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EVALUATION OF PHOSPHATE SOLUBILIZING MICROORGANISMS (PSMs) FROM RHIZOSPHERE SOIL OF DIFFERENT CROP PLANTS AND ITS ANTAGONISTIC ACTIVITY

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ABSTRACT

Indigenous rhizosphere soil samples were collected during study period (October 2011 – March 2012) of different crop plant from Thiruvannamalai District, Tamilnadu, India for the enumeration of Phosphate solubilizing microorganisms (PSMs). Efficient phosphate solubilizing bacteria, fungi and heterotrophic bacteria were enumerated. Maximum heterotrophic bacterial populations (19.4 $X10^5$), phosphate solubilizing bacteria (4.7 X 10^5) were recorded in the month of February and phosphate solubilizing fungi (3.9 X 10^2) were documented in the month of December in rhizosphere soil of ground nut. Minimum bacterial populations (14.3 X 10^5) were observed in rhizosphere soil of chilli in the month of March. Lowest phosphate solubilizing bacteria (1.2 $X10^5$) and phosphate solubilzing fungi (1.2 X 10^2) were observed in rhizosphere soil of paddy during the month of October. Phosphate solubilizing bacteria *Pseudomonas*sp. - BS1, *Bacillus* sp. – BS2, *Micrococcus* sp. – BS3 and fungi *Aspergillus* sp. – FS1, *Penicillium* sp. – FS2.and *Trichoderma* sp. – FS3 were identified. *Pseudomonas* sp. - BS1. exhibited maximum solubilizing efficiency (SE) and solubilizing index (SI) of 300.0 and 4.0 respectively. In fungi *Aspergillus* sp. – FS1 showed a maximum solubilizing efficiency (SE) and solubilizing index(SI) of 283.3 and 3.8 respectively. Antagonistic activity of P-solubilizing *Pseudomonas*sp. - BS1 was deliberated against selected fungal plant pathogens. Among pathogens studied *Aspergillus* sp. showed a maximum inhibition activity (16 mm) and minimum activity (12 mm) was observed against *Fusarium* sp. Moreover inhibition efficiency (IE) and inhibition index (II) of *Pseudomonas* sp. - BS1. also calculated base on the antagonistic activity. *Aspergillus* sp. exhibited highest inhibition efficiency and inhibition index of 166.6 and 3.6 respectively.

Keywords: Phosphate solubilization, microorganisms, antagonistic activity, plant pathogens

INTRODUCTION

Phosphorus is one of the major nutrients, next to nitrogen as mineral nutrient required by both plants and microorganisms and it plays an important role in plant metabolism by supplying energy required for metabolic processes **(Lal, 2002)**.Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major limiting factor for plant growth. The bioavailability of soil inorganic phosphorus in the rhizosphere soil divergesconsiderably with plant species, nutritional status of soil and ambient soil conditions**(Khan *et al.*,2006)**. Phosphorus, the second major plant nutrient is an integral part of plants generally deficient in soils **(Batjes,1997)** due to its speedy fixation.However approximately 95 -99% of soil phosphorous is present in the form of insoluble phosphates and hence cannot be utilized by the plants **(Vassileva *et al.*, 1998)**. Since plants cannot absorb insoluble forms of phosphorous and has to be converted into soluble forms by phosphatase enzyme such as acidic and alkaline phosphatase. The solubilization of phosphorus compounds may also be brought about by acids and enzymes of microbial origin **(Alexander, 1961; Skujins, 1968)**.Microorganisms having the phosphate solubilizing capacity can convert the insoluble phosphates into soluble forms through the production of organic acids **(Qureshi *et al.*, 2012)**. It has also been reported that siderophores, chelating compounds and mineral acids also responsible for phosphate solubilization **(Gyaneshwar *et al.*, 1998; Wu *et al.*,2005)**.
Terrestrial microorganisms play a vital role in the field of agriculture in order to promote the exchange of plant nutrients and reduce application of chemical fertilizers as much as possible.Severalsoil microoganisms,predominantly those belonging tophosphate solubilizing microoganisms (PSM)possess the ability to solubilize insoluble inorganic phosphate and make it available to plants. They are also known to produce a variety of essential amino acids, vitamins and growth promoting substances like indole acetic acid (IAA) and gibberellic acid (GA3) which help in better growth of plants.Phosphate solubilizing microorganisms have attracted the researchers to exploit their potential toutilize phosphate reserves in semi arid regions and toenhance the crop yields **(Khan*et al.*, 2006)**. Phosphate solubilizers are economical, eco-friendlyand have greater agronomic utility to compensatethe expensive inorganic sources of phosphate fertilizers.
The present investigation was carried out to study in detail about the distribution and population density of total heterotrophic bacteria (THB), phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) in the different rhizosphere soil crop plants (Brinjal, Chilli, Ground nut and Paddy) from Thiruvannamali District, Tamilnadu,India were enumerated. PSB isolates were also screened for their P-solubilization performance and antagonistic activity against fungal plant pathogens under *in vitro* conditions.

