International Journal of Plant and Environment
Vol. 4, No. 1 (January, 2018), 70-75

Phosphate Solubilizing Bacteria in Certain Agricultural Crop Soils of Delhi

Rhituporna Saikia and Ratul Baishya*

Department of Botany, University of Delhi, Delhi-110007, India

Publication Info

Article history:
Received: 02.12.2017
Accepted: 01.01.2018
DOI: https://doi.org/10.18811/ijpen.v4i01.11613

Key words:
Agricultural crop soils
Organic acids
Phosphatases
Phosphate solubilizing bacteria
Phosphate solubilizing efficiency

Corresponding author:
Dr. Ratul Baishya
Tel. No. : +91-11-27667573
Email: rbaishyadu@gmail.com

Abstract

Phosphate solubilizing bacteria (PSB) helps in the solubilization of insoluble phosphates and thus lead to increase in crop yields. A study was conducted to isolate and characterize biochemically PSB from different agricultural crop soils of Delhi such as Garlic, Radish, Chilli, Onion and Cabbage. PSB were isolated in Pikovskaya solid medium and formation of solubilization (halo) zone was measured. 16 PSB were isolated and identified. The selected PSB differed in phosphate solubilizing efficiency, production of organic acids and phosphatases. *Citrobacter* sp. and *Pseudomonas* sp. were dominant in all the crop plants. Among all the isolates, *Pseudomonas* sp. proved to be an efficient phosphate solubilizer.

1. Introduction

Phosphorus, an essential element for plants is the second most important element after nitrogen. However in some soils, depending on some environmental factors it can be the main limiting nutrient (Azziz *et al*., 2012). Phosphorus (P) is found in plants as part of proteins, nucleic acids, membranes and energy molecules such as ATP, GTP and NADPH (Azziz *et al*., 2012). Stunted growth of plants, delayed maturity and fruit setting are the symptoms of P deficiency. Plants generally absorb P in the forms of HPO_4^{2-} or $H_2PO_4^-$ (Rodríguez and Fraga, 1999).

Two kinds of phosphates exist in the soil, organic and inorganic. Inorganic phosphates which occur mainly after application of fertilizers are mineral complexes with minerals such as Calcium (Ca), Iron (Fe) and Aluminium (Al). 20-80% of soil phosphorus exist as immobilized phosphates in organic matter (Rodríguez *et al*., 2006).

While other nutrients are present in millimolar quantities, P is present in micro molar quantities. The less availability of soluble P is due to its high reactivity with calcium, iron or aluminium which leads to P precipitation (Gyaneshwar *et al*., 2002). Phosphorus is associated with Al and Fe compounds in acidic soils, whereas in calcareous soils, calcium phosphates are the main form (Gyaneshwar *et al*., 2002). Almost 98% of Indian soils contain low amounts of available P (Kumar and Narula, 1999). Due to the unavailability of phosphorus to plants, chemical fertilizers' application to agricultural crops has always been an important step. But, most of the added P fertilizer is precipitated by Fe, Al and Ca complexes present in the soils and therefore more amount of chemical fertilizers are added such that the crops get the adequate amount of P.

The use of Phosphate Solubilizing Bacteria (PSB) is one such measure which is less costly and at the same time eco-friendly (Chang and Yang, 2009). Organic acids and phosphatases are released by PSB. Mineral phosphate solubilization occurs due to the release of organic acids while mineralization of organic phosphorus is done by enzymes such as phosphatases (Rodríguez and Fraga, 1999).

PSB are more metabolically active in the rhizosphere (Vazquez *et al*., 2000). *Pseudomonas* and *Bacillus* are the common PSB (Rodríguez and Fraga, 1999).

The main aim of our research work was to isolate and characterize biochemically PSB from different agricultural crop soils from the banks of Yamuna, Delhi. Biochemical tests were performed to identify PSB upto genus level. The phosphate solubilization efficiency of bacteria was measured both qualitatively and quantitatively. Organic acid production and phosphatase activity by different PSB for solubilization of insoluble phosphates were estimated.

2. Materials and Methods

2.1. Study area

Delhi covering an area of 1,463 km^2 is located between 28º24'17" and 28º53'00''N latitudes and 76°45'30'' and 77º21'30'' E longitudes. The Yamuna river is the lifeline of Delhi. Delhi is situated on the banks of Yamuna. As per our study, we selected a farming field located near the old Yamuna bridge. The field is located between 28º39'46.80" and 28º39'48.96"N latitudes and 77º15'2.55" and 77º15'5.15"E longitudes. The soil is sandy in texture with an alkaline pH ranging 7-8.

