

Isolation and Screening of Efficient Phosphate Solubilizing Microorganisms From Different Soils



Microbiology

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ABSTRACT

Present investigation compares the phosphate solubilizing potential of fungi (PSF) and bacteria (PSB), isolated from different soil rhizosphere. Most phosphate solubilizing capacity was observed in case of *A. niger* and *B. subtilis* than *Penicillium* sp., and *P. aeruginosa*. The phosphate solubilizing efficiency of four isolates revealed, maximum reduction at pH at 6.0 on second day of incubation with *Bacillus* and *Pseudomonas* sp and on third day at pH 5, with *Aspergillus niger* & *Penicillium*. *B. subtilis* showed maximum phosphate solubilizing capacity (90%) and, *A. niger* found to be more efficient in solubilization (100%). A pot trial experiment was carried out using tomato plant seedlings. Two growth parameters % germination and height of seedlings over control after 30 days of sowing were studied. Maximum % germination and height of seedling was recorded in *A. niger* i.e. 100 % and 13 cm. followed by *B. subtilis* 90 % and 11 cm respectively. Control trials were found to be less as compared to all the tests is 60% and 5cm. Data from the present study support the hypothesis that liberation of organic acids is more with fungi than bacteria.

Introduction

Phosphorus, in Indian agriculture, occupies a unique position both in conventional as well as in alternative agriculture. It plays an important role in plants in many physiological activities such as cell division, photosynthesis and development of good root system and utilization of carbohydrate (Glick BR. 1995)¹ and (S. Sharma, 2011)².

Phosphorus is one of the major plant nutrients, second only to nitrogen in requirement. However, a greater part of soil phosphorus, approximately 95-99% is present in the form of insoluble phosphates and hence cannot be utilized by the plant, to increase the availability of phosphorus for plant, large amount of fertilizer are used on a regular basis. But after application, a proportion of fertilizer phosphorus is quickly transferred to the insoluble form (Omar, 1998)³. Very little percentage of the applied phosphorus is used, making continuous application necessary. It has been reported that many soil fungi and bacteria can solubilize phosphate. PSM play an important role in supplementing phosphorus to the plant, allowing a sustainable use of phosphate fertilizer. Application of PSM in the field has been reported to increase crop yield. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSM. Fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria (S. Sharma et al, 2011).

In the present study attempts had been made to isolate phosphate solubilizing microorganism i.e. bacterium and fungus from different soil rhizosphere, compare their solubilizing efficiency, addition of PSM inoculants in pot soil and study their effects on germination and growth of tomato seedlings.

The present investigation has been undertaken to isolate the efficient strain of the PSM for their phosphate solubilizing efficiency which could be a part of quest to increase the soil sustainability and ultimately increase in crop yield.

MATERIALS & METHODS

All the media, chemicals and reagent used in present study for different test were procured from Hi media, SD fine and SRL. Seeds of Tomatoes were procured from Ankur Seeds Pvt. Ltd. Co, Variety: PKM-1, Label: W7175825. Five different soil samples were collected from roots and rhizosphere area of plants like, soybean, mango, aloe vera, and park soil. Samples were collected in sterile plastic bags and immediately transferred to laboratory.

Isolation and identification of PSM: PSM (Phosphate solubilizing bacteria (PSB) and Phosphate solubilizing fungi (PSF) were isolated from each sample by serial dilution and spread plate method (Gour1990)⁴. One gram (1g) of soil sample was

dispersed in 9 ml sterile distilled water and was thoroughly shaken. Serial dilutions of soil samples were prepared in the range of 10² to 10⁸ dilutions. 0.1 ml of each dilution was spread on Pikovskaya's agar medium (PVK) containing insoluble tricalcium phosphate and incubated at 27^o-30^o C for 7 days. Colonies showing halo zones were picked and purified for further identification. All bacterial isolates were identified as per Bergey's Manual of Determinative Bacteriology, (1984)⁵ and fungi according to method described by (Gour1990). The colonies that show clearing of phosphate were purified separately on Pikovskaya's agar and were maintained on nutrient agar and potato dextrose agar slants, respectively for further studies Pikovskaya RI (1948)⁶.

Quantitative analysis of phosphate solubilization by isolated PSM: Quantitative analysis of phosphate solubilization by isolated organisms was carried out according to the method given by (Gaur, 1990). Pikovskaya's broth with known amount of phosphorous was prepared. Isolated bacteria and fungi, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Penicillium* spp. were inoculated aseptically in different flasks and were incubated at 37^oC for 24 hrs, whereas flask containing *Aspergillus niger*; and *Penicillium* spp. at 28-30^oC for 3-4 days. All broths were centrifuged at 5000 rpm for 15min. Aliquot (0.1-1ml) from supernatant or filtrate was mixed with 10 ml of ammonium molybdate and diluted the content to 45 ml. 0.25ml of chlorostanus acid was then added and final volume was made to 50 ml. Blue color intensity of solution was measured at 600 nm. Amount of soluble phosphorus is obtained with the help of standard curve. For standard curve, 100-ppm phosphorus solution was prepared by dissolving 0.439gm of KH₂PO₄ in 400ml distilled water with 25ml of (7N) H₂SO₄ and the volume was made up to 1 L.

