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The Biofertilizer Effect of Halophilic Phosphate Solubilising Bacteria on *Oryza sativa*

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Abstract: Phosphorus is one of the major essential macronutrients limiting plant growth owing to its low bioavailability in soils. Fertilizer phosphorus tends to be fixed soon after application and becomes mostly unavailable, low recovery by crops and a considerable phosphorus accumulation in soils. Several processes of the phosphorus cycle in soils remain obscure, despite large research efforts devoted to increasing the phosphorus availability to plants. The biofertilizer effect of phosphate solublizing bacteria on growth, yield and nutrient uptake of *Oryza sativa* was studied in a field experiment. Positive effect on plant growth, nutrient uptake, grain yield and yield components in *Oryza sativa* plants was recorded in the treatment inoculation of PSB. The PSB inoculated with *Oryza sativa,* increased significantly the average root length by 32.95%, average shoot length by 11.00%, numbers of roots by 38.46%, shoot biomass by 90.48%, root biomass by 3.77% as compared to control. The pigments increased the level of total chlorophyll by 68.70%, chlorophyll-a by 53.50%, chlorophyll-b by 80.47% and carotenoids by 45.83%, as compared to control and they also the level of carbohydrate by 33.33%, protein by 41.21% and amino acids by 30.23% as compared to control. Thus PSB is beneficial in raising vigorous of *Oryza sativa* under nursery and field conditions.

Key words: Biofertilizer · Chlorophyll pigments · Phosphate solubilising bacteria · *Oryza sativa* · Root biomass

INTRODUCTION

Phosphorus as an essential mineral nutrient for plant growth and development is the world's second highest chemical input in agriculture. Soluble P is often the limiting mineral nutrient for biomass production in agricultural ecosystems as well [1]. Plants utilize fewer amounts of phosphate fertilizers that are applied and the rest is rapidly converted into insoluble complexes in the soil. So this phenomenon encourages farmers to frequent application of phosphate fertilizers. Modern agriculture is severely modifying and polluting the natural environment, due to the widespread application of chemical fertilizers, herbicides and pesticides. Therefore, thinking about valid alternative for chemical fertilizers is too necessary. Phosphate solubilizing bacteria (PSB) are used as bio fertilizer since 1950's [2-4]. These microorganisms secrete different types of organic acids e.g. carboxylic acid [5] thus lowering the pH in the rhizosphere [6] and consequently dissociate the bound forms of phosphate like $Ca_3(PO_4)_2$ in calcareous soils.

The role of phosphate solublizing microorganism in their abilities to reduce the pH of the surroundings by the production of organic acids [7], production of acid and alkaline phosphatises [8] and to H^+ protonation [9]. These organic acids can either dissolve phosphates as a result of anion exchange or can chelate Ca, Fe or Al ions associated with the phosphates [10].

Various kinds of bacteria [8, 11, 12] have been isolated and characterized for their ability to solublize unavailable reduced phosphorus to available forms. Such transformations increase phosphorus availability and promote plant growth [8, 11, 13, 14]. The most abundant phosphates in the semiarid Argentinean pampas are

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bound to Calcium [15]. The continuous agricultural during more than 20 years caused considerable decreases in the soluble phosphorous and in similar degree in the total inorganic phosphorus [16].

Use of these microorganisms as environment friendly biofertilizer helps to reduce the much expensive phosphatic fertilizers. Phosphorus biofertilizers could help increase the availability of accumulated phosphate (by solubilization), efficiency of biological nitrogen fixation and increase the availability of Fe, Zn etc. through production of plant growth promoting substances [17]. Trials with PSB indicated yield increases in rice [18], maize [19] and other cereals [20, 21]. Hence, the present study aims to evaluate which extent a phosphate solubilizing bacteria strain has the ability of *Oryza sativa* 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 *Oryza sativa.*

MATERIALS AND METHODS

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 [22].

Preparation of Bacterial Inoculum: Identified phosphobacterial species of *Bacillus subtilis*, *Escherichia coli*, *Arthrobacter ilicis*, *Micrococcus roseus*, *Bacillus cereus*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Micrococcus luteus* were isolated from mangrove environments and were incoculated separately into 100ml of Pikocsky's broth 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 ($NaH_2Po_4.2H_2O$-32.2g, Na_2HPO4-28.39g in 100ml sterile distilled water) and washed repeatly with the buffer and were resuspended in the same buffer solution.

Phosphobacteria Induced Growth on *Oryza sativa:* To study the effect of phosphobacteria on the growth of coastal rice crop, an experiment was conducted. Certified seeds of paddy (IR36) were procured from Department of Agriculture, Thirupathisaram, Kanyakumari district. The seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes and the seeds were soaked for 1 hr in liquid cell suspension (10^8 cells ml^{-1}) separately and spreaded on to the soil moistured with sterile distilled water. Five replicates of 100 seeds of paddy were maintained for each bacterial species. The seeds without bacterial treatment served as control.

