

# Exploration of Native PSB in Saline and Semiarid Agroecosystems of Western Maharashtra

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## Abstract

The purpose of this work was to isolate and characterize phosphate-solubilizing bacteria (PSB) from semi-arid and saline soils in the Sangli and Kolhapur districts of Maharashtra to assess their potential as biofertilizers in stress-prone agricultural environments. Fifteen soil samples were collected from saline and drought-affected areas. Ten different PSB isolates (PSSB1-PSSB8, PSSG11 and PSSA1) were obtained and characterized based on colony features, Gram reaction, motility, biochemical traits and enzymatic activities. The isolates showed varying phosphate solubilization indices (PSI), with PSSB6, PSSB7, PSSB4, PSSB8 and PSSG11 being the most efficient.

These PSBs exhibited traits such as ammonia production, urease activity and acid phosphatase potential, indicating stress tolerance. The results support the development of PSB-based bioinoculants that are resistant to salt and drought, aiming to increase phosphorus availability, improving soil fertility and promoting sustainable agriculture in the challenging agroecosystems of Western Maharashtra.

**Keywords:** PSB, Saline soil, Agroecosystem.

## Introduction

Soil microorganisms are vital for soil health because they convert organic matter into nutrients that plants can absorb<sup>20,29</sup>. Soil microbial activity is essential for crop yields, sustainable land use and rehabilitating saline soils. Understanding how salinity and water content affect microorganisms helps them to provide necessary nutrients<sup>7</sup>. Many ions in salt-affected soils can block water absorption and harm different organisms. El-Gibaly et al<sup>8</sup> observed higher PSB populations in alkaline soil rhizosphere zones, indicating that phosphobacteria thrive in high-salinity environments and can dissolve stubborn phosphorus compounds. The study by El-Din and Saber<sup>9</sup> explores how inoculating phosphate-dissolving bacteria impacts barley P-uptake in salt-affected calcareous soil, showing that increased P-uptake can negatively affect soil microbial diversity. India could provide an affordable phosphate fertilizer solution to address soil phosphate deficiency which impacts plant growth, energy transfer and photosynthesis, potentially reducing crop yields<sup>26</sup>.

Low soil phosphorus levels reduce plant phosphorus content. Phosphate-solubilizing bacteria convert insoluble phosphorus into accessible forms, boosting productivity, but their effectiveness decreases in saline soils<sup>11</sup>. Soil bacteria and fungi can also solubilize phosphorus<sup>23</sup>. Phosphorus solubilization plays a key role in soil acidification, but most phosphate-solubilizing bacteria have low salinity tolerance, so the isolation of highly halophilic PSB is necessary for this purpose<sup>41</sup>.

Phosphate-solubilizing bacteria convert insoluble phosphorus compounds into forms that plants can absorb, increasing soil phosphorus availability, reducing reliance on chemical fertilizers and supporting sustainable agriculture by promoting improved plant growth and soil health<sup>37</sup>. These microorganisms are essential for providing phosphorus to plants and enabling the sustainable use of phosphate fertilizers<sup>23</sup>. Phosphorus availability declines in semiarid and saline soils due to low organic matter, high salt content and poor soil structure, which creates a need for developing salt-tolerant PBS to help plants tolerate these conditions<sup>1,4</sup>. Plant growth-promoting rhizobacteria (PGPR) are being researched as biofertilizers, aiding in nitrogen fixation, IAA production and the development of bio-inoculants to alleviate salt stress<sup>13,16</sup>.

Identifying and applying PSBs can encourage eco-friendly farming practices, but environmental stresses can considerably impact their effectiveness in the rhizosphere<sup>26</sup>. Understanding how salinity and water availability affect soil microorganisms is vital for crop production, sustainable land management and the restoration of saline soils<sup>40</sup>. PSB-based biofertilizers offer a sustainable, cost-effective and improved alternative to chemical phosphorus fertilizers, boosting crop nutrition and yields, especially for smallholder farmers facing soil phosphorus deficiencies<sup>28</sup>.

More than 6% of the world's land is salt-affected and bacteria in the rhizosphere have the potential to provide plant growth-promoting agents for agriculture<sup>22</sup>. Salt-tolerant rhizobacteria can reduce plant stress, leading to better growth, higher yields and increased disease resistance, thereby potentially enhancing crop productivity through microbial inoculants in an eco-friendly, affordable manner<sup>33</sup>. Drought stress, indicated by low soil moisture, severely hampers microbial survival and activity, causing microbial communities to shift toward drought-tolerant species<sup>5</sup>. This study explores the potential use of phosphate-solubilizing bacteria in agriculture to improve soil fertility and plant growth by examining their morphological, biochemical and salt stress responses.

