
Phosphate-solubilizing bacteria from upland rice (*Oryza sativa* L.) rhizosphere and their tricalcium phosphate solubilizing abilities

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Abstract Insoluble forms of phosphorus (P) may be converted to soluble P by phosphate-solubilizing organisms living different soil ecosystems to make them available for plant roots to be absorbed. In this study, isolation of rhizobacteria and screening of their ability for phosphate solubilization was done. A total of 25 isolates were obtained from upland rice rhizosphere in Isabela province, Philippines. Measuring the solubilizing efficiency revealed that seven out of 25 isolates were found to be promising phosphate-solubilizers showing clearing zone around the colony. Moreover, phosphorus solubilization index (PSI) of the isolates ranged from 1.25 to 1.60 and results obtained are higher than other observations indicating that strains of bacteria isolated are effective phosphate-solubilizers. Furthermore, these PSB could serve as efficient biofertilizer candidates for improving the P nutrition of the crop. This observation can be a promising contribution to cropping system of upland rice, which is constrained by drought leading to inefficient P acquisition. If further developed, this can be eco-friendly and prove to be cost effective strategy to improve upland rice production particularly in the Philippines.

Keywords: Phosphate-solubilizing bacteria, upland rice, solubilization index, rhizobacteria, isolation and screening

Introduction

Phosphorus (P) is one of the most limiting mineral nutrients for plant production in many low input agricultural systems. It is one of the essential macronutrients for plants and is applied to soil in the form of phosphatic fertilizers. Although, if available, this is the most performing practice, this should be accompanied by other measures; since it is known that majority of soluble inorganic phosphate applied as chemical fertilizer to the soil is immobilized rapidly and are made unavailable to plants (Ramaekers *et al.*, 2010; Goldstein, 1986).

The use of soil microorganisms may represent a sustainable solution to this problem. Microorganisms are vital part of the P cycle in soil as they

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are involved in a range of processes that affect the transformation of soil. Particularly, through solubilization and mineralization, microorganisms in soil are effective in releasing P both from inorganic and organic pools of total P in soil (Rodriguez and Fraga, 1999).

At present, the key resolution in managing soil P is to obtain optimum crop production at the same time minimizing loss of P from soils. Phosphate solubilizing microorganisms as soil inoculums have also recently gained the interest of agriculturists in enhancing growth and yield of crops (Cruz *et al.*, 2014; Cavite *et al.*, 2018; Young, 1994; Goldstein *et al.*, 1999). Plant growth-promoting rhizobacteria (PGPR) are heterogeneous group of rhizobacteria in association with roots which can directly or indirectly improve the quality or extent of plant growth (Glick, 2012), and P solubilization ability of the soil microorganisms is regarded as one of the most vital qualities associated with P nutrition in plants. Given the increasing costs and negative consequences of increasing use of chemical fertilizers, the use of PGPR is gainful alternative in sustainable crop production.

It is well-acknowledged that mineral phosphate solubilization mechanism by phosphate-solubilizing bacteria (PSB) strains is related with the release of low molecular weight organic acids (Rodriguez and Fraga, 1999). The cations bound to phosphate are chelated through their hydroxyl and carboxyl groups and converts it into soluble forms (Kpombekou and Tabatabai, 1994). However, many factors are dependent on this complex phenomenon of P solubilization which includes nutritional, physiological and growth conditions of the culture (Reyes *et al.*, 1999). Experimental evidence is present in mineral phosphate solubilization highlighting the role of organic acids (Halder *et al.*, 1990).

With the importance on potential PSB from upland rice rhizosphere, the present experiment was designed to isolate and screen rhizobacteria from upland rice of their ability for tricalcium phosphate solubilization.

