Characterization of Potential Phosphate Solubilizing Bacteria from the Local Paddy Fields in Perlis

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ABSTRACT

Phosphorus solubilizing bacteria (PSB) play an important role in the plant phosphorus nutrition by enhancing P availability. The study was conducted to isolate and characterize PSB from the rhizosphere of rice grown in MADA area. PSB isolates from soil were screened using selective National Botanical Research Institute Phosphate (NBRIP) media. Soluble P of the isolates was determined by Molybdenum Blue method. 16 isolates solubilizing index range from 1.16 - 1.87 were obtained. The P solubility efficiency of 10 selected isolates was found to be ranged between 7.23 mg/L to 87.36 mg/L. The capability of the P solubility of the isolates showed that they are competitive with the efficiency of the commercial Bacillus megaterium which produced 18.18 mg/L of soluble P. This result showed that there are diverse types of PSB exist in rice ecosystem in MADA area and this PSB isolates exhibited wide range of phosphate solubilizing capabilities and few of them were better than commercially B. megaterium. These PSB isolates have the potential to be developed into more effective bio-fertilizer.

INTRODUCTION

Phosphorus solubilizing bacteria (PSB) play an important role in phosphorus (P) nutrition by enhancing plant P availability. These PSB have been shown to improve growth and yield of rice [1]. Phosphorus is taken up by plant mostly as an anion of phosphate. However large proportion of phosphorus fertilizer applied to the soil is quickly converted into the insoluble forms by forming complexes with Al or Fe in acid soils or Ca in acidic soil [2] and become unavailable to the roots. It is established that PSB have the capability to transform the insoluble phosphorus to soluble forms by secreting organic acids from their metabolic activities [3]. Several soil bacteria, particularly those belonging to the genera Pseudomonas and Bacillus were reported to be involved in solubilizing phosphorus by secreting organic acids [4]. These acids will lower the pH and bring about the dissolution of bound forms of phosphate.

Isolation of potent PSB from the local paddy ecosystem would enhance bio-fertilizer efficacy which can complement the application of commercial in organic fertilizer. It is known that continuous application of chemical fertilizers can affect soil chemical and physical characteristic as well as crops yield. Hence, bio-fertilizer has been accepted as a complement to chemical fertilizer to increase soil fertility and crop production such as rice. The aim of this research is to isolate, identify and characterize local PSB strains from rhizosphere of rice plant grown in soils of Perlis paddy fields which subsequently be used for bio fertilizer formulation.

MATERIALS AND METHODS

Soils Sampling:

Soil samples were collected from wetland paddy fields, namely Simpang Empat and Beseri, Perlis. Simpang Empat is located in MADA irrigation project area while Beseri is located outside MADA area. The samples were taken based on two soil types (Marine Alluvium and Riverine Alluvium) and high yield profiles.

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Isolation of PSB from Rhizospheric Soil:

PSB were isolated from each sample by serial dilution and spread plate method. 10 g of soil sample was placed in an Erlenmeyer flask (250 ml) that containing 90 ml of autoclaved 0.9% saline solution and was thoroughly shaken. 1 ml of the above solution was again transferred to 9 ml of sterile saline solution to form 10⁻² dilution. Similarly, serial dilution was made until 10⁻⁶ for each soil sample. 0.1 ml of each dilution was spread on National Botanical Research Institute Phosphate (NBRIP) agar growth medium [5] containing: (Glucose 5 g, MgCl₂.6H₂O 5 g, MgSO₄.7H₂O 0.25 g, KCl 0.1 g, (NH₄)₂SO₄ 0.1 g, agar 15 g, dissolved in 1 L of distilled water supplemented by 5 g of Tricalcium Phosphate (TCP) as sole of phosphorus source that have the ability to releases soluble inorganic phosphate from TCP. The pH was adjusted to 7.0 and incubated at 30°C for 2 days.

Morphological Characterization and Gram Staining:

Morphological characterization of isolates including: shape, margin, elevation, size, texture, appearance, pigmentation (color), and optical property were observed for their characterization. The isolates were gram stained and examined for cellular morphology and arrangement according to the standard procedure [6]. The red colonies will show the gram negative bacteria and the purple colonies will show the gram positive bacteria.

