

## ESTIMATION OF AUXINS AND GIBBERELLINS SECRETED IN VITRO BY PHOSPHATE SOLUBILIZING *BACILLUS MEGATERIUM*

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**Key words:** Auxins, Gibberellins, phytohormone secretion, Phosphate solubilizing bacteria, Plant growth promotion, *Bacillus megaterium*

**Abstract** – Phosphate-solubilizing bacteria secrete the phytohormone essential for plant growth and crop productivity. In the present study, rhizospheric soil samples were collected to screen out the Phosphate-solubilizing bacteria having plant growth-promoting activities. A total of 12 phosphate-solubilizing bacteria were isolated, and those having a solubilization zone of 3-12 mm were chosen for Auxin and Gibberellin production studies. Auxin and Gibberellin production were confirmed qualitatively and quantitatively using the colorimetric method. All PSB isolates (PSB1 to PSB12) showed all plant growth promoting attributes, result positive. From all the isolates, PSB1 showed Maximum PGP production compared to the others, such as indole acetic acid (130 µg/ml) and gibberellic acid (720 µg/ml) strain PSB 1 was more effective for plant growth and sustainable agriculture. The ability of PSB to promote plant development was examined using a seed germination and pot assay study. Bacterial isolates with high potential of auxin and gibberellin production were found to show the enhanced 100% seed germination and plant growth promotion for wheat seeds, as compared with the untreated seed control. It enhanced both root and shoot length. After biochemical identification, it was observed that PSB 1 was *Bacillus megaterium*. Genus *Bacillus*, due to its enhanced hormone-producing abilities, stands out as a promising candidate for sustainable agricultural applications aimed at reducing chemical inputs, improving crop yield, and promoting environmental sustainability.

### INTRODUCTION

The rhizospheric bacteria having Plant growth-promoting Attributes (PGPA) support plant development (Rodríguez and Fraga, 1999). In the soil, Phosphorus is the second most important nutrient after nitrogen (400–1200 mg/kg) and cycles through a non-atmospheric (sedimentary) pathway involving microbes (Kumar and Mina, 2023). Phosphate solubilizing bacteria (PSB) have the capability to transform insoluble phosphorus, Such as dicalcium phosphate, tricalcium phosphate, hydroxyapatite, and rock phosphate, into a form that is easily available for use (Dhawase *et al.*, 2014; Ekin, 2010). Plant Growth Promoting Rhizobacteria (PGPR) are soil bacteria that can increase plant growth through various mechanisms, such as phosphate solubilization, biological nitrogen fixation, siderophore production, and other

beneficial processes (Benaissa, 2019). Plants produce phytohormones such as auxins, cytokinins, and gibberellins to regulate growth, enhance root nodulation, and solubilize essential minerals like phosphates and potassium (Savale *et al.*, 2024). This bacterium Secretes Phytohormone, which is essential for plant growth and plant productivity.

Auxins are a group of hormones produced in the shoot and root apices of a plant, from where they move to the region where elongation occurs. Indole Acetic Acid (IAA) is a naturally occurring auxin. Auxins promote cell enlargement and stem elongation. They play a vital role in various development processes like organ formation, vascular tissue differentiation, apical dominance, root initiation, and tropism (Khan and Nabi, 2023). The synthesis of indole acetic acid (IAA) by phosphate-solubilizing bacteria (PSB) is based on their potential to metabolize tryptophan.

Tryptophan, which is often found in root exudates of plants, acts as a precursor for IAA synthesis. The bacteria take up tryptophan and, through enzymatic pathways, convert it into indole acetic acid, a type of auxin (Myo *et al.*, 2019).

Gibberellins are a class of tetracyclic diterpenoid compounds based on four isoprene units, featuring the Ent-gibberellins ring structure. They act as plant growth regulators, affecting various developmental processes in higher plants, such as stem elongation, seed germination, dormancy release, flowering, sex determination, enzyme activation, shoot growth, aging of leaves and fruits, improving nutrient uptake, and enhancing stress tolerance (Castro-Camba *et al.*, 2022).

