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# Isolation and Characterization of Phosphate-Solubilizing Bacteria from Rhizospheric Soil in Suryapur, Bharatpur

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## ABSTRACT

**Objectives**: This study was conducted to isolate and characterize phosphate-solubilizing bacteria (PSB) from rhizospheric soils of Bharatpur and to evaluate their potential in solubilizing insoluble phosphate and promoting plant growth.

**Methods**: Soil samples were collected from nine different sites in Suryapur, Bharatpur. Pikovskaya's agar medium was used to screen for PSB based on halo zone formation. Isolates were characterized morphologically and microscopically. The Phosphate Solubilization Index (PSI) was calculated using halo zone and colony diameters. Pot experiments were conducted to evaluate the plant growth-promoting effects of selected PSB strains on shoot and root length.

**Results**: Out of nine sampling sites, PSB were successfully isolated from three (S1, S8, S9), yielding a total of nine isolates. Morphological and Gram-stain analyses identified both Gram-positive cocci and Gram-negative rods. PSI values ranged from 2.14 to 2.75, with isolate S9C showing the highest solubilization potential. Pot culture experiments revealed increased shoot lengths in PSB-treated plants (up to 9.5 cm) compared to controls (as low as 3.9 cm), suggesting enhanced phosphorus availability. The enhanced root length in treated plants suggests better nutrient and water absorption capability.

**Conclusion**: The study confirms the presence of effective phosphate-solubilizing bacteria in the rhizospheric soil of Bharatpur. These isolates have potential for use as bio-inoculants to enhance phosphorus availability and support sustainable agriculture.

**Keywords:** Phosphate-solubilizing bacteria, Pikovskaya's agar, PSI, rhizosphere, phosphorus bioavailability

## Introduction

Phosphate is a vital nutrient for plant growth and development, playing a crucial role in various biochemical processes, including energy transfer and nucleic acid synthesis. Despite its importance, phosphate availability in soils is often limited due to its low solubility and high fixation by soil particles. Studies have shown that 95-99% of soil phosphorus exists in forms that plants cannot readily absorb (Khan et al., 2007). To address this issue, phosphate-solubilizing bacteria (PSB) have emerged as a promising solution. These microorganisms play a crucial role in converting insoluble phosphate into soluble forms that plants can utilize, thereby enhancing soil fertility and supporting sustainable agriculture. Thus, the isolation of phosphate-solubilizing bacteria (PSB) from rhizospheric soil samples has emerged as a promising strategy for enhancing soil fertility and agricultural productivity. (Sudewi et al., 2020).

Rhizosphere soil, the zone of soil influenced by plant roots, harbors a diverse community of microorganisms, including bacteria that have evolved mechanisms to solubilize inorganic phosphate compounds (Seshadri et al., 2016). These phosphate-solubilizing bacteria are capable of converting insoluble phosphate compounds into forms that are more accessible to plants. This process is facilitated by the production of organic acids and enzymes that dissolve phosphate-bound minerals in the soil (Kumar et al., 2011).

Phosphate-solubilizing bacteria (PSB) are crucial microorganisms in agriculture, known for their ability to enhance phosphorus availability in soils. Phosphorus is an essential nutrient for plants, yet a significant portion in soils is bound in insoluble forms, making it inaccessible to plants. PSB facilitate the solubilization of these phosphate compounds, thereby improving soil fertility and supporting plant growth.

Phosphate deficiency is a common issue in agricultural soils, significantly limiting crop yield and growth. Despite the presence of phosphate in soil, it often exists in insoluble forms that are unavailable to plants, which exacerbates the challenge of nutrient management in agriculture (Khan et al., 2007). Conventional methods to address this deficiency involve the use of chemical phosphate fertilizers, which, while effective, pose environmental risks such as soil degradation, water pollution, and increased costs for farmers (Gupta et al., 2013).

In contrast, phosphate-solubilizing bacteria (PSB) present a more sustainable solution by converting insoluble phosphate compounds into soluble forms that plants can readily absorb. These microorganisms, found in the rhizosphere of plants, have shown the potential to enhance soil fertility and improve plant growth (Seshadri et al., 2016). However, the effectiveness of PSB can vary depending on the soil environment, bacterial strains, and specific conditions under which they operate.

