Characterization of PSB Isolates in Rhizosphere Soil of Rice Varieties Grown at OFRC, SKUAST Jammu

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A B S T R A C T

Phosphate is the key macronutrient required for the growth of plants. The soluble forms of phosphorus when applied to soil are rendered insoluble by undergoing chemical fixation. The soil collected from organic farm, SKUAST- Jammu was investigated for screening the phosphate solubilizing bacteria. In the present study, soil samples were collected from rice rhizosphere of sixteen varieties grown in OFRC, SKUAST- Jammu. This study revealed the existence of Phosphate Solubilizing bacteria in the rhizosphere soils of rice. These bacteria exhibited difference with respect to Gram property, nitrate reductase, oxidase, citrate utilization and catalase activities. Prominent halo zones were found in case of positive PSB isolates on Pikovskaya’s agar. Phosphate solubilization effectively took on sixth day. Based on Gram property, bacterial morphology and biochemical tests performed on various isolates, these were subjected to identification and placed in group I and VIII by referring to the separation outlined in Bergeys Manual of Systematic Bacteriology and confirms the dominance of genus Bacillus and Pseudomonas. The identification at species level will be further undertaken using 16S rRNA technique.

Keywords
Rice, Rhizosphere, Phosphorus, Phosphate-solubilizing bacteria (PSB), Isolation, Biochemical tests, Characterization, Identification

Introduction

It has been reported that phosphorus is the second most essential nutrient required by plants in adequate quantities for optimum growth. It has a very important role in affecting almost all major metabolic processes, including energy transfer, signal transduction, respiration, macromolecular biosynthesis, and photosynthesis (Anand et al., 2016). It is difficult for plants to absorb the phosphorous as 95–99% of phosphorus present is in insoluble, immobilized, or precipitated forms in the soil. Therefore, plants absorb phosphate only as monobasic (H2PO4−) and dibasic (HPO42−) ions. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (Yadav and Dadarwal, 1997). Therefore, the release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability. Phosphorus is the least mobile and available to plants in most soil conditions in comparison to other macro nutrients (El-
Azouni, 2008). Some soil microorganisms are able to mineralize and solubilize P from the organic and inorganic soil pools (Richardson, 2001). Soil bacteria mainly from genera *Bacillus* and *Pseudomonas* possess the ability to bring insoluble phosphates in the soluble forms by secreting organic acids. These acids lower the pH and bring about the dissolution of bound forms of phosphorous. The interest in PSB has increased due to the prospective use of efficient strains as bio-inoculants (biofertilizer) components in organic agriculture, which is emerging as an alternative to chemical inputs in intensive agriculture (Bashan and Holguin, 1998). Use of PSB inoculants is one of the strategies to combat the deficiency of phosphorous and meet the demands of expensive fertilizer. The present research was aimed to isolate and characterize PSB from the rhizosphere of rice varieties grown in research farm at OFRC.

**Materials and Methods**

**Soil sampling**

Soil samples were collected from rice rhizosphere at tillering stage from sixteen rice varieties grown at OFRC, SKUAST, Chatha and were replicated three times in Petri dishes for population count of total bacterial population using nutrient agar medium and phosphate solubilizers using Pikovskayas medium. The calculation for the total number of bacteria was done by plating soil dilution using plating enrichment and pour plate technique and incubating the plates for 7-10 days. The total count of the microorganisms presented in Table 2 was obtained by multiplying the number of cells per plate by the dilution factor viz. Bacteria/gm soil = Number of bacteria/ Wt. of soil x Dilution

**Isolation of PSB**

PSB were isolated on Pikovskaya medium (Pikovskaya, 1948) at tillering stage.

**Characterization of isolates**

Bacteria were studied for colony and cell morphology following microscopic examination and further identification by gram staining (Gram, 1884). The bacterial isolates were characterized using biochemical tests viz. Catalase test, Citrate utilization test, Nitrate reduction test and oxidase test (Table 3).

**Quantitative estimation of Phosphate solubilization**

It was done by following the method given by Selvi et al., (2011) and is presented in Table 4.

**Results and Discussion**

The study was proposed after taking into consideration the fact that no such previous work was undertaken on the said rice varieties. The aim of the study was therefore to screen the better rice cultivars grown in research farm at OFRC centre. With respect to total biomass and phosphate solubilizing rhizospheric bacteria
Enumeration of total biomass and P-solubilizers in rhizosphere soil

The total biomass and P-solubilizers estimated at $10^4$ dilution was 31 and 8 numbers respectively. The percentage of P-solubilizers was 20.80 (Table 2).

