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Organic acids production by Phosphate Solubilizing Bacteria leads to mineral phosphate solubilization

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Abstract-Phosphate Solubilizing Bacteria (PSB) produce organic acids for solubilization of insoluble mineral phosphates. PSB were isolated and their ability to produce organic acids was studied from soils of two crops of Delhi, Fenugreek and Cauliflower. Genera *Citrobacter*, *Pseudomonas*, *Staphylococcus* and *Bacillus* were isolated. The isolated PSB showed variation in the amount of organic acids production and pH reduction of the culture broth. *Pseudomonas* sp. produced the maximum amount of organic acids and also showed the highest amount of pH reduction.

Key words: Mineral phosphates, Organic acids, Phosphate solubilizing bacteria

1. INTRODUCTION

Phosphorus occur as Aluminium and Iron compounds in acidic soils and as calcium compounds in calcareous soils [3]. PSB solubilizes these insoluble mineral phosphates by the production of organic acids such as citric acid, succinic acid etc. ([10], [12]). PSB use these soluble phosphates for their growth and the surplus amount is absorbed by plants. When such PSB (*Pseudomonas* , *Bacillus* etc.) were applied around the roots of plants , it led to the promotion of plant growth and protected the plants from pathogens ([2]; [10]). When *Bacillus* was applied to Barley, it led to increase in grain and biomass yields of barley[11].In another case, when *Pseudomonas putida* was applied to Potato, it led to higher biomass and potato tuber[7].

Our aim was to isolate the PSB from two agricultural crop soils (Fenugreek and Cauliflower) and study amount of organic acids production by different PSB and the pH drop due to the production of organic acids.

2. MATERIALS AND METHODS

2.1. *Study area*

The study area was located between 28˚39'46.80" and 28˚39'48.96"N latitudes and 77˚15'2.55" and 77˚15'5.15"E longitudes (Yamuna bank, Delhi).The soil is sandy and alkaline (pH 7). The soil temperature during sampling ranged 12-15˚ C. The collection was made during the Rabi season, 2016. Fig 1 depicts the amount of rainfall that occurred during the year.

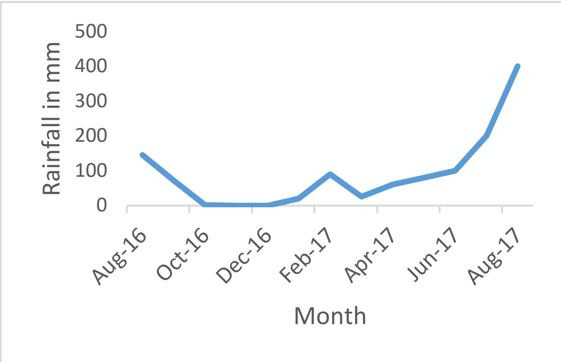

Fig.1. Rainfall pattern during 2016-17 [5]

2.2. *Collection of soil samples*

Soils were collected from the rhizosphere of two crops such as Fenugreek and Cauliflower. Samples thus collected were analysed within 2 weeks.

2.3. *Isolation of PSB*

Serial dilution method using Pikovskaya Agar containing tricalcium phosphates was used to isolate PSB. After incubation for 4 days at 30˚ C, colonies showing halo zone were considered as PSB ([8] ; [9]).

2.4. *Identification of PSB*

Biochemical tests mentioned in Bergey's Manual of Determinative Bacteriology were used for identification of PSB [4].

2.5. *Phosphate solubilizing efficiency (qualitative)*

Colony diameter and halozone diameter of colony was found out after inoculating the colony at the centre of plate for 7 days at 30° C.

PSE= (Colony diameter+ Halozone diameter)/Colony diameter [6]

PSE was measured for each PSB.

2.6. ***Reduction in pH of the pikovskaya medium***
The change in pH of the medium was noted after 7 days from an initial pH of 7.

2.7. ***Estimation of organic acids***
The method used for estimation of organic acids is from [1]. PSB was inoculated in Pikovskaya broth and kept in shaker for 7 days. It was then centrifuged at 10000 rpm for 15 min. In a flask, phenolphthalein was added to 2 ml of filtrate and titrated against 0.01N NaOH. 0.01N of NaOH consumed gave the amount of organic acids produced.

3. RESULTS

3.1. ***Isolation of PSB***
6 isolates were obtained from the 2 soil samples (Fenugreek and Cauliflower). Bacteria showing halo zone were isolated as PSB.

3.2. ***Identification of*** **PSB**
Biochemical tests helped in the identification of genera *Citrobacter*, *Pseudomonas, Bacillus* and *Staphylococcus* (Table 1 & Fig 2).
Citrobacter- Colony was white, round, smooth, shiny with entire margin ; Shape-Straight rods. *Citrobacter* was reported in Fenugreek and Cauliflower.
Pseudomonas- Colony was off-white, round, entire margin, smooth and shiny ; Shape -Straight or slightly curved rods. *Pseudomonas* was reported in Fenugreek and Cauliflower.
Staphylococcus-Colony was orange, round with entire margin and smooth ; Shape-Spherical. *Staphylococcus* was reported in Cauliflower.
Bacillus- Colony was dull white, dry with wavy margin; Shape-Rods. *Bacillus* was reported in Fenugreek.

