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Effect of halophilic phosphobacteria on *Avicennia officinalis* seedlings

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ABSTRACT

Phosphobacteria is one among the soil microorganisms which plays an important role in improving the chemical and physical nature of the soil, adding organic matter to soil, solubilizing the insoluble phosphate increasing availability and utilization of growth and yield. The phosphate solubilizing bacteria, which were inoculated with Avicennia officinalis seedlings, increased significantly the average root length by 43.43%, average shoot length by 40.00%, number of primary roots by 53.7%, number of secondary roots by 59.74%, shoot biomass by 69.39% and root biomass by 26.32%. The pigments also increased to the level of total chlorophyll by 54.22%, chlorophyll-a by 43.18%, Carotenoids by 90.00% and the biochemical constituents the level of carbohydrate by 61.88%, protein by 52.38% and amino acid by 27.85% increased. Thus, phosphate solubilizing bacteria is beneficial in raising vigorous seedling of Avicennia officinalis under nursery and field conditions.

Keywords: Avicennia officinalis, Mangroves, Phosphate solubilizing bacteria, Pigments, Protein.

INTRODUCTION

Phosphorus is an essential mineral nutrient that often limits plant growth because of its low solubility and fixation in the soil. Most of it is not readily available to plants due to its low solubility in the soil [1]. Phosphate solubilizing bacteria (PSB) are known to bring about mobilisation of insoluble phosphates and this can stimulate plant growth even under the conditions of phosphorus deficiency [2]. In many parts of the world, mangrove forests have been shown to naturally experience phosphorus deficiency that limit the growth of plants [3-6]. A number of fertilization experiments have shown dramatic increases in the growth and nutrient dynamics of mangroves grown in phosphorus deficient sites in response to the addition of phosphorus [6-8].

PSB are used as biofertilizer since 1950's [9,10]. These microorganisms secrete different types of organic acids e.g., carboxylic acid [11], thus lowering the pH in the rhizosphere [12], and consequently dissociate the bound forms of phosphate like $Ca_3(Po_4)_2$ in calcareous soils. Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expansive phosphatic fertilizers. The ability of microorganisms to solubilise phosphorus has been employed for improving crop yield in agriculture and horticulture [13]. Rhizosphere bacteria [14,15], and fungi [16], in many soil environments have been shown to improve plant growth by solubilizing sparingly soluble inorganic and organic phosphates.

Many species of PSB associated with mangrove roots and rhizosphere sediments [17,18]. The mechanism responsible for microbial phosphorus solubilisation in mangrove ecosystem is considered to involve the production of several organic acids [17]. Reports regarding inoculation of PSB on *Avicennia officinalis* are rare, especially in this study area. The major factors limiting establishment and early vigorous growth of the plants in the face of environmental extremes are infertility and poor germination. Rhizosphere microorganisms may allow plants to overcome these environmental extremes [19], particularly in mangrove seedlings, which show a serious problem of poor growth [20]. The objective of present study aims to evaluate which extent a phosphate solubilizing bacteria strain has the ability to colonize the rhizosphere of *Avicennia officinalis* plants fertilized with different phosphatase solubilizing bacteria and to determine the effect of inoculation with a phosphate solubilizing bacterial strain on the growth and yield of *Avicennia officinalis.*

MATERIALS AND METHODS

Collection of propagules

Healthy propagules of *Avicennia officinalis* (Forsk) Vierb., seeds were collected form Pichavaram mangrove forest, South East Coast of India (Lat. 11° 27' N and 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_4SO: 0.5g; $MgSO_4.7H_2O$: 0.1g; KCl: 0.2g; $MnSO_4$: trace; $FeSO_4$: 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 [21].

Preparation of bacterial inoculum

Identified phosphobacterial species of *Bacillus subtilis*, *Escherichia coli*, *Arthrobacter ilicis*, *Micrococcus roseus*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Micrococcus luteus* were inoculated separately into 100ml of Pikocsky's broth medium and were incubated at 28±1°C for 5 days in a shaker. The culture was centrifuged at 12,000 rpm for 15 min. The pellets were suspended in phosphate buffer

($NaH_2Po_4.2H_2O$: 32.2g; Na2HPO$_4$: 28.39g; sterile distilled water: 100ml) and washed repeatly with the buffer and were resuspended in the same buffer solution.

Phosphobacteria induced growth on *Avicennia officinalis*

100 ml (10^8 cells ml^{-1}) 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 *Avicennia officinalis* 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 [22], and Reddy [23]. The biochemical constituents *viz.*, carbohydrate [24], aminoacid [25], and protein [26].

