



ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2025): 4.97
IJAFA 2025; 7(9): 558-561
www.agriculturaljournals.com
Received: 08-07-2025
Accepted: 11-08-2025

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Isolation and characterization of phosphate solubilizing bacteria from western Maharashtra

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DOI: <https://www.doi.org/10.33545/2664844X.2025.v7.i9g.794>

Abstract

Phosphate-solubilizing bacteria (PSB) serve as eco-friendly biofertilizers by converting insoluble soil phosphorus—often bound with aluminum, iron, calcium, or magnesium—into plant-available forms. They achieve this by secreting low-molecular-weight organic acids that chelate metal ions, thus releasing phosphate ions for uptake. In a survey of soils from Satara and Sangli districts in Western Maharashtra, forty-two samples yielded ten PSB isolates selected based on phosphate solubilization index (SI ranging 1.00-4.08) and solubilization efficiency (PSE up to 308%). When cultured in Pikovskaya's broth containing tricalcium phosphate (TCP), phosphate release ranged from 52.7 to 180.5 mg/L, with isolate Tas 1 achieving the highest (180.5 mg/L). Morphological, cultural, and biochemical tests identified six isolates (Pal 1, Pal 2, Tas 1, Is 1, Pat 2, Ane 1) as *Bacillus* spp., and four (Kde 1, Wa 2, Sat 1, Kr 1) as *Pseudomonas* spp. All isolates tested positive for catalase, oxidase, and citrate utilization; several showed bromothymol blue reaction, ammonia production, and motility. Five isolates exceeded 100 mg/L phosphate solubilization, highlighting Tas 1 as a highly promising candidate for agricultural biofertilizer development.

Keywords: Isolation, Phosphate Solubilizing bacteria, Phosphate Solubilizing efficiency, Solubilization index

Introduction

Soil was created thousands of years ago by a combination of physical, chemical, and biological processes. A significant improvement in agricultural output is required to feed the expanding population. Numerous bacterial species, especially those present in the rhizosphere of plants, have the ability to favourably affect plant growth. *Pseudomonas*, *Serratia*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, and *Flavobacterium* are among the genera with strains of these bacteria, which are referred to as "plant growth-promoting rhizobacteria" (PGPR) (Rodríguez *et al.*, 1999) [9].

Many of these microorganism's function as phosphate-solubilizing agents, converting insoluble phosphorus into soluble forms (HPO_4^{2-} and H_2PO_4^-) by producing acids, facilitating ion exchange and acidification, chelating metal ions, and forming polymeric substances (Delvasto *et al.*, 2008) [12]. Consequently, they reduce dependency on chemical fertilizers and are increasingly seen as a sustainable alternative to conventional synthetic fertilization.

One essential macronutrient required for plant growth and development is phosphorus (P). According to Khan *et al.* (2009) [5], it is a component of biological molecules such as ATP, phospholipids, DNA, and RNA. By encouraging the growth of deeper roots, phosphorus is a crucial component that contributes significantly to plant nutrition. (Gaur 1990). Since the 1950s, phosphate-soluble bacteria (PSB) have been used as biofertilizers (Kudashev, 1956) [6]. One important factor affecting P availability in soils is the release of phosphorus (P) by PSB from insoluble and fixed/adsorbed forms.

A broad category of microorganisms known as phosphate solubilizing microorganisms (PSMs) help make soil phosphorus (P) accessible. Because they solubilize inorganic P minerals, mineralize organic P, and store large amounts of P in their biomass, they are essential to the soil P cycle. PSMs can transform soil P into soluble and plant-available orthophosphate forms, such as PO_4^{3-} , HPO_4^{2-} , and H_2PO_4^- , by releasing phosphatase enzymes

and organic acids, decreasing soil pH, and improving chelation activities with extra P adsorption sites. In current investigation soil samples were collected from western Maharashtra specially from Satara and Sangli region.

Materials and methods

Collection of soil sample

The soil samples were collected from Satara and Sangli region from the agricultural land. Soil samples were collected from rhizosphere of various soils and crops. Collected soil samples were stored in polythene bags aseptically and maintained at the laboratory for further study.