Colony morphology of P-solubilising bacteria

The PSB isolates from sixteen rice varieties were small, circular in morphology and were white, yellow and pink in color (Figs. 1a-c).

Characteristics of P-solubilizing bacteria

An appraisal of data presented in Table 3 revealed that P-solubilising bacteria isolated from rhizosphere soils of rice were rod shaped, gram positive and gram negative types (Figs. 2a-b). Biochemical tests (Catalase, Nitrate reduction, Citrate utilization and Oxidase test) performed showed positive results except few of them were negative (Table 3). Catalase production and activity was detected by adding substrate H$_2$O$_2$ to bacterial isolates. The enzyme was present in most cytochrome containing aerobic and facultative anaerobic bacteria. In the absence of enzyme, the toxic concentration cannot be degraded when these organisms are cultivated in the presence of oxygen (Srivastava, 2013).

In case of nitrate reduction test, in anaerobic conditions bacteria used NO$_3^-$ as a source of oxygen and reduced to nitrite i.e. nitrate reduction positive as reported by Karpagam and Nagalaxmi, (2014). This suggests they are capable of breaking down nitrate containing fertilizers in case of anaerobic conditions in soil. Most bacteria utilize the available oxygen in the medium for their growth and rapidly produced anaerobic conditions for further reaction suggesting that aerobic condition also do prevail in soil sample tested.

Bacteria used citrate as sole source of carbon and nitrogen and give the blue color to medium denoted citrate utilization positive as reported by Panhwar et al., (2012). This indicates that if the test is positive, the pH raises and there will be no acid in the end product as reported by Park et al., (2005).

The oxidase test is used to determine if a bacterium produces cytochrome c oxidases. The cytochrome system usually is present in aerobic organisms that are capable of using oxygen as the final hydrogen acceptor. Maximum isolates of diazotrophic bacteria require aerobic conditions except few of them can grow even in anaerobic conditions as indicated by oxidase test. Similar results are also reported by Karpagam and Nagalakshmi (2014).

Quantitative estimation of phosphate solubilization

Maximum amount of phosphate solubilization occurred on sixth day of experiment accompanied with its responsible organisms of genera Bacillus (2.44 mg/litre) and pseudomonas (2.18 mg/litre). Besides this, fourth day experiment showed the phosphate solubilization efficiency range from 0.34 to 2.40 (mg/50 ml) in Bacillus as compared to uninoculated conditions respectively in rhizosphere soil.

Similar effect was observed initially in second day of experiment. The sets of experiment with two days interval denote the comparatively phosphate solubilization effectively takes place in sixth day with bacteria belonging to genera Bacillus as compared to bacteria from genera Pseudomonas (Table 4).

The present study revealed the existence of phosphate solubilizing bacteria in the rhizospheric soil of sixteen varieties of rice.
Table 1 Rice varieties selected for PSB isolation

| S. No. | Variety       |
|--------|--------------|
| 1      | SJBR 95      |
| 2      | SJBR 97      |
| 3      | SJBR104      |
| 4      | SJBR109      |
| 5      | SJBR120      |
| 6      | SJBR121      |
| 7      | SJBR123      |
| 8      | SJBR129      |
| 9      | SJBR70       |
| 10     | Pb. Basmati 03 |
| 11     | Pusa 1121    |
| 12     | Pusa 1612    |
| 13     | Basmati370   |
| 14     | Basmati 564  |
| 15     | Basmati 1509 |
| 16     | Basmati Pak  |

Table 2 Enumeration of total biomass and P-solubilizers in rhizosphere (composite soil) of rice varieties at OFRC, SKUAST Jammu

| Dilution (x10^1.8) | Total Biomass | Phosphate solubilisers | % of P-solubilizers |
|--------------------|---------------|-------------------------|----------------------|
| 1                  | 98            | 16                      |                      |
| 2                  | 75            | 14                      |                      |
| 3                  | 48            | 11                      | 20.80                |
| 4                  | 31            | 8                       |                      |
| 5                  | 22            | 7                       |                      |
| 6                  | 12            | 4                       |                      |
| 7                  | 8             | 2                       |                      |
| 8                  | 4             | -                       |                      |
| Grand total        | 298           | 62                      |                      |
Table 3 Gram staining and characterization of P-solubilising bacteria at OFRC, SKUAST Jammu