Screening of the Isolates for Phosphate Solubilization:

Each bacterial isolate were aseptically streaked onto NBRIP agar that supplemented with TCP and incubated at 30°C for 7 days. The solubilization of phosphate was observed as a zone of clearance with a diameter that was measured in millimeters. The phosphate solubilizing ability of the isolates was analyzed by determining the phosphate solubilization efficiency [7]. The formula was expressed as below.

\[ SE = \frac{Total\ diameter\ of\ colony + clear\ zone (D)}{Colony\ diameter (d)} \]

Quantification of Phosphate Solubilization:

The isolates that showing large clear zone in solid media were further examined for their ability to released soluble phosphate in NBRIP broth media. B. megaterium was used as a controlled. The PSB cultures were grown in NBRIP broth for eight days with continuous shaking 150 rpm at 30°C. A 10 ml of each culture was taken in centrifuge tube and centrifuge for 15 minutes at 10,000 rpm. Supernatant was decanted and 5 ml of supernatant was added to 20 ml of AB-DTPA extracting solution. P in solution was extracted with Ammonium bicarbonate diethylene -triamine penta acetic acid (AB-DTPA) [8]. Broth P was determined by Molybdenum blue method [9]. 1 ml of broth sample extract was taken in 100 ml of conical flask and 9 ml of distilled water + 2.5 ml of freshly prepared color reagent. The optical density of the blue color developed after 15 minutes was measured at 880 nm by UV-VIS spectrophotometer and the concentration of available P (ppm) was measured. Initial pH and pH changes were measured at last day of incubation by digital pH meter.

RESULTS AND DISCUSSIONS

Isolation of PSB from Rhizospheric Soil and Gram Staining:

About 16 isolates were found to be able to solubilize phosphate. This isolates had the morphological characteristics like circular and irregular shape of colonies. These colonies mostly appeared in cream, yellow and colourless pigmentation. Upon gram staining, 4 isolates were identified as gram positive bacteria, while the 12 isolates shown as gram negative bacteria. Based on the results of the colouring gram, the gram negative rods bacteria is expected to be from Pseudomonas spp. and while the gram-positive rods bacteria is expected from Bacillus spp, due to the presence of endosphere. Soil bacteria are in cocci (sphere, 0.5µm), bacilli (rod, 0.5-0.3µm) and spiral (1-100µm) shapes [10]. Bacilli are common in soil, whereas spirili are very rare in natural environment. The result was expressed in the Table 1.

Screening of the Isolates for Phosphate Solubilization:

The solubilization efficiency of 16 isolates was measured at the end of 7 days incubation. The result was expressed in the Table 1. Among these isolates, PL 13 from Padang Lati, Perlis showed the highest phosphate solubilization efficiency index (1.96), followed by the isolate PL 16 (1.87) whereas all the other isolates showed lower efficiency which range from 1.16 to 1.69. This result proved that PL 13 was very efficient isolate in solubilizing TCP. In screening process, the PL 13 was showed the highest clearing zone because it released low molecular weight acid and it produced high amount of soluble P as compared to other isolates. However, all the selected isolates were able to solubilize TCP in NBRIP agar media. Generally, halo zone increased with increased in colony diameter [7]. The screened isolates were able to solubilize TCP on solid culture state by forming clear zone also depending on the type of organism involved.
Table 1: Gram staining and solubilization index of potential phosphate solubilizing bacteria in National Botanical Research Institute’s phosphate (NBRIP) growth medium.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Gram staining</th>
<th>Cell morphology</th>
<th>Colonies diameter (d) mm</th>
<th>Total diameter of colony + clear zone (D) mm</th>
<th>Solubilization Index/ efficiency (D/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE 1</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>17.0</td>
<td>25.0</td>
<td>1.47</td>
</tr>
<tr>
<td>SE 2</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>7.0</td>
<td>10.0</td>
<td>1.42</td>
</tr>
<tr>
<td>SE 3</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>13.0</td>
<td>19.0</td>
<td>1.46</td>
</tr>
<tr>
<td>SE 4</td>
<td>Purple, Gram positive</td>
<td>Rods</td>
<td>7.0</td>
<td>10.0</td>
<td>1.42</td>
</tr>
<tr>
<td>SE 5</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>8.0</td>
<td>11.0</td>
<td>1.37</td>
</tr>
<tr>
<td>SE 6</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>20.0</td>
<td>31.0</td>
<td>1.55</td>
</tr>
<tr>
<td>SE 7</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>7.5</td>
<td>11.0</td>
<td>1.46</td>
</tr>
<tr>
<td>SE 8</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>17.0</td>
<td>22.0</td>
<td>1.29</td>
</tr>
<tr>
<td>SE 9</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>20.0</td>
<td>30.0</td>
<td>1.50</td>
</tr>
<tr>
<td>SE 10</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>21.0</td>
<td>32.0</td>
<td>1.52</td>
</tr>
<tr>
<td>PL 11</td>
<td>Red, Gram negative</td>
<td>Rods</td>
<td>6.5</td>
<td>11.0</td>
<td>1.69</td>
</tr>
<tr>
<td>PL 12</td>
<td>Purple, Gram positive</td>
<td>Rods</td>
<td>19.0</td>
<td>28.0</td>
<td>1.47</td>
</tr>
<tr>
<td>PL 13</td>
<td>Red, Gram negative</td>
<td>Coccus</td>
<td>15.0</td>
<td>29.5</td>
<td>1.96</td>
</tr>
<tr>
<td>PL 14</td>
<td>Purple, Gram positive</td>
<td>Rods</td>
<td>20.0</td>
<td>25.0</td>
<td>1.25</td>
</tr>
<tr>
<td>PL 15</td>
<td>Purple, Gram positive</td>
<td>Rods</td>
<td>25.0</td>
<td>29.0</td>
<td>1.16</td>
</tr>
<tr>
<td>PL 16</td>
<td>Red, Gram negative</td>
<td>Coccus</td>
<td>16.0</td>
<td>30.5</td>
<td>1.87</td>
</tr>
</tbody>
</table>