2.2. Collection of soil samples

Sampling was carried out and soils were collected from the rhizospheric zone of 5 different crops during matured stage of Garlic, Radish, Chilli, Onion and Cabbage. Random soil samples were collected in triplicates from ten individuals of each species and analysis was carried within two weeks. Successive samples were collected every 10 days for revalidation of PSB identified.

2.3. Isolation of PSB

PSB was isolated from soil samples by serial dilution method in Pikovskaya Agar. The plates were incubated at 30°C for 4 days. Microbial colonies with a transparent zone developed. Such colonies were picked and used for further analysis.

2.4. Identification of PSB

PSB were identified using standard biochemical tests as listed in Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

2.5. Phosphate solubilizing efficiency

Spot inoculation of colony at the centre of plate was done and maintained at 30°C for 7 days. Colony diameter and halozone diameter were measured after 7 days (Fig. 1). Phosphate Solubilization Efficiency (PSE) was found out for each PSB.

PSE = (Colony diameter+ Halozone diameter)/Colony diameter

2.6. Change in pH of the medium

PSB were inoculated in Pikovskaya Broth and pH change was noted after 7 days.

2.7. Estimation of organic acid production

Organic acid production was estimated as described by Baliah *et al.* (2016). PSB were inoculated in Pikovskaya broth and grown for 7 days. Culture was centrifuged at 10000 rpm for 15 min. 2ml of filtrate was taken in a flask and few drops of phenolphthalein added to it. It was then titrated against 0.01N NaOH. Titratable acidity was expressed by ml of 0.01N of NaOH consumed.

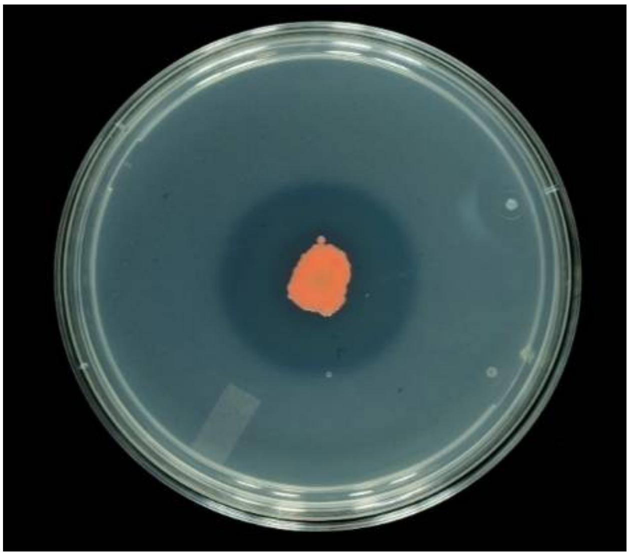

Fig. 1: Halo zone formed by *Staphylococcus* sp.

2.8. Estimation of available P

Phospho molybdate blue color method was used to determine available P (Allen *et al.*, 1974). PVK broth (100 ml) was poured in 250 ml flasks. In each autoclaved flask, 1 ml of each PSB were inoculated and placed on rotary shaker for 7 days. The culture was centrifuged at 10000 rpm for 15 min. The available phosphorous (P) was determined using spectrophotometer at 700 nm and calibrated with standard KH_2PO_4 curve.

2.9. Estimation of phosphatase production

It was determined following the method by Tabatabai and Bremner (1969). Broth Pikovskaya medium (100 ml) inoculated with PSB was placed on a rotary shaker for 7 days. The suspension was then centrifuged at 10000 rpm for 15 min. To the pellet 4 ml of Modified Universal Buffer, 0.25 ml of toluene and 1 ml of p-nitrophenyl phosphate solution were added and the flask swirled for a few seconds to mix the contents. The flask was placed in an incubator at 37°C. After 1 hr, 1 ml of 0.5M calcium chloride and 4 ml of 0.5M sodium hydroxide were added and swirled. It was then filtered through a Whatman filter paper. The absorbance was measured at 400nm.The p-nitrophenol content of the filtrate was calculated by reference to a calibration graph plotted from the results obtained with standards containing 0, 10, 20, 30, 40 and 50 µg of p-nitrophenol.

Table 1: Morphological and biochemical characteristics of the isolates.

