



## Isolation of plant growth promoting bacteria from rhizospheric soil and their effect on the seed germination and yield of *Triticum aestivum* L.

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### Abstract

The present study deals with the screening and isolation of phosphate solubilizing microbes from rhizospheric soil samples collected from cotton, maize, papaya, and sugarcane fields of the Shahada region and the determination of their potency on the seed germination and growth of *Triticum aestivum* L. Primary screening of the phosphate solubilizing microbes was performed on a Pikovskaya's medium. The most potent phosphate-solubilizing bacteria were analysed for the solubilisation of different forms of phosphates. The solubilisation index in the form of a halo zone formed around the growth was measured in mm and an increase in the zone diameter was observed from 24-72 h of incubation. When a consortium of different phosphate solubilizing isolates was applied for the growth promotion of wheat seeds, in the pot assay an increase in root and shoot length, the number of leaves and chlorophyll content, and an increased percentage of seed germination were observed with the bacterial consortia when compared with the non-inoculated as a control. Thus potential Phosphate solubilizing microbes can be an alternative to chemical phosphatic fertilizers.

**Keywords:** screening, isolation, phosphate solubilizing bacteria, wheat growth, pot assay

### Introduction

Continuous rise in populations demands an increase in food but industrialization and development limits food availability<sup>1</sup> and micronutrient deficiency has become one of the limiting factors for crop productivity in many parts of the world<sup>2</sup>. Phosphorus (P) is one of the biologically essential nutrients required for plant growth and development<sup>3</sup>. It is an integral component of every organism required in important metabolic pathways like nutrient uptake, biological oxidation, and energy metabolism<sup>4</sup> from bacteria to organisms of higher trophic levels. In plants P is involved in all physiochemical processes<sup>[5-7]</sup> such as respiration and photosynthesis, cell growth, seed germination, and root system development, nucleic acids and in phospholipids as it is a structural component. It also enhances stalk and stem strength, and higher-quality crop production. Phosphorus deficiency causes the leaves to turn brown, resulting in the generation of tiny leaves, a weak stem, and poor growth<sup>8</sup>. However the total P in soil accounts roughly for 0.04–0.1% (w/w), only a very tiny proportion of P can directly be assimilated by plants<sup>9</sup>, as the large portion of P in soils exists in the inorganic insoluble form [e.g.,  $\text{Ca}_3(\text{PO}_4)_2$ ] and organic insoluble/soluble form (e.g., phytate and nucleic acid)<sup>[10-12]</sup>. PSB are microorganisms that solubilize mineral-bound insoluble phosphate, mainly by the production of various organic acids PSB are the most effective organism in solubilizing calcium-bound P, rather than iron, aluminum, and magnesium-bound forms<sup>[13-14]</sup>. Thus phosphate solubilizing microbes are Plant growth-promoting rhizobacteria (PGPR)<sup>[15]</sup>.

Among various domains of PSMs, bacteria belonging to the genera *Bacillus*, *Pseudomonas*, *Burkholderia*, *Pantoea*, *Enterobacter*, *Erwinia*, *Azotobacter*, *Rhizobium*, *Acinetobacter*, *Flavo-bacterium*, *Klebsiella*, and *Micrococcus* have been reported as efficient phosphate solubilizers<sup>[16-18]</sup>.

In addition to phosphate mobilization, they are responsible to play a key role in carrying out the bioavailability of soil phosphorus, potassium, iron, zinc, and silicate to plant roots<sup>[19]</sup>. The present work was conducted on screening and isolation of phosphate solubilizing bacteria from agricultural soil and its application on the growth of wheat seeds, germination and morphology.

### Material and Methods

#### Isolation and Characterization

The soil samples from various agricultural fields of papaya, cotton, sugarcane, and maize were used for the isolation of bacteria having phosphate solubilization efficiency. About 1g of each soil was inoculated in 10 ml of sterile distilled water and diluted serially. The diluted samples were inoculated by spread plate technique on selective media of sterile Pikovskaya's agar so as to isolate the bacteria having the potential of solubilizing phosphate.