The problem lies in the limited understanding and practical application of PSB in diverse soil types and agricultural settings. There is a need to isolate and characterize PSB from rhizospheric soil samples to identify effective strains and understand their mechanisms of phosphate solubilization. This knowledge is crucial for developing targeted bio-inoculants that can improve phosphorus availability in soils, thereby enhancing agricultural productivity in an environmentally sustainable manner (Yadav et al., 2017).

## Materials And Method

**Sample Collection-** Rhizospheric soil samples were collected in a clean, sterile, autoclaved vessel from nine sites in Suryapur, Bharatpur. The samples were carried in an icebox to laboratory under the sterile condition Samples were processed for isolation using serial dilution and spread plate methods.

**Isolation of PSB-** Pikovskaya's agar was used to isolate PSB. Serial dilution was performed by transferring 1ml of stock solution into the test tube containing 9ml of distilled water. Dilution up to $10^{-6}$ were made for each sample. Then using a sterile pipette 1 ml of $10^{-4}$ and $10^{-6}$ dilution were poured on PVK media for isolation of phosphate-solubilizing bacteria. Plates were incubated at 30°C for 7 days. Colonies forming clear zones were selected for further study.

**Morphological and Microscopic Characterization-** Isolates were characterized based on colony morphology (size, shape, color, margin, elevation) and Gram staining.

**Phosphate Solubilization Activity-** The phosphate-solubilizing activity of the bacterial isolates was evaluated using Pikovskaya's agar medium, which contains insoluble tricalcium phosphate as the sole phosphorus source. When phosphate-solubilizing bacteria (PSB) grow on this medium, they release organic acids and other metabolites that solubilize the insoluble phosphate, resulting in the formation of a clear, transparent halo zone around their colonies. This halo zone is a visible indicator of the ability of the bacteria to convert insoluble phosphate into soluble forms.

To quantitatively assess this solubilizing capacity, the Phosphate Solubilization Index (PSI) was calculated for each isolate. PSI provides a ratio that reflects the efficiency of phosphate solubilization relative to the growth of the bacterial colony itself. The calculation is based on the formula:

**PSI = (Colony diameter + Halo zone diameter) / Colony diameter**

**Pot Culture Experiment-** Selected PSB isolates were inoculated into pots containing soil and seeds. Selected PSB isolates that showed significant phosphate solubilization activity on Pikovskaya's agar were used as inoculants. Seeds of a test plant (maize) were surface sterilized and sown in sterile soil-filled pots. Each treatment group received a bacterial inoculum, while a separate set of pots was maintained as the uninoculated control group to serve as a baseline for comparison. The inoculated pots were regularly monitored, and all groups were maintained under the same environmental and watering conditions to ensure uniform growth factors.

After a predefined growth period, typically around two to three weeks, the plants were carefully uprooted, and both shoot and root lengths were measured using a standard ruler and compared with uninoculated controls. These measurements were recorded to assess the impact of PSB on plant development.

## Results

In this study, nine isolates were obtained from three out of nine sites (S1, S8, S9). Site S1 yielded four isolates, S8 yielded two, and S9 yielded three.

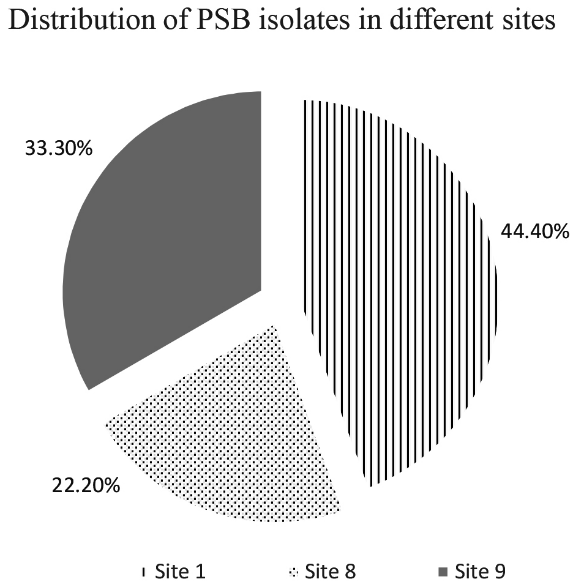


**Figure 1: Distribution of phosphate-solubilizing bacterial (PSB) isolates across positive sampling sites.**

The figure illustrates the distribution of PSB isolates recovered from three out of nine sampling sites: No PSB isolates were obtained from the remaining six sites (S2–S7). The distribution is uneven, or heterogeneous, with S1 contributing the largest number of isolates. The isolates varied in colony morphology. Most appeared creamy white or white with convex or flat elevation. Gram staining revealed five Gram-positive cocci and four Gram-negative rods.

