



## Bioinoculation of halophilic phosphobacteria for raising vigorous seedlings of *Rhizophora mucronata*

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### ABSTRACT

Phosphorous is an essential nutrient which is added to soil as soluble inorganic phosphate that, in a large portion, becomes insoluble and, therefore, unavailable to plants. Furthermore, this mineral is one of the most affected by the degrading processes of the soils. Numerous microorganism, especially those associated with roots, have the ability to increase plant growth and productivity. The PSB inoculated with *Rhizophora mucronata* seedlings, increased significantly the average root length by 19.09%, average shoot length by 21.26%, number of primary roots by 19.57%, number of secondary roots by 21.28%, shoot biomass by 47.33%, root biomass by 47.33%, leaf area by 44.76%, the level of total chlorophyll by 61.86%, chlorophyll-a by 41.86%, chlorophyll-b by 55.56%, and Carotenoids by 64.29%, the level of carbohydrate by 40.34%, protein by 43.56% and amino acid by 25.71% as compared to control. Thus, PSB is beneficial in raising vigorous seedling of *Rhizophora mucronata* under nursery and field conditions.

**Keywords:** Growth improvement, Mangroves, Phosphate solubilizing bacteria, *Rhizophora mucronata*.

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### INTRODUCTION

Phosphorous, one of the major nutrients limiting plant growth is rapidly immobilized after addition to soil as soluble fertilizer, and thus, it become less available to plant. Seed or soil inoculation with phosphate-solubilizing bacteria (PSB) such as *Bacillus* sp. can solubilize fixed soil P and applied phosphates, resulting in higher crop yields [1,2,3], and also increased inorganic P availability to plant by mineralization of organic P [4,5]. Further, this mineral is major plant nutrients, second only to nitrogen in requirement. However, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence

cannot be utilized by the plants [6]. To increase the availability of phosphorus for plants, large amounts of fertilizer are used on a regular basis. But after application, a large proportion of fertilizer phosphorus is quickly transferred to the insoluble form, [7,8]. Therefore, very little percentage of the applied phosphorus is used, making continuous application necessary [9]. It has been reported, that many soil fungi and bacteria can solubilize inorganic phosphates [10,11]. Phosphate solubilizing microorganisms play an important role in supplementing phosphorus to the plants, allowing a sustainable use of phosphate fertilizers.

Various kinds of bacteria [7,12,13], and fungi[14,15], have been isolated and characterized for their ability to solubilize unavailable reduced phosphorus to available forms. Such transformations increase phosphorus availability and promote plant growth. It is known that, soils have many of problems and we can overcome these problems via application of organic manures which have many advantages; *viz.*, improve soil physical properties and the availability of nutrients [16,17]. Jones Nirmalanath and Sreenivasa [18] observed that P-solubilizers in the rhizosphere of sunflower inoculated with *Pseudomonas* enhanced the yield. In the present study, aims to evaluate which extent a phosphate solubilizing bacteria strain has the ability to colonize the rhizosphere of *Rhizophora mucronata* plants fertilized with different phosphatase solubilising bacteria and to determine the effect of inoculation with a phosphate solubilizing bacterial strain on the growth and yield of *Rhizophora mucronata*.

## MATERIALS AND METHODS

### Collection of propagules

Healthy propagules of *Rhizophora mucronata* seeds were collected from Pichavaram mangrove forest, South East Coast of India (Lat. 11° 27' N, Long. 79° 46' E). The collected seeds were separated into different groups based upon their size and maturity.

### Isolation and identification of PSB

All the samples were subjected for Pikovkya's medium (glucose: 10g; tricalcium phosphate: 5g; NH<sub>4</sub>SO<sub>4</sub>: 0.5g; MgSO<sub>4</sub>.7H<sub>2</sub>O: 0.1g; KCl: 0.2g; MnSO<sub>4</sub>: trace; FeSO<sub>4</sub>: trace; yeast extract: 0.5g; Agar: 15.0g; aged seawater: 500ml; distilled water: 500ml; pH 7.2±0.2; autoclaved at 15lbs for 15 min). The plates were incubated at 28±2°C for 7 days. Morphologically different phosphobacterial species were identified by repeated streaking and identified by Bergey's Manual [19].

### Preparation of bacterial inoculum

Identified phospho bacterial species of *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Micrococcus luteus*, *Escherichia coli*, *Arthrobacter ilicis*, *Micrococcus roseus* and *Bacillus cereus* were inoculated separately into 100ml of Pikovsky's broth medium and were cultured at 28±1°C for 5 days in a shaker. The culture was centrifuged at 12,000 rpm for 15 minutes. The pellet were suspended in phosphate buffer (NaH<sub>2</sub>PO<sub>4</sub>.2H<sub>2</sub>O: 32.2g, Na<sub>2</sub>HPO<sub>4</sub>: 28.39g in 100ml sterile distilled water) and washed repeatedly with the buffer and were resuspended in the same buffer solution.

