Maliha Rashid. 2002. In vitro solubilisation of inorganic phosphate by phosphate solubilizing microorganisms PSM from maize rhizosphere. *International Journal of Agriculture and Biology*.4:454-458.

Sanjotha,P., Mahantesh ,P.,and Patil,C.S.2011. Isolation and Screening of efficiency of Phosphate Solubilizing Microbes. *International Journal of Microbiology Research.* 31:56-58.

Shruti Agrawal and R. K. Pathak.2012.Phosphate Solubilization by *Alcaligenes faecalis* over *Pseudomonas fluorescens. Agricultural Science Research Journals.* 22: 92-94.

Shekhar Nautiyal,C.1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters.* 170 . 265-270.

Sung-Man Woo, Min-Kyoung Lee, In-Soo Hong, S. Poonguzhali and Tong- Min Sa.2010. Isolation and characterization of phosphate solubilizing bacteria from Chinese cabbage.*19th World Congress of Soil Science, Soil Solutions for a Changing World.*56-59.

Tripti, Vipin Kumar and Anshumali.2012. Phosphate Solubilizing Activity of Some Bacterial Strains Isolated from Chemical Pesticide Exposed Agriculture Soil. *International Journal of Engineering Research and Development.* 39:01-06.

Uma Maheswar ,N., and G. Sathiyavani.2012. Solubilization of phosphate by Bacillus Sps, from groundnut rhizosphere *Arachis hypogaea* L. *Journal of Chemical and Pharmaceutical Research.* 48:4007-4011.

Velineni, S., and G. P. Brahmaprakash.2011. Survival and Phosphate Solubilizing Ability of *Bacillus megaterium* in Liquid inoculants under High Temperature and Desiccation Stress. *J. Agr. Sci. Tech*. 13: 795-802.

Venant Nihorimbre, Marc Ongena, Maite Smargiassi, Philippe Thonart.2010. Beneficial effect of rhizophere microbial community for growth and health.*Biotechnol.Agron.Soc.Environ*.15 2:327-337.