International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(1): 2874-2878

Received: 07-11-2019
Accepted: 09-12-2019

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

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**DOI:** https://doi.org/10.22271/chemi.2020.v8.i1ar.8706

## 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 $Ca^{2+}$ (Tricalcium phosphate), $Fe^{3+}$ (Strengite) and $Al^{3+}$ (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). *Burkholdaria cariabensis*, $PSB_1$: *B. cepacia, $PSB_2$*; Rock Phosphate (Udaipur): $RP_1$, Rock Phosphate (Purulia): $RP_2$; 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 $PSB_1$ treated soil as compared to $PSB_2$ whereas FYM and $PSB_1$ treated soil more decreased in pH over the VC and $PSB_2$. 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 $RP_1$ or $RP_2$ 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 $Ca^{2+}$ (Tricalcium phosphate), $Fe^{3+}$ (Strengite) and $Al^{3+}$ (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) [5, 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) [1]. 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) [8]. 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 25º15/40// N latitude and 78º02/42// 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, $PSB_1$ (*Burkholdaria cariabansis*) and $PSB_2$ (*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 vermicompost (VC) with phosphorus solubilizing bacteria (*Burkholdaria cariabensis* and *B. cepacia*). Treatment details, ($T_1$, Control; $T_2$, $PSB_1$+$RP_1$; $T_3$, $PSB_2$+$RP_1$; $T_4$, $PSB_1$+$RP_2$; $T_5$, $PSB_2$+$RP_2$; $T_6$, $PSB_1$+$PSB_2$+$RP_1$; $T_7$, $PSB_1$+$PSB_2$+$RP_2$; $T_8$, $PSB_1$ +FYM+$RP_1$; $T_9$, $PSB_2$ +FYM+$RP_1$; $T_{10,}$ $PSB_1$ +FYM+$RP_2$; $T_{11}$, $PSB_2$ +FYM+$RP_2$; $T_{12}$, $PSB_1$ +VC+$RP_1$; $T_{13}$, $PSB_2$ +VC+$RP_1$; $T_{14,}$ $PSB_1$ +VC+$RP_2$; $T_{15}$, $PSB_2$ +VC+$RP_2$; $T_{16}$, $PSB_1$+$PSB_2$ +FYM+VC+$RP_1$; $T_{17}$, $PSB_1$+$PSB_2$ +FYM+VC+$RP_2$). $PSB_1$: *Burkholdaria cariabensis*, $PSB_2$: B. cepacia; Rock Phosphate (Udaipur): $RP_1$, Rock Phosphate (Purulia): $RP_2$; 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 $ha^{-1}$) | 141.85 | 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 ($x10^{-4}$ $g^{-1}$ of oven dry soil) | 19 | 17 |
| Bacteria ($x10^{-6}$ $g^{-1}$ of oven dry soil) | 23 | 29 |
| Actinomycetes ($x10^{-5}$ $g^{-1}$ of oven dry soil) | 39 | 34 |
| Phosphate solubilizing Bacteria ($x10^{-6}$ $g^{-1}$ 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 %).

**Table 4:** Dates of incubation intervals

| S/R No. | Incubation interval days | Date | Temperature (°C) |
|---|---|---|---|
| 1. | 30 | 24/10/2019 to 7/11/2019 | 30-28 °C |
| 2. | 60 | 23/11/2019 to 7/12/2019 | 25-21 °C |

## 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 ($p \leq 0.05$) (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. Higher drop in pH was recorded in alluvial soil as compared to red soil. 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. On average the pH of soil was decreased with increasing time interval. Higher pH was dropped in $PSB_1$ treated soil as compared to $PSB_2$ whereas FYM and $PSB_1$ treated soil more decreased in pH over the VC and $PSB_2$. Other hand, the highly pH was dropped in $T_{16}$ as compared to other treatments (Table 5).