RESULTS AND DISCUSSION

The inoculation of different phosphobacterial species of PSB on the growth parameters of *Avicennia officinalis* reveals that, the *Bacillus subtilis* enhanced the average root length by 43.43% the *Pseudomonas aeruginosa* enhanced the shoot length by 40.0%, the *Bacillus subtilis* enhanced the shoot biomass by 69.39%, the *Pseudomonas aeruginosa* enhanced the root biomass was higher by 26.32%, the *Arthrobacter ilicis* enhanced the number of primary roots by 53.57% and the *Micrococcus luteus* enhanced the number of secondary roots by 59.74% over control. The leaf area was increased by 70.55% with the inoculation of *Bacillus subtilis* (Table 1).

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

PSB treated	Average root length	Average shoot length	Number of primary roots	Number of secondary roots	Shoot biomass	Root biomass	Leaf area
Bacillus subtilis	10.89 (43.43)	32.48 (35.34)	13.20 (50.20)	424 (41.51)	0.98 (69.39)	0.32 (12.50)	45.50 (70.55)
Escherichia coli	7.45 (17.32)	31.40 (33.12)	10.60 (38.68)	416 (40.38)	0.88 (65.91)	0.26 (-7.69)	23.80 (43.70)
Arthrobacter ilicis	5.48 (-12.41)	23.70 (11.39)	14.00 (53.57)	560 (55.71)	0.86 (65.12)	0.23 (-21.74)	19.50 (31.28)
Micrococcus roseus	9.84 (37.40)	25.00 (16.00)	11.60 (43.97)	456 (45.61)	0.91 (67.03)	0.27 (-3.70)	24.40 (45.08)
Bacillus cereus	7.11 (8.30)	24.20 (13.22)	9.20 (29.35)	368 (32.61)	0.88 (65.91)	0.25 (-12.00)	41.00 (67.32)
Bacillus megaterium	8.30 (25.78)	32.60 (35.58)	13.60 (52.21)	536 (53.73)	0.96 (68.75)	0.30 (6.67)	25.90 (48.26)
Pseudomonas aeruginosa	7.25 (15.03)	35.00 (40.00)	12.20 (46.72)	488 (49.18)	0.66 (54.55)	0.38 (26.32)	25.50 (47.45)
Enterobacter aerogenes	8.27 (25.51)	19.20 (-9.38)	12.00 (46.72)	480 (48.33)	0.90 (66.67)	0.29 (3.45)	28.00 (52.14)
Micrococcus luteus	6.39 (3.60)	24.90 (15.66)	12.40 (47.58)	616 (59.74)	0.76 (60.53)	0.25 (-12.00)	0.25 (-2.00)
Control	6.16 (0.00)	21.00 (0.00)	6.50 (0.00)	248 (0.00)	0.30 (0.00)	0.28 (0.00)	13.40 (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 54.22% with the addition of *Bacillus megaterium* than control. The *Bacillus cereus* increased the content of chlorophyll-a by 43.18%, the *Bacillus subtilis* increased the contents of chlorophyll-b by 69.77%, the *E. coli* increased the content of carotenoids by 90.00% over control (Table 2). The biochemical constituents study reveals that, carbohydrate were higher by 61.88% in *Micrococcus roseus* and *Bacillus megaterium* enhanced the protein by 52.38%, the amino acid content was higher by *Bacillus subtilis* and *Enterobacter aerogenes* by 27.85% over control (Table 3). In the present study, indicated the potential to enhance mangrove seedlings by inoculation of PSB to the current recommendation to amend soil with only an external source of phosphorus. The PSB applications were more effective in improving mangrove growth in phosphorus deficient soil and then each was applied alone. This study has also shown less expensive and PSB to promote the growth of mangrove seedlings [18,27].

Table 2. Effect of PSB on the pigments of Avicennia *officinalis* seedlings

PSB treated	Content of total chlorophyll	Content of chlorophyll-a	Content of chlorophyll-b	Content of carotenoids
Bacillus subtilis	0.082 (53.66)	0.038 (34.21)	0.043 (69.77)	0.074 (86.49)
Escherichia coli	0.068 (44.12)	0.034 (26.47)	0.033 (60.61)	0.010 (90.00)
Arthrobacter ilicis	0.067 (43.28)	0.036 (30.56)	0.031 (58.06)	0.050 (80.00)
Micrococcus roseus	0.080 (49.33)	0.042 (40.48)	0.033 (60.61)	0.050 (80.00)
Bacillus cereus	0.082 (53.66)	0.044 (43.18)	0.037 (64.86)	0.030 (66.67)
Bacillus megaterium	0.083 (54.22)	0.040 (37.50)	0.042 (69.05)	0.060 (83.33)
Pseudomonas aeruginosa	0.073 (47.95)	0.038 (34.21)	0.036 (63.89)	0.040 (75.00)
Enterobacter aerogenes	0.078 (51.28)	0.037 (32.42)	0.041 (68.29)	0.034 (70.59)
Micrococcus luteus	0.058 (24.00)	0.03 (0.00)	0.028 (53.57)	0.040 (75.00)
Control	0.04 (0.00)	0.03 (0.00)	0.01 (0.00)	0.01 (0.00)