MATERIAL AND METHODS

Sample collection

Rhizoshpere soil samples were collected during study period (October – 2011 to March- 2012) from different crop (Brinjal, Chilli, Ground nut and Paddy) in a depth of around 5cm in sterile poly ethylene bags separately. Soil samples were dried at room temperature for 3 days then subjected for the enumeration of heterotrophic bacteria, phosphate solubilizing bacteria and phosphate solubilizing fungi using different culture media.

Culture media

All chemicals and culture media were procured from Hi-media Laboratories Private Limited (Mumbai, India) were used for the isolation of phosphate solubilizing microorganisms and its antagonistic characteristic.

Enumeration of heterotrophic bacteria

Enumeration of heterotrophic bacteria was performed in nutrient agar (NA) medium containing peptone: 10g; beef extract: 10g; NaCl: 5g; agar: 18g; distilled water; 1000ml; pH: 7.2±0.2. One gm of air dried rhizosphere soil sample was electronically weighed and serially diluted and plated in triplicate on nutrient

agar mediumby pour plate method.The plates were incubated in an inverted position at 37°C for 24 – 48h. After incubation period the colonies were counted and recorded.

Isolation of phosphate solubilizing bacteria

One ml of serially diluted aliquots was plated in triplicates on Pikovskaya medium (glucose: 10g; tricalcium phosphate: 5g; NH_4SO: 0.5g; $MgSO_4.7H_2O$: 0.1g; KCl: 0.2g; Yeast extract: 0.5g; Agar: 18g; ditilled water: 1000ml; pH: 7.2±0.2) by pour plate method. The plates were incubated in an inverted position at 28± 2°C for 7 days.After incubation period the colonies were counted and morphologically different colony having halo zone were selected and re-streaked thrice on the same medium**(Pikovskaya, 1948)**. The potential halo formation (HF) candidate strainwas stored under 4°C for further characterization studies.Generic level identification of phosphate solubilizing bacteria was performed by *Bergey's Manual* of Determinative Bacteriology **(*Holt et al*.,*1994*)**. Based on the diameter of clear halo zones, solubilization efficiency (SE) and solubulization index (SI) were calculated followed by **Vazquez *et al.*(2000)**using the following formulas.

$$SE\% = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

$$SI = \frac{\text{Colony diameter + halozone diameter}}{\text{Colony diameter}}$$

Isolation of phosphate solubilizing fungi

Serially diluted aliquots was plated in triplicates on potato dextrose agar (PDA) medium (potato: 200g; peptone: 10g; dextrose: 40g; agar: 18g; distilled water: 100ml; pH: 6.5±0.2)amendedwith 0.5%(w/v) of calcium phosphate and 0.5% (w/v) of tricalcium phosphateby spread plate method. The plates were incubated in an inverted position at 22±2°C for 7 days. After incubation period the colonies were counted and morphologically different colony having halo zone around the colony were selected and re-streaked thrice on the same medium. Based on the diameter of clear halo zones, solubilization efficiency (SE) and solubulization index (SI) were calculated using above revealed formulas.The potential strain was stored under 4°C for further characterization studies. Phosphate solubilizing fungi were identified based on their colony morphology, spore formation and microscopic study **(Carranza Morse and Gilbertson, 1986)**.

Test pathogens

Test fungal pathogens such as *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp. *and Trichoderma* sp. were collected from Agriculture Science and Technology Institute (ASTI),Thiruvannamali District, Tamilnadu, India and maintained in our laboratory were chosen for the present study.

Antagonistic activity of P-solubilizing *Pseudomonas* sp. - BS1

Antagonistic activity of P-solubilizing *Pseudomonas* sp. - BS1 was undertaken by plate assay (PA) method against selected fungal plant pathogens. A spore suspension of 10^5 spores was seeded in triplicates on PDA plates and was incubated at 25°C for 48h. After incubation period 0.1ml aliquot spot was placed at the edge of mycelial growth and incubated at 30°C for 48h. After incubation the inhibition zone around the spotted area were measured and recorded in millimeter in diameter. Antifungal activity was expressed in terms of per cent inhibition efficiency (IE) and inhibition index (II)can be calculated by the following formulas.

