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Prevalence of phosphate solubilising bacteria in Muthupet Mangrove Reserve

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ABSTRACT

Mangrove ecosystems harbor diverse microbial communities that play crucial roles in nutrient cycling and ecosystem sustainability. Phosphorus is an essential macronutrient for plant growth; however, a large proportion of soil phosphorus exists in insoluble forms that are unavailable for plant uptake. Phosphate-solubilizing bacteria (PSB) enhance phosphorus availability by converting insoluble phosphate into plant-accessible forms. This study investigated the prevalence and phosphate-solubilizing potential of bacteria associated with the rhizospheric soil of selected mangrove species in the Muthupet Mangrove Reserve, Tamil Nadu, India. A total of 45 soil samples were collected from five mangrove sites at three different depths and analyzed for physicochemical characteristics, including soil pH and texture. Soil pH ranged from 7.0 to 8.3, indicating neutral to moderately alkaline conditions. Soil texture analysis revealed that Sample 2 was well-graded (Cu = 14.84; Cc = 1.64), whereas the remaining samples were poorly graded. Isolation on Pikovskaya's agar medium yielded 167 phosphate-solubilizing bacterial isolates, of which 112 pure cultures were obtained and 38 isolates were selected for further screening. Three isolates (O1, O2, and O3) exhibited superior phosphate-solubilization activity with phosphate solubilization index (PSI) values of 2.67 ± 0.21, 2.63 ± 0.25, and 2.47 ± 0.31, respectively. Morphological and biochemical characterization revealed that all three isolates were Gram-positive bacteria with distinct physiological traits. The findings demonstrate the abundance of efficient native PSB in mangrove sediments and highlight their potential application as eco-friendly bio-inoculants for sustainable agriculture and improved phosphorus management.

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INTRODUCTION

Phosphorus (P) is one of the major macronutrients essential for plant growth and development as it plays a crucial role in several physiological and biochemical processes including protein synthesis, cell division, root and shoot development, energy transfer and metabolic regulation (Sundareshwar *et al.*, 2003; Khan *et al.*, 2010; Khan *et al.*, 2009). Although phosphorus is abundantly present in soil, nearly 95-99% exists in insoluble forms, making it unavailable for direct uptake by plants (Sundareshwar *et al.*, 2003). Only a small fraction of total soil phosphorus is available in orthophosphate form for plant utilization (Alori *et al.*, 2017). Consequently, agricultural productivity largely depends on the application of phosphatic fertilizers to satisfy plant phosphorus requirements. However, inorganic phosphates supplied through fertilizers are rapidly immobilized in soil and become unavailable to plants (Mehta and Nautiyal, 2001).

Microorganisms present in soil play an indispensable role in nutrient cycling and maintenance of ecosystem sustainability. Among them, phosphate solubilizing bacteria (PSB) contribute significantly to phosphorus mineralization and solubilization through the production of organic acids, phosphatases and plant growth-promoting substances such as indole-3-acetic acid (IAA) (Ponmurugan and Gopi, 2006). These microorganisms convert insoluble phosphates into soluble forms such as $H_2PO_4^-$ and HPO_4^{2-}, thereby increasing phosphorus bioavailability for plants (Lal, 2002). PSB belonging to genera such as *Bacillus*, *Pseudomonas*, *Flavobacterium*, *Corynebacterium* and *Serratia* have been reported from terrestrial, aquatic and coastal ecosystems, including mangrove sediments (Widawati, 2011). In marine ecosystems, phosphate solubilizing microorganisms are involved in biogeochemical interactions linking phosphorus and carbon cycling (Thingstad and Rassoulzadegan, 1995; Das *et al.*, 2007).