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## Material and Methods

**Collection of Soil Sample:** Soil samples were collected from 15 sites in Maharashtra's Sangli and Kolhapur districts, including Jat, Dighanchi, Aatpadi, Kumbhari, Ravalgundewadi, Devnal, Birnal and Sangli. The samples were divided into five homogeneous units, cleaned of surface debris and placed in containers. After mixing, the sample weight was reduced to half a kilogram and each was labeled with the collection number, date, site and soil type. The samples were stored in polythene bags in the research laboratory at low temperatures until needed<sup>10,15,27</sup>. Based on climate and annual rainfall, semi-arid areas were selected for isolating drought-tolerant bacteria, while saline lands with poor crop growth were chosen for salt-tolerant bacteria in the western belt of Maharashtra.

**Enrichment and isolation of Phosphate-Solubilizing Bacteria (PSB):** Multiple sub-samples were combined, each derived from individual soil samples. Serial dilutions were then performed on the mixed samples, reaching up to  $10^{-6}$ . From the  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  dilutions, a volume of 0.1 mL was taken. Cultures were grown on Pikovskaya medium, with the pH adjusted to 7.2. All plates were incubated at 30°C for 7 days. PSB isolates were selected and purified based on their distinct halos.

**Characterization of bacterial isolates:** The isolates were examined for morphological, cultural and biochemical characteristic tests such as IMViC, Oxferm test, nitrate reduction test and fermentation of glucose, fructose, sucrose, lactose and mannitol etc. Enzymatic tests included amylase, caseinase, lipase, lecithinase, catalase and oxidase, among others<sup>26</sup>.

**Study on phosphate solubilization index:** P-solubilization on Pikovskaya agar medium was used to evaluate the effectiveness of different isolates. The samples were spot-inoculated on Pikovskaya agar plates and incubated at 28°C for 5 days. The presence of a halo zone surrounding the colony, along with growth, indicates the bacteria's phosphate solubilizing activity. The zone of phosphate solubilization around the colonies was measured and the solubilization index (SI) was calculated using the formula:

$$SI = \frac{\text{Diameter of colony} + \text{Diameter of halozone}}{\text{Diameter of colony}}$$

**Study on the effect of pH on the growth of the isolates:** Bacterial cultures were inoculated into 25 mL of LB broth medium with different pH values, ranging from 7 to 12. Cultures were inoculated and incubated for 02 days at 28 °C on a rotary shaker at 150 rpm and the optical density was measured at 530 nm<sup>11</sup>.

**Study on the effect of salt on the growth of the isolates (1%–7.5%):** Bacterial cultures were separately inoculated on LB broth medium with different NaCl concentrations (1% to 7.5%) to examine the effect of salt on the growth of the

isolates<sup>11</sup>. Cultures were inoculated and incubated for 02 days at 28 °C on a rotary shaker at 150 rpm and the optical density was measured at 530 nm<sup>11</sup>.

**Study on drought stress on the growth of isolates:** Nutrient agar medium was sterilized by autoclaving and supplemented with different concentrations of PEG 6000 (10%, 15%, 20%, 25% and 30% w/v) to mimic osmotic stress and assess drought stress resistance in microbial cultures. The screened microbial cultures, including a PEG-free control, were streaked on each plate and incubated for three to seven days at 28 to 30°C. The growth of the cultures was observed after incubation. Consistent growth indicates tolerance, while a decline in growth with increasing PEG concentrations shows susceptibility to drought stress<sup>25</sup>.

**Study on Plant Growth Promoting Properties:** The bacterial isolates that showed tolerance were then used for the study on plant growth-promoting traits.

**Nitrogen Fixation:** The isolates were inoculated on Yeast Extract Mannitol Agar plates containing congo red, then incubated for 48 hours at room temperature to determine the nitrogen fixation ability of the isolates<sup>2,35</sup>.

**Zinc Solubilization:** The isolates were inoculated into a minimal salt medium containing 0.1% zinc oxide and incubated for 48 hours at room temperature. Zinc solubilization was indicated by the formation of clear halos<sup>34</sup>.

**Production of Indole Acetic Acid (IAA):** The bacterial isolates were cultured for 72 hours at room temperature after being inoculated in a broth that contained 5 milliliters of peptone yeast extract broth. After incubation, 1.5 mL of the culture was centrifuged at 13000 × g for 10 minutes. The supernatant was let to rest at room temperature in the dark for an hour after 1 mL of Salkowski reagent (50 mL of 35% perchloric acid and 1 mL of 0.5 M FeCl<sub>3</sub> solution) was added. Formation of red colour indicates IAA production<sup>31</sup>.

**Production of Siderophore:** After being spot-inoculated onto Chrome Azurol S (CAS) agar plates, isolates were cultured for 48 hours at room temperature. The presence of an orange halo zone indicated the development of siderophores<sup>32</sup>.

**Ammonia Production:** Isolates were cultivated for 72 hours at room temperature after inoculation with 4% peptone broth. Following incubation, 1 mL of Nessler's reagent was added. The appearance of a yellow to brown precipitate indicated ammonia production<sup>2,38</sup>.