After 20 days of treatment, the plant growth characteristics *viz.* average root length, average shoot length, number of roots, root and shoot biomass were analyzed. The pigments such us chlorophyll, chlorophyll-a, chlorophyll-b and carotenoides [23] which were extracted in 80% ice cold acetone from leaves, were measured by following respectivey the methods [24, 25]. The biochemical constituents *viz.* carbohydrate, aminoacid and protein [26, 27].

RESULTS

The inoculation of different phosphobacterial species of PSB on the growth parameters of *Oryza sativa* reveals that, the *Micrococcus roseus* enhanced the root length by 32.95%, the *Bacillus megaterium* and *Enterobacter aerogenes* enhanced the shoot length by 38.46%, the *Escherichia coli, Arthrobacter ilicis, Micrococcus roseus, Bacillus cereus* and *Enterobacter aerogenes* enhanced the root biomass 3.77% and *Arthrobacter ilicis*, *Escherichia coli* enhanced the average shoot length and shoot biomass was increased by 11.00% and 90.48% respectively (Table 1).

The content of total chlorophyll, chlorophyll-a and chlorophyll-b in *Oryza sativa* seedlings were found to be increased by 68.70%, 53.50% and 80.47% respectively by the inoculation of the bacterial species of *Enterobacter aerogenes*, the content of carotenoids was increased by 45.83% by the inoculation of *Micrococcus luteus*.The content of carbohydrate was increased by 33.33% by *Bacillus cereus* and the content of protein was increased maximum by 41.21% by the inoculation of *Bacillus megaterium*, the amino acid content was increased by 30.23% by *Bacillus subtilis* over control (Table 2).

Table 1: Effect of PSB on the average root length, shoot length, number of roots, shoot biomass and root biomass of *Oryza sativa* seedlings as percentage increase of decrease over control.

PSB treated	Average root length	Average shoot length	Number of roots	Shoot biomass	Root biomass
Bacillus subtilis	8.70 (5.75)	17.31 (5.41)	11.00 (27.27)	0.161 (2.48)	0.157 (2.55)
Escherichia coli	10.02 (18.16)	18.20 (9.78)	6.00 (-33.33)	1.650 (90.48)	0.159 (3.77)
Arthrobacter ilicis	10.64 (22.93)	18.45 (11.00)	6.00 (-33.33)	1.600 (90.19)	0.159 (3.77)
Micrococcus roseus	12.23 (32.95)	18.18 (9.68)	7.00 (-14.29)	0.159 (1.26)	0.159 (3.77)
Bacillus cereus	10.69 (23.29)	17.32 (5.20)	11.00 (27.27)	0.168 (6.55)	0.159 (3.77)
Bacillus megaterium	8.90 (7.89)	16.86 (2.61)	13.00 (38.46)	0.167 (5.99)	0.158 (3.16)
Pseudomonas aeruginosa	10.52 (22.05)	17.07 (3.81)	9.00 (11.11)	0.159 (1.26)	0.156 (1.92)
Enterobacter aerogenes	11.31 (27.50)	18.39 (10.71)	13.00 (38.46)	0.174 (9.77)	0.159 (3.77)
Micrococcus luteus	9.18 (10.68)	16.53 (0.67)	9.00 (11.11)	0.159 (1.26)	0.156 (1.92)
Control	8.20 (0.00)	16.42 (0.00)	8.00 (0.00)	0.157 (0.00)	0.153 (0.00)

Values are parentheses are percent increase over control

Table 2: Effect of PSB on the chlorophyll-a, chlorophyll-b, carotenoid, carbohydrate, protein and aminoacids of *Oryza sativa* seedlings as percentage increase of decrease Over control.

PSB treated	Total content of chlorophyll	Content of chlorophyll-a	Content of chlorophyll-b	Carotenoids	Carbohydrate	Protein	Amino acids
Bacillus subtilis	0.198 (27.27)	0.087 (-6.90)	0.109 (54.13)	0.09 (-44.44)	0.091 (25.27)	0.160 (39.38)	0.043 (30.23)
Escherichia coli	0.181 (20.44)	0.113 (17.70)	0.067 (25.37)	0.14 (7.14)	0.079 (13.92)	0.119 (18.49)	0.039 (23.08)
Arthrobacter ilicis	0.193 (25.39)	0.072 (-29.17)	0.119 (57.98)	0.03 (-333.33)	0.075 (9.33)	0.112 (13.39)	0.037 (18.92)
Micrococcus roseus	0.167 (13.77)	0.103 (9.71)	0.060 (16.67)	0.02 (-550.00)	0.073 (6.85)	0.129 (24.81)	0.036 (16.67)
Bacillus cereus	0.190 (24.21)	0.096 (3.13)	0.094 (46.81)	0.13 (0.00)	0.102 (33.33)	0.161 (39.75)	0.038 (21.05)
Bacillus megaterium	0.296 (51.35)	0.106 (12.26)	0.187 (73.26)	0.16 (18.75)	0.094 (27.66)	0.165 (41.21)	0.041 (26.83)
Pseudomonas aeruginosa	0.241 (40.25)	0.108 (13.89)	0.130 (61.54)	0.08 (-62.50)	0.076 (10.53)	0.101 (3.96)	0.034 (11.76
Enterobacter aerogenes	0.460 (68.70)	0.200 (53.50)	0.256 (80.47)	0.21 (38.39)	0.086(20.93)	0.160 (39.38)	0.036 (16.67)
Micrococcus luteus	0.349 (58.74)	0.193 (51.81)	0.153 (67.32)	0.24 (45.83)	0.098 (30.61)	0.132 (26.52)	0.034 (11.76)
Control	0.144 (0.00)	0.093 (0.00)	0.050 (0.00)	0.13 (0.00)	0.068 (0.00)	0.097 (0.00)	0.030 (0.00)