Isolation of strain

For isolation of Phosphate Solubilizing Microbes, 1g rhizosphere soil was suspended in 100ml of distilled water. An aliquot (100^{-1}) from decimal dilutions was inoculated on Pikovskaya s medium by pour plate technique and incubated at 30°C. Colonies showing phosphate solubilizing zone around them were considered as PSB. Single colonies appearing on Pikovskaya s agar plates were transferred in liquid broth of Pikovskaya s and on agar slants for further study. Aneja 2003 ^[1].

Analysis of phosphate solubilization ability

From the isolates, larger halo zone producing strains were selected for further study. The qualitative as well as quantitative analyses of phosphate solubilizing activity of the selected isolates were conducted by plate screening method and broth culture method.

Qualitative method

All the suspected colonies were screened for phosphate solubilization on Pikovskayas medium. Isolates showing phosphate solubilizing ability were spot inoculated at the centre Pikovskaya s plate and incubated at 37°C. Diameter of clearance zone was measured successively after 24 hours, up to 7 days. The Phosphate Solubilization Efficiency (PSE) is the ratio of total diameter. i.e. clearance zone including bacterial growth and the colony diameter. (Premono *et al.*, 1996) ^[8].

$$\text{Solubilization Index (SI)} = \frac{\text{Colony diameter} + \text{Holo zone diameter}}{\text{Colony diameter}}$$

$$\text{Phosphate solubilisation efficiency (SE) \%} = \frac{\text{Halo zone diameter}}{\text{CD}} \times 100$$

All the observations were recorded in triplicate. Strains developing clear zones around their colonies could easily identify as PSB.

Quantitative method

Pikovskaya s broth medium (100 ml) with Tricalcium phosphate (0.3g/100ml) was prepared and sterilized; 1ml of each isolates was inoculated into the broth medium. Then the inoculated sample were incubated for 5 days on rotatory shaker 37°C after incubation, culture broth was centrifuged at 10,000 rpm for 30 min. Uninoculated broth served as control. The available Phosphorous was determined using calorimetrically at 410 nm with standard KH_2PO_4 .

Assesment of pH change

Isolated PSB culture were inoculated in Pikovskaya's broth pH on inoculated day was recorded. The progressive reduction in pH on 3rd, 6th and 9th day was recorded.

Result and discussion

Isolation and identification of PSB

In Pikovskaya's agar medium, the phosphate-solubilizing microbes in the soil samples were assessed. Using insoluble tri-calcium phosphate (TCP) from soil in western Maharashtra, 42 phosphate-soluble bacterial colonies were recovered on Pikovskaya's agar medium. Ten isolates with the highest Phosphate Solubilization Index (PSI), ranging from 2.11 to 4.08, out of 42 microbial isolates were chosen for more research. Phosphate solubilization was identified in colonies that displayed distinct halo zones surrounding the microbial growth.

Following a series of morphological and biochemical reactions, the bacterial isolates were further identified as *Pseudomonas sp.* and *Bacillus sp.* the biochemical and physical traits of these isolates. Table 1

Analysis of phosphate solubilizing activity

Larger strains that produced halo zones were chosen from the isolates for more investigation. The chosen isolates' phosphate solubilizing activity was analyzed both qualitatively and quantitatively using the broth culture and plate screening methods.

Qualitative method

All the selected isolates were found to be potent phosphate solubilizers showing clear zone around their colonies. Out of 42 microbial isolates 10 isolates showed highest Phosphate Solubilization Index (PSI) ranged from 2.11-4.08 were selected for further studies. These findings are consistent with those of Karpagam and Nagalakshmi (2014) ^[4] and Roy *et al.* (2013) ^[10]. Among these 10 potent isolates, 3 strains showed maximum PSI of Tas-1, Pal-1 and Pal-2 in agar plates. The PSI measurement was shown in the Table 2.

Quantitative method

Pikovskaya s broth medium (100 ml) with Tricalcium phosphate (0.3g/100ml) was prepared and sterilized; 1ml of each isolates was inoculated into the broth medium. It was observed that isolates Tas-1, Pal-1 and Pal-2 showed highest percent P solubilization Panda *et al.* (2013) ^[7] showed similar result. The results were showed in the Table 3 and figure 1.