Characteristics	*Citrobacter* sp.	*Pseudomonas* sp.	*Staphylococcus* sp.	*Bacillus* sp. 1	*Bacillus* sp. 2
Colony morphology	White, round, smooth, shiny, entire margin	Off-white, round, entire margin, smooth, shiny	Orange, round, entire margin, smooth	Dull white, dry, wavy margin	Dull white, dry, wavy margin
Shape	Straight rods	Straight or slightly curved rods	Spherical	Rods	Rods
Gram Reaction	-	-	+	+	+
Lactose fermentation	+	-	+	+	+
Mannitol fermentation	+	+	+	+	+
Sucrose fermentation	+	-	+	+	+
Citrate utilization test	+	+	+	+	+
Gelatin hydrolysis test	-	+	+	+	+
Indole test	-	-	-	-	-
Methyl red test	+	-	+	-	-
Vogues-Proskauer test	-	-	+	-	+
Oxidase test	-	-	-	-	-
Catalase test	+	+	+	+	+
Nitrate reduction test	+	+	+	-	+
Isolated from crop soils	Garlic, Radish, Cabbage, Onion, Chilli	Garlic, Radish, Cabbage, Onion, Chilli	Garlic, Radish, Cabbage	Garlic, Radish, Chilli	Radish

3. Results

3.1. Isolation of PSB

Total 16 isolates were obtained from 5 soil samples (Garlic, Radish, Chilli, Onion and Cabbage) based on their ability to form a halo zone on Pikovskaya medium.

3.2. Identification of PSB

Identification of PSB was done by various biochemical tests. Four genera were identified namely, *Citrobacter*, *Pseudomonas, Bacillus* and *Staphylococcus. Citrobacter* and *Pseudomonas* were present in all the crops. Radish had the maximum kinds of PSB followed by Garlic (Table 1).

3.3. Phosphate solubilizing efficiency (PSE) and estimation of available P

There was significant difference in PSE and available P in different PSB isolated from various crop soils. *Pseudomonas* sp. and *Bacillus* sp. showed the highest amount of phosphate solubilizing efficiency (3.9-4). *Pseudomonas* sp. and *Bacillus* sp. showed the highest amount of solubilization of insoluble phosphates (3.87-4.04 mg l^{-1}) (Table 2).

3.4. Reduction of pH, organic acid and phosphatase production

Production of organic acids by PSB to solubilize insoluble phosphates lowers the pH. There was significant difference in ability to reduce pH by different PSB isolated from various crop soils. *Pseudomonas* sp. showed the maximum pH drop, followed by *Bacillus* sp. pH drop was least in case of *Citrobacter* sp. Production of organic acids also showed a similar pattern to pH reduction. Amount of organic acids and phosphatases produced was significantly different for different PSB. *Pseudomonas* sp. showed the maximum amount of organic acids production ranging from 2.6 to 2.8 ml. *Citrobacter* sp. displayed the least amount of organic

Table 2: Characterization of PSB isolates.

Crops	PSB	PSE	Estimation of available P (mg l^{-1})	Reduction of pH (Initial pH – 7)	Organic acid (ml)	Phosphatase activity ($\mu g\ ml^{-1} hr^{-1}$)
Garlic	*Citrobacter* sp.	3.5	3.46	5.31	1.4	100.56
	Pseudomonas sp.	4.0	3.94	3.83	2.6	139.23
	Staphylococcus sp.	3.8	3.80	4.16	2.0	102.76
	Bacillus sp.1	4.0	3.88	4.03	2.0	111.10
Radish	*Citrobacter* sp.	3.5	3.51	5.32	1.5	100.26
	Pseudomonas sp.	3.9	4.04	3.85	2.8	138.38
	Staphylococcus sp.	3.6	3.85	4.15	2.0	102.42
	Bacillus sp.1	3.9	3.71	4.10	2.0	109.66
	Bacillus sp.2	3.6	3.89	4.12	2.2	111.27
Chilli	*Citrobacter* sp.	3.5	3.58	5.29	1.4	101.01
	Pseudomonas sp.	3.9	3.95	3.74	2.6	138.38
	Bacillus sp.1	3.9	3.87	4.10	2.0	102.75
Onion	*Citrobacter* sp.	3.4	3.45	5.36	1.4	97.74
	Pseudomonas sp.	4.0	3.98	3.89	2.7	136.35
Cabbage	*Citrobacter* sp.	3.5	3.45	5.33	1.6	101.92
	Pseudomonas sp.	3.9	3.89	3.83	2.7	141.52
	Staphylococcus sp.	3.6	3.77	4.21	2.2	103.10

acid production (1.4-1.5 ml). *Pseudomonas* sp. showed the highest amount of phosphatase production ranging from 136.35 to139.23 $\mu g\ ml^{-1}\ hr^{-1}$. While *Citrobacter sp.* depicted the lowest amount of phosphatase production ranging from 97.74 to101.92 $\mu g\ ml^{-1}\ hr^{-1}$ (Table 2).

