

Solubilization of Phosphorus by Phosphate Solubilizing Bacteria Isolated from Rhizosphere Soil of *Mangifera indica*

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Abstract:

Background: Soil plays a potent role in supporting the growth of plants by providing macro and micronutrients, and water essential for development. It consists of microorganisms, organic materials, minerals, water and air. Soil deficient in phosphorus is one of the serious obstacles to attaining the practices of sustainable agriculture. Phosphorus is utilized for the growth and cellular processes but is often limited in soil through its continuous fixation. The phosphate-solubilizing bacteria (PSB) found in rhizosphere soil can transform insoluble phosphate into soluble form and make it available for plant uptake. The objective of present study was Isolation, screening and characterization of phosphate solubilizing bacteria from the rhizosphere soils of Mango plants in order to enhance phosphorus bioavailability was the primary focus of this study.

Materials and Methods: Rhizosphere soil samples from *Mangifera indica* orchards were tested for their physicochemical properties, such as pH measurement, electrical conductivity, organic carbon content, nitrogen content, and potassium content. Bacteria were isolated from soil, and morphological and biochemical tests were carried out for Identification purposes. The isolated bacterial culture was quantitatively and qualitatively tested for its phosphate solubilization capability.

Results: Total nineteen bacteria were isolated and morphologically and biochemically characterized. These bacterial strains were screened using a clear zone method. Among these two strains were showed significant potentialities for phosphate solubilization, as evidenced by phosphate solubilizing index of ≥ 3 . These two strains proved their remarkable efficiency in insoluble tricalcium phosphate solubilization with soluble phosphorus levels ranging 49.60 $\mu\text{g/ml}$ and 285.48 $\mu\text{g/ml}$. The morphological and biochemical tests confirmed that these bacteria belong to *Bacillus* sp. and *Proteus* sp.

Conclusion: The present study found that PSB isolates have the potential to enhance soil phosphorus availability and hence, could be useful for sustainable agricultural practices as they have the ability to improve the fertility of soil, including promotion of plant growth.

Key Word: Phosphate solubilizing index; *Mangifera indica*; Phosphate solubilizing bacteria; Phosphorus

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I. Introduction

Soil is the most dynamic, naturally made, complex living ecosystem, which aids as an essential reserve for vital nutrients, organic matter, air and water. It plays a critical role in plant development and growth (Kumari *et al.*, 2025). The soil is considered home to diverse microorganisms that transform these vital nutrients, making them accessible to plants. Soil naturally has organic matter, macronutrients such as nitrogen (N), potassium (K), phosphorus (P), and micronutrients essential for the growth and metabolism of plants and soil microbiota. Despite being present in large amounts, there are deficiencies of these nutrients in the soil medium, especially for phosphorus. Such limitation is largely due to the process of its fixation, where phosphate ions combine with metal ions like calcium, iron, and aluminum to precipitate into insoluble forms (Sati and Pant, 2018). These fixed phosphorus forms are not available to plants, leading to inefficient uptake of nutrients and growth retardation, particularly in highly weathered tropical and subtropical soils. Phosphorus deficiency is thus a prime limiting factor in realizing maximal crop yields and sustainable agriculture productivity (Johan *et al.*, 2021). In response to phosphorus deficiency, the extensive use of artificial phosphate fertilizers has become widespread. Although the fertilizers can raise phosphorus levels in the soil temporarily, they are not without their drawbacks. Chemical fertilizer production and use are usually costly and lead to environmental degradation. With time, overuse has the potential to cause nutrient imbalances, soil organic matter depletion, acidification, and alteration