### Phosphobacteria induced growth on *Rhizophora mucronata*

100 ml (10<sup>8</sup> cells ml<sup>-1</sup>) of suspended culture of phosphobacterial species were separately added in to 1Kg of soil (sterilized at 12°C for 1 hr) and were kept in sterilized poly bags. Propagules of *Rhizophora mucronata* were planted into soil and were irrigated with sterile

water (100 ml per bag Kg of soil). After 60 days of treatment, the root and shoot, growths characteristics were ascertained, which were extracted in 80% ice cold acetone from leaves, were measured by following respectively the methods of Arnon [20], and Reddy [21]. The biochemical constituents *viz.*, carbohydrate [22], aminoacid [23], and protein [24].

## RESULTS AND DISCUSSION

The inoculation of different phosphobacterial species of PSB on the growth parameters of *Rhizosphora mucronata* reveals that, the *Micrococcus luteus* enhanced the average root length by 19.09% the *Bacillus megaterium* enhanced the shoot length by 21.26%, the shoot biomass was higher by 47.33% and the root biomass was higher by 47.33% over control. But the *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* enhanced the number of primary roots by 19.57%, number of secondary roots by 21.28% over control. The leaf area was increased by 44.76% with the inoculation of *Enterobacter aerogenes* (Table 1).

**Table 1. Effect of PSB on the root length, shoot length, number of primary roots, number secondary roots, shoot biomass, root biomass and leaf area of *Rhizophora mucronata* seedlings**

PSB treated	Average root length	Average shoot length	Number of primary roots	Number of secondary roots	Shoot biomass	Root biomass	Leaf area
<i>Bacillus subtilis</i>	7.38 (3.52)	27.90 (15.05)	9.2 (19.57)	460 (19.57)	1.23 (35.77)	0.80 (37.50)	78.66 (41.01)
<i>Escherichia coli</i>	6.44 (-10.56)	28.60 (17.13)	8.6 (13.95)	430 (13.95)	1.43 (44.76)	0.37 (-35.14)	72.10 (35.64)
<i>Arthrobacter ilicis</i>	8.3 (14.22)	27.00 (12.22)	8.2 (9.76)	410 (9.76)	1.30 (39.23)	0.75 (33.33)	71.00 (34.65)
<i>Micrococcus roseus</i>	2.94 (-42.18)	20.40 (-16.18)	7.0 (-5.71)	350 (-5.71)	0.98 (19.39)	0.34 (-47.06)	72.10 (36.09)
<i>Bacillus cereus</i>	8.38 (15.04)	26.80 (11.57)	8.2 (9.76)	410 (9.76)	1.10 (28.18)	0.40 (-25.00)	70.10 (33.81)
<i>Bacillus megaterium</i>	8.11 (12.21)	30.10 (21.26)	9.2 (19.59)	470 (21.28)	1.50 (47.33)	0.85 (41.18)	69.00 (32.75)
<i>Pseudomonas aeruginosa</i>	8.44 (15.64)	26.50 (10.57)	9.2 (19.59)	460 (19.57)	0.89 (11.24)	0.70 (28.57)	60.00 (22.67)
<i>Enterobacter aerogenes</i>	7.32 (2.73)	30.08 (21.21)	9.2 (19.59)	460 (19.57)	0.94 (15.96)	0.42 (-9.05)	84.00 (44.76)
<i>Micrococcus luteus</i>	8.8 (19.09)	28.36 (16.43)	9.0 (17.78)	460 (7.50)	0.86 (8.14)	0.39 (-28.21)	70.10 (33.81)
Control	7.12 (0.00)	23.70 (0.00)	7.4 (0.00)	370 (0.00)	0.79 (0.00)	0.50 (0.00)	46.40 (0.00)

Values are parentheses are percent increase over control

The effect of bacterial inoculation of phosphate solubilizing bacteria on the photosynthetic pigments shows that the total chlorophyll content was increased by 61.86% with the addition of *Micrococcus luteus* than control. The *Bacillus megaterium* increased the content of chlorophyll-a by 41.86% the content of chlorophyll-b by 55.56% over control. The levels of carotenoids pigment was found higher by 64.29% by the addition of *Bacillus subtilis* (Table 2). Among the bacterial species, *Bacillus megaterium* increased the content of carbohydrate by 40.34%, protein by 43.56% and amino acid by 25.71% respectively over than the other bacterial species (Table 3). Phosphorous deficiency is the major constraint on crop production, as reported by many researchers [25,26,27].