## EPS Production

**Qualitative Method for EPS:** Each bacterial strain was inoculated onto 5 mm diameter paper discs placed in a medium containing 2% yeast extract, 1.5% K<sub>2</sub>HPO<sub>4</sub>, 0.02% MgSO<sub>4</sub>, 0.0015% MnSO<sub>4</sub>, 0.0015% FeSO<sub>4</sub>, 0.003% CaCl<sub>2</sub>,

0.0015% NaCl and 1.5% agar, adjusted with 10% saccharose and had a pH of 7.5. The size of the halo zone and its slimy appearance were characteristic of EPS producers. The presence of EPS was confirmed by combining a portion of the mucoid material with 2 ml of 100% cold ethanol, resulting in the formation of a precipitate.

**Quantitative Method for EPS:** A mineral salt medium with 12.6% K<sub>2</sub>HPO<sub>4</sub>, 18.2% KH<sub>2</sub>PO<sub>4</sub>, 10% NH<sub>4</sub>NO<sub>3</sub> and 1% MgSO<sub>4</sub> was used to cultivate EPS-producing bacteria. One liter of distilled water containing 0.6% MnSO<sub>4</sub>·7H<sub>2</sub>O, 1% CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.06% FeSO<sub>4</sub>·2H<sub>2</sub>O, 1% sodium molybdate, 1.5% NaCl and 0.2% glucose was prepared for ten days. The bacterial broth cultures were centrifuged at 10,000 rpm for 20 minutes at 4°C after a 10-day incubation period. Twice as much ice-cold ethanol (95%) was added to the supernatant to extract the EPS. Complete precipitation was achieved by chilling the solution to 4°C<sup>39</sup>.

## Results

**Collection of Soil Sample:** A total fifteen soil samples were collected from agricultural fields of Sangli and Kolhapur districts. The sample codes, source, place and description of each sample are presented in the table 1. Sangli, India's semiarid region, is prone to aridification and affects specialized microbial populations. The region has high temperatures, low rainfall and 60% humidity. Major microbial species are found in various locations such as Akiwat, Majarewadi, Narsobawadi, Arjunwad, Kurundwad and Rajapur from Kolhapur and these climates contribute to the microbial ecosystems in Sangli and Kolhapur, varying in temperature, humidity and soil structure.

**Isolation and Screening of PSB:** A total of ten morphologically distinct bacterial isolates (PSSB1-PSSB8,

PSSG11 & PSSA1) with a phosphate solubilization zone were isolated from semiarid soil samples.

**Characterization of phosphate solubilizing bacteria:** Ten microbial isolates were examined for colony characteristics with most forming round colonies with smooth edges. However, some had irregular margins and opaque colonies, while others had a regular shape with mucoid consistency. The colony traits may reflect the isolates' physiological or functional characteristics.

The study analyzed the morphological, physiological and biochemical characteristics of ten phosphate-solubilizing bacterial isolates (PSSB1 to PSSA1). Most were Gram-negative rods, with two classified as Gram-negative coccobacilli. Most strains were motile, indicating their ability to colonize the rhizosphere. They were excellent fermenters of glucose, fructose, sucrose and mannitol, with some producing acid and gas. Lactose fermentation varied, with some strains producing indole. Citrate utilization was generally positive across all strains. Hydrolytic enzyme production was inconsistent, with only PSSG11 and PSSA1 producing amylase. Some strains possessed different amino acid metabolism and stress adaptation.

**Phosphate Solubilization Index:** The phosphate solubilization index (PSI) of bacterial isolates indicates their efficiency in solubilizing insoluble phosphate, crucial for plant growth and soil fertility. PSSB6, PSSB7, PSSB4, PSSB8 and PSSG11 showed the highest PSI values, indicating their potential for use in biofertilizer formulations to enhance phosphorus availability in soils under nutrient-limited or stress-prone agricultural conditions (Table 3, Figure 1).

**Table 1**  
**Sample code, source, place and description of samples**

S.N.	Sample code	Source	Place	Description
1	SJ	Agricultural field	Sangli	Semiarid region
2	SD1	Agricultural field	Sangli	Semiarid region
3	SA	Agricultural field	Sangli	Semiarid region
4	SK	Agricultural field	Sangli	Semiarid region
5	SR	Agricultural field	Sangli	Semiarid region
6	SD2	Agricultural field	Sangli	Semiarid region
7	SB	Agricultural field	Sangli	Semiarid region
8	SS	Agricultural field	Sangli	Medium saline soil
9	SM	Agricultural field	Sangli	Medium saline soil
10	KA1	Agricultural field	Kolhapur	Saline land
11	KM	Agricultural field	Kolhapur	Saline land
12	KN	Agricultural field	Kolhapur	Medium saline soil
13	KA2	Agricultural field	Kolhapur	Medium saline soil
14	KK	Agricultural field	Kolhapur	Saline land
15	KR	Agricultural field	Kolhapur	Medium saline soil

