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Coexistence of Phosphate solubilizing bacteria isolated from *Sorghum bicolor* rhizosphere soil inoculated with arbuscular mycorrhizae fungi (*Glomus sp*)

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ABSTRACT

The presence of phosphorus in plant material is abundant while the soil phosphorus availability is limited for plants due to their quick transformation to the insoluble form. There are many soil micro-organisms which could enhance the solubility of insoluble phosphate in soils and make it available for plant growth. This study investigates the coexistence of phosphate solubilizing bacteria in the rhizosphere soil of *sorghum bicolor* pre-inoculated with mycorrhizal fungi (AMF)*Glomus* sp. The rhizosphere soil sample was collected and the phosphate solubilizing bacteria was enumerated followed by screening for their efficiency to solubilize the insoluble phosphate using pikovskaya's medium. Based on the solubilization index five bacterial isolates were chosen for further study. All the five phosphate solubilizing bacteria were subjected to morphological and various biochemical tests and were identified as *Acinetobacter* sp, *Bacillus* sp, *Pseudomonas fluorescence*, *Pseudomonas aeruginosa* and *Micrococcus* sp. The maximum solubilization index was shown by *Bacillus sp* (1.75cm) followed by *Micrococcus* sp (1.75cm) on 6^{th} day of incubation and the total soluble inorganic phosphorus production was estimated caloriemetrically which showed the maximum production by *Bacillus* sp (186 µg/ml) followed by *Micrococcus* sp (184µg/ml) on 6^{th} day of incubation. The five PSB isolates were associated with the drop in pH with the production of soluble inorganic phosphorus.

Introduction

Phosphorous is normally the most limiting nutrient in soil, the transition of the insoluble forms of phosphorous to an accessible form to the plants is an important attribute of phosphate solubilizing bacteria and arbuscular mycorrhizal fungi. Soil microbiota develops important fractions in the ecosystem and the phosphate solubilizing microorganisms (PSB) and the arbuscular mycorrhizal fungi (AMF) are the most important groups of soil microbial community which plays vital role in the plant growth. These microorganisms have the ability to solubilize and mineralize P from inorganic and organic pools of total soil P, making the element available for plants (Gyaneshwar *et al.*, 2002).

AMF improve the absorption of P and other nutrients by plants increasing the contact surface and the explored soil volume (Clark and Zeto 2000) and possibly facilitating nutrient transport among plants (Chen *et al*. 2005). Moreover, plants colonized by AMF have alternative mechanisms to meet their nutritional demands and to maintain their physiological functions under abiotic stress conditions, such as drought stress (Ruiz-Lozano *et al*. 2001) and salinity (Azcón and El-Atrash 1997).

Although potentials of AMF and PSB clearly exists and their widespread application remains limited mainly by a poor understanding of microbial ecology and population dynamics in soil. Therefore, it is interesting to quantify these organisms in rhizosphere soil for their possible use as combined inoculum, in order to obtain the maximum benefit to the development of plants. The purpose of the study is to investigate the presence phosphate solubilizing bacteria in the AMF (*Glomus* sp) inoculated rhizosphere soil of *Sorghum bicolor*.

Materials and methods:

Enumeration of Phosphate solubilizing bacteria from rhizosphere soil sample: (Pikovskaya's, 1948)

The rhizosphere soil sample was collected from the *Sorghum bicolor* inoculated with *Glomus sp*. The samples were processed and enumerated for PSB using pikovskaya's medium with tricalcium phosphate. The screening was carried out several times to obtain efficient phosphate solubilizers with pikovskaya's medium incorporated with tricalcium phosphate. Further the solubilization eficiency of the PSB isolates was determined by the zone of clearance in the pikovskaya's agar plates and calculated the solubilization index (SI) (Premono *et al*., 1996) using the formula given below:

Solubilization Index = colony diameter + Diameter of the halozone / colony diameter.

Based on the solubilization index five bacterial isolates were selected for further study

Identification of phosphate solubilizing bacteria: (Cappucino and Sherman, 1999)

The five efficient PSB isolates were identified based on the standard morphological and biochemical characteristics and the results were compared with bergey's manual of determinative bacteriology.