The experiment was done in triplicate
*SE= Simpang Empat Phosphate Solubilizing Bacteria
*PL= Padang Lati Phosphate Solubilizing Bacteria

Quantification of Phosphate Solubilization:
Test for available P in NBRIP broth were also conducted to quantify the phosphate solubilizing activity of the isolates. The ability of PSB to measured inorganic P and pH of the broth media was also measured up to 8 days incubation. The amount of soluble P released in the NBRIP broth by each of the 16 isolates was quantitatively measured by Molybdenum Blue method describes by Watanabe and Olsen in 1965. In quantitative estimation, range of soluble P released between 7.23 mg/L to 87.36 mg/L which is shown in Figure 1. SE9 (25.35 mg/L) is not significantly different to PL12 (22.73 mg/L) but significantly different to B. megaterium and SE1 (19.16 mg/L). B. megaterium used as control was solubilized TCP only 18.18 mg/L. Result showed the maximum soluble P produced by PL 13 (87.36 mg/L), PL 16 (78.52 mg/L), PL 11 (44.26 mg/L), SE 6 (39.89 mg/L) and SE 10 (36.55 mg/L) after 8 days incubation. 8 isolates were better than commercially B. megaterium. P solubilization is a complex phenomenon, which depend on many factors such as nutritional like carbon and nitrogen source for their metabolic activity, physiological and growth condition of the culture like temperature and time of incubation [11]. Measurement of the pH was measured at the end of the incubation day. After 8 days, pH was decreased 5.84 to 4.18. 5 bacteria isolates which were SE 3, SE 6, SE 10, PL 12 and PL 16 have significantly lower than B. megaterium. It was revealed that pH was gradually decreased with the increased incubation time. Generally, bacteria secreting low molecular weight of organic acid from their metabolic process [12] and may due to acidic condition in NBRIP broth. Thus, in present study PL 11, PL 13 and PL 16 were found to be more efficient bacterial isolates to solubilize TCP.

Each value is expresses as mean (n = 3)
Bars graph not connected by same letters are significantly different (P < 0.05)
Conclusion:

This result showed that diverse types PSB exist in rice ecosystem in MADA area and this PSB isolates exhibited wide range of phosphate solubilizing capabilities and few of them were better than commercially available B. megaterium. It can be conclude that, all selected PSB has the capacity to solubilized Tricalcium phosphate (TCP) that supplemented in NBRIP medium. P released by the PSB was associated with reduction in pH of the medium. PL 11, PL 13 and PL 16 were found more efficient bacterial isolates to solubilize TCP. PL 13 is the most efficient strains on the basis on their phosphate solubilizing activity. Based on morphological characteristics, PL 13 was confirmed as a bacteria because it have coccus shaped and gram negative characteristic. These PSB isolate have the potential for development more effective bio-fertilizer.

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