of indigenous microbial communities. The large amount of applied phosphorus is not utilized by plants and reacts with metal ions present in the soil, making it unavailable for plants. This results in wasteful fertilizer application and higher input costs for farmers. Against this backdrop, there is increasing interest in the possibility of finding sustainable and environmentally friendly ways to increase phosphorus availability in soils. One such potential answer is through the application of PSB. These pragmatic bacteria are capable of transforming insoluble phosphorus forms into plant-available soluble forms using different biochemical processes. These involve organic acid secretion, enzyme synthesis, and proton extrusion, which contribute to a decrease in pH and the mobilization of bound phosphate compounds. By increasing phosphorus availability biologically, PSBs not only enhance plant nutrient uptake but also help to promote overall soil ecosystem, health and fertility. Its use as a biofertilizer is an inexpensive, environment friendly way to boost crop yields without much reliance on chemical fertilizers. The study was conducted with the aim of isolating, characterizing, and identifying phosphate-solubilizing bacteria from rhizosphere of *Mangifera indica*. The rhizosphere, a small soil area immediately surrounding roots, where microbial activity is significantly enhanced by root secretions, is a rich source of microbial biodiversity, of which PSBs are a part. Isolation of effective PSB strains from this habitat yields promising candidates for inclusion in formulations of biofertilizers.

II. Materials and Methods

Collection of rhizosphere soil

Soil samples were collected from orchards of *Mangifera indica* from two different sites of Purbi Champaran, Bihar, India. Samples were collected at a depth of 15-20 cm from the rhizosphere. The soil samples were collected in sterilized polyethene bags. Samples were transported to the Botany Department, Mahatma Gandhi Central University, Motihari, East Champaran, Bihar for further experiments. For isolation of rhizobacteria, a portion of this sample was plated. The remaining sample was stored in a refrigerator at 4°C.

Study of soil physicochemical properties

To check the physicochemical properties of the collected soil sample, a portion of stored soil samples was taken, air-dried at room temperature, and sieved through 2 mm sieve. The physicochemical analysis was performed following standardized laboratory protocols such as pH measurement of soil with a calibrated digital pH meter- "Eutech" Instrument pH 700 and soil electrical conductivity (EC) examination by using a Systronics Conductivity meter (Model 306). The total soil organic carbon content was quantified using the wet oxidation method with $K_2Cr_2O_7$ by Walkley and Black (1934). Soil total nitrogen content was examined by the Aerobic incubation method, soil available phosphorus was determined spectrophotometrically, with a Systronics® Double Beam Spectrophotometer model 2203 by extraction with sodium bicarbonate method (Olsen *et al.*, 1954), and total potassium content of soil was determined by flame photometry, using the ELICO® CL 378 FLAMEPHOTOMETER. Detailed representation of soil physicochemical properties played a critical role in assessing the nutrient status, chemical balance and overall well-being of the soil environment.

Bacteria Isolation from rhizosphere soil

Bacteria isolation was performed by applying the traditional method through transferring 1 gram of soil from stored rhizosphere soil samples into reagent bottles of 250 ml, consisting of autoclaved distilled water. The reagent bottle was vortexed thoroughly and diluted the prepared culture up to 10^{-5} using standard serial dilution method. 1 ml of the serially diluted samples were placed in to the autoclaved petri dishes. As agar is the most prominent medium for bacterial growth and expansion which is then used for proliferation of the desired bacteria. Therefore, on those petri-dishes nutrient agar medium was added and then these plates were swirled and allowed to solidify. Enzymatic and biochemical reactions of bacterial cell worked efficiently on optimum temperature and pH. Hence, to maintain the optimum temperature of $35\pm 2^\circ\text{C}$ for 72 hours, those inoculated petri-dishes were placed in an incubator. After incubation period, bacterial colony appeared on nutrient agar plates. Isolated bacterial colonies from the nutrient agar plate and purified them for further experiments. The pure bacterial cultures were stored in the refrigerator at 4°C for further use.

Isolates morphological characterization

The bacterial isolates were examined for their colony morphology on nutrient agar plates. According to their phenotypic appearance, including size, shape, color or pigmentation, elevation, type of edge, opacity, and texture of surface, were carefully recorded to differentiate between isolates. Besides colony morphology, microscopic analysis was performed to determine the cellular morphology of the bacterial strains. Gram staining was used as a differential staining technique to classify bacteria as either Gram-negative or Gram-positive, depending on the nature of their cell walls. Following staining, microscopic examination was conducted under light microscope to assess the structure of bacterial strains, including their shape, size, and arrangements.