Quantitative analysis of soluble inorganic phosphorus:

P-solubilization in broth cultures as described by Koenig *et al*., (1942). Five phosphate solubilizing bacteria (PSB) isolates were inoculated into 100 ml Pikovskaya's medium incorporayed with tricalcium phosphate and incubated at 28+2 ^{0}C in rotary shaker at 200 rpm. The experiments were conducted in triplicate and the cultures were harvested on every alternate day, centrifuged at 10000 rpm for 15 minutes and the cell free culture

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filtrates were subjected for caloriemetric estimation of soluble inorganic phosphorus at 430nm.

Measurement of pH:

A change in the pH of the medium inoculated with the PSB strains was measured with a pH meter at a regular interval of 24 hrs till 7th day of incubation.

Results:

The rhizosphere soil sample collected from *S. bicolor* is processed for the isolation of phosphate solubilizing bacteria (PSB) using pikovskaya's medium. Further the PSB isolates having the potentials to solubilize complex phosphorous were identified based on their morphological and biochemical characteristics. The results for the morphological and biochemical characteristics of the five PSB isolates are recorded and tabulated in Table 1 and 2.

The solubilization index of the five PSB isolates were determined and recorded at a regular interval of 24 hrs till 7th day of incubation (Table 3).

Quantitative analysis of soluble inorganic phosphorus by the PSB isolates were determined caloriemetrically at different intervals from 0th day to 7th day at 430nm and the results were recorded (Table4).

The change in pH of the five PSB strains with the production of soluble inorganic phosphorus was measured with pH meter from 0th day to 7th day of incubation and the results were tabulated (Table4).

Discussion:

Current trends in agriculture are focused on the reduction of the use of pesticides and inorganic fertilizers, forcing the search for alternative ways to improve a more sustainable agriculture. The best way to replace is by soil microorganisms which are able to solubilize phosphate ions from sparingly soluble inorganic or organic P compounds in vitro (Barea *et al*., 1983). It is likely that the phosphate solubilized by the bacteria could be more efficiently taken up by the plant through a mycorrhizae-mediated bridge between roots and surrounding soil that allows nutrient translocation from soil to plants (Jeffries and Barea, 1994).

The efficiency of bacterial strains isolated from *Sorghum bicolor* rhizosphere soil indicated that all the strains solubilize inorganic phosphate (tri calcium phosphate) effectively in the Pikovskaya's medium. Various researches show that the solubility efficiency of different bacteria varies considerably (Mahalingam and Thilagavathy, 2012).

Table 1: Morphological characteristics of the bacterial isolates

Name of the Isolate	Colony morphology	Gram's reaction	Cell shape	Motility
PSB 1	Opaque white	+	Cocco bacilli	Non motile
PSB 2	Waxy white	+	Rod	Non motile
PSB 3	Yellowish white	-	Rod	Motile
PSB 4	Thin white	-	Rod	Motile
PSB 5	Soft, smooth, yellow	+	Cocci	Non motile

Table 2: Biochemical characteristics of PSB isolates

Name of the Isolate	Indole	Methyl Red	Voges Proskauer	Citrate	H_2S	Urease	Growth in selective media	Name of the PSB isolates
PSB 1	-	-	-	+	-	-	Macconkey agar (without Nacl)	*Acinetobacter* sp
PSB 2	-	-	-	-	-	-	Skim milk agar	*Bacillus* sp
PSB 3	-	-	-	+	-	-	King's B	*Pseudomonas fluorescens*
PSB 4	-	-	-	+	-	-	King's B	*Pseudomonas aeruginosa*
PSB 5	-	-	-	+	-	+	Mannitol salt agar	*Micrococcus* sp

