In vitro solubility of P from the different type rock phosphate along with PSBs and organic substrates

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Abstract
P is an important primary nutrient for the crop growth as root development, seed quality and energy currency. P use efficiency generally ranges within 15-20%. Commercially available P fertilizers immobilized in soil owing to formation of insoluble mineral complexes with Ca²⁺ (Tricalcium phosphate), Fe³⁺ (Strengite) and Al³⁺ (Variscite) making it unavailable to plants after application. Organic form of P constitutes 20–80% of total soil P and remaining form is in inorganic form (apatite, strengite and varicite). Burkholderia cariabensis, PSB: B. cepacia, PSB; Rock Phosphate (Udaipur): RP₁; Rock Phosphate (Purulia): RP₂; FYM (dosage) 200 Q/ha and Vermi-Compost (VC): 5 t/ha. were applied to the soils. Lowest pH (6.44) was observed at 60 days after incubation in alluvial soil whereas the encountered lowest pH value (4.29) at 60 days incubation period in red soil. Higher pH was dropped in PSB: treated soil as compared to PSB: whereas FYM and PSB: treated soil more decreased in pH over the VC and PSB. Phosphorus solubilization was resulted lower in case of red soil as compare to alluvial soil, in other hand phosphorus solubilization was increased with lapse of time interval. Udaipur rock phosphate was recorded higher solubilization of phosphorus over the purulia rock phosphate. Coinoculation of PSBs along with RP₁ or RP₂ was resulted lower in phosphorus solubilization as compared to Coinoculation of PSBs with FYM or VC.

Keywords: Solubilization, rock phosphate, phosphorus solubilizing bacteria, farm yard manure, vermicompost

Introduction
Most important role of phosphorus (P) in plant growth as an energy transfer (ATP and ADP), root development etc. Whereas, in soil solution undergoes several fates such as fixation, adsorption, ligand exchange and anion exchange. P use efficiency generally ranges within 15-20%. Commercially available P fertilizers immobilized in soil owing to formation of insoluble mineral complexes with Ca²⁺ (Tricalcium phosphate), Fe³⁺ (Strengite) and Al³⁺ (Variscite) making it unavailable to plants after application (Malik et al., 2012) [13]. Organic form of P constitutes 20–80% of total soil P and remaining form is in inorganic form (apatite, strengite and varicite). Readily available (Olsen P and Bray Kurtz P) form of P is 1.5 to 11 % out of total soil. While, major portion of it found in primary minerals, adsorbed, precipitated or in organically-complexed forms (Candron et al., 2005; Pierzynski et al., 2005; Stutter et al., 2012) [18, 21]. Phosphorus solubilising microorganisms (PSMs) solubilize the P from organic P and mineral P by the process of i.e., mineralization, Chelation, Fe & Mn reduction and Acidification. Phosphate solubilizers convert insoluble P form to soluble P form by acidification, ligand-exchange reaction, secretion of low mass molecular organic acids and chelation (Barroso et al., 2006) [11]. Plant establishment under low P availability is facilitated by soil resident phosphorus solubilizers. PSB is widely spread in soil and it constituted 40% of the culturable bacterial population (Kucey, 1983; Jorquera et al., 2008) [11]. Correction of P deficiency through application of commercially available P fertilizers is economically constrained and ecologically owing the higher P fertilizer requirement and greater P fixing capacity soils (Hinsinger, 2001) [3]. Soil P bank develop in some soils under continued long-term application of fertilizers and manures (Kamprath, 1967) [10]. Technological intervention in increasing P use efficiency under diverse soil types need to be explored (Menezes-Blackburn
et al., 2017) [15]. Effective utilization of naturally occurring P sources (Rock-phosphates) under red and alluvial soils need to be explored. Co-inoculation of P solubilizers along with carbon rich sources (FYM and Vermicompost) under red and alluvial soils would be great interest to study. Keeping the beyond facts in mind, the present exploration has been planned to study the effect of PSB (two isolates), rock phosphates (two sources) under organic inputs (vermicompost and farmyard manure) in red and alluvial soil to quantify the effective performance microbial solubilisation and chemically make available of fixed and organic phosphorus as well as is adsorbed as backward from soil.