Qualitative screening of isolates for phosphate solubilization efficiency

The qualitative screening of bacterial isolates for phosphate solubilizing efficiency was examined by the plate assay method on Pikovskaya's agar (PVK) medium with 0.4% bromothymol blue. The freshly grown bacterial cultures were spot onto Pikovskaya's agar plates. Plates were incubated at a temperature of 30°C for 10 days. After 3rd day of incubation, the inoculated agar plates were observed for clear zones or a change in the color of the media from green to orange surrounding the colony, indicating the solubilization of phosphate by PSBs. The inoculated colonies of bacteria that created clear, distinct zones or changes in color of media were identified as possible phosphate solubilizer and subculture on fresh PVK agar plates (Pikovskaya 1948; Mehta and Nautiyal 2001). Subculture plates were incubated at a temperature of 30 ± 2°C for 10 days. A clear zone growth or color change around the colony is an indication of inorganic phosphate solubilization. The subculturing PVK agar plates were monitored at intervals on 3rd day, 5th day, 7th day, and 10th day for measurements of colony diameter as well as growth of the clear zone. These measured values of colony diameter and clear zone growth were used for calculation phosphate solubilizing index (PSI). The PSI was calculated applying the mentioned formula of Pande *et al.* (2017) i.e., Diameter of Colony + Clearing zone measurements/Diameter of Colony. Strains with a range of PSI ≥3 were highly efficient and were selected for further experiments.

Available phosphorus estimation by efficient bacterial phosphate solubilizer

The promising PSB strains were thereafter grown in PVKs' broth medium to determine their capability of phosphate solubilization under lab conditions. The freshly grown bacterial culture, 1 ml aliquot was transferred to 100 ml of sterile PVK broth, supplemented with 0.5% tri-calcium phosphate (TCP) as the sole insoluble source of phosphorus. To monitor solubilization of phosphorus over time, 5 ml of bacterial aliquots were aseptically withdrawn from the culture broth to centrifuge tubes every 3 days- i.e., on the 3rd, 6th, 9th, 12th, 15th, 18th and 21st day after inoculation. Each withdrawn sample was centrifuged at 10,000 rpm for 15 minutes at 4°C using Neutron iFuge M24PR Refrigerated Centrifuge. After centrifugation, the clear supernatant was carefully decanted and collected for the estimation of soluble phosphorus. Concentration of solubilized phosphorus in the supernatants was determined spectrophotometrically using the ascorbic acid method (Olsen *et al.* 1954; Watanabe and Olsen 1965). For the colorimetric assay, 1ml sample of the supernatant was mixed with the reaction solution (freshly prepared) consisting of ammonium molybdate solution and ascorbic acid. The reaction mixture was incubated at room temperature for 30 minutes to facilitate full formation of molybdenum blue complex, optical density (OD) was recorded at 660 nm with Systronics® Double Beam Spectrophotometer 2203. Phosphorus solubility was quantified using a standard phosphorus solution of KH₂PO₄, with concentrations varying from 0 to 10 µg ml⁻¹ of phosphorus.

Biochemical characterization of efficient bacterial phosphate solubilizers

Isolates were distinguished by differences in colony appearance and formation of halo zone due to TCP solubilization on PVK agar petri dishes. Among nineteen isolates, only three bacterial isolates were screened as promising phosphate solubilizers for agricultural practices. The bacterial isolates were further evaluated for catalase, oxidase, casein hydrolysis and urease activities. Each isolate was reviewed in triplicate. To analyze the potential of PSBs to utilize starch as a carbon source, they were cultured on plates of starch agar and maintained at 30°C±2°C for 24 hours. Indole production was indicated by a color change from yellow to cherry red in the top layer of the inoculated Tryptone broth tubes after adding Kovac's reagent. MR-VP tests were also performed for the isolates. Isolates' citrate utilization was determined using Simmon's citrate agar slants, where sodium citrate acted as the exclusive carbon and energy source. 1 ml of 1% bromothymol blue (BTB) solution was added to Simmon's citrate agar medium. Simmon's citrate agar slants, were inoculated and kept in incubator at 35°C. A significant color shift from green to blue indicated positive test for citrate utilization after 7 days of incubation (Surange *et al.*, 1997). Carbohydrate fermentation tests were also carried out for the characterization of bacterial isolates.