\*Note: SJ -Jath, SD1- Dighanchi, SA- Aatpadi, SK- Kumbhari, SR - Ravalgundewadi, SD2 - Devnal, SB - Birnal, SS – Sangli, SM - Miraj from Sangli District

KA1 - Akiwat, KM - Majarewadi, KN - Narsobawadi, KA2 - Arjunwad, KK - Kurundwad, KR – Rajapur from Kolhapur District

**Table 2**  
**Characterization of phosphate solubilizing bacteria**

S.N.	Characteristics	PSSB1	PSSB2	PSSB3	PSSB4	PSSB5	PSSB6	PSSB7	PSSB8	PSSG11	PSSA1
1	Gram Nature	Gram negative rods	Gram negative rods	Gram negative rods	Gram negative cocobacilli	Gram negative rods	Gram negative rods	Gram negative cocobacilli	Gram negative rods	Gram positive rods	Gram positive rods
2	Motility	Motile	Motile	Motile	Motile	Motile	Motile	Motile	Motile	Non motile	Non motile
3	Oxferm test	++	++	++	++	++	++	++	++	++	-
4	Nitrate reduction test	++	++	++	++	++	++	++	++	++	+
5	Glucose fermentation	<sup>a</sup> ++	++	<sup>a</sup> ++	<sup>a</sup> ++	++	++	<sup>a</sup> ++	++	<sup>a</sup> ++	-
6	Fructose fermentation	<sup>a</sup> ++	++	++	+	<sup>a</sup> ++	++	+	+	-	-
7	Sucrose fermentation	<sup>a</sup> ++	++	++	+	++	++	+	+	++	-
8	Lactose fermentation	++	--	--	+	--	++	+	+	-	-
9	Mannitol fermentation	<sup>a</sup> ++	++	++	+	++	++	+	+	+	-
10	Indole	+	+	+	-	+	+	-	-	+	-
11	MR	-	-	-	-	-	-	-	-	+	-
12	VP	-	-	-	+	-	-	+	+	+	-
13	Citrate Utilization	+	+	+	+	+	+	+	+	+	+
14	Amylase	-	-	-	-	-	-	-	-	+	+
15	Caseinase	+	+	+	-	+	-	-	-	+	-
16	Lipase	+	-	+	+	-	-	+	+	+	+
17	Lecithinase	-	-	-	+	-	+	+	-	+	-
18	Catalase	+	+	+	+	+	++	+	+	+	+
19	Oxidase	-	+	-	-	-	+	-	-	+	+
20	Ammonia production	+	-	+	+++	-	+	+	+++	+++	+++
21	Urease	-	+	-	+	+	+	+	+	+	-
22	Arginine dihydrolase	+	-	-	-	-	-	-	-	+	-
23	Ornithin decarboxylase	-	-	+	+	+	+	+	-	+	+
24	Lysine decarboxylase	+	-	+	-	+	-	+	+	-	+
25	Phenyl alanine Deaminase	+	+	-	-	+	-	-	-	-	-

\*Note: <sup>a</sup> represents acid and gas formation

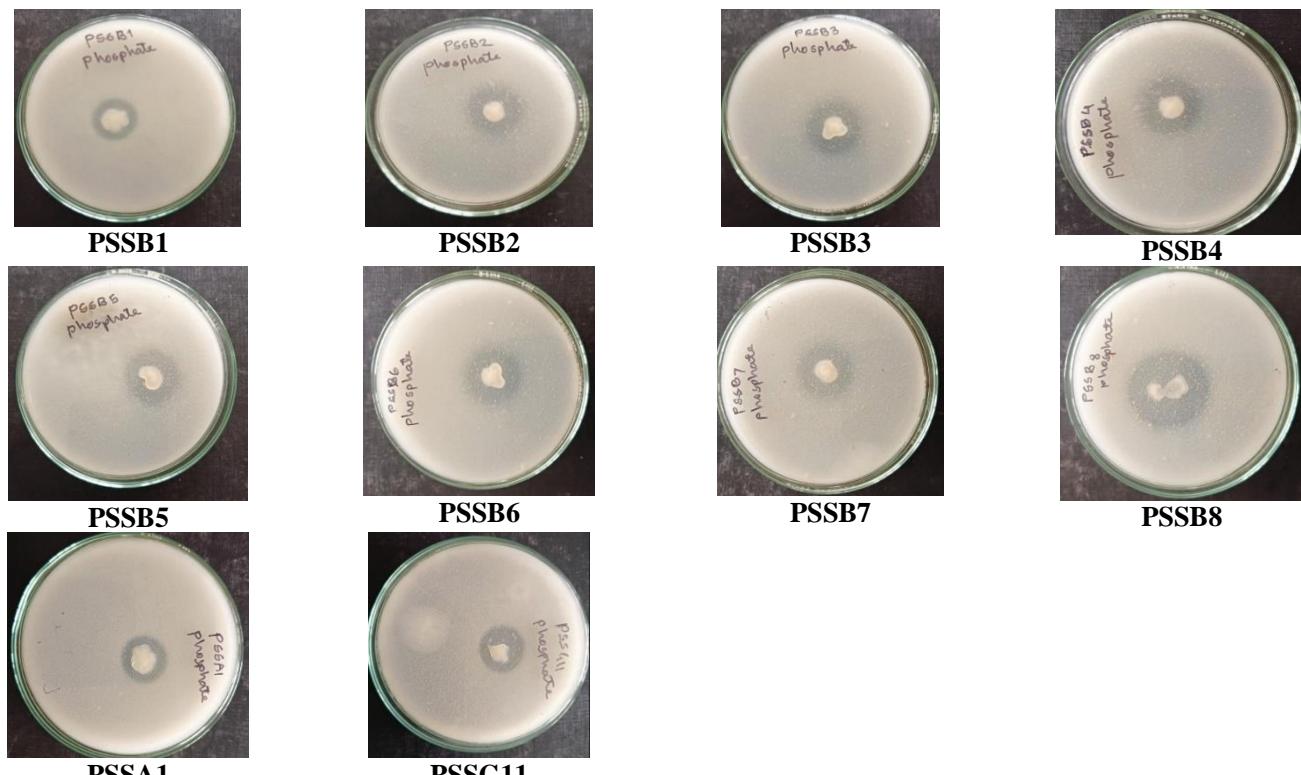
**Study on the effect of pH on the growth of the isolates:** The study found that bacterial isolate's adaptability to different pH conditions varies. Isolates like PSSG11 and PSSB8 showed the highest OD values at neutral pH 7, while PSSB2 and PSSB5 showed enhanced growth at alkaline pH 9. At acidic pH 5, some isolates maintained high OD values, while others showed limited growth. PSSG11 was the most pH-tolerant strain, suggesting that it better suited for soils with variable pH, enabling the development of bioinoculants (Table 4).