#### Phosphate Solubilisation

The Phosphate solubilization ability of each isolate was checked again using Pikovskaya's agar which contains calcium phosphate as an insoluble phosphate. The pre-grown cultures were spot inoculated on sterile Pikovskaya's agar plates. Each plate was incubated for 5-7 days at 28°C. After incubation the plates were observed for a clear halo zone around the colony on Pikovskaya's agar. The solubilization efficiency of the isolates was also determined. The halo zone diameter was measured after every 24 hours for consecutive four days.

#### Culture Preservation and Maintenance

The colony of the organism showing zone of phosphate solubilization was purified further and streaked onto sterile nutrient agar slants and stored at 4°C. The preserved cultures were sub-cultured after every month. A loopful of

respective cultures were pre-grown in fresh nutrient broth and incubated for 24 hours before each experiment.

### Morphological and Biochemical Characterization

The isolates were subjected to microscopic analysis so as to determine their morphology. The isolates were grown on the respective media and the colony characteristics were determined. The isolates were also analyzed by various biochemical tests.

### Seed germination test

The efficiency of the isolate for plant growth promotion was determined by seed germination assay and percent germination was determined. The seeds of wheat (*Triticum aestivum L.*) were coated with the bacterial culture and subjected to germination. The seeds of wheat (*Triticum aestivum L.*) were surface sterilized using 0.1% mercury chloride solution for 1 minute and washed thrice with sterile distilled water, and immersed in the 24 hours old culture of the respective isolate for 5-6 hours. After 5-6 hours of coating, the seeds were dried and subjected to germination by the paper towel method (10 seeds/plate). The seeds were provided 12 hours of light and a 12-hour dark cycle. The results were analyzed after every 24 hours to determine the percentage of germination and the rate of germination. A control was kept by using the seeds immersed in sterile distilled water. Percent germination was calculated by number of seeds germinated to the total number of seeds were sown.

### Plant growth promotion test by pot assay

Pot assay was carried out to determine the effects of phosphate solubilizing isolates on the growth and yield of the seeds of wheat (*Triticum aestivum L.*). The pots were initially washed and treated with 70% alcohol to avoid contamination. The sieve passed soil having uniform particle size was sterilized and filled in the plastic pots of uniform size. Ten seeds coated with each PSM isolate were sown into each pot. The bacterial consortia of PSM were inoculated in one pot. The pots were given an equal volume of water and light and dark cycles. After 20-25 days the plants were harvested and they were analyzed for root length, root number, shoot length, and chlorophyll content.

The chlorophyll content was determined by the method mentioned by Jayaraman<sup>20</sup>. The chlorophyll from the leaves was extracted in acetone. The leaves were cut and incubated overnight in acetone for 24 hours at 4°C. After 24 hours the optical density was recorded at 663 nm and 645nm. The chlorophyll content was determined by the following formula:

$$(20.0X \text{ optical density at } 645 \text{ nm}) + (8.02X \text{ optical density at } 643 \text{ nm})$$

The comparison was done with the control that was not coated by the phosphate solubilising isolates.

### Results and Discussion

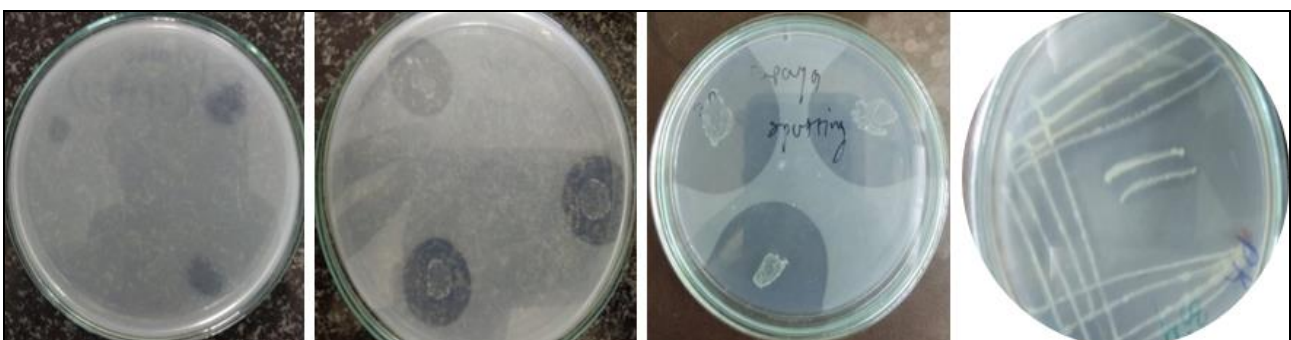
Agricultural soil samples from various areas of the Shahada region were collected for screening and isolation of phosphate-solubilizing bacteria. Physico-Chemical characteristics of various soil samples were found as smooth and moist texture, with black and brown color, and pH range between 6.9 to 7.1