Identification of PSB

Isolated PSB were identified as described in Bergey's Manual (Systematic Bacteriology, 2nd ed., vol. 5, eds., 2012) and Bergey's Manual of Determinative Bacteriology respectively (Holt *et al.*, 1994).

Statistical analysis

Software of Microsoft Excel 2016 was used to analyze and summarize the laboratory findings. The values represent the means ± standard error from repeated observational data. IBM SPSS Statistics 20 software was used for statistical analysis using one-way ANOVA test to evaluate the variations among qualitative and quantitative phosphate solubilization ability.

III. Result

Physicochemical analysis of rhizosphere soil

A total of two rhizosphere soil samples were collected from two different sites of Purbi Champaran. The physico-chemical analysis was performed for both soil samples. This process is important because the physicochemical properties of soil directly affect the presence of bacterial populations and agricultural productivity. Soil pH plays a very important role in phosphate solubilization. The soil bacterial community and the specific functions they perform are affected by the gradient of soil pH. Table 1 shows the pH values of rhizospheric soils collected from two different locations. Soil samples taken in our study are of a slightly alkaline nature, with pH values for Parsa were 7.40 and for Dekhahan were 7.79, which is the optimal pH level for the growth of phosphate-solubilizing bacteria. Electrical conductivity (EC) of soil is directly related to the salinity of the soil. As the EC of soil increases, the salinity of the soil also increases, resulting in a limitation of phosphate solubilization rates. In this study, the soil samples EC ranged between 0.57 ds m⁻¹ and 0.79 ds m⁻¹ which is considered suitable for most agricultural crops and the growth of microorganisms.

Table 1. Geographical locations and physicochemical analysis of soil samples collected from rhizosphere of *Mangifera indica*

Soil Sample	GPS location	pH	EC (ds m ⁻¹)	OC (%)	N (Kg ha ⁻¹)	P (Kg ha ⁻¹)	K (Kg ha ⁻¹)
Parsa	26° 47' 19.5" N 84° 41' 08.9" E	7.40	0.57	0.75	146	23	145
Dekhahan	26° 34' 17.1" N 84° 59' 29.9" E	7.79	0.79	0.31	224	35	158

Soil organic carbon (SOC) provides an essential resource of organic matter that bacteria utilize for their growth and metabolism. In this study, the two tested soil samples were found to have SOC levels ranging from 0.31% to 0.75%, which is considered good for agricultural practices. For soil fertility and active participation of bacteria or microbes, balanced concentrations of Nitrogen (N), Phosphorus (P) and Potassium (K) are necessary. The nutrient availability in soil at the optimum range enhances crop production and also increases bacterial performance for phosphate solubilization and other nutrient solubilization or fixation. The available Nitrogen, Phosphorus and Potassium in rhizosphere soil sample was shown in Table 1. Nitrogen levels in soil can significantly depend on various soil's geographical location and agricultural fields. In our study, the tested soil samples showed Nitrogen ranges from 146 to 224 Kg ha⁻¹. Potassium (K) is necessary for bacterial growth and phosphate solubilization. The tested soil samples in our study showed K levels from 145 to 158 Kg ha⁻¹. The Phosphorus availability in all studied soil samples was between 23 to 35 Kg ha⁻¹. At location site of Dekhahan, rhizosphere soil exhibited high concentration of available phosphorus, i.e., 35 Kg ha⁻¹, at an estimated pH of 7.79 and SOC level of 0.31%. While, at pH 7.40 and SOC of 0.75%, the amount of phosphorus in the soil was found to be 23 Kg ha⁻¹ only. The results of this study provided important information about the soil condition, which directly impacts the types of bacteria, their metabolic functions, and the most efficient isolation technique to apply.

Bacterial isolation and estimation of phosphate solubilization

Nineteen bacterial cultures were isolated from two rhizosphere soil samples on Nutrient Agar Medium Plate using the viable count method. The isolated bacterial cultures were further characterized based on their morphological appearance, such as size of colony, shape, color, margin, elevation, surface and opacity of bacterial colonies.