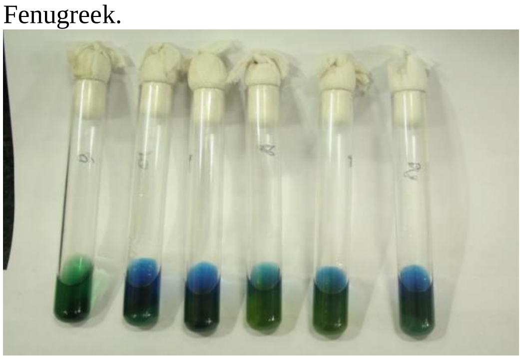

Fig. 2. Citrate utilization test

3.3. ***Phosphate Solubilization Efficiency (PSE)***

PSE varied among PSB (Table 2 & Fig 3). *Pseudomonas* showed the highest PSE followed by *Bacillus*. *Citrobacter* showed the least amount of PSE.

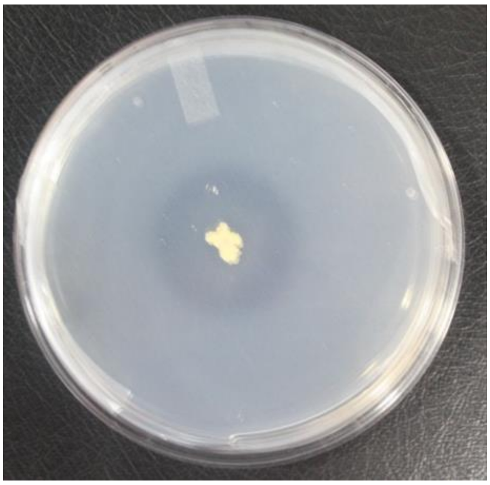

Fig. 3. Halozone produced by *Bacillus* (Day 7)

3.4. ***Reduction of pH, organic acid estimation***

Organic acids production varied in different PSB (Table 2). Organic acids increase the acidity of the broth and lowers the pH of broth. *Pseudomonas* produced the highest amount of organic acids and also showed the maximum drop in pH. *Citrobacter* showed the least production of organic acids and also the least drop in pH.

4. DISCUSSION

From our study, we found out that the crop soils (Fenugreek and Cauliflower) of Delhi is dominated by various kinds of PSB such as *Pseudomonas, Citrobacter*, *Staphylococcus* and *Bacillus*. Genera *Pseudomonas, Bacillus* are the common phosphate solubilizers ([10]; [14]).In our study we too could isolate *Pseudomonas* and Bacillus as PSB. We could also isolate *Staphylococcus* and *Citrobacter* which showed phosphate solubilizing ability. *Citrobacter* and *Pseudomonas* were dominant in all the crop soils. The PSB isolated showed variation in the production of amount of organic acids and the lowering down of PSB. They too varied in phosphate solubilization efficiency. *Pseudomonas* showed the highest PSE followed by *Bacillus* and *Staphylococcus*. *Citrobacter* showed the least PSE. The main mechanism of solubilization of mineral phosphates is the production of organic acids. We can see that both in the Pikovskaya agar and broth, the PSB produced organic acids which solubilized the insoluble phosphates. The halo or transparent zone is formed due to the solubilization of phosphates. In case of the broth culture, organic acids are produced which lower down the pH and we could note down the pH change from an initial pH of 7. *Pseudomonas* produced the highest amount of organic acids and had the highest pH drop. *Citrobacter* produced the lowest amount of organic acids and the least pH drop. [13] and [1] also stated that the maximum drop in pH due to the production of organic acids was by *Pseudomonas*. The increase in organic acids lower the pH. From all the parameters, we can see that *Pseudomonas* is an efficient solubilizer of insoluble phosphates.

5. CONCLUSION

We could isolate various kinds of PSB from the Yamuna bank, Delhi. Thus, bacteria isolated from the Yamuna banks have efficient phosphate solubilizing abilities. These could be used as biofertilizers in agricultural fields and reduce the use of chemical fertilizers

Table 1. Biochemical tests performed to identify PSB

PSB	Gram reaction	Lactose fermentation	Mannitol fermentation	Sucrose fermentation	Citrate utilization test	Gelatin hydrolysis test
PSB 1	-	+	+	+	+	-
PSB 2	-	-	+	-	+	+
PSB 3	+	+	+	+	+	+
PSB 4	+	+	+	+	+	+
	Indole test	Methyl red test	Vogues – Proskauer test	Oxidase test	Catalase test	Nitrate reduction test
PSB 1	-	+	-	-	+	+
PSB 2	-	-	-	-	+	+
PSB 3	-	+	+	-	+	+
PSB 4	-	-	-	-	+	-

PSB 1- *Citrobacter* ; PSB 2- *Pseudomonas;* PSB 3-*Staphylococcus;* PSB 4 –*Bacillus*

Table 2: PSE, Organic acids production and pH reduction by various PSB

PSB	**Crops**	**Phosphate solubilization efficiency**	**Organic acids**	**pH Reduction**
Citrobacter	Fenugreek	3.5	1.4	5.31
	Cauliflower	3.5	1.4	5.31
Pseudomonas	Fenugreek	4	2.6	3.81
	Cauliflower	4	2.6	3.83
Staphylococcus	Cauliflower	3.8	2	4.16
Bacillus	Fenugreek	3.9	2	4.15

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