$$IE\ \% = \frac{\text{Inhibition diameter – colony diameter}}{\text{Colony diameter}} \times 100$$

$$II = \frac{\text{Colony diameter + inhibition diameter}}{\text{Colony diameter}}$$

RESULTS

Total bacterial population, phosphate solubilizing bacteria and phosphate solubilizing fungi were examined from different crop rhizosphere soil during study period (October 2011 – Marc 2012). Maximum bacterial populations (19.4 $X10^5$) were recorded in ground nutrhizosphere soil in the month of February. Minimum bacterial populations (14.3 X 10^5) were observed in rhizosphere soil of chilli in the month of March. There is no much variation of bacterial populations of different crop rhizosphere soil during the study period (Table 1). Highest phosphate solubilizing bacteria (4.7 X 10^5) were noticed in rhizosphere soil of ground nut during the month of February. However lowest phosphate solubilizing bacteria (1.2 $X10^5$) were observed in rhizosphere soil of paddy duringthe month of December and January. Phosphate solubilizing fungi also examined during the study period. Utmost phosphate solubilizing fungi (3.9 X 10^2) were documented in the month of December in rhizosphere soil of ground nut. On the other hand least populations of phosphate solubilzing fungi (1.2 X 10^2) were recognized in the month of October in rhizosphere soil of paddy.

Table 1 Population density of total heterotrophic bacteria, phosphate solibulizing bacteria and phosphate solubilizing fungi of different crop soil

Month of collection	Seasons	Population density of total bacteria (CFU X 10^5/g)				Population density of phoaphate solubilizing bacteria (CFU X 10^5/g)				Population density of phosphate solubilizing fungi (CFU X 10^2/g)			
		Brinjal	Chilli	Groundnut	Paddy	Brinjal	Chilli	Groundnut	Paddy	Brinjal	Chilli	Groundnut	Paddy
October 2011	Monsoon	18.1	16.0	16.8	15.2	2.3	2.2	3.9	1.6	2.0	2.1	3.3	1.2
Novenber 2011		18.3	16.4	17.4	15.6	2.6	2.3	4.2	1.7	2.2	2.2	3.6	1.4
December 2011		19.2	17.4	18.2	17.0	3.2	3.0	3.6	1.2	2.3	2.5	3.9	1.7
January 2012	Non-monsoon	18.2	17.3	18.2	19.3	3.4	3.1	4.6	1.2	2.2	2.4	3.5	1.5
February 2012		18.4	17.2	19.4	18.3	3.2	2.2	4.7	1.6	2.0	2.3	3.4	1.4
March 2012		17.2	14.3	18.2	16.3	2.8	2.0	4.0	1.4	1.8	2.0	3.1	1.3

The data shown are the average of triplicates

The P-solubilizing bacteria were identified as *Pseudomonas* sp. - BS1, *Bacillus* sp. - BS2 and *Micrococcus* sp. -BS3 P-solubilizing fungi also isolated and identified as *Aspergillus* sp. – FS1, *Penicillium* sp. – FS2 and *Trichoderma* sp. – FS3 based on the colony morphology,formation of exospores and microscopic studies. Phosphate solubilization efficiency (SE) and phosphate solubilization index (SI) of isolates were calculated based on the phosphate solubilizing competency. Among the bacteria studied *Pseudomonas* sp. - BS1 exhibited maximum solubilizing efficiency and solubilizing index of 300.0 and 4.0 respectively. Minimum solubilizing efficiency and solubilizing index were noticed 200.0 and 3.0 correspondingly by the strain *Micrococcus* sp. - BS3. In fungi *Aspergillus* sp. – FS1 showed a maximum solubilizing efficiency and solubilizing index of 283.3 and 3.8 respectively. However *Penicillium* sp. – FS2 perceived null solubilizing efficiency and solubilizing index (Table 2).

Table 2 Zone of halo formation (HF), solubilization efficiency (SE) and solubilization index(SI) of phosphate solubilizing isolates.

Phosphate solubilizing isolates	Zone of halo formation (HF)	Solubilization efficiency (SE)	Solubilization index (SI)
Bacteria			
Pseudomonas sp. - BS1	18	300.0	4.0
Bacillus sp. - BS2	16	266.0	3.6
Micrococcus sp. – BS3	12	200.0	3.0
Fungi			
Aspergillus sp. – FS1	17	283.3	3.8
Penicillium sp. – FS2	0.0	0.0	0.0
Trichderma sp. – FS3	10	166.6	2.6

The data shown are the average of triplicates

Table 3 Antagonistic activity, inhibition efficiency (IE) and inhibition index (II) of *Pseudomonas* sp. - BS1 against selected plant pathogens