Materials and Methods

Isolation of Bacteria from the Rice Rhizosphere

Plant sample collection: Six upland rice plant samples of the ‘Balatinaw’ variety were randomly collected from Brgy. Sindon Bayabo, Ilagan City, Isabela, Philippines [17° 7’ 19.497’’ N 122° 0’ 25.206’’ E (DMS)]. These were carefully dug out from the soil making sure not to disturb the entire root system (Cruz and Paterno, 2014). Samples were then placed in clean plastic pails.

Rhizosphere sample collection: Isolation of bacteria from the rhizosphere was performed based on the method of Cruz and Paterno (2014). It was carried out by carefully removing the entire root system completely

out of the ground. Then, gently tapped to remove soil adhering to the roots. For each plant, 10 g of root samples were transferred into a 250-mL Erlenmeyer flask with 100 mL sterile distilled H₂O and shaken for 24 hr.

Serial dilution and spread plate method: The root-water mixture was diluted and made into a series of four ten-fold dilutions. Then, 0.1 mL of 10⁻³ and 10⁻⁴ dilutions was spread on duplicate Burks agar plates (Cruz and Paterno, 2014). The plates were incubated at room temperature and different colonies appearing morphologically on the medium were isolated and sub-cultured for phosphate solubilization assay.

Media used: Burks medium was used for isolation and cultivation of rhizobacteria from upland rice rhizosphere. This medium is in dehydrated form and prepared only when needed. Media were sterilized for 20 min at 121° C before use.

Purification of isolated bacteria: To purify isolated bacteria, colonies formed were examined first in terms of their color. Then, a loopful of each morphologically different colony was streaked on duplicate Burks agar plates. Pure cultures were used for further experiments. A record of the list of purified bacterial isolates was kept by assigning codes at every colony purified to facilitate identification.

Screening for Phosphate Solubilization

Bacterial isolates were screened and tested for phosphate solubilization. The medium used in the assay was sterilized in an autoclave for 20 min at 121° C before use.

Phosphate solubilization assay: To test phosphate solubilization activity, isolates were grown in modified Pikovskaya's medium containing 5.0 g Ca₃(PO₄), 0.2 g NaCl, 0.2 g KCl, 0.1 g MgSO₄ · 7H₂O, 0.00025 g MnSO₄ · 7H₂O, 0.00025 g FeSO₄ · 7H₂O, 0.5 g (NH₄)₂SO₄, 0.5 g yeast extract, 10.0 g glucose, 20.0 g agar and 1,000 mL distilled H₂O. Bacterial isolates were spot-inoculated on surface of the agar and incubated for five days. Clearing zone around the bacterial growth or colony indicated phosphate solubilization (Cruz and Paterno, 2014; Shahab *et al.*, 2009).

Determination of phosphate-solubilizing capacity: Then, phosphate-solubilizing capacity of isolates was semi-quantitatively determined in terms of its phosphorus solubilization index (PSI) using the formula below by Islam and Hossain (2012). Total diameter of halo zone was measured by getting length of the halo zone from one edge to the other. Same procedure was done also in measuring length of colony diameter.

$$\text{PSI} = \frac{\text{Total diameter of the halo zone (mm)}}{\text{colony diameter (mm)}}$$

Results

Isolation of Bacteria from the Rice Rhizosphere

A total of twenty-five bacterial isolates were obtained from upland rice rhizosphere. Isolates were observed as having heavy and thick growth and produced thick layer of mucus. Different colors were observed such as white, yellow, orange and purple. Isolate codes were represented by the location of where these were collected (I for Isabela province), medium used in isolation (B for Burks medium), variety (B for Balatinaw) and colors colony colors observed (either white, yellow, orange or purple).

Screening for phosphate solubilization

Phosphate solubilization assay: Solubilizing efficiency of twenty-five bacterial isolates was measured using modified Pikovskaya's agar medium containing tricalcium phosphate. Only seven out of twenty-five isolates were able to produce clearing zone around the colony which is attributed to phosphate solubilization in its vicinity (Figure 1). Results further revealed that the 7 isolates produced different lengths of colony diameters as well as of the halo zones. Generally, halo zones increased with increase in colony diameter.