Table 2. Morphology of isolated bacterial colony from rhizosphere of *Mangifera indica*

Strain Name	Size (mm)	Shape	Color	Margin	Elevation	Surface	Opacity
RSM1	2	Circular	White	Entire	Convex	Smooth	Opaque
RSM2	1	Circular	Cream	Entire	Flat	Smooth	Opaque
RSM3	1	Circular	Cream	Entire	Raised	Smooth	Opaque
RSM4	2	Irregular	White	Undulate	Flat	Smooth	Opaque
RSM6	1	Irregular	White	Entire	Raised	Smooth	Opaque
RSM8	2	Circular	Light yellow	Entire	Convex	Smooth	Opaque
RSM9	1	Circular	Yellow	Entire	Raised	Smooth	Opaque
RSM10	1	Circular	White	Entire	Raised	Smooth	Opaque
RSM11	1	Circular	White	Entire	Raised	Smooth	Opaque

RSM12	2	Irregular	Cream	Undulate	Flat	Smooth	Opaque
RSM14	2	Irregular	Cream	Entire	Raised	Smooth	Opaque
RSM16	2	Irregular	Cream	Undulate	Raised	Smooth	Opaque
RSM21	2	Irregular	Cream	Undulate	Flat	Smooth	Opaque
RSM23	2	Circular	Yellow	Entire	Raised	Smooth	Opaque
RSM24	2	Irregular	White	Curled	Raised	Smooth	Opaque
RSM25	3	Irregular	Cream	Undulate	Wrinkled	Rough	Opaque
RSM26	1	Circular	Bright orange	Entire	Raised	Smooth	Opaque
RSM27	1	Circular	Yellow	Entire	Raised	Smooth	Opaque
RSM28	1	Circular	White	Entire	Flat	Smooth	Opaque

The screening of isolated bacterial culture for phosphate solubilization was carried out on sterilized petri-dishes containing Pikovskaya's agar medium by the streak plate method. Out of nineteen bacterial cultures, two bacteria RSM3 and RSM21 were found to have positive results for phosphate solubilization on Pikovskaya's agar medium with a Phosphate solubilizing index (PSI) ≥ 3 (Table 3). Both these strains proved their capability to solubilize insoluble calcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) by producing a clear zone with indices ranged between 3-4 on PVK agar plates. Based on screening data, bacterial culture RSM3 and RSM21 were tested for quantitative estimation of phosphate solubilization. These bacterial isolates were found to be efficient as they solubilize insoluble $\text{Ca}_3(\text{PO}_4)_2$ at a rate of 181.22 $\mu\text{g/ml}$ by RSM3, and 259.24 $\mu\text{g/ml}$ by RSM21 after 15 days of incubation (Figure 1). These strains proved their capability to solubilize $\text{Ca}_3(\text{PO}_4)_2$ on PVK medium by increasing the available content of phosphorus that ranged between 49.60 $\mu\text{g/ml}$ and 259.24 $\mu\text{g/ml}$. The observed value was noted and analyzed with IBM SPSS Statistics 20 software. One-way ANOVA was conducted to compare the PSI between samples. The data indicated a statistically significant difference in PSI, with a p -value of less than 0.05 (p -value < 0.05).

Table 3. Visual screening of phosphate solubilization by bacteria isolated from rhizosphere soil

Strain Name	Colony diameter (cm) (At 10 th day)	Clear zone diameter (cm) (At 10 th day)	*Halo diameter (cm) (At 10 th day)	Phosphate Solubilizing Index (PSI)
RSM1	0.38 $\pm$ 0.04	NA	NA	–
RSM2	0.34 $\pm$ 0.03	0.29 $\pm$ 0.01	0.63 $\pm$ 0.04	1.86 $\pm$ 0.06
RSM3	1.23 $\pm$ 0.07	2.47 $\pm$ 0.09	3.70 $\pm$ 0.15	3.00 $\pm$ 0.06
RSM4	*NG	NA	NA	–
RSM6	0.27 $\pm$ 0.02	NA	NA	–
RSM8	*NG	NA	NA	–
RSM9	0.63 $\pm$ 0.04	NA	NA	–
RSM10	0.81 $\pm$ 0.02	0.67 $\pm$ 0.02	1.48 $\pm$ 0.03	1.82 $\pm$ 0.04
RSM11	0.33 $\pm$ 0.04	NA	NA	–
RSM12	*NG	NA	NA	–
RSM14	*NG	NA	NA	–
RSM16	*NG	NA	NA	–
RSM21	0.57 $\pm$ 0.03	1.67 $\pm$ 0.09	2.23 $\pm$ 0.07	3.97 $\pm$ 0.33
RSM23	*NG	NA	NA	–
RSM24	*NG	NA	NA	–
RSM25	0.27 $\pm$ 0.02	NA	NA	–
RSM26	0.50 $\pm$ 0.03	NA	NA	–
RSM27	*NG	NA	NA	–
RSM28	0.23 $\pm$ 0.02	NA	NA	–

