Isolation of phosphate solubilizing bacteria from acacia tree rhizophere soil

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ABSTRACT

Phosphorous is an essential plant nutrient whose deficiency severely restricts crop yield and it is the least available nutrient to plants. Phosphate solubilizing bacteria play an essential role in the phosphorus nutrition cycle by enhancing its availability to plants through release from inorganic and organic soil phosphate pools. In this study, we sought to isolate phosphate solubilizing bacteria (PSB) from soil of the Acacia plant rhizophere. Candidate colonies were grown on National Botanical Research Institute’s phosphate (NBRIP) screening medium with 0.5% tricalcium phosphate (TCA) to determine phosphate liberation capabilities. The colonies were tested to temperatures (27°C, 37°C, 47°C and 57°C) and pH (from 4 to 9) compatibility, as well as heavy metal exposure (0-0.12 µg/ml HgCl(II)). We learned that PSB colonies isolated from the Acacia plant rhizophere soil demonstrated high phosphate liberating activity (on NBRIP medium containing TCA) and survived temperatures to 47°C over a pH range of 4.5 to 8.5, and up to .02 µg/ml HgCl(II). These results provide a strong baseline source for application in agriculture when a bio-fertilizer is required.

Key words: Phosphate solubizing, Bacteria, Acacia, Tri-calcium-phosphate.

INTRODUCTION

Phosphorous is essential for survival, growth and productivity of plants (1). Organically bound phosphorous enters the soil during the decay of natural vegetation, of dead animals, and from animal excretions (2). Assimilation of phosphate from organic compounds by plants and microorganisms take place through the broad "phosphatase" enzyme family which is present in a wide variety of soil microorganisms (3). However, plants can absorb phosphate only in a soluble form. Hence, transformation of insoluble phosphate into its soluble form is needed to be carried out by a number of microbes present in the soil. A large fraction of soil microbes can dissolve insoluble inorganic phosphates present in the soil and make them available to the plants (4). Because of the negative charge of phosphate ions, they are quickly adsorbed after weathering of clays or detritus particles, forming insoluble forms of aluminum, calcium, or iron phosphates, all unavailable to plants (5). Phosphorus (P) is sequestered by adsorption to the soil surface and by precipitation reactions with soil cations, particularly iron, aluminium, and calcium. P availability additionally impaired in alkaline and calcareous soil due to the formation of poorly soluble calcium phosphate minerals (6).

Phosphate functions in a cell cannot be performed by any other nutrient, and an adequate supply of phosphate is required for survival and reproduction of plants. Phosphorus is classified as a major nutrient, meaning that it is

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frequently deficient for crop production and is required by crops in relatively large amount (7). Unfortunately, the majority of soil P remains interlocked in various forms not available for plant use (8). Therefore, a large amount of P fertilizer has been used to increase plant growth, which is likely to cause negative impact in respects to both environment and economy (9). Chemical fertilizers added to the soils to circumvent the problem of P deficiency further complicates the situation by the fact that almost 75-90% of added P fertilizer is precipitated by Fe, Al and Ca complexes present in the soils (10).

Phosphorus bio-fertilizers in the form of micro-organisms can help increase the availability of accumulated phosphates for plant growth through bio-solubilization of inorganic phosphates (11). Individual or co-inoculation of phosphate solubilizing bacteria (PSB) with other groups of microorganisms may enhance the plant growth by increasing the efficiency of phosphate solubilization or the availability of other trace elements (12). Insoluble phosphate compounds can be bio-solubilized by organic acids and phosphatase enzymes produced by microorganisms. PSB have been shown to enhance the solubilization of insoluble P compounds through the release of organic acids and phosphatase enzymes, thereby increase the availability of P to plants (13). These bacteria also increase prospects of using phsphatic rocks in crop production instead of phosphate fertilizers. Increased efficiency of P solubilizing bacteria is possible through co-inoculation with other beneficial bacteria and mycorrhizae (7) and a considerable number of bacterial species are able to exert a beneficial effect upon plant growth. They are used as bio-fertilizers or control agents for agriculture improvement, and for environment conservation of agricultural regions. It has been reported that different bacterial species have the ability to solubilize inorganic phosphate compounds, such as tricalcium phosphate (TCP), dicalciumphosphate, hydroxyapatite and rock phosphate (14). Therefore, this study focuses on isolation of TCP solubilizing bacteria from Acacia tree rhizosphere soil which is an important agro-forestry plant species.