**Methods and Materials**

Alluvial soil was collected from rice section field of Bihar Agricultural University, Sabour, located on the bank of river ganga at 25°15'40" N latitude and 78°02'42" E longitude with an elevation of 45.57 meters above the sea level. Red soil was collected from Banka district situated at 24° 30' to 25° 8'N latitude and 86° 30' to 87° 12' East latitude in the south west part of Bihar. Soil samples were air dried under shadow the ground with the help of 2 mm sieve. Inoculants, PSB1 (*Burkholdaria cariabensis*) and PSB2 (*B. cepacia*) procured by Biofertilizer unit, BAU, Sabour. FYM (Farm Yard Manure) from Dairy unit and VC (Vermicompost) from Vermicompost unit, BAU, Sabour. The general characteristics of soil were determined by the following standard methods as following standard procedure. 50 g of both soils were filled in 100 mL of test tubes and used further research. The microbiological parameters were analyzed following standard procedures. The different parameters including pH, EC, organic carbon, cation exchange capacity (CEC), available nitrogen, phosphorus, potassium, phosphorus solubilizing power and phosphatases activity along with microbial population (bacteria, actinomycetes, fungi and phosphorus solubilizing bacteria) were assessed. The experiment was conducted at 0, 30 and 60 days of incubation interval. Application of rock phosphates (from Udaipur and purulia), farm yard manure (FYM) and vermecompost (VC) with phosphorus solubilizing bacteria (*Burkholdaria cariabensis* and *B. cepacia*). Treatment details, (T1, Control; T2, PSB1+RP; T3, PSB1+RP; T4, PSB1+RP; T5, PSB1+RP; T6, PSB1+PSB1+RP; T7, PSB1+PSB1+RP; T8, PSB1+FYM+RP; T9, PSB2+FYM+RP; T10, PSB1+FYM+RP; T11, PSB2+FYM+RP; T12, PSB1+VC+RP; T13, PSB2+VC+RP; T14, PSB1+VC+RP; T15, PSB2+VC+RP; T16, PSB1+PSB2+FYM+VC+RP; T17, PSB1+PSB2+FYM+VC+RP). PSB1: *Burkholdaria cariabensis*, PSB2: *B. cepacia*; Rock Phosphate (Udaipur): RP1, Rock Phosphate (Purulia): RP2; FYM (dosage) 200 Q/ha and Vermi-Compost (VC):5 t/ha.

**Morphological characteristics of isolates**

Morphological characteristics test was carried out by bergey’s manual of systemic bacteriology (Buchanan, 1974).

### Table 1: Colony characteristics of phosphorus solubilizing bacterial isolates

| Isolate | Shape | Edge | Opacity | Colour | Elevation | Texture | Gram staining |
|---------|-------|------|---------|--------|-----------|---------|---------------|
| PSB1    | Round | Undulate | Opaque | Creamy | Raised | Mucoid | Negative |
| PSB2    | Round | Undulate | Opaque | White   | Raised | Mucoid | Negative |

### Table 2: Initial chemical and physical properties of experimental soils

| Parameter                        | Red Soil | Alluvial Soil |
|----------------------------------|----------|---------------|
| pH                               | 4.51     | 7.00          |
| EC (dSm⁻¹)                       | 0.08     | 0.20          |
| Cation Exchange Capacity (CEC), [Cmol (+) kg⁻¹] | 14.5 | 26.45 |
| Oxidisable organic carbon (g kg⁻¹) | 2.10 | 7.10 |
| Available N (kg ha⁻¹)            | 149.85   | 169.85 |
| Available P (kg ha⁻¹)            | 5.75     | 16.74         |
| Available K (kg ha⁻¹)            | 91.44    | 104.72        |
| DTPA Extractable Fe (mg kg⁻¹)    | 1.57     | 0.070         |
| Texture class                    | Sandy loam texture | Silty loam texture |
| Maximum water holding capacity (%) | 27.69 | 44.76 |