Mangrove ecosystems are highly productive coastal wetlands distributed in tropical and subtropical regions and are characterized by saline, waterlogged and nutrient-limited conditions (Sofawi *et al.*, 2017). These ecosystems support diverse microbial communities that contribute to nutrient transformation and ecological balance. Mangrove sediments contain substantial quantities of organic and inorganic phosphorus, and microbial mineralization processes are essential for maintaining phosphorus availability in these environments (Das *et al.*, 2014). The Muthupet mangrove reserve located in the Cauvery delta region of Tamil Nadu represents one of the ecologically important mangrove ecosystems in India and harbors diverse mangrove species including *Avicennia marina*, *Excoecaria agallocha*, *Rhizophora mucronata* and *Acanthus ilicifolius* (Arunprasath and Gomathinayagam, 2014).

Several studies have reported the occurrence and phosphate-solubilizing potential of bacteria from agricultural and terrestrial soils (Mehta and Nautiyal, 2001; Chen *et al.*, 2006; Pande *et al.*, 2017). Although PSB diversity has also been investigated in certain coastal ecosystems (Widawati, 2011), limited information is available regarding the prevalence and characterization of phosphate solubilizing bacteria associated with the rhizospheric soil of mangrove species in the Muthupet mangrove reserve. In particular, comparative studies involving different mangrove plant species and varying soil depths remain scarce. Moreover, the identification of efficient native PSB strains from mangrove ecosystems for potential use as bio-inoculants in sustainable agriculture has not been extensively explored.

Therefore, the present study was undertaken to isolate and characterize phosphate solubilizing bacteria from the rhizospheric soil of selected mangrove species in the Muthupet mangrove reserve. The study further aimed to determine soil pH and texture, evaluate phosphate solubilization efficiency through phosphate solubilization index (PSI), and identify efficient bacterial isolates with potential application as bio-inoculants for plant growth and development.

MATERIALS AND METHODS

Study area and soil sample collection

Soil samples were collected from the Muthupet Mangrove Reserve located in Thiruvarur District, Tamil Nadu, India. Sampling was carried out beneath the rhizosphere region of selected mangrove trees using the pit-drilling method following the procedure described by Baskara *et al.* (2020). Soil was collected from three different depths, namely surface soil, 30 cm, and 60 cm, at approximately 2 m distance from the base of each mangrove tree. Five different mangrove species were selected for sampling, resulting in a total of 45 soil samples.

The geographical coordinates of each sampling location were recorded using a GPS device and are presented in Table 1. Plant specimens associated with each sampling site were collected and authenticated by the Botanical Survey of India (BSI), Tamil Nadu Agricultural University Campus, Coimbatore.

Table 1. Geographical coordinates of sampling sites in Muthupet mangrove reserve

Sampling site	GPS Coordinates	Direction
Sample 1	10.34901°N, 79.54008°E (±6 m)	SW
Sample 2	10.34496°N, 79.54053°E (±9 m)	N
Sample 3	10.35797°N, 79.53291°E (±4 m)	NE
Sample 4	10.36122°N, 79.51940°E (±5 m)	W
Sample 5	10.36596°N, 79.51588°E (±7 m)	SW

Collection and identification of mangrove plant species

Mangrove plant species associated with the collected rhizospheric soil were documented and taxonomically identified. The mangrove species selected for rhizospheric soil sampling are presented in Fig. 1. Plant specimens were authenticated by Dr. M.U. Sharief, Scientist 'F' and Head of Office, Botanical Survey of India (BSI), Tamil Nadu Agricultural University Campus, Coimbatore. The identified mangrove species and their taxonomic details are listed in Table 2.

Determination of soil pH

Soil pH was determined using the soil–water suspension method recommended by FAO (2021). Approximately 10 g of soil sample was mixed with 25 mL of double-distilled water and agitated for 60 min. The suspension was allowed to settle, and pH was measured using a calibrated digital pH meter. Soil pH was classified as acidic, neutral, or alkaline based on the obtained values.