Values are parentheses are percent increase over control

DISCUSSION

Use of phosphate solubilising bacteria has been reported promising in reducing phosphate fixation and increasing the phosphorous availability from soluble and insoluble phosphatic fertilizers. Beneficial effect of inoculation of phosphate solubilizers on the uptake of nutrients and on the yield of crops has been reported by many workers. Gerretson [28] was the first to demonstrate that plants take up more phosphate from insoluble phosphatic fertilizers in the presence of microorganisms with these ideas in view present investigation was undertaken to see the effect of phosphate solubilising microorganisms on growth and yield of rice crops [29, 30]. Ravikumar [31] found that rhizosphere bacteria have greater metabolic activity and suggested that they might contribute significantly to the phosphate economy of the plant. Laboratory studies reviewed have shown that the microbial solubilising of soil phosphate in liquid medium studies has often been due to the excretion of organic acids as a result of which a decrease in pH was affected. A few reports have indicated the phosphate solubilising activity of some nitrogen fixers [32, 33].

The maximum and significantly high shoot length was observed in *Bacillus* sp. with phosphate solubilising bacteria. The inoculation of PSB has also increased the shoot growth. The control plants have recorded the lowest shoot length. PSB was observed on block pepper and tomato [34]. The inoculation of PSB recorded significantly high root length over control. The control plants produced less root mass. Similar studies also showed that phosphorene (as a source of phosphate solubilising bacteria) at 0.1% with phosphate fertilizers had an incrementally beneficial effect on growth and phosphate uptake on olive seedlings.

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 [31, 35-38]. It was previously observed that mangrove seedlings usually grow better after inoculation with the diazotrophic filamentous cyanobacteria [39], *Azospirillum* and *Azotobactor* [40]. PSB have positive effective on the growth characteristics, biochemical constitutions and pigments of *Oryza sativa*. This promontory effect may be attributed to ability of the

PSB and making it available to the growing coastal crops. 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 *Oryza sativa* [41]. Similar findings already have been reported that the inoculation of *Azospirillum* sp. and *Azotobactor* sp. enhanced the level of pigments in mangrove seedlings. Hegazi *et al*. [42] reported an increase in total nitrogen content of rice due to inoculation of *Azospirillum* and *Azotobacter*. Elshanshoury [43] reported that dual inoculation of *Azospirillum brasilense* with *Azotobacter chroococcum,* in sterilized soil resulted in significant stimulation of their populations in rhizosphere of wheat seedlings. These PSB inoculations also significantly increased the plant growth, content of chlorophyll and biochemical constients in coastal rice crops.

Enhancement of seedling growth due to seed treatment with phosphate solubilizing bacteria like *B. subtilis* and *B. megaterium* may be due to release of plant growth promoting substances. Plant growth promoting microbes are an important contributor to biofertilization of agricultural crops. Production of growth regulators by phosphate solubilizing bacteria has been studied by Ponmurugan and Gopi [44]. There is increasing evidence that phosphobacteria improve plant growth due to biosynthesis of plant growth substances rather than their action in releasing available phosphorus. Chaykovskaya, *et al*. [45] reported that treatment with phosphate solubilizing bacteria resulted in increased yield of pea and barley. Several results suggest that phosphate-solubilizing bacteria have the ability to solubilize phosphate thereby increasing its availability to plants [46]. Results of this study suggest that inoculation of PSB could enhance the maximum growth as well as result increased productivity. Among the PSB, will be more effective in increasing growth with inoculation of PSB will give better results as compared to control. Hence use of these phosphate-solubilizing bacteria, as biofertilizer should be promoted.

CONCLUSION

In conclusion, organic fertilizer and chemical fertilizers could increase the phosphate level available in soil. Present investigation was therefore carried out on phosphate solubilising bacteria and their biofertilizer effect on coastal rice crops. This result revealed many interesting and important facts about the halophilic PSB, as well as the role of P-availability, for crop production and agricultural sustainability.

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