### Screening and Isolation

At the primary screening on Pikovskaya's agar many colonies showing halo zone of clearance were found with the respective dilution of soil sample. The colonies showing the largest zone of clearance were selected and again spot inoculated on sterile Pikovskaya's agar plates. The plates were incubated at 28°C for 5-7 days. The isolates were distinguished on the basis of colony characteristics, morphology, and biochemical characteristics. After that, the selected colonies showing the highest zone of clearance were selected and were given names according to the soil as Cotton soil PSM 1, Papaya soil PSM 2, Maize soil PSM 3, and sugarcane soil PSM 4 isolate.

**Table 1:** Zone of phosphate solubilisation by isolated bacteria

Sample	24 h	48 h	72 h	96 h
Cotton soil isolate PSM 1	0.7 cm	1.1 cm	1.8 cm	2 cm
Papaya soil isolate PSM 2	0.5 cm	1.2 cm	1.7 cm	2.1 cm
Maize soil isolate PSM 3	0.4 cm	0.9 cm	1.5 cm	2.2 cm
Sugarcane soil isolate PSM 4	0.8 cm	1.2 cm	1.8 cm	2.4 cm



**Fig 1:** Increase in the zone diameter by isolates from 24-96 hours and growth of phosphate solubilizing isolate

### Seed Germination Test

Data obtained table 2 on seed germination test revealed that 100 per cent seed germinations were observed in the consortium of the isolates while 80 to 90 per cent seed germinations were observed with single isolates as compared to control, indicated that PSM isolates

significantly enhances seed germination in comparison with control.

### Pot assay

The pot assay was carried out using the seeds of wheat (*Triticum aestivum L.*) with 10 seeds of each isolate. After

20-25 days of incubation, the plantlets were harvested and various analyses were done.

All the pots inoculated with isolates of PSM 1-4 showed the good result in the root length, root number, shoot length, whole plant dry weight, dry weight of shoot, and chlorophyll content was found to be more than the control. Among the four PSM isolates, isolate 2 was found to be most potent plant growth promoting Rhizobacteria. Consortia of all PSM inoculated pot showed increased in plant height and all growth parameters as compared to individual inoculation and control pot.



**Fig 2:** Pot assay of wheat seeds inoculated with consortia of PSM isolates

**Table 2:** Morphological Properties of Plants after inoculation of PSM isolates and Consortia of isolates

Seeds	Root length (cm)	Root number	Shoot length (cm)	Number of leaves	Plant height (cm)	Chlorophyll	Seed germination	Dry wt. of the plant gm
Wheat+ PSM 1	15.5 ± 0.3	17	2.8 ± 0.4	12	10.5 ± 0.3	0.95 ± 0.2	80	165
Wheat+ PSM 2	12.3 ± 0.5	20	3.0 ± 0.3	15	12.5 ± 0.24	1.1 ± 0.4	90	175
Wheat+ PSM 3	10.5 ± 0.2	15	2.1 ± 0.2	16	12.5 ± 0.2	0.7 ± 0.3	90	178
Wheat+ PSM 4	11.6 ± 0.4	16	2.2 ± 0.4	14	13.5 ± 0.2	0.8 ± 0.4	90	186
Consortia	18.5 ± 0.5	28	3.9 ± 0.3	22	19.5 ± 0.3	1.5 ± 0.1	100%	206
Control	10.5 ± 0.2	06	2.4 ± 0.2	6	10.3 ± 0.4	0.82 ± 2.2	40%	45

### Discussion

Phosphorous is one of the major nutrients required for plant growth, although large amount of Phosphorous is present in soil but it is in insoluble form. Applications of chemical fertilizers causes environmental problems hence phosphate solubilizing microorganisms are best alternative to chemical phosphatic fertilizers.

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### Conflict of Interest

There is no any conflict of Interest

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