Determination of soil texture

Soil texture analysis was performed using the sieve analysis method described by Nagendra *et al.* (2020). Soil samples were oven-dried for 72 h and mechanically pulverized. The dried samples were passed through a stack of standard sieves ranging from 4.75 mm to 75 µm using a mechanical sieve shaker. The percentage of soil retained on each sieve was recorded, and particle size distribution curves were constructed. Based on the coefficient of curvature (Cc) and coefficient of uniformity (Cu), soil samples were categorized as well-graded or poorly graded according to ASTM standards.

Isolation of phosphate solubilizing bacteria (PSB)

Serial dilution and spread plate techniques were employed for the isolation of phosphate-solubilizing bacteria. One gram of soil sample was suspended in 100 mL sterile distilled water to prepare the initial dilution. Serial dilutions up to 10^{-7} were prepared, and 100 µL aliquots from each dilution were spread onto Pikovskaya's agar (PKA) medium containing tricalcium phosphate as the sole phosphorus source.

The composition of PKA medium included dextrose (10 g/L), ammonium sulfate (0.5 g/L), yeast extract (0.5 g/L), magnesium sulfate (0.1 g/L), potassium chloride (0.2 g/L), manganese sulfate (0.0001 g/L), ferrous sulfate (0.0001 g/L), agar (15 g/L), and tricalcium phosphate (5 g/L). Plates were incubated at 33°C for 7 days. Colonies producing clear halo zones around bacterial growth were considered positive for phosphate solubilization. Selected colonies were purified by repeated streaking and maintained on nutrient agar slants at 4°C for further analysis.

Sample 1 Sample 2 Sample 3 Sample 4 Sample 5

Fig 1. Mangrove tree species selected for rhizospheric soil sampling in Muthupet mangrove reserve

Table 2. Mangrove species collected from Muthupet mangrove reserve (Mahathalana and Jeeva, 2018)

Sl	Common name	Botanical name	Family	Habit
1	Vellai kandai	*Avicennia alba* Blume	Avicenniaceae	Tree
2	Karunkanthal	*Avicennia marina* (Forssk.) Vierh.	Avicenniaceae	Tree
3	Thillai	*Excoecaria agallocha* L.	Euphorbiaceae	Tree
4	Kandal	*Rhizophora mucronata* Poir.	Rhizophoraceae	Tree
5	Koli mulli / Neer mulli	*Acanthus ilicifolius* L.	Acanthaceae	Shrub

Phosphate solubilization index (PSI)

The phosphate-solubilizing efficiency of bacterial isolates was evaluated on Pikovskaya's agar medium. Pure bacterial cultures were spot-inoculated at the center of the agar plates and incubated at 33 ± 2°C for 15 days. Formation of clear halo zones around colonies indicated phosphate solubilization activity.

The phosphate solubilization index (PSI) was calculated using the following equation:

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

All measurements were recorded in triplicate, and isolates exhibiting higher PSI values were selected for further characterization.

Morphological and biochemical characterization

Morphological characterization of selected isolates was performed using Gram staining and microscopic observation. Colony morphology, Gram reaction, and cellular shape were recorded. Biochemical characterization included oxidase, catalase, urease, citrate utilization, methyl red (MR), Voges–Proskauer (VP), and indole tests following standard microbiological procedures described by Isenberg (1998). IMViC reactions were used to differentiate bacterial isolates belonging to Enterobacteriaceae and related groups.

RESULTS AND DISCUSSION

Soil pH analysis

Soil pH is one of the important physicochemical parameters influencing nutrient availability, microbial activity and plant growth in mangrove ecosystems. In the present study, the pH of the collected mangrove soil samples ranged from neutral to moderately alkaline conditions (Fig. 2). Sample 1 and Sample 3 recorded a neutral pH of 7.0, whereas Sample 2 showed a slightly alkaline pH of 7.4. Comparatively higher alkaline conditions were observed in Sample 4 and Sample 5 with pH values of 8.1 and 8.3, respectively.