Effect on pH in broth medium after inoculation of PSB isolates

These results reinforce the hypothesis that organic acid production, leading to medium acidification, plays a central role in mobilizing insoluble phosphates by lowering the pH and chelating cations such as calcium, iron, or aluminum that bind phosphate. Isolates like Tas-1 and Pal-1 with robust acid-producing capacity, hold promise for bioformulation development aimed at enhancing phosphorus availability in acidic and calcareous soils. These findings concur with those of Ma. Esther Sanchez-Gonzalez *et al.* (2023) ^[11]. The results were showed in the Table 4 and figure 2.

Table 1: Morphological and biochemical characters of PSB isolates

Sr. No	Characters	Tas-1	Pal-1	Pal-2	Is-1	Kde-1	Wa-2	Sat-1	Kr-1	Pat-2	Ane-1
1	Size	Medium	Small	Medium	Medium	Small	Medium	Small	Medium	Very small	Small
2	Shape	Irregular	Round	Round	Round	Irregular	Irregular	Round	Round	Irregular	Irregular
3	Colour	Creamy white	Creamy white	Dull white	Creamy white	Creamy white	White	Dull white	Yellow	Creamy white	White
4	Margine	Irregular	Irregular	Regular	Regular	Irregular	Irregular	Regular	Irregular	Regular	Irregular
5	Catalase test	+	+	+	+	+	+	+	+	+	+
6	Oxidase	+	+	+	+	+	+	+	+	+	+
7	Citrate utilization	+	+	+	+	+	+	+	+	+	+
8	NH ₃ production	+	-	-	+	+	-	-	+	-	+
9	BTB	+	+	+	-	+	-	+	-	+	-
10	Motility	+	+	+	+	-	+	-	+	+	+

Table 2: Phosphate solubilizing activity of selected PSB isolates

Sr. No.	Isolates	Colony Diameter	Holo zone diameter	Phosphate solubilization index	Phosphate solubilization efficiency
1	Tas-1	6.2	19.1	4.08	308.6
2	Pal-1	7.4	15.6	3.10	210.81
3	Pal-2	0.1	0.2	3.00	200.00
4	Is-1	5.1	5.3	2.04	103.92
5	Kde-1	5.5	6.5	2.18	118.18
6	Wa-2	6.3	12.7	3.02	201.59
7	Sat-1	6.2	6.5	2.05	104.84
8	Kr-1	4.3	5.7	2.33	132.56
9	Pat-2	14.8	17.6	2.19	118.92
10	Ane-1	7.1	7.9	2.11	111.92

Table 3: Phosphate solubilization quantitative ability of PSB isolates

Sr. No.	Isolates	Soluble 'P' conc. mg/L
1	Tas-1	180.5
2	Pal-1	174.6
3	Pal-2	160.3
4	Is-1	63.2
5	Kde-1	138.1
6	Wa-2	146.2
7	Sat-1	83.8
8	Kr-1	97.4
9	Pat-2	74.4
10	Ane-1	52.7