**Effect of Salt Stress on PSB Growth:** The study investigated how different salt levels impact the growth of phosphate-solubilizing bacteria (PSB). Results showed a decline in growth as salinity increased, although some isolates displayed notable tolerance. At 1% NaCl, all isolates had high OD values, while at 2.5%, there was a slight decrease. At 5% NaCl, most isolates experienced a significant reduction in growth, but B7, G11 and B8 demonstrated better tolerance. These strains could be useful in saline soil environments (Table 5, Figure 4).

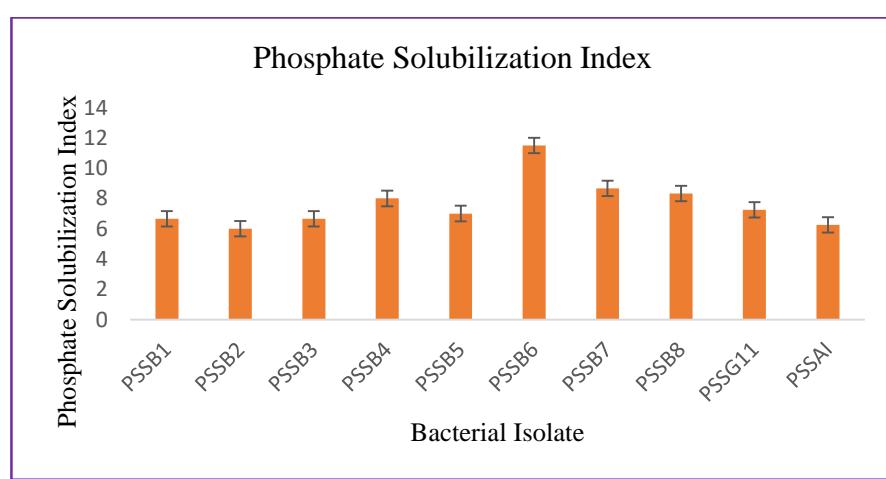
**Study on drought stress on growth of the isolates:** The effect of drought stress simulated with polyethylene glycol (PEG), on phosphate solubilization by various bacterial strains was evaluated at different concentrations (10% to 30%). All isolates (PSSB1 to PSSA1) showed growth and phosphate solubilization activity at 20% PEG, indicating tolerance to moderate drought stress.

However, at higher drought stress levels of 25% and 30% PEG, none of the isolates grew, suggesting a threshold beyond which their activity is blocked. This pattern emphasizes the isolates' potential for use in moderately drought-affected soils while also indicating limitations under extreme drought conditions.

**Plant Growth Promoting Traits of the Isolates:** Table 6 presents the plant growth-promoting (PGP) traits of selected microbial isolates, evaluating their multifunctional abilities relevant to sustainable agriculture. All six isolates (PSSB4, PSSB6, PSSB7, PSSB8, PSSG11 and PSSBA1) showed positive activity across a broad spectrum of beneficial traits including solubilization of phosphate, silicate and zinc, along with the production of ammonia, siderophores, indole acetic acid (IAA) and exopolysaccharides (EPS). Each isolate consistently demonstrated the ability to solubilize essential minerals such as phosphate, silicon and zinc, indicating their potential to enhance nutrient availability in soil.