MATERIALS AND METHODS

Sample Site and Sample Collection
Acacia tree grown on hill side where there is a possible loss of soluble phosphate as the result of washing out to the depression down side of the hill was selected. Approximately, 20 cm long roots were taken from the Acacia tree grown in the Tewdros campus, University of Gondar, Ethiopia. The root samples were suspended in 200ml distilled water and vortexed for an hour (Figure 1). Root soil solution was prepared by 10-fold serial dilutions and plated on National Botanical Research Institute’s phosphate (NBRIP) screening medium at 37°C. Following culture on the medium supplemented with10g glucose, 5g Ca₃(PO₄)₂, 5g MgCl₂·6H₂O, 0.25g MgSO₄·7H₂O, 0.2g KCl, 0.1g (NH₄)₂SO₄, and 1.5% agar/l at pH 7.0, bacterial colonies were observed. These bacterial colonies were examined for their phosphate solubilizing ability. After 24hrs of culture, colonies appeared, some of which showed clearing zone. These isolates were selected for their phosphate solubilizing ability from the medium supplemented with Ca₃(PO₄)₂.
To test pH and temperature tolerance, nutrient broth with peptone as the source of nitrogen, yeast extract as mineral growth factors and sodium chloride as maintenance of osmotic pressure was prepared and autoclaved. A loopful of colonies selected previously for their halo zone formation on NBRIP was inoculated in a broth of pH ranging from 4.0–9.0. Bacterial growth at 37°C, 47°C and 57°C at pH 7 was also performed to determine optimum temperature.

For heavy metal tolerance screening, concentrations (0–0.12 µg/ml) of HgCl(II) were prepared and added to broth medium. Then, PSB colonies were incubated for 24hrs at 37°C. After 24hrs cultures from each mercuric concentration were pour plated on to the agar medium to recover the surviving bacterial colonies from the test tube cultures containing mercuric chloride (II).

**RESULTS**

Bacterial isolates were identified based on their phosphate solublization efficiency of TCP present in the NBRIP medium. The isolates were found to be promising for solubilization of inorganic tri-calcium phosphate (Figure 2).

The isolates having a halo zone were grown on NBRIP agar medium after 24 hours were tested for pH tolerance. They have shown a wide range of pH tolerance from 4.5 to 8.5 and the maximum OD \( \text{OD}_{600} \) was observed at pH 8 (Figure 3).
These colonies were also observed to tolerate a mercuric concentration of 0.01µg/ml. The minimum bactericidal concentration (MBC) was 0.02µg/ml even if OD<sub>600</sub> was positive for a concentration of 0.02µg/ml, no colony was recovered from this mercuric chloride concentration on nutrient agar. The isolates having a halo zone were grown in NBRIP agar medium after 24 hours were also tested for temperature tolerance and able to tolerate a temperature of 47°C (mesophilic) (Table 1).

**Table 1. Growth of bacterial colonies over different temperature and different mercuric chloride concentrations**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>HgCl&lt;sub&gt;2&lt;/sub&gt; (µg/ml)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>1</td>
<td>Growth</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Temperature range</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Bio-solubilizing of nutrients for crop availability can have a large impact in terms of the economic and production of food growth. Here we tested and identified microbe species with such capabilities in the roots of the important forest plant, Acacia. To identify these microbes, NBRIP medium was used for testing phosphate bio-solubility. It serves as a selective medium for phosphate solubilizing bacteria isolation due to the presence of tri-calcium phosphate (15), which is known for its halozone formation (13). Our results were found to be similar with the works of Sharma (13) and we demonstrated that micro-organisms exist that could solubilize and make phosphorus available for the Acacia root, thereby contributing to its success as an agriculturally significant plant. These findings were consistent with the works by (16), in which native phosphate solubilizing bacteria (PSB) were isolated from some areas of Ethiopia. Similarly, Kannapiran and Ramkumar (17) have isolated phosphate solubilizing *Pseudomonas sp.* with a maximum phosphate solubilization potential. According to (18), production of halo zones on solid media and proficient release of phosphate in solution is attributed to the release of organic acids viz. citric, glyoxalic, malic, keto butyric, and succinic. Likewise, in our work, the clear zone was formed in the same way probably due to acid production. The major mechanism of P solubilization by these PSB is considered to involve pH reduction through the production of these organic acids. As such, it is important, and consistent with our results, that these isolates were capable of growing at low pH.

**CONCLUSION**

Rhizosphere of the Acacia tree contains phosphate solubilizing bacteria that form halo zones when cultured on NBRIP medium containing tri-calcium phosphate. This demonstrates that the cells can solubilize inorganic tri-calcium phosphate and is a promising microbial resource for the development of phosphate solubilization for plant nutrient availability. Treatment at different temperature and pH indicated that they were capable of growing up a temperature of 47°C and over a pH range of 4.5 to 8.5 with pH 8 being the optimum where maximum growth was achieved at 37°C after 24 hrs. These colonies were also capable of resisting a 0.01 µg/ml mercuric chloride (II) and concentration of 0.02µg/ml was the minimum bactericidal concentration (MBC) for these colonies.

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REFERENCES