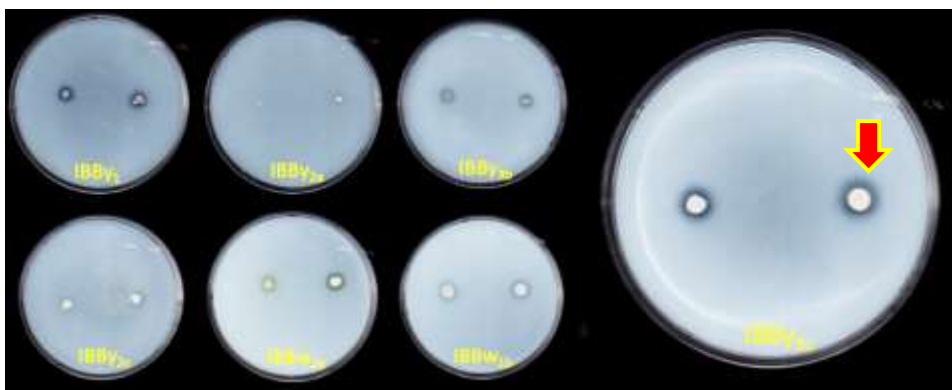


Figure 1. Halos formation around colonies of phosphate-solubilizing bacterial isolates

Determination of phosphate-solubilizing capacity: The total diameter of the halo zone and colony diameter were recorded after five days of incubation at room temperature to determine the isolate's phosphorus solubilization index (PSI). The general trend can be seen that halo zones increased with increase colony diameter. Phosphorus solubilization index (PSI) of the isolates ranged from 1.25 to 1.60 (Table 1). Highest efficiency was exhibited by isolate IBBY₁ (1.60), while lowest efficiency was shown by isolate IBBW_{1b} (1.25). No general trend can be seen between PSI and

diameters of halo zone and the colony diameter since not even the longest diameter of halo zones and colony yielded the highest PSI – this was shown by isolate IBBw_{2e}. The results reveal that at same halo zones, higher efficiency is obtained with shorter colony diameters.

Table 1. Phosphate-solubilizing bacteria and their solubilization efficiency

Isolate	Halo Zone Diameter (mm)	Colony Diameter (mm)	Phosphorus Solubilization Index (PSI)
IBBy ₁	10.0	6.0	1.60
IBBy _{2a}	6.0	4.0	1.50
IBBy _{3b}	8.0	6.0	1.30
IBBy _{2c}	8.0	6.0	1.30
IBBy _{3c}	11.0	7.0	1.50
IBBw _{2e}	12.0	8.0	1.50
IBBw _{1b}	10.0	8.0	1.25

Discussion

Phosphorus (P) is an essential macronutrient for plant growth. Due to its conversion into calcium or magnesium salts in soils, a large portion of P from chemical fertilizers becomes insoluble and thus become unavailable to plants. Certain soil microorganisms influence the subsequent availability of phosphate to plant roots by transforming insoluble forms of P into soluble forms (Illmer and Schinner, 1995).

Phosphate-solubilizing bacteria (PSB) are important components of soil and directly or indirectly influence the soil's health through their useful activities. It is known that rhizospheric microbes mediate many soil processes such as decomposition, nutrient mineralization and nitrogen fixation (Pradhan and Sukla, 2009). In this study, seven isolates were able to produce clearing zone around the colony which is attributed to phosphate solubilization in its vicinity (Figure 1). Cruz *et al.* (2014) used Pikovskaya's media and reported similar results. These results are also in agreement with those of Tripti Kumar and Anshumali (2012), Awais (2017), Lavakush *et al.* (2012), Mohan and Radhakrishnan (2012) and Madhan Chakkaravarthy *et al.* (2010) since present PSB isolates solubilized tricalcium phosphate. Tripti Kumar and Anshumali (2012) also described that zone of formation could be due to activity of enzyme phosphatase by bacterial isolates.

