International Journal of Horticulture and Food Science 2025; 7(8): 112-113


**E-ISSN:** 2663-1067
**P-ISSN:** 2663-1075
NAAS Rating (2025): 4.74
www.hortijournal.com
IJHFS 2025; 7(8): 112-113
Received: 09-07-2025
Accepted: 11-08-2025

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# Phosphate-solubilizing bacteria from agricultural soils of Western Maharashtra: Isolation, characterization, and potential for sustainable phosphorus management

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**DOI:** https://www.doi.org/10.33545/26631067.2025.v7.i8b.375

**Abstract**
Phosphorus is a critical macronutrient for plant growth, yet much of it in soil exists in insoluble forms, rendering it unavailable to plants. Phosphate-solubilizing bacteria (PSB) are recognized as a sustainable alternative to chemical fertilizers, capable of mobilizing fixed soil phosphorus through acid production, enzymatic activity, and other biochemical processes. This study systematically collected 42 agricultural soil samples from Satara and Sangli districts of Western Maharashtra, representing diverse soil types and cropping patterns. Forty-two PSB isolates were screened qualitatively using Pikovskaya’s agar and quantitatively in Pikovskaya’s broth. Ten high-performing isolates were characterized for cultural, morphological, and biochemical traits, pH reduction, titrable acidity, and phosphatase enzyme activity. Among these, isolates Tas-1, Pal-1, and Pal-2 exhibited superior phosphate solubilization efficiency, with Tas-1 showing the highest solubilization index (4.08), phosphate solubilization efficiency (308.06%), and soluble phosphorus (180.5 mg/L). These isolates also demonstrated robust acid production (pH reduction to 3.7-3.9; titrable acidity up to 1.7%) and high phosphatase activity, highlighting their potential for biofertilizer development. The findings are consistent with earlier reports on *Bacillus* and *Pseudomonas* spp., and reinforce the promise of PSB for sustainable phosphorus management in agriculture.

**Keywords:** Phosphate-solubilizing bacteria, biofertilizer, Pikovskaya’s medium, *Bacillus* spp., *Pseudomonas* spp., sustainable agriculture

## Introduction
Phosphorus (P) is an essential element for plant growth, playing a vital role in energy transfer, photosynthesis, and signal transduction. However, in most soils, a substantial portion of phosphorus exists in insoluble mineral complexes bound with calcium, iron, or aluminum, making it inaccessible to plants. Conventional management of phosphorus deficiency relies heavily on chemical phosphate fertilizers. While effective in the short term, such fertilizers are costly, often inefficient (due to fixation in the soil), and environmentally detrimental.
Phosphate-solubilizing bacteria (PSB) represent an eco-friendly alternative, mobilizing soil phosphorus through organic acid production, chelation, and enzymatic activity (Sharma *et al*., 2013; Alori *et al*., 2017) [10, 1]. Several genera, particularly *Bacillus* and *Pseudomonas*, have been identified for their ability to improve phosphorus availability and contribute to overall soil health. In India, PSB-based biofertilizers have shown promise in increasing crop yield and reducing dependence on synthetic fertilizers (Kumar *et al*., 2014) [5].
Western Maharashtra, with its diverse agro-ecological zones and intensive agricultural practices, presents a unique opportunity for identifying efficient PSB strains adapted to local soils. This study aims to isolate, characterize, and evaluate PSB from Satara and Sangli districts, integrating findings with existing literature to assess their potential as sustainable biofertilizers.

## Soil Sampling and Isolation of PSB
A total of 42 agricultural soil samples were collected from various cropping systems (sugarcane, wheat, gram, groundnut, jawar, etc.) across Satara and Sangli districts. Sampling covered both black and red soils, ensuring broad ecological representation.

Each sample was geo-tagged and coded. PSB isolation was performed on Pikovskaya's agar medium, where phosphate solubilization was detected by halo zone formation around bacterial colonies. This method remains a widely accepted preliminary screening tool due to its simplicity and correlation with actual solubilization potential (Karpagam & Nagalakshmi, 2014) [4].

**Qualitative and Quantitative Phosphate Solubilization**

Among the 42 isolates, 10 demonstrated exceptional phosphate-solubilizing abilities. The standout performer, Tas-1, achieved a solubilization index (SI) of 4.08 and phosphate solubilization efficiency (PSE) of 308.06%, producing a 19.1 mm halo zone. Pal-1 and Wa-2 also recorded high SI values (> 3.0) and PSE (> 200%).

Quantitative estimation in Pikovskaya's broth showed Tas-1 producing 180.5 mg/L soluble P, followed closely by Pal-1 (174.6 mg/L) and Pal-2 (160.3 mg/L). This range is comparable to values reported by Panda *et al*. (2013) [7], where high-efficiency PSB strains solubilized between 150-200 mg/L under *in vitro* conditions.

**Morphological and Cultural Characterization**

The 10 high-performing isolates exhibited diverse colony morphologies: sizes from 0.5-4.0 mm, shapes ranging from round to irregular, and colors including creamy white, dull white, white, and yellow. Gram staining classified them into Gram-positive (Pal-1, Pal-2, Tas1, Is-1, Pat-2, Ane-1) and Gram-negative (Kde-1, Wa-2, Sat-1, Kr-1) groups, suggesting affiliations with *Bacillus* and *Pseudomonas*. This aligns with earlier reports where these genera dominated PSB populations in agricultural soils (Jayamma *et al*., 2013) [3].

**Biochemical Characterization**

All isolates tested positive for catalase, oxidase, and citrate utilization. Ammonia production a trait beneficial for plant nitrogen supply was observed in Tas-1, Is-1, Kr-1, Ane-1, and Kde-1. Motility was confirmed in eight isolates, enhancing their potential for rhizosphere colonization. Such traits contribute to their classification as plant growthpromoting rhizobacteria (PGPR), combining phosphorus solubilization with other growth benefits (Glick, 2012) [2].

**Mechanisms of Phosphate Solubilization**

**Acidification of Growth Medium**

Initial broth pH (6.8) decreased significantly in most isolates after 9 days, with Tas-1 and Pal-1 reaching pH 3.7-3.9. Organic acid production is a primary PSB mechanism, lowering pH and chelating cations bound to phosphate (Rodríguez & Fraga, 1999) [8].

**Titrable Acidity**

Tas-1 showed the highest titrable acidity (1.7%), followed by Pal-1 (1.4%). High acid levels correlated with elevated phosphate solubilization, consistent with Sharma *et al*. (2021) [11].

**Phosphatase Enzyme Activity**

Both acid and alkaline phosphatase activities were highest in Tas-1 and Pal-1. Tas-1 recorded 12.76 µg PNP/ml/hr. (acid) and 17.87 µg PNP/ml/hr (alkaline), indicating ability to mineralize organic phosphorus under varying soil pH conditions. This dual capability enhances versatility in different agricultural soils.

**Conclusion**

The study identifies Tas-1, Pal-1, and Pal-2 as promising PSB strains from Western Maharashtra, with robust *in vitro* phosphate-solubilizing abilities supported by multiple plantbeneficial traits. Their adaptability to local soils and potential for biofertilizer application could significantly enhance phosphorus use efficiency in agriculture. Future work should focus on molecular identification, large-scale formulation, and field validation.

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