The neutral to alkaline nature of the soil may be attributed to tidal water intrusion, salinity and accumulation of mineral deposits in mangrove sediments. Mangrove soils are generally characterized by fluctuations in pH due to decomposition of organic matter, microbial transformations and saline water influence (Sofawi *et al.*, 2017). Soil pH greatly affects phosphorus solubility and microbial phosphate mineralization processes. Slightly alkaline conditions often reduce phosphorus availability because phosphates become fixed in insoluble forms. Under such conditions, phosphate solubilizing bacteria play a crucial role in converting insoluble phosphorus into plant-available forms through the secretion of organic acids and phosphatase enzymes (Alori *et al.*, 2017).

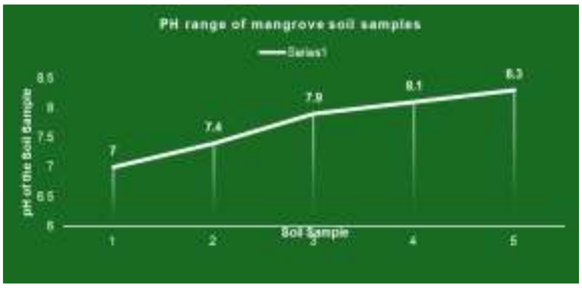


Fig. 2. Soil pH determination

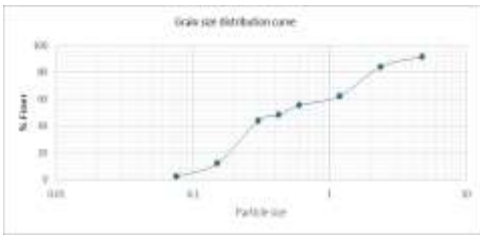
The variation in pH among the sampling sites also indicates differences in sediment composition and mangrove vegetation. Similar observations on pH variability in mangrove sediments have been reported earlier from coastal ecosystems where nutrient dynamics and microbial diversity are closely associated with sediment chemistry (Das *et al.*, 2014).

Soil texture analysis

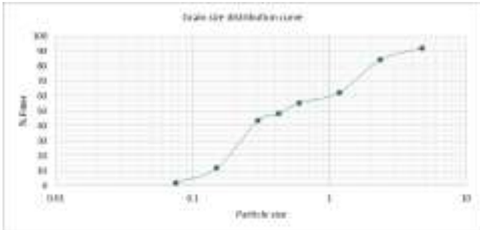
The particle size distribution curves obtained through sieve analysis revealed considerable variation in soil texture among the sampling sites (Fig. 3a–e). The coefficient of curvature (Cc, 1.64) and coefficient of uniformity (Cu, 14.84) values indicated that Sample 2 possessed well-graded soil characteristics, whereas Samples 1, 3, 4 & 5 were poor grade soils.

Poorly graded soils contain particles of nearly uniform size and generally exhibit reduced water retention and nutrient holding capacity. In contrast, well-graded soils contain a wider distribution of particle sizes that facilitate improved aeration, drainage and nutrient availability. The well-graded nature of Sample 2 may provide a more favorable microenvironment for microbial colonization and phosphate solubilization activity.

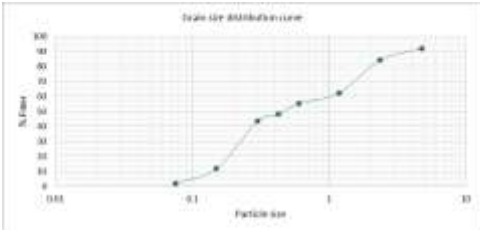
Mangrove sediments are highly heterogeneous due to tidal movement, organic matter deposition and sediment transport processes. The variation observed in soil texture among the samples may therefore influence the distribution and abundance of phosphate solubilizing bacteria. Similar findings have been reported by Nagendra *et al.* (2020), where soil particle distribution significantly affected nutrient mobility and microbial interactions within the soil matrix.