**Table 1: Phosphate Solubilization Activity and PSI**

| Isolate Code | Colony Size (mm) | Clear Zone (mm) | PSI = (C+Z)/C |
|---|---|---|---|
| S1A | 3 | 5 | 2.67 |
| S1B | 3 | 5 | 2.67 |
| S1C | 14 | 16 | 2.14 |
| S1D | 5 | 6 | 2.20 |
| S8A | 5 | 7 | 2.40 |
| S8B | 15 | 22 | 2.47 |
| S9A | 11 | 13 | 2.18 |
| S9B | 7 | 8 | 2.14 |
| S9C | 4 | 7 | 2.75 |

Nine phosphate-solubilizing bacterial (PSB) isolates were assessed on Pikovskaya's agar by measuring colony size and clear zone diameter. The Phosphate Solubilization Index (PSI) was calculated using the formula (Colony + Halo zone)/Colony size, with values ranging from 2.14 to 2.75**,** indicating variation in solubilization efficiency.

Among S1 isolates, PSI ranged from 2.14 (S1C) to 2.67 (S1A, S1B)**.** S8 isolates showed relatively high PSI values of 2.40 (S8A) and 2.47 (S8B**)**, with S8B showing a large clear zone. S9 isolates had PSI values from 2.14 (S9B) to 2.75 (S9C), the latter being the highest overall despite a small colony size.

In general, isolates with smaller colonies and larger clear zones, like S9C, showed greater phosphate solubilizing potential, suggesting their effectiveness in mobilizing phosphorus in soil environments.

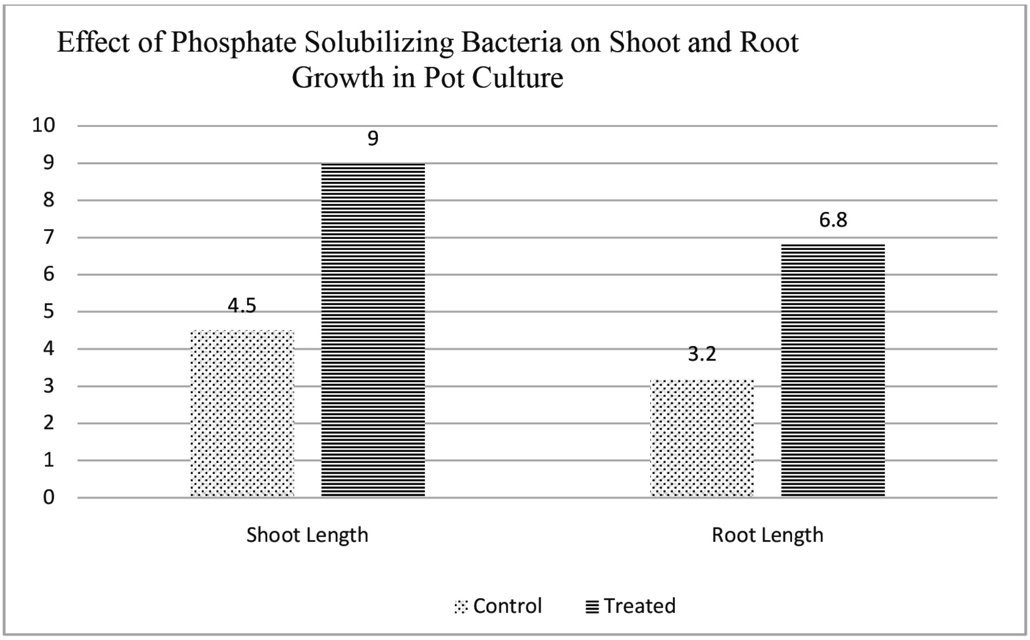


**Figure 2: Effect of Phosphate Solubilizing Bacteria on Shoot and Root Growth in Pot Culture**

The study measured the shoot and root lengths (in cm) of plants under two conditions: treated (with phosphate-solubilizing bacteria) and control (no treatment). The treated plants show significantly greater shoot and root lengths compared to the control plants. This indicates that the treatment positively influenced plant growth by promoting both shoot and root development. The enhanced root length in treated plants suggests better nutrient and water absorption capability, while the increased shoot length indicates improved overall growth and vigor. These results suggest that the treatment, likely involving phosphate-solubilizing bacteria, effectively supports plant growth compared to untreated plants.