*NG = No Growth; NA = Not Applicable; – = No Phosphate Solubilization activity; *Halo diameters = Colony diameter + Clear zone diameter

Values shows the average of three replicates mean $\pm$ SE. These data are statistically significant (p -value ≤ 0.05).

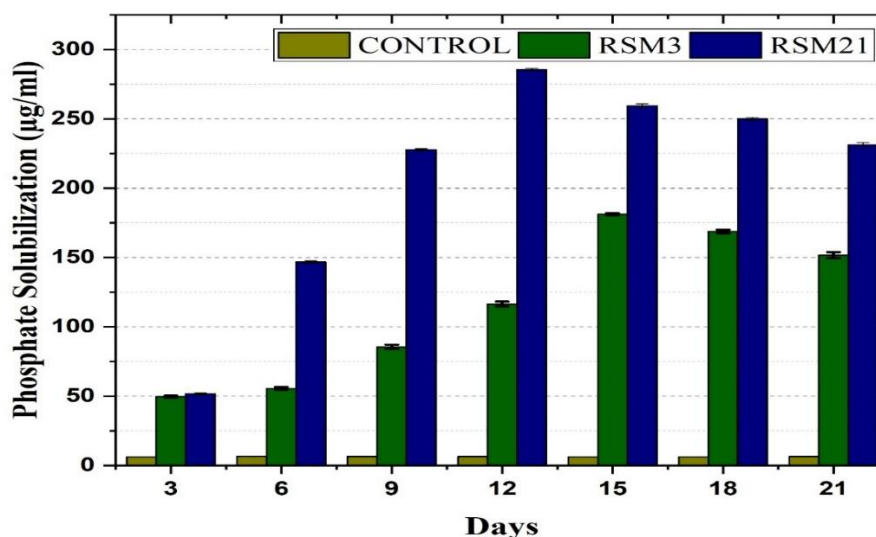


Figure 1. Quantitative assessments of phosphate solubilization on PVK broth
Identification of strains involved in phosphate solubilization

The bacterial cultures RSM3 and RSM21 which show promising phosphate solubilization capacity, were then tested on biochemical parameters for identification. The biochemical tests including Gram staining, catalase test, urease test, iMViC tests, starch hydrolysis test, cell-wall degrading enzyme production activity and carbohydrate utilization tests shown in Table 4. On the basis of morphological, microscopic observation, and biochemical characterization, the strain RSM3 was found to be Gram positive, rod, catalase, starch hydrolysis, voges-proskauer, citrate-utilization, oxidase, and carbohydrate fermentation tests positive, while negative for urea hydrolysis, indole production, etc. The bacterial isolate RSM21 was found to be Gram-negative, rod-shaped, catalase, urea hydrolysis, MR, citrate utilization, xylose and glucose fermentation tests positive, while negative for oxidase, starch hydrolysis, casein hydrolysis, indole, Voges-Proskauer, sucrose, maltose, and mannitol fermentation test. When these mentioned characteristics compared to Bergey's manual of systematic Bacteriology, the bacterial strain RSM3 was identified as *Bacillus* sp. and Bacterial isolate RSM21 was identified as genera *Proteus* sp.