## MATERIALS AND METHODS

### Sample Collection

The rhizospheric soil samples were collected from the various region of Amravati, directly associated with Plants root. Intact system was dug out and the rhizospheric soil samples were carefully taken in zip-lock plastic bags. Soil samples were collected for the isolation of phosphate solubilizing bacteria.

### Isolation of phosphate solubilizing bacteria

The isolation of phosphate solubilizing bacteria was done from the rhizospheric soil samples by serial dilution technique in Pikovskaya's Agar media. Take 1g of Soil Sample in 9ml of double-distilled Water. Serial dilution was carried out, and the appropriate dilution was spread on sterile Pikovskaya's agar plates by the spread plate method technique. After that incubated it for 24-48 h at 37 °C. After incubation, colonies that showed zone of solubilization were considered as phosphate solubilizing bacteria. 12 isolates of Phosphate solubilizing bacteria were isolated from a rhizospheric soil sample. These isolates were purified by repeated streaking and made a stock culture in sterile nutrient medium and kept at 4 °C for further use (Kumar *et al.*, 2012).

### Production of Auxins

#### Qualitative Estimation of Indole acetic acid

For auxin production, phosphate-solubilizing bacterial (PSB) isolates were inoculated into sterile nutrient broth supplemented with 0.7% tryptophan and incubated at 37°C for 7 days. An uninoculated medium served as the control. Following

incubation, cultures were centrifuged at 3000 rpm for 20 minutes, and the supernatant was collected. To each sample, two drops of orthophosphoric acid along with 4 ml of Salkowski reagent (prepared by mixing 50 ml of 35% perchloric acid with 1 ml of 0.5 M FeCl<sub>3</sub>) were added. Auxin production was observed by a change in color from yellow to pink/red (Thakur *et al.*, 2015).

#### Quantitative Estimation of Indole acetic acid

Quantitative estimation of auxin was carried out by measuring the optical density (OD) of the reaction mixture at 530 nm using a colorimeter. A standard calibration curve was constructed using known concentrations of indole-3-acetic acid (IAA) ranging from 10 to 150 µg/ml. The concentration of IAA produced by the bacterial isolates was determined by comparing their absorbance values against the standard curve, and results were expressed in µg/ml (Thakur *et al.*, 2015; Hariharan *et al.*, 2014).

### Production of Gibberellins

#### Qualitative and Quantitative Estimation of Gibberellic acid

Gibberellins were secreted by Phosphate-solubilizing bacteria (PSB). All isolates of PSB were inoculated in Luria broth and incubated overnight. Centrifuged it at 3000 rpm for 20 min. After centrifugation, take 15 ml supernatant, add 2 ml of zinc acetate reagent, and 2 ml of potassium ferrocyanide was added after 2 min, then centrifuged again for 15 min at 2000 rpm. Take 5 ml supernatant, add 5 ml of 30% HCL, and incubate it at 20°C for 75 min. After that, for quantitative estimation, absorbance was read at 254 nm. For blank, 5 ml of 5% HCL was used, and the concentration of gibberellins was calculated by preparing a standard curve by using gibberellic acid as a standard (10-00 µg ml<sup>-1</sup>) (Sharma *et al.*, 2018; Patil and Patil, 2012).

### Seed Germination

Among the all isolates of Phosphate-solubilizing bacteria (PSB), the isolate showing the highest secretion of phytohormones (i.e, Auxin, Gibberellin and Siderophore) was inoculated in sterile nutrient broth. 0.1% H<sub>2</sub>O<sub>2</sub> was used to sterilize seeds (Wheat), then coated with a jaggery solution. After that, the seeds were soaked in a broth of selected PSB isolates for 2-3 minutes. Then, the PSB-coated seeds and control seeds (non-PSB-coated seeds) were sown in the pot for the Pot assay.