**Figure 1: Phosphate solubilizing zone surrounding the screened bacterial isolates individually on Pikovskaya agar plates**



**Figure 2: Phosphate solubilizing zone surrounding the screened bacterial isolates individually on Pikovskaya agar plates**

**Table 3**  
**Phosphate Solubilization Index of Isolates**

Bacterial Isolate	Colony diameter (mm)	Halozone diameter (mm)	Phosphate Solubilization Index
PSSB1	3	17	6.66
PSSB2	4	20	6.0
PSSB3	3	17	6.66
PSSB4	3	21	8.0
PSSB5	3	18	7.0
PSSB6	2	21	11.5
PSSB7	3	23	8.66
PSSB8	3	25	8.33
PSSG11	4	22	7.25
PSSA1	4	22	6.25

**Table 4**  
**Effect of pH on PSB isolates O.D. at 530 nm**

Isolate	pH at 5	pH at 7	pH at 9
PSSB1	0.17	0.19	0.30
PSSB2	0.17	0.20	0.33
PSSB3	0.18	0.21	0.17
PSSB4	0.43	0.18	0.29
PSSB5	0.13	0.21	0.33
PSSB6	0.18	0.21	0.23
PSSB7	0.24	0.25	0.30
PSSB8	0.28	0.37	0.18
PSSG11	0.31	0.37	0.31
PSSA1	0.20	0.26	0.21

**Table 5**  
**Effect of salt (NaCl) on phosphate solubilization (OD at 530 nm)**

Isolates	1%	2.5%	5%	7.5 %
PSSB1	0.36	0.32	0.17	0.17
PSSB2	0.46	0.36	0.25	0.24
PSSB3	0.36	0.36	0.13	0.14
PSSB4	0.48	0.39	0.29	0.19
PSSB5	0.41	0.43	0.22	0.20
PSSB6	0.46	0.40	0.17	0.15
PSSB7	0.43	0.38	0.33	0.31
PSSB8	0.42	0.39	0.27	0.24
PSSG11	0.38	0.29	0.27	0.27
PSSA1	0.36	0.29	0.25	0.24

**Table 6**  
**PGP traits of the selected isolates**

Isolate	Phosphate solubilization	Silicate solubilization	Zinc solubilization	Ammonia production	Nitrogen fixation	Siderophore production	Indole Acetic Acid production	EPS production
								g/L
PSSB4	+	+	+	+	+	+	+	1.2
PSSB6	+	+	+	+	+	+	+	1.6
PSSB7	+	+	+	+	+	+	+	1.6
PSSB8	+	+	+	+	+	+	+	1.8
PSSG11	+	+	+	+	+	+	+	0.5
PSSBA1	+	+	+	+	+	+	+	0.6