4. Discussion

Various PSB were isolated from rhizosphere soils of Garlic, Radish, Chilli, Onion and Cabbage. PSB isolated from these soils were *Citrobacter* sp*., Pseudomonas* sp*., Staphylococcus* sp*. and Bacillus* sp*.* Among the various PSB*, Pseudomonas* sp*.* and *Citrobacter* sp*.* were the most dominant, present in all the crop soils. According to Rodríguez and Fraga (1999) bacterial strains from the genera *Pseudomonas, Bacillus* are the common phosphate solubilizers. *Pseudomonas* are often the dominating PSB in the rhizosphere (Gulati *et al.*, 2007). Our result supports these statements as we can see from our study that *Pseudomonas* is the common PSB found in all crop soils. The crop soils did not vary much in the kinds of PSB. This may be due to the collection of soils from the crops from the same area facing same kind of environmental conditions and having same properties of soil. So, we can say there was no genus found specific to any plant species. But the PSB isolated showed significant differences in all the other parameters studied and we were able to differentiate these PSB on the ability to solubilize phosphates. *Pseudomonas* sp. and *Bacillus* sp. showed the highest amount of phosphate solubilizing efficiency, followed by *Staphylococcus* sp. and *Citrobacter* sp. *Pseudomonas* had the highest efficiency in all the crop soils. This is supported by the statement of Rodríguez and Fraga (1999), who recognizes *Pseudomonas* as a powerful solubilizer of insoluble phosphates. The main mechanism behind the formation of halo zone is the production of organic acids which solubilizes tri calcium phosphate present in the media.

During our study, it was noticed that *Staphylococcus* sp. was solubilizing P at a faster rate at the beginning, but it slowed down later. While, *Pseudomonas* sp. did not develop such a fast rate in the beginning but it continued solubilizing phosphate at the same rate. This also proves that *Pseudomonas* is an efficient PSB. Highest amount of solubilization of insoluble phosphates was shown by *Pseudomonas* sp. and *Bacillus* sp., while *Citrobacter* sp*.* showed the lowest amount of P solubilization. The solubilization of P in the liquid medium was due to the production of organic acids. According to Kundu *et al*. (2009), insoluble phosphates are converted into soluble orthophoshates ($H_3PO_4^{-1}$, HPO_3^{-1} and PO_4^{-3}). *Pseudomonas* sp. was dominant in all the crop soils and also showed the highest efficiency in phosphate solubilization. This was supported by the statement of Rodríguez and Fraga (1999), who recognizes *Pseudomonas* as a powerful solubilizer of insoluble phosphates. *Pseudomonas* sp. solubilizes good amount of insoluble phosphates (Tripti and Kumar, 2012; Baliah *et al*., 2016). Even though *Citrobacter* sp. was present in all the crop soils, the ability to solubilize phosphates was significantly less than *Pseudomonas*. Initial pH of the media before inoculation of PSB was 7. However, after incubation with PSB for 7 days, the pH of the media decreased. Reduction of pH of the Pikovskaya broth occurred due to the production of organic acids.

Different kinds of acids are produced to solubilize the insoluble phosphates. In our study, *Pseudomonas* sp. showed the maximum pH drop, followed by *Bacillus* sp. *Citrobacter* sp. showed the least pH drop. Tripti and Kumar (2012) and Baliah *et al*. (2016) also showed that the maximum reduction in pH was by *Pseudomonas* sp. Reduction of pH can be negatively correlated with the production of organic acids i.e. the more amount of organic acids produced, pH will decrease accordingly. Since, we have seen that *Pseudomonas* have solubilized the highest amount of insoluble phosphates so it is understood that it will also produce the highest amount of organic acids which will lower the pH. Sharma *et al*. (2013) stated that organic acids are the metabolic products of microbes by oxidative respiration or by fermentation of carbon sources. These organic acids help in the solubilization of insoluble mineral phosphates.