Table 4. Biochemical characterization of bacterial isolates screened for quantitative analysis of phosphate solubilization

Bacterial isolates	Gram staining	Shape of colony	Catalase activity test	Urea hydrolysis	Starch hydrolysis test	Indole production test	Methyl-Red test	Voges-Proskauer test	Citrate utilization test	Casein hydrolysis test	Oxidase test	Carbohydrate fermentation test				
												Glucose	Sucrose	Maltose	Xylose	Mannitol
RSM3	+	Rod	+	-	+	-	-	+	+	-	+	+	+	+	+	+
RSM21	-	Rod	+	+	-	-	+	-	+	-	-	+	-	-	+	-

IV. Discussion

PSB acts as the most vital player in unlocking insoluble phosphorus and making it utilizable for plants and other soil microbes. The growth of PSBs depend upon various conditions of the soil in which they reside. Conditions like soil pH, EC, TOC, and the concentration of nutrient such as nitrogen, phosphorus, potassium, etc. transform the efficacy of PSB. Among these, soil pH is one of the main factors. It tells a lot about the

structural characteristics of the soil bacterial population and is strongly associated with the diversity of the soil microflora. The bacterial population look very different when there is a comparison between acidic soil with neutral ones. This shift also affects phosphorus solubilization (Nicol *et al.*, 2008). At low pH, activity and diversity of microbes is also less. Neutral soil, or slightly alkaline, usually shows the opposite that is more microbial types, and more activity. Therefore, the management of soil pH properly not only enhance bacterial performance but also improves soil health, which in turn enhance sustainable crop yields. PSB-*Pantoea agglomerans* was inoculated in semi-arid soil by Saadoui *et al.* (2021) and they found drop in soil pH, followed by a growth in available P content of the soil. The inoculation of these bacteria also transformed structure and the composition of soil bacterial community, as well as increase in the comparative richness of firmicutes. It's been suggested by Läubli and Grattan, (2017) that a pH between 6.0 and 8.0 is ideal for both plant growth and bacterial performance. Within this range, nutrients stay available, while at pH values lower than 5.5 or higher than 7.5, P tends to get locked away by aluminum, calcium, iron, etc., making it difficult for plants to access. These locked insoluble P can be unlocked by PSBs again. In our study, the soils showed pH levels of 7.40 and 7.79, which fits comfortable in the zone where PSB activity is expected to be strong.

Soil EC is another factor which gives us a rough idea of salt concentration, which has a direct impact on bacteria, crop performance, and soil fertility. A good range for bacterial growth lies somewhere between 0.2 ds/m and 2.0 ds/m (Patel *et al.*, 2022). When salinity climbs, phosphate solubilization may slow down mainly because organic acid production drops and soil pH shifts in odd ways (Vignesh *et al.*, 2021). Extra salts can also lead to cations like sodium competing with calcium on soil particles, which reduces phosphate accessibility for plants. In our case, soil EC values were between 0.57 and 0.79, a healthy range that supported the growth and function of PSBs. The results ranged below 1.0 dS m⁻¹ indicating non-saline soil conditions and also suitable for the phosphate solubilization by PSBs. SOC percentage supports phosphate solubilization, soil nutrient cycling, and increased, phosphorus concentration (Wan *et al.*, 2021). The continuous application of biofertilizers such as PSBs resulted in higher content of SOC in cultivated soils, as compared to non-cultivated soil. Hence, organic carbon contributes to the overall fertility of the soil, enhancing the performance of bacteria and other microbes present in soil, which indirectly supports the phosphate solubilization and soil nutrient cycling (Wan *et al.*, 2021). Nitrogen acts as a crucial limiting factor for plants, following carbon, hydrogen, and oxygen, playing a key role in the overall growth of plant throughout their life cycle. Nitrogen is tied to photosynthesis, hormone regulation, and overall growth of plants, but it also shapes soil structure by helping with aggregation, aeration, and water retention. It supports microbial diversity too (Wani *et al.*, 2015). Potassium acts as an enzyme cofactor that keeps osmotic balance in bacterial cells, and helps microbes stay stable and functional (Stautz *et al.*, 2021). So, having enough potassium in the soil not only feeds bacteria but also improves nutrient cycling, including phosphate availability.