| S. No. | Sample No. | Gram staining | Colony Colour | Colony size | Bacterial Cell Shape | Isolate code | Catalase test | Citrate test | Nitrate test | Oxidase test |
|--------|-------------|---------------|---------------|-------------|-----------------------|--------------|--------------|--------------|--------------|--------------|
| 1      | R1-1        | +             | White         | Small       | Rod shaped            | P1           | +            | +            | -            | +            |
| 2      | R1-2        | +             | White         | Small       | Rod shaped            | P2           | +            | -            | +            | +            |
| 3      | R1-3        | -             | White         | Small       | Rod shaped            | P3           | -            | -            | +            | +            |
| 4      | R1-4        | -             | White         | Small       | Rod shaped            | P4           | -            | +            | +            | +            |
| 5      | R1-5        | +             | White         | Small       | Rod shaped            | P5           | -            | -            | -            | +            |
| 6      | R1-6        | +             | Pink          | Small       | Rod Shaped Chain structure | P6           | +            | -            | -            | -            |
| 7      | R1-7        | -             | White         | Small       | Rod shaped            | P7           | +            | +            | -            | -            |
| 8      | R1-8        | +             | White         | Small       | Rod shaped            | P8           | +            | +            | +            | +            |
| 9      | R1-9        | -             | White and Yellow | Medium     | Rod shaped            | P9           | +            | +            | +            | +            |
| 10     | R1-10       | +             | Yellow and white | Medium     | Rod shaped            | P10          | +            | -            | +            | +            |
| 11     | R1-11       | \+            | Pink          | Small       | Rod shaped            | P11          | -            | +            | -            | -            |
| 12     | R1-12       | -             | White         | Small       | Rod shaped            | P12          | -            | -            | -            | -            |
| 13     | R1-13       | -             | Pink, Yellow and white | Medium     | Rod shaped            | P14          | -            | -            | +            | +            |
| 14     | R1-14       | -             | White         | Small       | Rod shaped            | P15          | +            | +            | +            | +            |
| 15     | R1-15       | +             | Purple which changes into pink | Medium | Rod shaped chain structure dispersed | P16          | +            | +            | +            | +            |
| 16     | R1-16       | -             | White         | Small       | Rod Shaped            | P16          | +            | +            | +            | +            |
Table 4 Quantitative estimation of phosphate solubilization in Pikovskaya’s medium

| Microorganism     | Available phosphate (mg/50ml) |
|-------------------|-------------------------------|
|                   | 2\textsuperscript{nd} day | 4\textsuperscript{th} day | 6\textsuperscript{th} day |
| Uninoculated      | 0.32                         | 0.34                        | 0.34                        |
| Genus Bacillus    | 0.70                         | 2.40                        | 2.44                        |
| Genus Pseudomonas | 0.46                         | 2.28                        | 2.18                        |

Fig. 1 White (a) and yellow (b) Fig. 2 G +ve (a) and G-ve (b) bacteria isolated from rhizosphere soil of rice varieties and pink (c) colonies of PSB
Similar studies have been carried by many other workers on rice as well as other rhizospheric soil of other plants. The PSBs have been isolated from Vigna mungo L. (Qureshi et al., 2012), Festuca arundinacea (Monk et al., 2009), legume plants (Khan et al., 2010) rice grown in acidic soil (Thakuria et al., 2004), rice crop of eastern Uttar Pradesh (Shahi et al., 2009).

Present study revealed the existence of Phosphate Solublizing Bacteria in the rhizosphere soils of rice. These bacteria exhibited difference with respect to Gram property, nitrate reductase, oxidase, citrate utilization and catalase activities. Prominent halo zones were found in case of positive PSB isolates on Pikovskaya’s agar. Phosphate solubilization effectively took on sixth day with Bacillus than Pseudomonas. Based on Gram property, bacterial morphology and biochemical tests viz. nitrate reduction, oxidase, citrate utilization and catalase performed on various isolates these were subjected to identification and placed in group I and VIII by referring to the separation outlined in Bergeys Manual of Systematic Bacteriology and confirms the dominance of genera Bacillus and Pseudomonas. The identification at species level will be further undertaken by using 16SrRNA technique.

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