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Isolation of Phosphate Solubilizing Microorganism from Rhizospheric Medium Black Soil of Yavatmal District (MH), India

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A B S T R A C T

Plants acquire phosphorus from soil solution as phosphate anion. It is the least mobile element in plant and soil contrary to other macronutrients. In the present study phosphate solubilizing bacteria were isolated from rhizosphere of four different crops on Pikovskaya's agar medium. The potential isolate was selected on the basis of reduction in pH of media and formation of halo zone. Its biochemical study was conducted which conclude that the isolate belong to *Bacillus sp*.

Keywords

Soil phosphorus, Solubilization, Nodulation, Organic acids, pH, *Bacillus*, Chick pea

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Introduction

Chick pea occupies a unique position in pulse crops but its production is very low. Chick pea (*Cicer arietinum* L.) is more efficient than other pulses in taking up P from soil, as it secrets more acid which helps in solubilizing P. Phosphorus is one of the most essential elements for plant growth after nitrogen. Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al.*, 2002). Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition. However, the availability of this nutrient for plants is limited by different chemical reactions especially in medium black soil of Yavatmal district. Large amount of P applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils (Gyaneshwar *et al.*, 2002; Hao *et al.*, 2002). It has been reported that a great proportion of phosphorus in chemical fertilizers becomes unavailable in the soil due to the formation of strong bonds of phosphorus with calcium and magnesium in alkaline pH and the same bonds with iron and aluminium in acidic soil. Insoluble phosphate compounds can be solubilized by organic acids and phosphatase enzymes produced by plants and microorganisms. The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and keto gluconic acids in addition to lowering the pH of rhizosphere. The pH of rhizosphere is lowered through biotical production of proton / bicarbonate release (anion / cation balance) and gaseous (O2/CO2) exchanges. For example, PSB have been shown to enhance the solubilization of insoluble P compounds through the release of organic acids and phosphatase enzymes (Sharma *et al.,* 2005). Phosphate solubilizing bacteria are considered among the most effective plant assistants to supply phosphorus at a favourable level. Phosphate deficiency in soil can severely limit plant growth, productivity of legumes, deleterious effect on nodule formation (Alikhani *et al.,* 2006). However, a meagre work has been reported on P solubilisation compared to nitrogen fixation and its effect on nodulation. Therefore the present investigation was aimed to isolate and screen potential phosphate solubilising microbes from Rhizospheric medium black soil collected from Yavatmal district of Maharashtra state.

Materials and Methods

Collection of soil samples

Soil samples around Rhizosphere of Wheat, Chickpea, Safflower, Cotton crops were collected from the fields of Yavatmal districts. The samples were then air-dried, powered and mixed well to represent a single sample. The sample was then taken for the study.

Isolation of PSB

PSB were isolated from each sample by serial dilution and spread plate method. One gram (1g) of soil sample was dispersed in 9 ml of autoclaved distilled water and was thoroughly shaken. 1 ml of the above solution was again transferred to 9ml of sterile distilled water to form 10^{-2} dilution. Similarly 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} serials were made. 0.1ml of each dilution was spread on Pikovskaya's agar medium (PVK) Pikovskaya RI (1948) containing insoluble Tricalcium phosphate and incubated at 27 - 30^{0} C for 7 days. Colonies showing halo zones were picked and purified by 5 times subculture method on Pikovskaya's (PVK) agar medium for studying colony morphology Goenadi *et al.,* (2000).

Morphological characterization

Morphological characteristics of isolates viz. shape, size, elevation, surface form, margins and surface texture, color were observed for their characterization. Lal (2002).

Phosphate solubilization index

Bacterial isolates were selected from the colonies based on their ability to form a clear halo zone on PVK agar. The isolates were aseptically spot-inoculated onto the center of the PKV agar plate. All plates were incubated at 28° ± 2ºC for 7 days. Clear halo were marked as positive for phosphate solubilization. It was measured and the phosphate SI was evaluated using the following formula (Edi-Premono *et al.,* 1996).

Solubilizing index = [(colony diameter +halo zone diameter)/colony diameter]

The phosphate solubilization efficiency (SE) of the isolates was calculated using following formula (Nguyen *et al.,* 1992).

Solublizing efficiency (SE) = (Halo zone diameter/Colony diameter) X 100

Change in pH of medium

Principal mechanism in of phosphate solubilisation was lowering of pH of media by microbial production of organic acids and mineralization of organic P by acid phosphatase. Isolates were inoculated on Pikovskaya's broth medium with neutral pH and observation of change in pH was recorded.

Morphological and Biochemical characterization

The isolate was characterized for its morphological study and gram staining. The potential isolate was subjected to fifteen different biochemical test namely Catalase, Urease, Hydrogen sulphite, Potassium hydroxide solubility, Gelatin liquefaction, Nitrate reduction, Citrate utilization, Lysine utilization, Ornithine utilization, Phenylalanine deamination, Motility, Triple sugar iron, Adonitol, Arabinose, Sorbitol following standard procedure.