### Table 3: Initial microbial properties of both soils samples

| Microbial status | Red Soil | Alluvial Soil |
|------------------|----------|---------------|
| Fungi (x10⁶ g⁻¹ of oven dry soil) | 19 | 17 |
| Bacteria (x10⁶ g⁻¹ of oven dry soil) | 23 | 29 |
| Actinomycetes (x10⁵ g⁻¹ of oven dry soil) | 39 | 34 |
| Phosphate solubilizing Bacteria (x10⁵ g⁻¹ of oven dry soil) | 12 | 09 |
| Acid phosphatase activity (µg PNP g⁻¹ soil hr⁻¹) | 39.31 | 30.12 |
| Alkaline phosphatase activity (µg PNP g⁻¹ soil hr⁻¹) | 10 | 65 |
| Phosphate Solubilizing power (mg P 15 mg⁻¹ insoluble P 0.15 g⁻¹ of carbon substrate) | 0.15 | 0.22 |

**Chemical properties of P substrates**

The properties of organic and inorganic substrates i.e., farm yard manure, vermicompost and rock phosphate (RP) have been used in experiment, Table 4. (FYM & VC) and 4.5 (rock phosphate). The experimental FYM and VC used were chemical analysed that having medium content in total N, P, and K. Rock phosphate (udaipur) having more (34 %) in total P content compared to purulia rock phosphorus (10.87 %).
Statistical analysis
Factorial completely randomized design was used for the analysis of variance. The significant differences among the treatment means were calculated at 5% probability levels (p<0.05) (Gomez and Gomez, 1984) [7].

Results
Changes in soil pH (Soil reaction)
Effect of different type of substrates (organic & inorganic) with phosphorus solubilizing bacteria (PSB) on pH of soils at 30 and 60 days of interval had been presented in Table 5.

Effect of organic and inorganic substrates on the P solubilization
The change in soluble phosphorus of the both red and alluvial soils from organic and inorganic substrates with passing the time interval. Statistically significant P solubilization was observed in alluvial soil as compared to red soil. The highest solubilization of phosphorus (P) was recorded at 60 days of interval followed by 30 days of interval in alluvial soil compared to red soil, illustrated in Table 6. Whereas, maximum solubilization of P was recorded at 60 days of interval in red soil (18.83 g P kg⁻¹) and in alluvial soil (23.56 g P kg⁻¹). Statistically significant solubilization of P was observed in both experimental soils (red and alluvial), soil was treated with PSB1, RP1, and FYM alone as well as combination over the other treatments. The solubilization of P was documented 30.11% more in T16 (PSB1 +PSB2+FYM+VC+RP1) over the T0 (PSB1+PSB2+RP1), in case of red soil whereas in alluvial soil 19.26% P solubilization in same comparison (Table 6). Phosphorus solubilization was resulted lower in case of red soil as compare to alluvial soil, in other hand phosphorus solubilization was increased with lapse of time interval. Udaipur rock phosphate was recorded higher solubilization of phosphorus over the purulia rock phosphate. Coinoculation of PSBs along with RP1 or RP2 was resulted lower in phosphorus solubilization as compared to Coinoculation of PSBs with FYM or VC.

| Treatment | Red Soil | Alluvial Soil |
|-----------|----------|---------------|
|           | 30 days  | 60 days       | 30 days | 60 days |
| T1        | 3.53     | 3.60          | 9.02    | 8.99    |
| T2        | 7.10     | 7.27          | 14.62   | 16.16   |
| T3        | 6.50     | 6.66          | 12.34   | 14.55   |
| T4        | 6.70     | 6.71          | 11.32   | 13.56   |
| T5        | 6.46     | 6.60          | 10.63   | 14.16   |
| T6        | 11.18    | 13.16         | 16.63   | 19.02   |
| T7        | 10.12    | 10.92         | 14.99   | 16.61   |
| T8        | 12.84    | 13.27         | 19.28   | 20.54   |