## RESULTS AND DISCUSSIONS

The soil samples were collected from the rhizospheric soil of the Amravati region and serially diluted. Bacterial colonies that formed clear zones on Pikovskaya's agar were isolated and sub-cultured using the point inoculation method. 12 phosphate-solubilizing bacteria (PSB1–PSB12) were selected, purified, and sub-cultured on nutrient agar medium.

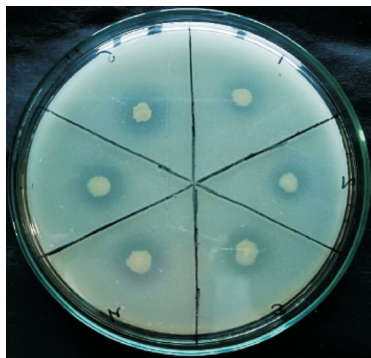


Fig. 1. Point inoculation of PSB isolates

### Production of Auxin

#### Qualitative Estimation of Indole acetic acid

The production of the phytohormone auxin by phosphate-solubilizing bacteria was confirmed on the basis of a change in color from yellow to pink-red, as shown in Fig. 3.

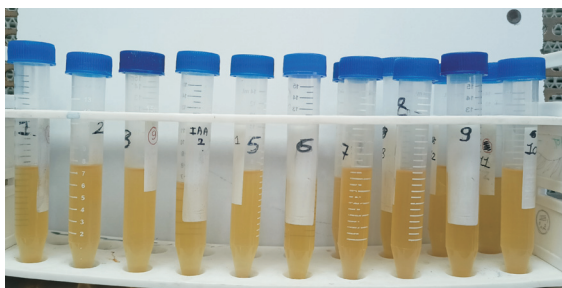


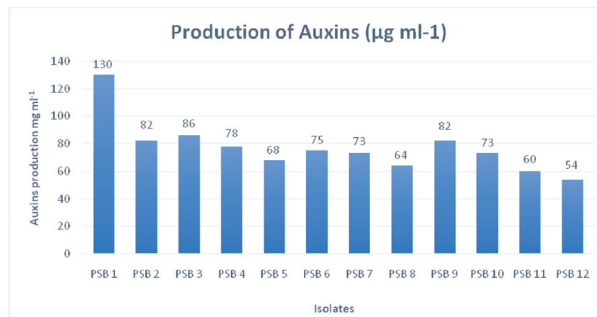
Fig. 2. Indole acetic acid production before incubation



Fig. 3. Indole acetic acid production after incubation

### Quantitative Estimation of Indole acetic acid

Graph 1 presents the differential production of auxin by selected bacterial isolates, using a standard graph for comparison.



Graph 1. Production of indole acetic acid by PSB isolates

Based on the recorded values in Table 1, each isolate exhibited varying levels of auxin synthesis. Among them, strain PSB 1 showed the highest auxin production at 130 µg ml<sup>-1</sup>, followed by PSB 3 at 86 µg ml<sup>-1</sup>, while strain PSB 12 produced the least. These findings suggest that PSB 1 is particularly effective in enhancing plant growth.

Table 1. Production of indole acetic acid in µg ml<sup>-1</sup>

Isolates	Production
PSB1	130 µg ml <sup>-1</sup>
PSB2	82 µg ml <sup>-1</sup>
PSB3	86 µg ml <sup>-1</sup>
PSB4	78 µg ml <sup>-1</sup>
PSB5	68 µg ml <sup>-1</sup>
PSB6	75 µg ml <sup>-1</sup>
PSB7	73 µg ml <sup>-1</sup>
PSB8	64 µg ml <sup>-1</sup>
PSB9	82 µg ml <sup>-1</sup>
PSB10	73 µg ml <sup>-1</sup>
PSB11	60 µg ml <sup>-1</sup>
PSB12	54 µg ml <sup>-1</sup>