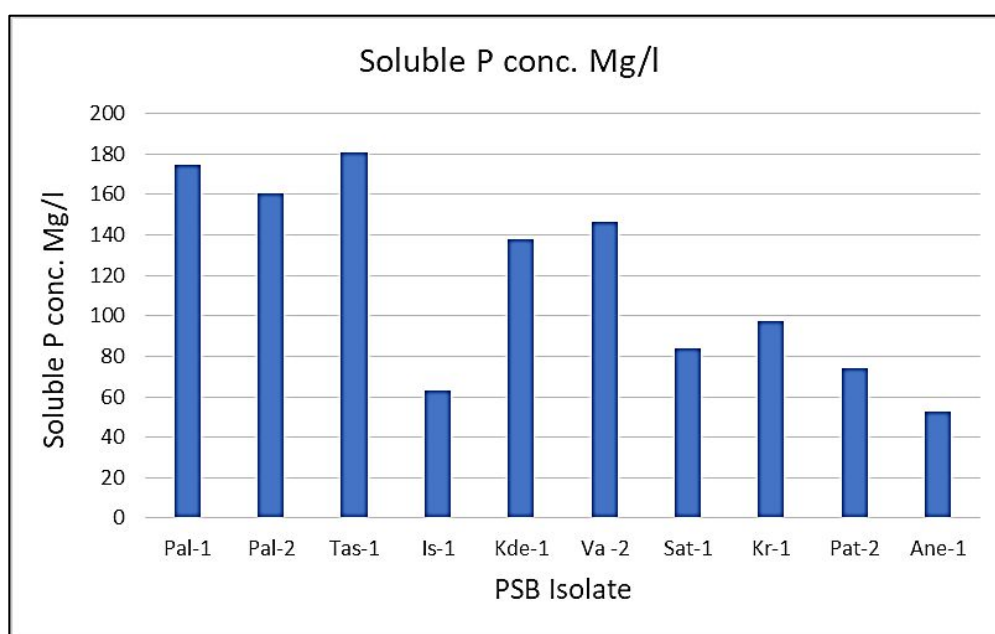
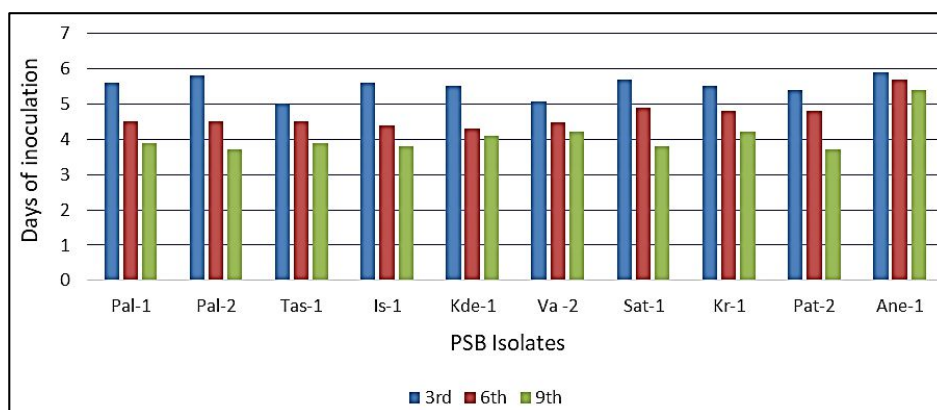
**Fig 1:** Phosphate solubilization quantitative ability of PSB isolates

Table 4: Effect on pH after 3rd, 6th and 9th day of inoculation of PSB isolates

Sr. No.	Isolates	pH on			
		Inoculated day	3 rd DAI	6 th DAL	9 th DAI
1	Tas-1	6.8	5.0	4.5	3.9
2	Pal-1	6.8	5.6	4.5	3.9
3	Pal-2	6.8	5.8	4.5	3.9
4	Is-1	6.8	5.6	4.4	3.8
5	Kde-1	6.8	5.5	4.3	4.1
6	Wa-2	6.8	5.1	4.5	4.2
7	Sat-1	6.8	5.7	4.9	3.8
8	Kr-1	6.8	5.5	4.8	4.2
9	Pat-2	6.8	5.4	4.8	3.7
10	Ane-1	6.8	5.9	5.7	5.4

**Fig 2:** pH change of PSB culture on 3rd, 6th, 9th day of inoculation

Conclusion

The study found that PSB, especially Tas-1, Pal-1 and Pal-2 had a lot of potential for releasing soluble phosphate into the soil and allowing plants to use it.

Based on cultural, morphological and biochemical characteristics, it was concluded that Kde-1, Wa-2, Sat-1 and Kr-1 were *Pseudomonas* species while Pal-1, Pal-2, Tas-1, Is-1, Pat-2 and Ane-1 was *Bacillus* species.

Acknowledgement

The authors would like to thank Mahatma Phule Krishi Vidyapeeth Rahuri, Biological Nitrogen fixation scheme and Department of Plant Pathology, College of Agriculture Pune for providing necessary research facilities.

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