**Table 2. Effect of PSB on the total chlorophyll, chl-a, chl-b and carotenoids of *Rhizophora mucronata* seedlings**

PSB treated	Content of total chlorophyll	Content of chlorophyll-a	Content of chlorophyll-b	Content of carotenoids
<i>Bacillus subtilis</i>	0.072 (51.39)	0.036 (38.86)	0.036 (55.56)	0.056 (64.29)
<i>Escherichia coli</i>	0.042 (16.67)	0.026 (13.46)	0.017 (5.88)	0.02 (0.00)
<i>Arthrobacter ilicis</i>	0.038 (7.89)	0.021 (-2.74)	0.163 (1.84)	0.01 (-100.00)
<i>Micrococcus roseus</i>	0.04 (12.50)	0.026 (16.67)	0.021 (23.81)	0.03 (33.33)
<i>Bacillus cereus</i>	0.048(27.98)	0.027 (18.18)	0.026 (38.46)	0.03 (33.33)
<i>Bacillus megaterium</i>	0.074 (52.70)	0.038 (41.86)	0.036 (55.56)	0.02 (0.00)
<i>Pseudomonas aeruginosa</i>	0.049 (28.57)	0.027 (16.67)	0.021 (23.81)	0.02 (0.00)
<i>Enterobacter aerogenes</i>	0.061 (42.62)	0.036 (37.50)	0.026 (38.46)	0.04 (50.00)
<i>Micrococcus luteus</i>	0.09 (61.11)	0.026 (13.46)	0.013 (-23.08)	0.03 (33.33)
Control	0.035 (0.00)	0.022 (0.00)	0.016 (0.00)	0.02 (0.00)

Values are parentheses are percent increase over control

**Table 3. Effect of PSB on the carbohydrate, protein and amino acid of *Rhizophora mucronata* seedlings**

PSB treated	Carbohydrate	Protein	Amino acid
<i>Bacillus subtilis</i>	2.20 (21.36)	1.60 (42.50)	0.70 (25.71)
<i>Escherichia coli</i>	2.34 (26.07)	1.50 (38.67)	0.47 (-10.64)
<i>Arthrobacter ilicis</i>	2.40 (27.92)	1.48 (37.84)	0.49 (-6.12)
<i>Micrococcus roseus</i>	1.70 (-1.76)	1.30 (29.23)	0.55 (5.45)
<i>Bacillus cereus</i>	1.95 (11.28)	1.47 (37.41)	0.56 (7.14)
<i>Bacillus megaterium</i>	2.90 (40.34)	1.63 (43.56)	0.70 (25.71)
<i>Pseudomonas aeruginosa</i>	1.24 (-39.52)	1.45 (36.55)	0.51 (-1.96)
<i>Enterobacter aerogenes</i>	2.55 (32.16)	1.58 (41.77)	0.57 (8.77)
<i>Micrococcus luteus</i>	1.81(4.42)	1.35 (31.85)	0.50 (-4.00)
Control	1.73 (0.00)	0.92 (0.00)	0.52 (0.00)

Values are parentheses are percent increase over control

A number of other studies also pointed out, that application of biofertilizer increased the plant height in [28,29]. The positive effect of PSB enhanced root growth by synthesizing promoting substances resulted in more nutrient uptake and decreased cell division and expansion [30]. Atiyeh [31], reported that, effect of PSB enhanced growth of tomato plants. The present study observed that the halophilic bacterial species of phosphobacteria particularly *Bacillus megaterium* and *Bacillus subtilis* enhanced the maximum number of plant growth parameters *Rhizophora mucronata*.

Phosphorus plays a vital role in physiological and developmental process in plant life and favourable effect of this important nutrient might have accelerated the growth process that increases N uptake in plants [32]. These results suggest that, treatment with PSB is beneficial as a general increase in growth and length as compared to control was observed in all cases. Enhancement of growth in *Rhizophora mucronata* seedlings might be due to treatment with PSB so as to enable to release the available phosphorous to the plants. Several results suggest that PSB have the ability to solubilise rock phosphate thereby increasing availability to plants [33,34].

The bacterial species that facilitate phosphate solubilisation by inoculation with mangroves are not well characterized, although some of the organisms involved in the inoculation processes have been identified [35-37]. It was previously observed that mangrove seedlings usually grow better after inoculation with the diazotrophic filamentous cyanobacteria [38],

*Azospirillum* and *Azotobacter* [37]. Based on this observation, it was reasoned that mangrove seedlings might also benefit by being inoculated with plant growth promoting bacteria [39]. PGPBs have been reported to stimulate regeneration of temperate forests [40-42]. Phosphobacterial species are well known PGPBs that facilitate the growth of terrestrial plant species [43,44]. But there are only few reports describing the inoculation of halophilic phosphobacteria on to mangrove plants. Hence, the present study has been carried out to find out the effect of nine halophilic phosphobacteria on the growth of *Rhizophora mucronata* and coastal crops. It reveals that all the nine phosphobacterial species. A total of nine phosphobacterial species enhanced the growth and physiology of *Rhizophora mucronata* seedlings.

In the present study, halophilic phosphobacteria had positive effects on the pigments, organic contents and growth characteristics of *Rhizophora mucronata*. It was also found that, the halophilic phosphobacteria enhanced the level of photosynthetic pigments in *Rhizophora mucronata* seedlings. PSB have positive effective on the growth characteristics, biochemical constitutions and pigments of mangroves. This promontory effect may be attributed to ability of the PSB and making it available to the growing seedlings of mangroves. In this present study, all of the nine bacterial species of PSB also synthesizing the phytohormone, which are required for better growth and pigment production of mangrove seedlings [45,46]. Similar findings already have been reported that the inoculation of *Azospirillum* sp. and *Azotobacter* sp. enhanced the level of pigments in mangrove seedlings.

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