### Production of Gibberellin

#### Qualitative and Quantitative Estimation of Gibberellic acid

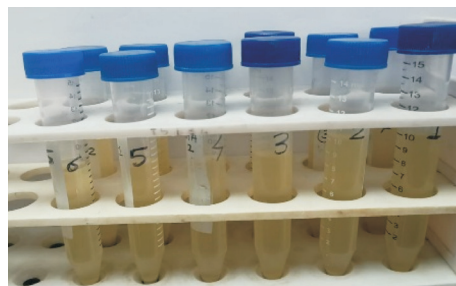


Fig. 4. Gibberellic acid production Before incubation

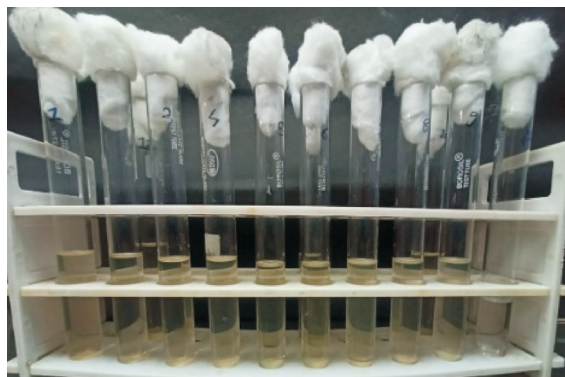
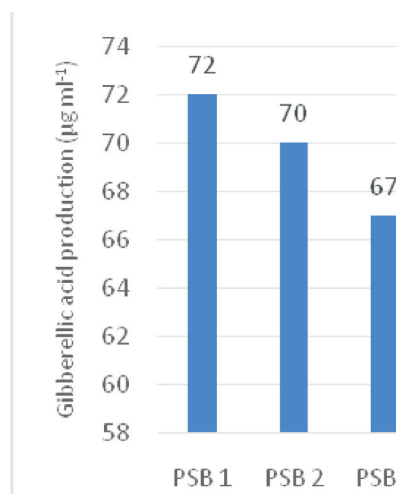


Fig. 5. Gibberellic acid production after incubation

The production of gibberellins by selected bacterial isolates is presented in Graph 2, using a standard graph for comparison.



Graph 2. Production of Gibberellic acid by PSB isolates

Based on the recorded values in Table 2, each isolate exhibited varying levels of gibberellin synthesis. Among them, strain PSB 1 and PSB 7

Table 2. Production of Gibberellic acid in µg ml<sup>-1</sup>

Isolates	Production
PSB1	72 µg ml <sup>-1</sup>
PSB2	70 µg ml <sup>-1</sup>
PSB3	67 µg ml <sup>-1</sup>
PSB4	70 µg ml <sup>-1</sup>
PSB5	70 µg ml <sup>-1</sup>
PSB6	70 µg ml <sup>-1</sup>
PSB7	72 µg ml <sup>-1</sup>
PSB8	70 µg ml <sup>-1</sup>
PSB9	70 µg ml <sup>-1</sup>
PSB10	67 µg ml <sup>-1</sup>
PSB11	63 µg ml <sup>-1</sup>
PSB12	65 µg ml <sup>-1</sup>

showed the highest auxin production, i.e., 72/µg ml<sup>-1</sup>, followed by PSB 2, PSB 4, PSB 5, PSB 6, PSB 8, and PSB9 at 70/µgml<sup>-1</sup>, while strain PSB 11 produced the least. These findings suggest that PSB 1 is particularly effective in enhancing plant growth.

### Seed Germination

Phosphate solubilizing bacteria secrete a phytohormone which is essential for plant Growth. Among all 12 PSB isolates, PSB 1 shows the highest production of phytohormones, that is, Auxin, Siderophore and Gibberellin. Isolate PSB 1 is selected for the seed germination. PSB 1 shows 100% seed germination compared to the control, where 50% seeds germinated. By measuring the shoot length, root length, shoot dry weight, root dry weight, shoot wet weight, root wet weight, PSB1 shows the highest shoot and root length as compared to the control.