Results and Discussion

Solubilizing index/ Solubilizing efficiency

Comparative study isolate PSB -1 shows maximum Solubilizing index/ Solubilizing efficiency i.e. (2.62) /(161.90%) followed by PSB-2, PSB-3, PSB-9, PSB-6, PSB-10, PSB-11, PSB-5, PSB-7, PSB-8 and PSB-4 (Table 1) (L. Moreno-Ramírez *et. al.*, 2015).

Change in pH of medium

Comparative study of PSB isolates shows that the isolate PSB -1 shows maximum acidic pH i.e. (4.40) followed by PSB-2, PSB-3, PSB-5, PSB-9, PSB-7, PSB-8, PSB-10, PSB-6, PSB-11 and PSB-4 (Whitelaw 2000).

Morphological and biochemical characterization

From above eleven isolates best isolate PSB-1 was used for studied for its morphological, and biochemical characteristics. In gram staining isolate PSB-1 showed gram +ve result (Table 2 and 3).

Table.1 Measurement of halo zone, change in pH, Solubilizing index and Solubilizing efficiency

Isolates	Halo zone(cm)		Change in pH	Solubilizing index [(colony diameter+halo zone diameter) / colony diameter]	Solubilizing efficiency (Halo zone diameter/Colony diameter) x 100
	Colony diameter	Halo zone diameter			
PSB-1	2.1	3.4	4.40	2.62	161.90
PSB-2	1.7	2.5	4.64	2.47	147.05
PSB-3	1.6	2.1	4.70	2.31	131.25
PSB-4	1.7	1.0	5.39	1.59	58.52
PSB-5	1.65	1.2	4.71	1.73	72.72
PSB-6	1.68	1.8	4.90	2.07	107.14
PSB-7	1.72	1.2	4.74	1.70	69.76
PSB-8	1.75	1.2	4.76	1.69	68.57
PSB-9	1.71	2.0	4.73	2.17	116.95
PSB-10	1.69	1.4	4.79	1.83	82.84
PSB-11	1.73	1.3	4.90	1.75	75.14

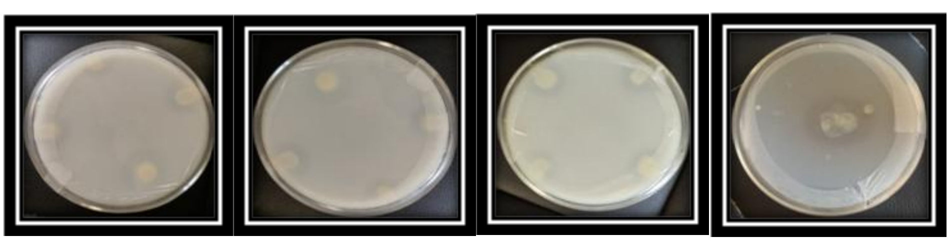
Table.2 Study of external morphology of PSB-1 isolate

Sr. No.	Character	PSB-1
1	Shape	Circular
2	Size of colony	4.0cm
3	Colour/pigmentation	Whitish Pink
4	Elevation	Elevated
5	Margin	Regular
6	Motility	Motile
7	Bacterium shape	Rod

Table.3 Biochemical characterization of PSB-1 isolate

Sr. No.	TEST	Phosphate Solubilizing Bacteria
		PSB1
1	Catalase test	+
2	Urease test	-
3	Hydrogen sulphite test	-
4	Potassium hydroxide solubility test	+
5	Gelatin liquefaction test	+
6	Nitrate reduction test	+
7	Citrate utilization test	+
8	Lysine utilization test	-
9	Ornithine utilization test	-
10	Phenylalanine deamination test	-
11	Motility test	+
12	Triple sugar iron test	+
13	Adonitol test	-
14	Arabinose test	+
15	Sorbitol test	+

Plate No. 1: Comparison of zone of clearance of PSB isolates



Biochemical characterization

Biochemical tests viz. Catalase test, Potassium hydroxide solubility test, Gelatin test Nitrate reduction test, Citrate utilization test, Motility test, Triple sugar ion test, Arabinose test and Sorbitol test were found to be positive, whereas Urease test, Hydrogen

Sulphite test, Lysine utilization test, Ornithine utilization test, Phenylalanine deamination test and Adonitol test were found to be negative on the basis of biochemical reactions this isolate was identified as *Bacillus sp*.

In conclusion, phosphate solubilizing bacterial strains were isolated successfully on Pikovskaya's (PVK) agar medium. There is a close relationship between the phosphate solubilizing activity and low pH levels in the growth medium. This suggests that phosphate solubilization could be the results of organic acids released from bacterial metabolism, as reported in literature. As per biochemical tests the PSB-1 isolate was found to be related to *Bacillus sp*.

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