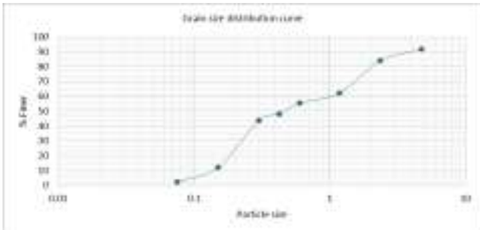
Sample 1



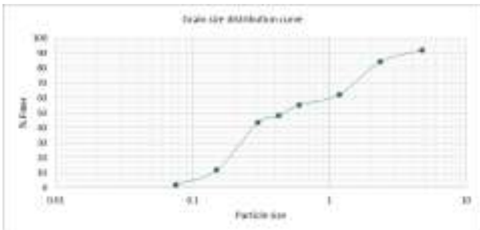
Sample 2



Sample 3



Sample 4



Sample 5

Fig. 3. Grain size distribution curve of the soil samples

Isolation of phosphate solubilizing bacteria

Phosphate solubilizing bacteria were successfully isolated from all the collected mangrove soil samples using Pikovskaya's agar medium. Colonies exhibiting clear halo zones around bacterial growth were considered positive for phosphate solubilization activity. A total of 167 phosphate solubilizing bacterial isolates were obtained from the 45 soil samples collected from different mangrove rhizospheres (Fig. 4).

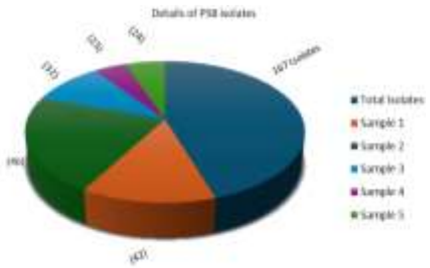


Fig. 4. Representing the number of PSB obtained from the 5 sampling places

The occurrence of a large number of PSB isolates indicates that mangrove sediments support a rich microbial diversity capable of participating in phosphorus cycling. Mangrove ecosystems are known to harbor diverse microbial populations because of their high organic matter content and nutrient-rich rhizospheric environment (Das *et al.*, 2007). The halo zone formation observed around the colonies was due to the microbial conversion of insoluble tricalcium phosphate into soluble phosphate forms through acidification and enzymatic activity.

The variation in the number of isolates obtained from different sampling sites may be associated with differences in soil pH, sediment texture and mangrove vegetation type. Rhizospheric zones of mangrove plants generally support greater microbial populations because of root exudates that stimulate microbial growth and metabolic activity. Similar observations have been reported in coastal and mangrove ecosystems where phosphate solubilizing bacterial populations contribute significantly to phosphorus mineralization and nutrient cycling (Widawati, 2011).

Phosphate solubilization index (PSI) of bacterial isolates

Among the 167 initial isolates, 112 pure cultures were successfully obtained after repeated subculturing. Based on halo zone formation and phosphate solubilization efficiency, 38 isolates were selected for further screening. Three isolates designated as O1, O2 and O3 exhibited comparatively higher phosphate solubilization indices and were therefore selected for detailed characterization (Table 3).

Isolate O1 showed the highest phosphate solubilization activity with a colony diameter of 2.25 ± 0.02 cm, solubilization zone diameter of 3.73 ± 0.15 cm and PSI value of 2.67 ± 0.21. Isolate O2 recorded a PSI value of 2.63 ± 0.25, while isolate O3 exhibited a PSI value of 2.47 ± 0.31. The clear halo zones surrounding these colonies indicate efficient solubilization of tricalcium phosphate present in the medium.

The higher PSI values obtained in the present study suggest strong phosphate mobilization ability of the selected isolates. Production of organic acids such as citrate, oxalate and gluconate by PSB lowers the pH of the surrounding medium and facilitates dissolution of insoluble phosphate compounds (Ponmurugan and Gopi, 2006). Similar phosphate solubilization efficiencies have been reported in earlier studies involving mangrove and agricultural soil bacteria (Chen *et al.*, 2006; Pande *et al.*, 2017).