Plant pathogens	Zone of inhibition mm in diameter	Inhibition efficiency (IE)	Inhibition index (II)
Aspergillus sp.	16	166.6	3.6
Penicillium sp.	14	133.3	3.3
Fusarium sp.	12	100.0	3.0
Trichoderma sp.	12	100.0	3.0

The data shown are the average of triplicates

Antagonistic activity of *Pseudomonas* sp. - BS1 was deliberated against selected fungal plant pathogens was showed in Table 3. Among the pathogens studied*Aspergillus* sp.showed a maximum inhibition activity (16 mm). However minimum inhibition activity (12 mm) was observed for the tested pathogens*Fusarium* sp. and *Trichderma* sp. by the P-solubilizing antagonistic *Pseudomonas* sp. - BS1. Moreover inhibition efficiency (IE) and inhibition index (II) of *Pseudomonas* sp. - BS1 also calculated base on the antagonistic activity against test pathogens. Among the tested pathogens *Aspergillus* sp. exhibited highest inhibition efficiency and inhibition index of 166.6 and 3.6 respectively. Whereas least inhibition efficiency and inhibition index of 100.0 and 3.0 respectively was noticed for the tested pathogens *Fusarium* sp. and *Trichderma sp*. by the P-solubilizing antagonistic *Pseudomonas* sp. - BS1.

DISCUSSION

The population density of heterotrophic bacteria and phosphate solubilizing microorganisms were showed significant difference in different rhizosphere soil of crop plant. The total heterotrophic bacteria and phosphate solubilizing microbial population intensity was higher in the rhizosphere soil of groundnut followed by brinjal and chilli.**Ponmurugan and Gopi (2006)** endorsed that total phosphate solubilizing population level was higher in the rhizosphere soil of groundnut when compared to other rhizosphere soil. This might be due to root exudates produced by ground nut plant and enhance the growth of bacterial population. Moreover the phosphobacterial strain GP02 which was isolated from groundnut rhizosphere soil had higher phosphate solubilizing activity than the strain SP03 isolated from other soil.Phosphate solubilizing bacteria having potential of phosphate solubilization for growing crop more available phosphate by the production of organic acids which act like chelates and solubilized insoluble phosphorus **(Zaidi *et al.*,2004; Khan *et al.*,2006)**.

Phosphate solubilizing efficiency and phosphate solubilizing index of bacteria and fungi were estimated in the present study. From the results it revealed that bacteria possess distinguished phosphate solubilizing efficiency and solubilizing index than the fungi. This might be due to the extracellular production of acid phosphatase and alkaline phosphatase enzymes.**Shah *et al.*(2001)** anticipated our results that bacterial inoculation having high phosphate solubilization extent (SE and SI) and phosphorus application enhanced nutrient uptake efficiency.

The phosphate solubilizing efficiency of isolated strains of PSB indicated that all the strains weresolubilized inorganic phosphate contents effectively in the Pikovskaya's medium. The result shows that *Pseudomonas* sp. - BS1 was potentially efficient phosphate solubilizer with solubilization efficiency of 300.0. **Kannapiran and Sri Kumar (2011)**reported that *Pseudomonas* sp. was most efficient phosphate solubilizer on Pikovskaya's agar plates with solubilization index 228±6.12 after 7 days incubation. This result agreed with the present study of phosphate solubilizing *Pseudomonas* sp. - BS1.**Qureshi *et al.*(2012)**endorsed that the effect of phosphate solubilizing *Bacillus* sp. on the growth of cotton and fluctuation of available P in soil under cotton crop. But in the present investigation *Pseudomonas* sp. - BS1 shows efficient P solubilization followed by *Bacillus* sp. – BS2 and *Micrococcus* sp. – BS3.**Noori and Saud (2012)** reported that phosphate solubilizing *Pseudomonas* sp. isolated from rhizosphere soil of paddy shows antagonistic activity against tested fungal pathogen. In the present investigation phosphate solubilizing *Pseudomonas* sp. - BS1 effectively inhibiting selected fungal plant pathogens.

CONCLUSION

The present research clearly demonstrated phosphate solubilising microorganisms can play a significant role by converting insoluble form of phosphate into soluble form by secreting extracellular enzymes and organic acids lead to enhance the available P in soil. The findings of the present investigation highlighted that phosphate solubilizing microorganisms from rhizozsphere soil of crop plants could be easily isolated and may be exploited as bio-fertilizer and bio-control agent to improve the crop productivity. This research opens a new avenue in the sector of agriculture to improve the crop productivity by enhancing phosphate availability to growing crop plants and reduce the pathogenicity of plant pathogens. However, further field applications of these isolates are required to explore the exact contribution of phosphate solubilization,promotion of plant growth and the antagonistic activity.

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