Pot trial experiment:

To find out the phosphate solubilizing action of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Penicillium* spp. in soil, a pot trial experiment was carried out. All the organisms were grown in pikovskaya's broth in 20 ml each, at corresponding temperature. A sterilized soil was filled up in the pot. Then 10 seeds were sown into each pot. Then broth was added into each pot (Table-1). These treatments were replicated three times. The germination of the seeds was recorded at regular time interval 24 hours.

RESULTS

Isolation and Identification of PSB and PSF from soil samples

The soil samples collected for screening of PSB and PSF from different rhizospheric and root regions and inoculated on Pikovskaya's agar plates have shown clear zone around it indicating phosphate solubilization (Plates 1,2,3,4). Rhizospheric and root regions are predominated by phosphate solubilizing micro-

organisms (PSM) P. Vazquez, G. et al, (2000)7. S. Sharma, Vijay Kumar and Ram Babu Tripathi (2011) collected soil samples for their study from rhizosphere of four different types of plants of Aloe vera, Mango, Graveyard, also included park soil, from which they isolated *Pseudomonas fluorescens*, and *Bacillus megatherium*.

Plate 1: Zone of phosphate solubilization by A1 culture



Plate 2: Zone of phosphate solubilization by A2 culture



Plate 3: Zone of phosphate solubilization by A niger



Plate 4: Zone of phosphate solubilization by Penicillium sp



Identification of PSB isolates: The microscopic observation of the isolates, A1, A3, A5, A7, A9 were confirmed to be Gram positive motile bacilli, whereas isolates A2, A4, A6, A8, A10 were confirmed to be Gram negative, motile bacilli.

The cultural characteristics of all PSB isolates on different media are presented in (Table-2). From the table it has shown that cultures (A1, A3, A5, A7, and A9) showed large, luxuriant colonies on *Bacillus* differentiating agar medium and (A2, A4, A6, A7, A8, and A10) showed green colored colonies on *Pseudomonas* isolation agar medium.

Richardson (2001)⁸ found that the predominant soil bacteria involved in phosphate solubilization include *Bacillus* and *Pseudomonas*. Vijay Kumar and Ram Babu Tripathi (2011) collected soil sample for their study from rhizosphere of four different types of plants of Aloe vera, Mango, Graveyard, also included park soil, from which they isolated *Pseudomonas fluorescens*, and *Bacillus megatherium*. Predominant PSB isolates identified were *P aeruginosa* and *Bacillus subtilis* in our studies.

Table 2: Colony characteristics on different agar media

Isolates	Medium	Appearance	Microbial species
A1, A3, A5, A7, A9	<i>Bacillus</i> differentiating agar	Large, luxuriant	<i>Bacillus subtilis</i>
A2, A4, A6, A8, A10	<i>Pseudomonas</i> isolation agar	Small, mucoid, entire with greenish sheen	<i>Pseudomonas aeruginosa</i>

Biochemical characteristics of PSB isolates: The results of biochemical characteristics of PSB isolates are shown in (Table-3).

Table 3: Biochemical characteristics of PSB:

S. N	I	G	L	S	M	In	MR	V	C	TSI	Cat	U
1	A1	-	-	-	-	-	-	-	-	-	+	-
2	A2	-	-	-	-	-	-	-	+	-	+	-

Keys: + Positive, - Negative, I – Isolate, G – Glucose, L – Lactose, S – Sucrose, M – Mannitol, In – Indole, MR – Methyl Red Reaction, V – Voges-Proskauer, C – Citrate utilization, TSI – Triple Sugar Iron Agar, Cat – Catalase, U – Urease

All isolates utilize sugars oxidatively, forming acid only. Indole, MR, VP, are negative except that A2 is citrate positive and A1 as citrate negative. On TSI the slant show alkaline reaction and H₂S test are negative. Both are catalase positive and urease negative. On the basis of above observation it was found that A1 to A9 had identical morphological, biochemical and cultural characteristics like that *Bacillus subtilis*, and A2 to A10 as *Pseudomonas aeruginosa*.

Identification of PSF: Identification of fungi was done on the basis of morphological characteristics, which include staining and cultivating on potato dextrose agar. F1 culture showed conidiospores along with conidia and presence of conidiophores under microscope whereas on potato dextrose agar aerial black sporulated colonies were found to be identified as *A niger*. The culture F2 showed conidiospore with branched or septate hyphae. On Potato Dextrose Agar colonies were bluish green, and centrally raised and identified as *Penicillium*. Fungi were found to be more predominant in rhizosphere regions. Predominance of *Aspergillus* spp. in rhizosphere of different crops is reported by Ali and Gaffar (1991)⁹.