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

| Treatment | Red Soil | | Alluvial Soil | |
|---|---|---|---|---|
| | 30 days | 60 days | 30 days | 60 days |
| $T_1$ | 4.49 | 4.48 | 6.78 | 6.78 |
| $T_2$ | 4.38 | 4.38 | 6.71 | 6.70 |
| $T_3$ | 4.38 | 4.38 | 6.73 | 6.71 |
| $T_4$ | 4.40 | 4.38 | 6.72 | 6.69 |
| $T_5$ | 4.42 | 4.38 | 6.73 | 6.71 |
| $T_6$ | 4.37 | 4.35 | 6.65 | 6.63 |
| $T_7$ | 4.38 | 4.36 | 6.66 | 6.64 |
| $T_8$ | 4.33 | 4.30 | 6.52 | 6.51 |
| $T_9$ | 4.33 | 4.32 | 6.53 | 6.53 |
| $T_{10}$ | 4.33 | 4.32 | 6.52 | 6.51 |
| $T_{11}$ | 4.33 | 4.32 | 6.53 | 6.52 |
| $T_{12}$ | 4.34 | 4.31 | 6.55 | 6.52 |
| $T_{13}$ | 4.33 | 4.33 | 6.58 | 6.53 |
| $T_{14}$ | 4.35 | 4.35 | 6.56 | 6.55 |
| $T_{15}$ | 4.34 | 4.35 | 6.62 | 6.57 |
| $T_{16}$ | 4.33 | 4.29 | 6.46 | 6.44 |
| $T_{17}$ | 4.34 | 4.30 | 6.48 | 6.48 |

### 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 $PSB_1$, $RP_1$ and FYM alone as well as combination over the other treatments. The solubilization of P was documented 30.11% more in $T_{16}$ ($PSB_1$ +$PSB_2$+FYM+VC+$RP_1$) over the $T_6$ ($PSB_1$+$PSB_{2+}$+$RP_1$), 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 $RP_1$ or $RP_2$ was resulted lower in phosphorus solubilization as compared to Coinoculation of PSBs with FYM or VC.

**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

| Treatment | Red Soil | | Alluvial Soil | |
|---|---|---|---|---|
| | 30 days | 60 days | 30 days | 60 days |
| $T_1$ | 3.53 | 3.60 | 9.02 | 8.99 |
| $T_2$ | 7.10 | 7.27 | 14.62 | 16.16 |
| $T_3$ | 6.50 | 6.66 | 12.34 | 14.55 |
| $T_4$ | 6.70 | 6.71 | 11.32 | 13.56 |
| $T_5$ | 6.46 | 6.60 | 10.63 | 14.16 |
| $T_6$ | 11.18 | 13.16 | 16.63 | 19.02 |
| $T_7$ | 10.12 | 10.92 | 14.99 | 16.61 |
| $T_8$ | 12.84 | 13.27 | 19.28 | 20.54 |

| | | | | |
|---|---|---|---|---|
| $T_9$ | 10.13 | 11.29 | 15.28 | 18.77 |
| $T_{10}$ | 11.38 | 12.10 | 14.93 | 17.52 |
| $T_{11}$ | 9.99 | 10.27 | 14.99 | 18.59 |
| $T_{12}$ | 8.66 | 9.48 | 16.94 | 17.23 |
| $T_{13}$ | 8.36 | 8.91 | 15.17 | 16.71 |
| $T_{14}$ | 7.87 | 9.01 | 16.65 | 17.60 |
| $T_{15}$ | 7.69 | 8.57 | 16.17 | 16.82 |
| $T_{16}$ | 16.95 | 18.83 | 22.40 | 23.56 |
| $T_{17}$ | 14.86 | 14.49 | 19.17 | 21.00 |

## 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 $Ca_3(PO_4)_2$ from 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 $C_1$ (3.08) and $H_6$ (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. $PSB_1$ was solubilized more P from $RP_1$ compared to $RP_2$ 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 $T_{16}$ ($PSB_1$ +$PSB_2$+FYM+VC+$RP_1$) over the $T_6$ ($PSB_1$ +$RP_1$), 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 SVUNM16 (74.3mg $l^{-1}$) from Mica followed by SVUNM9 (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). $PSB_1$ was higher P solubilizer as compared to $PSB_2$ owing to higher secretion of organic acid (more drop in pH) in former isolate than later one. Generally P solubilization was higher in case of $RP_1$ (Udaipur) than $RP_2$ (Purulia rock phosphate) due to higher amount of impurities present in $RP_2$.

## 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 *Burkholdaria 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.

## Acknowledgement

The authors are thankful to the Vice Chancellor, Bihar Agricultural University (BAU), Bhagalpur, Bihar, India for providing necessary facilities.

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