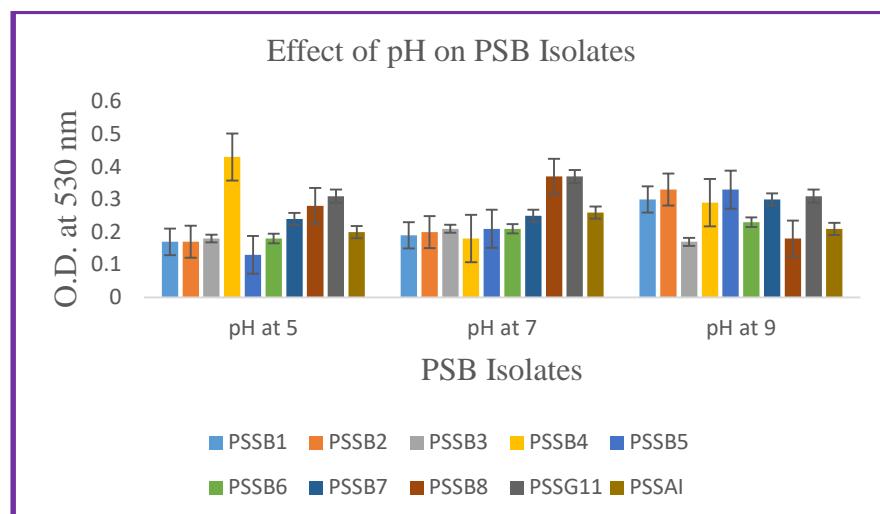


Figure 3: Effect of pH on PSB isolates O.D. at 530 nm

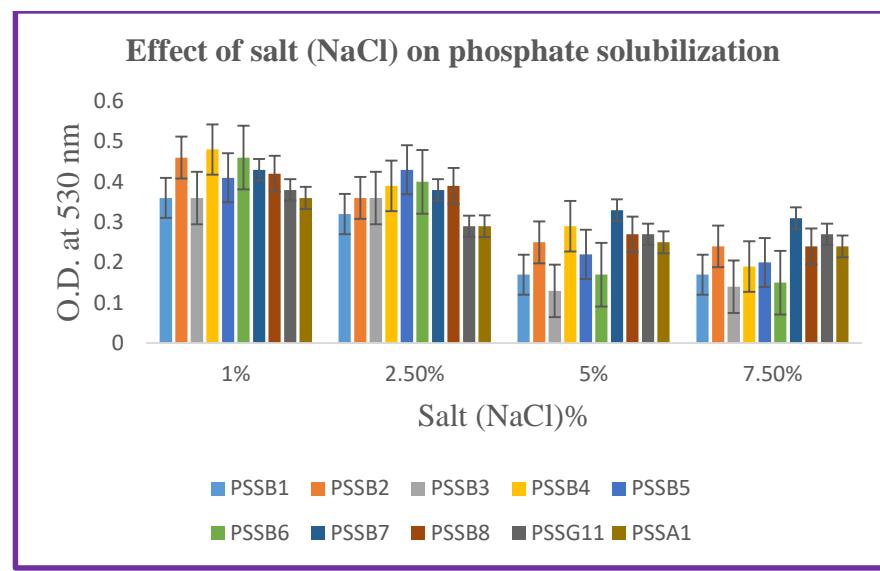


Figure 4: Effect of salt (NaCl) on phosphate solubilization (OD at 530 nm)

They also tested positive for ammonia production and nitrogen fixation, suggesting that they can contribute to nitrogen enrichment in the rhizosphere. Siderophore production by all isolates indicates a strong capacity to chelate iron which may increase iron availability for plants and may help to suppress soil-borne pathogens through competitive exclusion. Additionally, the production of indole acetic acid (IAA), a key phytohormone involved in root development, was confirmed in all isolates, emphasizing their direct role in promoting plant growth.

Additionally, exopolysaccharide (EPS) production, measured in g/L, varied among the isolates. PSSB8 showed the highest EPS production (1.8 g/L), followed by PSSB6 and PSSB7 (both 1.6 g/L), PSSB4 (1.2 g/L) and lower levels in PSSBA1 (0.6 g/L) and PSSG11 (0.5 g/L). High EPS production improves soil aggregation and microbial survival under stress conditions, making these isolates especially suitable for use in harsh or degraded soils. Overall, the data confirms that these isolates possess multiple PGP traits, making them strong candidates for development into

effective bioinoculants for improving soil fertility and promoting plant health in sustainable agricultural systems (Table 6).

## Discussion

The study identifies phosphate-solubilizing bacteria in soil samples from Kolhapur and Sangli districts of Maharashtra, revealing moderate fertility, alkaline pH and sufficient nutrients for crop growth. Karpagam and Nagalakshmi<sup>14</sup> analyzed ten microbial isolates of PSBs from agricultural soil samples, showing their round, translucent colonies, efficient fermentation of glucose, fructose, sucrose and mannitol and high catalase activity, making them promising candidates for biofertilizer development in saline and drought-affected conditions. Similarly, Patel et al<sup>26</sup> collected samples from rhizospheric soil and studied the isolation and characterization of PSB. Karpagam and Nagalakshmi<sup>14</sup> isolated 37 bacteria showing halo zone phosphate solubilization from agricultural soils. López-Gutiérrez et al<sup>18</sup> analyzed soil-isolated PSBs and found high phosphate solubilization indices in several isolates,

suggesting potential for biofertilizer formulations to improve phosphorus availability in agriculture. Studies by Kumar et al<sup>17</sup>, López-Gutiérrez et al<sup>18</sup> and Mohamed et al<sup>21</sup> also focused on phosphate solubilization activity of these isolates. Additionally, Patel et al<sup>26</sup> studied the impact of salt and drought stress on microbial growth, revealing *Enterobacter*, *Bacillus*, *Pseudomonas* and *Azotobacter* as effective phosphate solubilizers, though further field trials are necessary.

The present study isolated phosphate-solubilizing bacteria (PSB) from soil samples in the Kolhapur and Sangli districts of Maharashtra, analyzing them under salt and drought stress. The soil showed moderate fertility but had low levels of iron, manganese, zinc and copper, which could limit plant growth. Similar to our study, Karpagam and Nagalakshmi<sup>14</sup> isolated PSBs from agricultural soils and examined their physicochemical characteristics. This study evaluated ten microbial isolates (PSSB1 to PSSB8, PSSG11 and PSSA1) for colony features and assessed their morphological, physiological and biochemical traits. Most isolates were Gram-negative rods with strong colonization potential. They efficiently fermented glucose, fructose, sucrose and mannitol, though lactose fermentation varied.