Phosphate solubilization Efficiency: The estimation of solubilized phosphate was done by the method reported by (Gaur 1990) in vitro. The four isolates were subjected to estimate their phosphate solubilization activity in Pikovaskaya’s medium in *Bacillus subtilis*, and *Pseudomonas aeruginosa*, recorded maximum reduction at pH at 6.0 on second day of incubation with 1.0 % phosphate concentration. On the other hand, on

third day of incubation the reduction in 1.0 % phosphate concentration was observed with *Aspergillus niger* & *Penicillium* at pH 5.0. In case of PSB, *B. subtilis* showed maximum phosphate solubilizing capacity i.e. 0.90 ppm and *P. aeruginosa* i.e. 0.40 ppm whereas in case of PSF, *A. niger* found to be more efficient in solubilization 1.04 ppm followed by *Penicillium* spp. i.e. 0.46 ppm. Results are shown in (Figure 1). Similar findings have been observed by Das (1963)10, Cerezine et al., (1988)11 and S., P. Mahantesh and C.S. Patil (2011)12.

Further comparative study in regard to phosphate solubilization revealed that efficiency of *A niger* is greater followed by *B. subtilis*, *Penicillium* spp. and *Pseudomonas aeruginosa*.

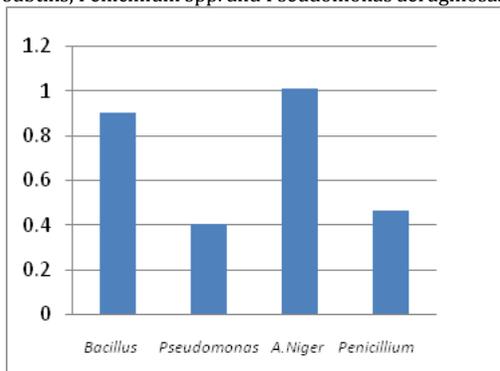


Fig 1: Comparison of phosphate solubilization efficiency of various isolates

PSM	Percent Germination	Seedling Height
Control	60%	5 cm
<i>A. niger.</i>	100%	13 cm
<i>Bacillus</i> spp.	90%	11 cm
<i>Penicillium</i> spp.	80%	8 cm
<i>Pseudomonas</i> spp.	80%	7 cm

Pot experiments: A pot trial experiment was carried out with 6 treatments. i.e. Control, *B. subtilis*, *P. aeruginosa*, *A. niger*, and *Penicillium* spp. using tomato plant seedlings. The results are shown in table 4. Two growth parameters were studied as % germination and height of seedlings over control after 30 days of sowing. Maximum % germination and height of seedling was recorded in *A. niger* i.e. 100 % and 13 cm. followed by *B. subtilis* i.e. 90 % and 11 cm respectively, whereas in *Penicillium* spp. it was 80% and 8cm followed by in *P. aeruginosa* i.e. 80% and 7cm.

Treatment with *A. niger* has given maximum percentage of germination, i.e. 100% than *B. subtilis* 90 % and SSP 90%. It was followed by *Penicillium* spp. 80% and *Pseudomonas aeruginosa* 80 %.The maximum height of seedling was recorded in *A. niger* is 13cm followed by *B. subtilis* and SSP i.e.11cm. In case of *Penicillium* spp it was 8 cm. and in case of *Pseudomonas aeruginosa* it was 7cm. Control trials was found to be less as compared to all the tests is 60% and 5cm.

An increase in P availability to plants through the inoculation of phosphate-solubilizing bacteria (PSB) has been reported in pot experiments and under field conditions; Hameeda, B., et al (2008)¹³ and Zaida et al., 2003)¹⁴.

Table 4:Percentage germination and height of tomato seedlings

PSM	Percent Germination	Seedling Height
Control	60%	5 cm
<i>A. niger.</i>	100%	13 cm
<i>Bacillus</i> spp.	90%	11 cm
<i>Penicillium</i> spp.	80%	8 cm
<i>Pseudomonas</i> spp.	80%	7 cm

Similar result was reported by Das (1963), Rogers et.al. (1999)¹⁵ and Sharma (2011) and revealed the superiority of *Bacillus* spp. in soybean than other plants. *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop. Phosphate solubilizing bacteria enhanced the seedling length of *Cicer arietinum* while co-inoculation of PSM and PGPR reduced Phosphate application by 50 % without affecting corn yield (Sharma, 2011). In our studies *A. niger* revealed to be superior than bacteria in terms of germination rate and height.

Conclusion

In the present investigation, quantitative phosphate solubilization by different PSB and PSF helped to find the most efficient strain of PSM. Most phosphate solubilizing capacity was observed in case of *A. niger* and *B. subtilis* than *Penicillium* spp., *P. aeruginosa*. The study has shown that the fungi have greater efficiency to solubilize phosphate than bacteria. From the above results it is concluded that potentials of fungi could be effectively exploited in the future for the production of eco-friendly phosphate solubilizing biofertilizer for sustainable agriculture.

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