When we tested strains in plate assays, the PSI varied quite a bit. This is likely because each strain secretes different mixes of polysaccharides, enzymes, or acids into the medium (Amri *et al.*, 2023). Selvi *et al.* (2017) also explained that the variation might be tied to how fast different acids diffuse across the plate. In our study, phosphate solubilization ranged between 49.60 µg/ml and 285.48 µg/ml after 12 days. Based on biochemical work, the isolates were identified as *Bacillus* sp., and *Proteus* sp. Among these two strains, *Proteus* sp. was the strongest, hitting 285.48 µg/ml. Interestingly, our values were even higher than those recorded for bacteria from Chinese fir tissues that was 44.29-195.61 µg/ml (Chen *et al.*, 2021). Other studies have shown wide variability: PSBs from rice rhizospheres reached 2777.87 µg/ml (Chaiarn and Lumyong, 2009), while maize PSBs solubilized up to 487.67 µg/ml (Qiao *et al.*, 2017). *Acinetobacter* sp. WR922 managed 888 µg/ml (Ogut *et al.*, 2010), and *Burkholderia cepacia* was able to solubilize around 305 µg/ml thanks to acids like citric and gluconic (Pande *et al.*, 2017). In accordance with Wang *et al.* (2020), the strain of *Bacillus subtilis* BPM12 was determined to solubilize Ca₃(PO₄)₂ approximately 330.7 µg/ml. Different isolates, different mode of actions and different timeframes too, from just a few days to two weeks or more (Sridevi and Mallaiah, 2009; Paul and Sinha, 2017). The PSBs adopted various mechanisms for phosphate solubilization. Janati *et al.* (2022) reported that organic acids, carboxylates, enzymes, siderophores, antibiotics, and hydrogen cyanide released by PSBs play a significant role in phosphate solubilization. *Bacillus* species are well known not only for solubilizing phosphorus but also for breaking down petroleum residues, dyes, and aromatic hydrocarbons, and producing hormones like auxins that stimulate plant growth (Harirchi *et al.*, 2022). RSM21 (*Proteus* species), in our study is highly efficient bacteria with phosphate solubilizing ability. These traits could explain its dominance in alkaline soil. Rodriguez *et al.* (2006) explained that phosphate solubilization is tied to enzyme-encoding genes, while Zhao *et al.* (2025) pointed out specific genes such as *glpQ*, *phoA*, *phoD*, *phnA*, *ppx-gppA*, *phoR* regulates how microbes break down phosphates, phosphonates, and phosphate esters, especially during phosphorus starvation. Prieto-Correal *et al.* (2015) suggested that sometimes soluble phosphorus levels drop not because it isn't released, but because microbes are absorbing it into their own biomass. The contributions performed by PSB are very much aligned with plant growth promoting rhizobacteria due to several biochemical pathways like solubilization of K, fixation of N and specifically for the production of siderophores besides phosphorus.

V. Conclusion

Phosphorus is the second most necessary macronutrient found in soil, which play a significant role in plant life and microbial metabolic functions. In spite of this, it often gets locked in soil with complex cations, which results its inaccessibility for plant uptake. To tackle this subject, the application of PSBs as biofertilizer is advantageous. PSBs serve positive function in soil fertility and sustainable promotion of plant growth. PSBs shift from laboratory trials to practical agricultural practices marks a significant progression in sustainable agriculture, with fewer environmental impacts and higher crop productivity. The primary work of the present study was the isolation, characterization, and assessment of PSB strains with the ability to efficiently phosphate solubilization. The two mentioned strains showed their potential to solubilize tricalcium phosphate on PVK medium by enhancing the available content of phosphorus. From the findings, the isolated strains from this study possess the capabilities of phosphate solubilization. In conclusion, we suggested the application of PSB with high potential as biofertilizers in the field of agriculture. In order to attain the ambiguous art of these naturally occurring bacteria, further research must be explored on their molecular mechanism, and microbial-mineral interfaces. Future cutting-edge study should be directed toward the strategic utilization of microbial biotechnology in agricultural practices with the objective of identification of other unexplored PSBs for their application in consortia to possess effective microbial inoculants in sustainable culture production systems under different conditions. This strategy seeks to create efficient microbial inoculants that improve sustainable agricultural production systems under varied environmental conditions. Investigating and utilizing a broader set of PSBs, makes us capable to improve nutrient cycling, limits our reliance on chemical fertilizers, and ensure stronger agricultural ecosystems. Such initiatives are crucial in achieving increasing worldwide food demand while ensuring ecological stability and healthy soils.

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