Catalase activity was high in all isolates, suggesting their potential for biofertilizer development in saline and drought-prone agricultural environments. Similarly, Patel et al<sup>26</sup> collected rhizospheric soil samples for PSB isolation and characterization. Karpagam and Nagalakshmi<sup>14</sup> isolated 37 bacteria demonstrating halo zone phosphate solubilization from agricultural soils. López-Gutiérrez et al<sup>18</sup> studied the morphological and biochemical features of PSBs isolated from soil. PSSB6, PSSB7, PSSB4, PSSB8 and PSSG11 showed high phosphate solubilization indices, indicating their potential for biofertilizer formulations to improve phosphorus availability.

Research by Kumar et al<sup>17</sup>, López-Gutiérrez et al<sup>18</sup> and Mohamed et al<sup>21</sup>, revealed that phosphate solubilization activity varies with pH levels, with PSSG11 and PSSB8 showing the highest optical density at neutral pH 7, while PSSB2 and PSSB5 thrive at alkaline pH 9. Similar results were reported by Kumar et al<sup>17</sup> and Mohamed et al<sup>21</sup>. The study found that salt levels influence PSB growth, with some strains tolerant at 1% NaCl, while others show significant decline at 5% NaCl.

Jiang et al<sup>11</sup> analyzed stress tolerance by varying NaCl and Na<sub>2</sub>CO<sub>3</sub> levels and pH. Johri et al<sup>12</sup> presented evidence that LCR3 increases phosphate solubilization at low salt levels. Under stress, organisms grow more slowly, reducing phosphorus solubilization. Despite this, *Enterobacter* species from agricultural farms solubilized 1.6-2.5 times more phosphate under stress than previously reported. Malboobi et al<sup>19</sup> and Nautiyal et al<sup>24</sup> showed similar trends. This study examined drought effects on bacterial growth and phosphate activity, finding all isolates tolerating moderate

drought stress at 20% PEG, but none grew at 25% or 30%. Patel et al<sup>26</sup> explored salt and drought effects on microbial growth, finding *Enterobacter*, *Bacillus*, *Pseudomonas* and *Azotobacter* species as effective phosphate solubilizers, although field trials are still needed.

The study found that microbial isolates, particularly PSSB6 and PSSB8, showed significant phosphate solubilization efficiency, surpassing the positive control, enhancing nutrient availability in damaged soils. According to Chen et al<sup>6</sup>, effective phosphate-solubilizing bacteria increased phosphorus bioavailability through organic acid secretion and most isolates maintained solubilizing activity under NaCl and PEG stress, indicating functional tolerance. Studies by Upadhyay et al<sup>36</sup> suggest that adaptive acidification mechanisms enable halotolerant PSB to retain their solubilizing capacity under salt stress. Similarly, drought-tolerant PGPR can withstand osmotic stress while continuing to promote plant nutrient uptake, as noted by Sandhya et al<sup>30</sup>.

The ANOVA analysis revealed significant variation in solubilization among isolates, suggesting PSSB6 and PSSB8 as promising bioinoculants for agroecosystems, particularly in salinity and drought-prone environments. Six tested isolates (PSSB4, PSSB6, PSSB7, PSSB8, PSSG11 and PSSA1) exhibited multiple plant growth-promoting traits, highlighting their potential for sustainable agriculture. These traits include phosphate, silicate and zinc solubilization, ammonia production, nitrogen fixation, siderophore production and EPS production. Similarly, Yadav et al<sup>39</sup> focused on plant growth-promoting rhizobacteria, aiming to screen exopolysaccharide (EPS)-producing bacteria from PGPR isolates and assess their effects on okra plant growth.

Seven bacterial strains were tested for drought tolerance, PGPR traits and EPS production, with *Pseudomonas aeruginosa* and *Bacillus coagulans* identified as the most effective. Okra seeds were treated with different combinations of EPS-producing bacteria, leading to maximum seed germination, plant height, leaf area, root length and leaf weight observed in treatment T7.

The study by Ait et al<sup>2</sup> reveals that saline soils naturally harbor halotolerant microbes with PGP traits and salt-sensitive plants and non-saline soils can also serve as good reservoirs for halotolerant PGPR. The presence of culturable halotolerant PGPR in both saline and non-saline soils underscores the importance of soil heterogeneity and microstructure in creating diverse soil micro-niches. The four halotolerant bacterial strains with the most PGP traits belong to genera that include many commercially available biofertilizers.

The study suggests further investigation into the ability of halotolerant PGPRs to withstand high osmotic pressures when selecting biofertilizer candidates. Banerjee et al<sup>3</sup>, Patel et al<sup>26</sup> etc. studied PGP, screening phosphate-solubilizing

bacteria and testing their ability to solubilize insoluble phosphates such as ferric phosphate and aluminum phosphate. Strains with high solubility demonstrated plant growth promotion and biocontrol activities including indole acetic acid production.

## Conclusion

The study on phosphate-solubilizing bacteria (PSB) from semiarid soils suggests they can improve phosphorus availability in saline and degraded soils. However, further research is needed to fully explore their application in sustainable agriculture, including large-scale field trials, microbial consortia development, molecular studies and commercialization.

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