The principal mechanism for the formation of clearing zone by the seven isolates is the secretion of organic acids. Sarker *et al.* (2004) have verified this effect on a similar assay, both in solid and culture broth media, which interestingly recorded a decrease in pH value of the broth medium used. Although, solid agar medium was utilized in this assay, this result agrees with the above-mentioned findings. The secretion of organic acids by phosphate-solubilizing bacteria (PSB) have also been well-documented by

Rodriguez and Fraga (1999). On the other hand, phosphatase enzyme activity contributes to the solubilization of organic phosphates in soil (Al-Amoodi *et al.*, 2005). Normally the pH of agricultural soils ranges between acidic to neutral, so acid phosphatases are thought to play the major role in phosphate solubilization (Rodriguez and Fraga, 1999; Duponnois *et al.*, 2005). However, alkaline phosphatase also plays a role in phosphate solubilization (Sahu and Jana, 2000). It has often been noted that the distribution and activity of PSB and their subsequent effect on phosphate solubilization is regulated by exogenous P level (Rodriguez and Fraga, 1999).

The PSI results obtained in the solid medium are higher than other observations and supported with Nostrati *et al.* (2014), indicating that the strains of bacteria isolated from upland rice rhizosphere are effective phosphate-solubilizers. These results are also in agreement with the findings of Madhan Chakkaravarthy *et al.* (2010) in similar studies on agar plates. Furthermore, these PSB could serve as efficient biofertilizer candidates for improving the P nutrition of the crop.

In crop production, PSB are also used as inoculants. Use of these bacteria as inoculants simultaneously increases P uptake and crop yield of plant. Among the most powerful phosphate-solubilizers are strains from genera *Pseudomonas*, *Bacillus* and *Rhizobium*. Enzyme acid phosphatases play major role in mineralization of organic phosphorous in soil (Rodriguez and Fraga, 1999).

Phosphorus is abundant in most soils and is one of the macronutrients that limits plant growth. With phosphate fertilizer application, overall efficiency is low because of insoluble complexes formed (Vassilev and Vassileva, 2003). This necessitates for a frequent application of insoluble forms of inorganic P for crop production which ultimately would leach to the ground water resulting to eutrophication of aquatic systems (Del Campillo *et al.*, 1999). With understanding on environmental concerns and present advances in sustainability, researches are geared towards strengthening crop technologies that lessen costly, though less bio-available sources of plant nutrients such as rock phosphate. Additionally, by application of PSB, the agronomic effectiveness of crops can be enhanced (Whitelaw, 2000).

Use of PSB as bio-inoculants results in an increase availability of P in soil, minimizes P fertilizer application, reduces environmental pollution and promotes sustainable agriculture (Rodriguez and Fraga, 1999). Phosphate solubilizing bacteria also have the potential to remediate metal contaminated land by enhancing phosphate-induced immobilization of metals (Ma *et al.*, 1994; Cotter-Howells and Caporn, 1996).

It is concluded from the present study that upland rice rhizosphere could be a good source of phosphate-solubilizing bacteria. Solubilizing efficiency revealed that seven out of 25 isolates were found to be promising

phosphate-solubilizers showing clearing zone around the colony. Moreover, phosphorus solubilization index (PSI) of the isolates are higher than other observations indicating that strains of bacteria isolated are effective phosphate-solubilizers. Furthermore, these PSB could serve as efficient biofertilizer candidates for improving the P nutrition of the crop. This observation can be a promising contribution to cropping system of upland rice, which is constrained by drought leading to inefficient P acquisition. However, more studies are warranted to understand the mechanism of isolated PSB on a biochemical and molecular level. If further developed, this can be eco-friendly and prove to be cost effective strategy to improve upland rice production particularly in the Philippines.

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