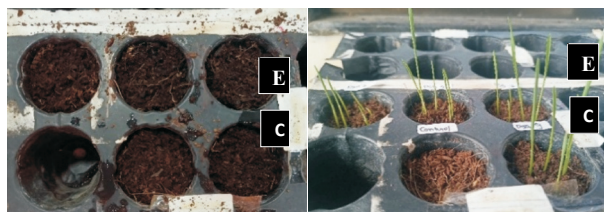


Fig. 6. Day 1

Fig. 6. Day 5



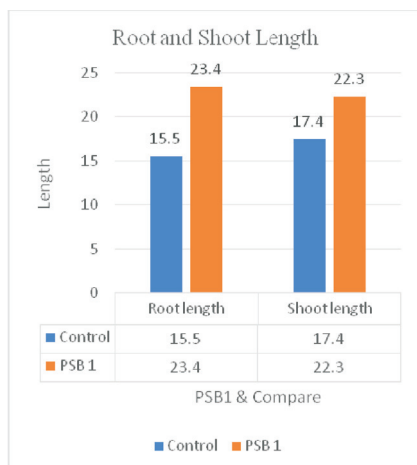
Fig. 6. Day 15

Plant growth analysis on different days

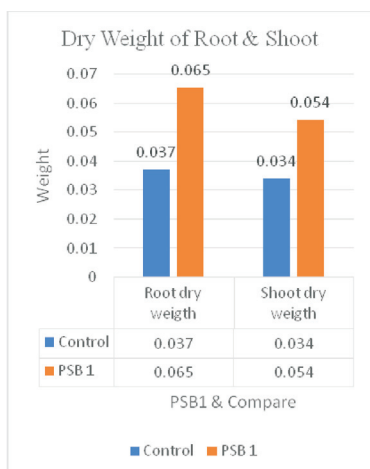
C = Control (Plant without phosphatic biofertilizer)

E = Experimental (Plant with phosphatic biofertilizer of PSB 1 isolate).

Parameters	Control	PSB 1
Root Length	15.5 mm	23.4 mm
Shoot Length	17.4 mm	22.3 mm
Dry weight of Root	0.037 g	0.065 g
Dry weight Shoot	0.034 g	0.054 g



Graph 3. Root and Shoot Length



Graph 4. Dry Weight of Root and Shoot

## CONCLUSION

This experiment was carried out for the evaluation of the plant growth-promoting abilities of phosphate-solubilizing bacteria (PSB), aiming to identify potential candidates of phosphate-solubilizing bacteria (PSB) from different isolates for use in sustainable agriculture. The isolated PSB strains were screened out from the rhizosphere. Phosphate-solubilizing bacteria (PSB), able to produce key phytohormones, namely auxins and gibberellins, are present in the soil. These compounds known to enhance root development, stimulate plant growth, seed germination, soil fertility, and improve nutrient acquisition, etc.

The production of these phytohormones was confirmed through qualitative and quantitative analysis of phytohormones. The quantitative estimation provides a comparative measure of hormone secretion levels among different isolates of

phosphate-solubilizing bacteria (PSB). Based on the data obtained, it was concluded that *Bacillus megaterium* exhibited the highest production of all three phyto hormones, that is, Auxins and Gibberellins. This superior secretion capacity indicates a strong potential of *Bacillus megaterium* to promote plant health, seed germination, and growth more effectively than the other isolates.

During the process of seed germination, *Bacillus megaterium* treatment has been observed to significantly enhance both root and shoot length when compared to the uninoculated seeds (control). Additionally, the seeds treated with *Bacillus megaterium* demonstrated a 100% germination rate compared to the control, indicating their strong potential in promoting early plant growth and improving overall germination efficiency.

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**Conflict of Interest-** None

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