The efficient isolates identified in the present study may therefore serve as potential bio-inoculants for sustainable agriculture and eco-friendly phosphorus management strategies.

Morphological and biochemical characterization of selected isolates

Morphological and biochemical characterization revealed that all the selected isolates were Gram-positive bacteria (Table 4). Isolates O1 and O3 were rod-shaped, endospore-forming bacteria, whereas O2 appeared as cocci arranged in grape-like clusters.

Biochemical assays showed that isolates O1 and O3 were positive for oxidase, catalase, Voges-Proskauer and citrate utilization tests, while negative for indole, urease and methyl red tests. In contrast, isolate O2 showed positive reactions for urease, catalase and citrate tests but negative responses for oxidase, indole, Voges-Proskauer and methyl red tests.

Table 3. Representing the PSI of obtained bacterial isolates

Bacterial isolates	Colony diameter (cm)	Zone of solubilization (cm)	PSI
O1	2.25 ± 0.02	3.73 ± 0.15	2.67 ± 0.21
O2	0.63 ± 0.25	1.03 ±0.25	2.63 ± 0.25
O3	1.63 ± 0.15	2.7 ± 0.2	2.47 ±0.31

Table 4. Biochemical characterization of the obtained isolates

Bacterial isolates	Cell shape	Gram's staining	Oxidase	Indole	Urease	Catalase	VP	MR	Citrate
O1	Rod-shaped, forming endospores	+	+	-	-	+	+	-	+
O2	Cocci, clusters like grapes	+	-	-	+	+	-	-	+
O3	Rod-shaped, forming endospores	+	+	-	-	+	+	-	+

The observed biochemical variability indicates physiological diversity among the selected phosphate solubilizing bacterial isolates. The positive catalase and oxidase activities suggest aerobic metabolic capability and adaptability to oxygen-rich rhizospheric conditions. Endospore formation in O1 and O3 may additionally provide survival advantages under fluctuating environmental conditions commonly observed in mangrove ecosystems.

The present findings are in agreement with earlier reports demonstrating the dominance of Gram-positive phosphate solubilizing bacteria in mangrove and coastal soils (Widawati, 2011; Chen *et al.*, 2006). The selected isolates possessing higher phosphate solubilization efficiency and favorable biochemical characteristics may therefore have promising applications as biofertilizers for improving phosphorus availability and promoting plant growth under saline and nutrient-limited environments.

CONCLUSION

The present study demonstrated the prevalence and diversity of phosphate solubilizing bacteria in the rhizospheric soil of mangrove species from the Muthupet mangrove reserve. Variations in soil pH and texture among the sampling sites indicated the heterogeneous nature of mangrove sediments, which may influence microbial distribution and phosphorus availability. A total of 167 phosphate solubilizing bacterial isolates were obtained using Pikovskaya's agar medium, confirming that mangrove ecosystems serve as an important reservoir of beneficial microorganisms involved in phosphorus cycling.

Among the obtained isolates, three bacterial strains designated as O1, O2 and O3 exhibited comparatively higher phosphate solubilization indices and distinct biochemical characteristics. The formation of clear halo zones around the colonies demonstrated their ability to convert insoluble phosphate into plant-available forms, suggesting their potential role in improving phosphorus bioavailability under nutrient-limited conditions. The predominance of Gram-positive isolates further indicates the adaptability of these microorganisms to the saline and fluctuating environmental conditions of mangrove ecosystems.

The findings of the present study highlight the ecological significance of phosphate solubilizing bacteria in mangrove sediments and their possible application as eco-friendly bio-inoculants for sustainable agriculture. However, further studies involving molecular identification through 16S rRNA sequencing, quantitative phosphate solubilization analysis, phosphatase activity, optimization of environmental parameters and field-level evaluation are necessary to establish the agricultural potential of these isolates. The utilization of native phosphate solubilizing bacteria from mangrove ecosystems may contribute to reducing dependency on chemical phosphatic fertilizers and promote environmentally sustainable crop production systems.

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