Table 3: Solubilization index of bacterial isolates

Incubation period	Solubilization index of the five PSB isolates (cm)				
	Acinetobacter sp	*Bacillus* sp	*Pseudomonas fluorescens*	*Pseudomonas aeruginosa*	*Micrococcus* sp
1st day	Nil	Nil	Nil	Nil	Nil
2nd day	Nil	Nil	Nil	1.23	1.33
3rd day	Nil	1.33	Nil	1.33	1.66
4th day	1.33	1.50	1.25	1.33	1.66
5th day	1.33	1.50	1.25	1.40	1.66
6th day	1.42	1.75	1.60	1.42	1.75
7th day	1.37	1.66	1.50	1.37	1.57

Table 4: Total soluble inorganic phosphorus released by five PSB isolates

Name of the PSB isolates	Total soluble inorganic phosphorus (µg/ml)						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Acinetobacter sp	140	148	156	172	178	182	164
Bacillus sp	136	144	162	168	174	186	164
Pseudomonas fluorescens	124	146	152	164	178	166	154
Pseudomonas aeruginosa	138	144	162	176	184	180	166
Micrococcus sp	120	136	148	156	176	184	170

Table 5: Change in pH at different time interval for the five PSB isolates

Name of the PSB isolates	pH at different time interval						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Acinetobacter sp	5.26	5.13	5.01	4.90	4.82	4.71	4.53
Bacillus sp	4.12	4.06	3.98	3.84	3.75	3.70	3.64
Pseudomonas fluorescens	5.10	5.01	4.93	4.84	4.66	4.52	4.31
Pseudomonas aeruginosa	5.23	5.11	5.02	4.95	4.81	4.71	4.53
Micrococcus sp	4.23	4.12	4.02	3.86	3.71	3.60	3.52

The five PSB isolates were identified based on the morphological, biochemical characteristics, selective medium (Table 1 and table 2) and they are *Acinetobacter* sp, *Bacillus* sp, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Micrococcus* sp. The ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate was reported by Goldstein (1986). Phosphate solubilizing ability is widespread among many bacterial genera which include *Pseudomonas*, *Micrococcus*, *Bacillus*, *Rhizobium*, *Achromobacter*, *Agrobacterium*, *Flavobacterium*, *Aerobacter*, *Burkholderia*, and *Erwinia* (Madigan *et al*., 2000).

The five PSB isolates showed a diverse range of inorganic phosphorus production in pikovskyas medium containing tricalcium phosphate. The solubilization of tricalcium phosphate in the medium was determined by the solubilization index (Table 3) and it found to be increased from 2nd day incubation in *Pseudomonas aeruginosa*, *Micrococcus sp* followed by 3rd day of incubation in *Acinetobacter* sp, *Bacillus* sp and 4th day of incubation in *Pseudomonas fluorecence*. The maximum solubilization was observed on 6th day of incubation by *Bacillus* sp (1.75cm), followed by *Micrococcus* sp (1.75cm), *Pseudomonas fluorescence* (1.60cm), *Pseudomonas aeruginosa* (1.42cm), *Acinetobacter* sp (1.42cm) respectively. The solubilization starts to diminish on 7th day of incubation. Similar results were reported by various researchers (Gaur, 1990, Gyaneshwar *et al*., 1998, Madhan Chakkaravarthy *et al*., 2010, Maloy Kumar Sahu *et al*., 2007).

The quantification of soluble inorganic phosphorus (Fig 1) in the pikovskya's medium with tricalcium phosphate was observed to be maximum in *Bacillus sp* (186µg/ml) followed by *Micrococcus sp* (184µg/ml), *Acinetobacter sp* (182µg/ml), *Pseudomonas aeruginosa* (180µg/ml), and *Pseudomonas fluorescence* (166µg/ml) the results were supported by Nautiyal et al., 2000. The pH drop in pikovskayas culture broth was observed from 2nd day of incubation and it continues to drop till 7th day of incubation which proportionally indicates the inorganic phosphorus production by the five PSB isolates. The pH drop in liquid cultures have been reported in several researches which supports the pH changes in the present study (

The S*orghum bicolor* rhizosphere soil dominates more number of microbial populations which is capable of solubilizing insoluble phosphorus. This study indicates Mycorrhizae and soil bacteria have a synergistic association that could manifest plant growth.

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