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 X 10^5), phosphate solubilizing bacteria (4.7 X 10^3) were documented in the month of December in rhizosphere soil of ground nut. Minimum bacterial populations (14.3 X 10^3) were observed in rhizosphere soil of chilli in the month of March. Lowest phosphate solubilizing bacteria (1.2 X 10^3) and phosphate solubilizing 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. Phosphomous 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. 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 generally a major limiting factor for plant growth. The bioavailability of soil inorganic phosphorus in the rhizosphere soil diverges considerably 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 phosphorus 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 (Gyaneswvar 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. Several soil microorganisms, predominantly those belonging to phosphpohosphate solubilizing microorganisms (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 to utilize phosphate reserves in semi arid regions and to enhance the crop yields (Khan et al., 2006).

Phosphate solubilizers are economical, eco-friendly and have greater agronomic utility to compensate the 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 Thiruvannamalai District, Tamilnadu, India were enumerated. PSF 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

Rhizosphere 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.
agrar medium try 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 were plated in triplicates on Pikovskaya medium (glucose: 10g; tricalcium phosphate: 5g; NH₄Cl: 0.5g; MgSO₄.7H₂O: 0.1g; KCl: 0.2g; Yeast extract: 0.5g; Agar: 18g; distilled 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 can be calculated by the solubilization index (SI) of isolates were calculated based on the 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 solubilization index (SI) were calculated followed by Vazquez et al. (2000) using the following formulas.

\[
\text{Solubilization diameter} = \text{Colony diameter + halozone diameter} \\
\text{SE} = \frac{\text{Solubilization diameter}}{100} \\
\text{SI} = \frac{\text{Solubilization diameter}}{\text{Colony diameter}} \\
\]

**Isolation of phosphate solubilizing fungi**

Serially diluted aliquots were plated in triplicates on potato dextrose agar (PDA) medium (potato: 200g; peptone: 10g; dextrose: 40g; agar: 18g; distilled water: 1000ml; pH: 6.5±0.2) amended with 0.5% (w/v) of calcium phosphate and 0.5% (w/v) of tricalcium phosphate 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 solubilization 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).

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

<table>
<thead>
<tr>
<th>Month of collection</th>
<th>Season</th>
<th>Brinjal</th>
<th>Chilli</th>
<th>Groundnut</th>
<th>Paddy</th>
<th>Brinjal</th>
<th>Chilli</th>
<th>Groundnut</th>
<th>Paddy</th>
<th>Brinjal</th>
<th>Chilli</th>
<th>Groundnut</th>
<th>Paddy</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 2011</td>
<td>Monsoon</td>
<td>18.1</td>
<td>16.0</td>
<td>16.8</td>
<td>15.2</td>
<td>2.3</td>
<td>2.2</td>
<td>3.9</td>
<td>1.6</td>
<td>2.0</td>
<td>2.1</td>
<td>3.3</td>
<td>1.2</td>
</tr>
<tr>
<td>November 2011</td>
<td>Monsoon</td>
<td>18.3</td>
<td>16.4</td>
<td>17.4</td>
<td>15.6</td>
<td>2.6</td>
<td>2.3</td>
<td>4.2</td>
<td>1.7</td>
<td>2.2</td>
<td>2.2</td>
<td>3.6</td>
<td>1.4</td>
</tr>
<tr>
<td>December 2011</td>
<td>Monsoon</td>
<td>19.2</td>
<td>17.4</td>
<td>18.2</td>
<td>17.0</td>
<td>3.2</td>
<td>3.0</td>
<td>3.6</td>
<td>1.2</td>
<td>2.3</td>
<td>2.5</td>
<td>3.9</td>
<td>1.7</td>
</tr>
<tr>
<td>January 2012</td>
<td>Non-monsoon</td>
<td>18.2</td>
<td>17.3</td>
<td>18.2</td>
<td>19.3</td>
<td>3.4</td>
<td>3.1</td>
<td>4.6</td>
<td>1.2</td>
<td>2.2</td>
<td>2.4</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>February 2012</td>
<td>Non-monsoon</td>
<td>18.4</td>
<td>17.2</td>
<td>19.4</td>
<td>18.3</td>
<td>3.2</td>
<td>2.2</td>
<td>4.7</td>
<td>1.6</td>
<td>2.0</td>
<td>2.3</td>
<td>3.4</td>
<td>1.4</td>
</tr>
<tr>
<td>March 2012</td>
<td>Non-monsoon</td>
<td>17.2</td>
<td>14.3</td>
<td>18.2</td>
<td>16.3</td>
<td>2.8</td>
<td>2.0</td>
<td>4.0</td>
<td>1.4</td>
<td>1.8</td>
<td>2.0</td>
<td>3.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

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 exoospores 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.

<table>
<thead>
<tr>
<th>Phosphate solubilizing isolates</th>
<th>Zone of halo formation (HF)</th>
<th>Solubilization efficiency (SE)</th>
<th>Solubilization index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas sp. - BS1</td>
<td>18</td>
<td>300.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Bacillus sp. - BS2</td>
<td>16</td>
<td>266.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Micrococcus sp. – BS3</td>
<td>12</td>
<td>200.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus sp. – FS1</td>
<td>17</td>
<td>283.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Penicillium sp. – FS2</td>
<td>0.0</td>
<td>83.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Trichoderma sp. – FS3</td>
<td>10</td>
<td>166.6</td>
<td>2.6</td>
</tr>
</tbody>
</table>

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 pathogen isolates.

<table>
<thead>
<tr>
<th>Plant pathogens</th>
<th>Inhibition mm in diameter</th>
<th>Inhibition efficiency (IE)</th>
<th>Inhibition index (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus sp.</td>
<td>16</td>
<td>166.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>14</td>
<td>133.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>12</td>
<td>100.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>12</td>
<td>1000.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

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 Trichoderma 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 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 Trichoderma sp. by the P-solubilizing antagonistic Pseudomonas sp. - BS1.

CONCLUSION

The present research clearly demonstrated phosphate solubilizing microorganisms can play a significant role by converting insoluble forms 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 rhizosphere 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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REFERENCES