In our study, *Pseudomonas* sp. produced the maximum amount of organic acids. Baliah *et al*. (2016) also showed the production of maximum amount of organic acids by *Pseudomonas*. Various kinds of organic acids such as Citric acid, Malic acid, Gluconic acid etc. are produced by *Pseudomonas* (Fankem *et al*., 2006; Khan *et al*., 2007). *Staphylococcus* sp. and *Bacillus* sp. showed the same amount of organic acids production. *Citrobacter* sp. produced the least amount of organic acids. The production of organic acids lowers the pH of the media. Thus, pH and organic acids are negatively correlated. Mineralization of organic phosphates is mediated by phosphatases by hydrolysis of phosphoester or phosphoanhydride bonds. In our present study, *Pseudomonas* sp. showed the highest amount of phosphatase production, while *Citrobacter* sp. produced the lowest amount of phosphatase. Efficient producers of phosphatases (alkaline/acidic) are *Pseudomonas*, *Bacillus* (de Freitas *et al*., 1997; Rodriguez and Fraga, 1999; Baliah *et al*., 2016).

5. Conclusion

Thus, bacteria isolated from the Yamuna banks have efficient phosphate solubilizing abilities. The PSB were able to produce organic acids and phosphatases which helps in the solubilization of insoluble phosphates. Further field trials using these bacteria will help us to finalize the PSB which can be used as biofertilizers. These genera can be used as potent biofertilizers in agricultural crops facing phosphorus immobilization and thus reducing excessive use of chemical fertilizers.

Acknowledgment

The authors acknowledge the financial support received from university of Delhi in the form of R&D grants and UGC Non-NET fellowship.

References

Allen, S. E., Grimshaw, H.M., Parkinson, J.A. and Quarmy, C. 1974. *Chemical analysis of ecological materials*. Blackwell Scientific Publications. pp. 565.

Azziz, G., Bajsa, N., Haghjou, T., Taulé, C., Valverde, Á., Igual, J. and Arias, A. 2012. Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–pasture rotations in a no-tillage regime in Uruguay. *Applied Soil Ecology* **61**:320-326.

Baliah, N.T., Pandiarajan, T. and Kumar, B.M. 2016. Isolation, identification and characterization of phosphate solubilizing bacteria from different crop soils of Srivilliputtur Taluk, Virudhunagar District, Tamil Nadu. *Tropical Ecology* **57**(3):465-474.

Chang, C. and Yang, S. 2009. Thermo-tolerant phosphate-solubilizing microbes for multi-functional biofertilizer preparation. *Bioresource Technology* **100**:1648-1658.

de Freitas, J.R., Banerjee, M.R. and Germida, J.J. 1997. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). *Biology and Fertility of Soils* **24**(4):358-364.

Fankem, H., Nwaga, D., Deubel, A., Dieng, L., Merbach, W. and Etoa, F.X. 2006. Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. *African Journal of Biotechnology* **5**:2450-2460.

Gulati, A., Rahi, P. and Vyas, P. 2007. Characterization of Phosphate-Solubilizing Fluorescent

Pseudomonads from the Rhizosphere of Seabuckthorn Growing in the Cold Deserts of Himalayas. *Current Microbiology* **56**:73-79.

Gyaneshwar, P., Kumar, G.N., Parekh, L.J. and Poole, P.S. 2002. Role of soil microorganisms in improving P nutrition of plants. *Food Security in Nutrient-Stressed Environments: Exploiting Plants' Genetic Capabilities.* Springer Netherlands, pp. 133-143.

Holt, J., Krieg, N., Sneath, P., Staley, J. and Williams, S. 1994. *Bergey's Manual of Determinative Bacteriology.* 9th ed. Philadelphia: Lippincott, pp. 787.

Khan, M., Zaidi, A. and Wani, P. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture — A review. *Agronomy for Sustainable Development* **27**:29-43.

Kumar, V. and Narula, N. 1999. Solubilization of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants. *Biology and Fertility of Soils* **28**:301-305.

Kundu, B., Nehra, K., Yadav, R. and Tomar, M. 2009. Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. *Indian Journal of Microbiology* **49**:120-127.

Rodríguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances* **17**:319-339.

Rodríguez, H., Fraga, R., Gonzalez, T. and Bashan, Y. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil* **287**:15-21.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. and Gobi, T.A. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus* **2**:587.

Tabatabai, M. and Bremner, J. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry* **1**:301-307.

Tripti, V. and Kumar, A. 2012. Phosphate Solubilizing Activity of Some Bacterial Strains Isolated from Chemical Pesticide Exposed Agriculture Soil. *International Journal of Engineering Research and Development* **3**(9):1-6.

Vazquez, P., Holguin, G., Puente, M., Lopez-Cortes, A. and Bashan, Y. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils* **30**:460-468.