Values are parentheses are percent increase over control

Table 3. Effect of PSB on the biochemical constituents of *Avicennia officinalis* seedlings

PSB treated	Carbohydrate	Protein	Aminoacid
Bacillus subtilis	1.49 (42.95)	1.39 (35.25)	0.79 (27.85)
Escherichia coli	1.11 (23.42)	1.51 (40.40)	0.60 (4.20)
Arthrobacter ilicis	1.20 (29.17)	1.37 (34.31)	0.57 (12.31)
Micrococcus roseus	2.23 (61.88)	1.76 (48.86)	0.77 (4.20)
Bacillus cereus	1.72 (50.58)	1.54 (41.56)	0.74 (25.29)
Bacillus megaterium	1.59 (46.54)	1.89 (52.38)	0.80 (22.97)
Pseudomonas aeruginosa	1.08 (21.30)	1.23 (26.83)	0.71 (19.72)
Enterobacter aerogenes	1.38 (38.41)	1.81 (50.28)	0.79 (27.85)
Micrococcus luteus	1.02 (16.67)	1.35 (33.33)	0.75 (24.00)
Control	0.85 (0.00)	0.90 (0.00	0.65 (0.00)

Values are parentheses are percent increase over control

Phosphate solubilizing microorganism has been reported promising in reducing phosphorus fixation and increasing the phosphorous availability from soluble and insoluble phosphate fertilizers. Katznelson and Bose [28], found that rhizosphere bacteria have greater metabolic activity and suggested that they might contribute significantly to the phosphate economy of the plant. The inoculation of PSB releases more available phosphorus from insoluble source and water soluble source of phosphorus [29]. The bacterial species that facilitate phosphate solubilizing by inoculation with mangroves are not well characterizes, although some of the organism involved in the inoculation processes have been identified [30-32]. It was previously observed that mangrove seedlings usually grow better after inoculation with the diazostrophic filamentous cyanobacteria [33], *Azospirillum* and *Azotobacter* [32]. Based on this study observation, it was reasoned that mangrove seedlings might also benefit by being inoculated with PSB.

PSB was observed on block pepper [34] and tomato [35]. The inoculation of PSB recorded significantly high root length over control. The control plants produced less root mass. Gerretson [36], was the first to demonstrate that plants take up more phosphate from insoluble phosphatic fertilizers in the presence of micro organisms with these idea in view present investigations was undertaken to see the effect of phosphate solubilizing microorganism on growth and yield of mangroves. Similar studies by Ahmed [37], also showed that combining *phosphorene* (as a source of phosphate solubilizing bacteria) with phosphate fertilizers had an incrementally effect on growth and phosphorus uptake on olive seedlings. Most of the work on phosphate solubilizing have been carried out in relation to agricultural environments and only a few studies have focused on the ability of PSB originating from mangrove roots and sediments to soluble forms of phosphates [18,38], and to promote the growth of mangrove seedlings [39]. Although phosphorus fertilization has been employed to enhance mangrove establishment in Flerid [40], Panama [7], Belize [7,6]. Hence the present study reveals that, *Bacillus megaterium* and *Bacillus subtilis* could enhance the maximum number of mangrove plant growth parameters.

In the present study, all the halophilic bacterial species of phosphobacteria are capable of solubilizing the inorganic phosphorous. It was also found that, the halophilic phosphobacteria enhanced the level of photosynthetic pigments in *Avicennia officinalis* 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 [41,42]. Similar findings already have been reported that the inoculation of *Azospirillum sp.* and *Azotobactor sp.* enhanced the level of pigments in mangrove seedlings. Hence the present study has been carried out to find out the effect of nine halophilic phosphobacteria on the growth of *Avicennia officinalis*. It reveals that, a total of nine phosphobacterial species enhanced the growth and physiology of *Avicennia officinalis* seedlings. The application of PSB can improve and sustain significant growth rate of *Avicennia officinalis.*

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