Table 5: Effect of inorganic and organic substrates on soil pH at 30 and 60 interval of days

Table 6: Phosphorus solubilization (mg P kg⁻¹ of soil) from organic and inorganic substrates at 30 and 60 days of interval in two different soils
Discussion

Influence of organic and inorganic along with PSBs

Lowest pH (6.44) was observed at 60 days after incubation in alluvial soil whereas the encountered lowest pH value (4.29) at 60 days of incubation period in red soil. The maximum phosphate solubilization was reported by Dordevic et al., 2014 [6], at 144 hours of incubation with a maximum drop in pH (3.2) of the Pikovskya’s broth. Dropping of pH under FYM and vermicompost added soil could be explained by release of low molecular weight organic acids during progress of decomposition. Reduction in pH of both experimental soils might be due to secretion of low molecular weight of organic acids by PSB and protonation of FYM and VC caused the effect of donated proton to the soil solution and which in turn, created soil acidity. Pande et al., 2017 [17] also studied the change in pH of liquid medium (broth) that containing Ca₅(PO₄)₃ to pH 7.11 to 3.08 after 8 days of inoculation individually and combined but thereafter no change in pH had been observed. The inoculation of phosphorus solubilizing bacteria (PSB), Burkholderia cepacia (C1) and Alcaligenes aquatilis (H6) produced maximum pH by using combined inoculation followed by C₁ (3.08) and H₆ (3.82).

Changes in Olsen and Bray No.-1 Phosphorus in soil solution

Highest solubilized phosphorus (P) was recorded at 60 days of interval compared to 30 days of interval in alluvial soil, illustrated in Table 6. Whereas the maximum solubilized P was recorded at 60 days of interval in red soil and alluvial soil. PSB₁ was solubilized more P from RP₁ compared to RP₂ at 30 and 60 days interval in red and alluvial soil. FYM and RP treated soils was recorded more solubilization of P over the VC and RP or RP individually treated soils. It might be due to higher dose of FYM as compared to VC. Solubilization of P was recorded higher in T₁₀ (PSB₁ +PSB₂+RP₁) over the T₆ (PSB₁ +RP₁), due to might be application of FYM and VC that supply C source for PSB and activity higher. FYM having organic P and released various low molecular weight organic acids which in turn chelate Ca, Fe and Al, thereby releasing P from Ca-P, Fe-P and Al-P. The efficiency of three phosphorus solubilizing bacteria (PSB) strains released maximum phosphorus from rock phosphate containing broth (mica) at 28 days of incubation compared to 14 days. There was maximum quantity of P released in inoculated soil under SVUMN16 (74.3mg l⁻¹) from Mica followed by SVUMN9 (66.50 mg l⁻¹), reported by Sreenivasulu et al., 2014. Available P increased upto 60 days of incubation period in alluvial soil whereas in red soil also increment was found upto 60 days. It might be due to continuously increments in the release of low molecular weight organic acids by PSBs. Application of rock phosphate to the soil for 100 days of incubation period Begum et al., 2003 [2] reported that Olsen’s extract i.e., available phosphorus (P) of soil was enhanced in subsequent 2 weeks. Then available P was gradually declined in succeeding 65 days and followed by a strident drop afterward. Gradual increase of available P both in alluvial and red soil was due to secretion of weak organic acids (reduction in pH) via microbial action and decomposition of organic inputs (FYM and Vermicompost). PSB₁ was higher P solubilizer as compared to PSB₂ owing to higher secretation of organic acid (more drop in pH) in former isolate than later one. Generally P solubilization was higher in case of RP₁ (Udaipur) than RP₂ (Purulia rock phosphate) due to higher amount of impurities present in RP₂.

Conclusions

Insoluble forms of P can be make available in soil system by using organic substrates along with PSBs. Based on present study, can suggest to farmers for the application of Burkholderia cariabensis (PSB) isolate as PSBs biofertilizers. Rock phosphate Udaipur performed better than Rock phosphate Purulia while FYM is a good source of energy for PSBs over the VC.

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