## Discussion

The study successfully isolated nine phosphate-solubilizing bacteria (PSB) from three out of nine sampling sites (S1–S9), with the greatest number of isolates found at S1. This heterogeneous distribution is consistent with findings by Zhang et al. (2022), who observed marked site-to-site variation in PSB abundance and diversity influenced by soil properties and management practices (Zhang et al., 2022).

The PSI values for our isolates ranged from 2.14 to 2.75, with isolate S9C showing the highest PSI despite a small colony size. This inverse relationship aligns with screening studies, which often identify high halo/colony ratios as indicators of strong solubilization via organic acid secretion [Singh & Kapoor, 1999; Alori et al., 2017]. PSB releases gluconic, citric, and oxalic acids to chelate metal ions and mobilize phosphate (Rodríguez et al., 2006; Alori et al., 2017). S9C likely functions similarly.

The nine isolates were split between Gram-positive cocci (5) and Gram-negative rods (4), presenting varied colony morphology (creamy white, convex, or flat). Such diversity echoes similar surveys indicating effective PSBs are frequently found across genera such as *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Enterobacter* (Alori et al., 2017; Jyothisri et al., 2018).

Pot culture experiments showed that PSB treatment nearly doubled shoot length (≈8.7 cm vs. ≈4.5 cm) and more than doubled root length (≈6.8 cm vs. ≈3.2 cm) compared to untreated controls. This aligns with multiple studies reporting enhanced root/shoot development following inoculation with efficient PSB strains (e.g., *Pseudomonas* and *Bacillus*) in maize, tomato, and wheat under greenhouse conditions (Safari et al., 2021; Singh & Kapoor, 1999; Jyothisri et al., 2018). Further, PSB-enhanced root architecture improves phosphorus uptake, plant vigor, and stress resilience (Safari et al., 2021).

Using PSB as biofertilizers offers a sustainable alternative to chemical phosphorus fertilizers, mitigating risks like fixation and runoff (Alori et al., 2017; Safari et al., 2021). PSB can also boost P availability from rock phosphate, especially when formulated with carriers like biochar or as microbial consortia, demonstrating synergistic effects on soil health and plant growth (Safari et al., 2021; Safari et al., 2021).

Although PSI provides a quick preliminary screen, liquid culture assays measuring soluble phosphorus are essential for confirming mineral solubilization—halo zones may not accurately reflect actual P release (Wang et al., 2024). Moving forward, isolates such as S9C, S1A/B, and S8B should be tested quantitatively in liquid media, genetically characterized for organic acid production, and ultimately evaluated in field trials, possibly in combination with rock phosphate or mycorrhizal inoculants, to verify efficacy at agricultural scale (Wang et al., 2024; Safari et al., 2021).

**Conclusion**

This study successfully isolated and characterized nine phosphate-solubilizing bacterial (PSB) strains from three out of nine sampling sites, with the highest number of isolates obtained from Site S1. The isolates exhibited diverse colony morphologies and belonged to both Gram-positive and Gram-negative groups. Phosphate Solubilization Index (PSI) values ranged from 2.14 to 2.75, with isolate S9C showing the highest solubilization potential despite its small colony size.

Pot culture experiments demonstrated that inoculation with PSB significantly enhanced both shoot and root lengths compared to untreated controls, confirming their plant growth-promoting potential. These findings suggest that PSB, particularly isolates like S9C, S1A, and S8B, can serve as effective biofertilizers for improving phosphorus availability and promoting sustainable plant growth.

Further research, including quantitative phosphate solubilization assays, molecular identification, and field-level evaluation, is recommended to fully validate the efficiency and application potential of these isolates in agriculture.

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