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# Efficiency of Plant Growth Promoting Rhizobacteria Isolated from Sand Dunes of Chennai Coastal Area

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**Abstract:** Plant Growth Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize the plant root and enhance the plant growth. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides and supplements. In the present study, PGPR were isolated from 18 different rhizosphere soil samples of coastal sand dune plants, belonging to the genus *Ipomoea* sp. collected from the Chennai coastal area. For isolation of bacteria from soil samples, pour plate technique was followed. The rhizobacterial population was ranged from $4.4\times10^{6}$-$7.5\times10^{7}$ CFU $g^{-1}$. From that, 46 morphologically different bacterial strains were isolated. Among 46, 18 strains exhibited the production of Indole Acetic Acid. (IAA). When screened for phosphate solubilzing activity, six strains showed maximum activity. All these selected six strains were screened for seed germination among which these two strains (AMET1136 and AMET 1148) showed remarkable increase in the seed germination of black gram and green gram. For plant growth promotion, three types of treatments namely, seed bacterization, soil drenching and mixed (seed+soil) were carried out to check the potential of these two strains. Among that one strain which was identified as *Pseudomonas* sp. AMET1148 showed remarkable and significant increase in shoot length and root length of the tested plants. The study concluded that PGPR from coastal sand dund plants can be developed as plant growth promoters in agricultural crops.

**Key words:** PGPR, *Pseudomonas*, coastal sand dune plants, growth promotion, *Ipomoea* sp.

## INTRODUCTION

Bacteria that colonize the rhizosphere and plant roots and enhance plant growth by any mechanism are referred to as Plant Growth Promoting Rhizobacteria (PGPR) and it include the genera like *Pseudomonas*, *Burkholderia*, *Agrobacterium*, *Erwinia*, *Azospirillum*, *Bacillus*, *Rhizobium* (Siddiqui, 2005; Raj *et al*., 2005; Dursun *et al*., 2008). Plant Growth-Promoting Rhizobacteria (PGPR) have the ability to improve growth, vigour, establishment and therefore yield, in a number of plant species (Sharma and Johri, 2003) by (i) the ability to produce the plant hormones like indoleacetic acid (Mordukhova *et al*., 1991) gibberellic acid, cytokinins (Tien *et al*., 1979) and ethylene (Glick *et al*., 1995); (ii) asymbiotic $N_2$ fixation (Boddey and Dobereiner, 1995; Kennedy *et al*., 1997); (iii) inhibit the phytopathogenic microorganisms growth (Fridlender *et al*., 1993) by production of siderophores (Scher and Baker, 1982), β-1,3-glucanase (Fridlender *et al*., 1993), chitinases (Renwick *et al*., 1991) and cyanide (Flaishman *et al*., 1996) and (iv) solubilization of mineral phosphates and other nutrients (De Freitas *et al*., 1997). Another major benefit of PGPR is to produce antibacterial compounds that are effective against certain plant pathogens and pests (Herman *et al*., 2008; Minorsky, 2008; Thakuria *et al*., 2004). There has been much research interest in PGPR and there is now an increasing number of PGPR being commercialized for crops. Over the years the PGPR have gained worldwide importance and acceptance for agricultural benefits and these microorganisms are the potential tools for sustainable agriculture and the trend for the future. Scientific researchers involve multidisciplinary approaches to understand adaptation of PGPR to the rhizosphere, mechanisms of root colonization, effects of plant physiology and growth, biofertilization, induced systemic resistance, biocontrol of plant pathogens, production of determinants etc.

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Coastal sand dunes are common in different parts of the world. These are natural structures which protect the coastal environment by absorbing energy from wind, tide and wave action. Coastal sand dunes are the very least studied among the marine ecosystem and it has variety of microenvironments due to substrate mobility and physical processes (Jayaprakashvel *et al*., 2010). There is very little information regarding the use of PGPR from the sand dune samples. Therefore, the present study was undertaken to screen the PGPR strains isolated from the sand dune samples of Chennai coastal area for their plant growth promotion activities such as production of Indole Acetic Acid (IAA) and solubilization of phosphate. The potential strains were also tested for their efficacy in enhancing the seedling growth of some crop plants.

## MATERIALS AND METHODS

**Isolation of rhizobacteria:** Totally eighteen different rhizosphere soil samples of sand dune plants, belongs to the genus *Ipomoea* sp. were collected from Chennai coastal area. For isolation of rhizobacteria, pour plate technique was followed, Each 10 g of sample was suspended in 90 mL of 50% aged sea water blank and the flasks were shaken for 10 min on a rotary shaker. They were serially diluted up to $10^{-4}$, 1 mL of sample from $10^{-4}$ and $10^{-5}$ were poured in petri plates after that 20 mL of sterile King's B medium (Peptone-20 g, Glycerol-20 mL, Dipotassium hydrogen phosphate-20 g, Magnesium sulphate-15 g, Agar-20 g, Distilled water-1000 mL, pH-7.2±0.2) was poured and the plates were kept for incubation for 24 h. After incubation period the bacterial colonies were counted and the morphologically different colonies were pure cultured and stored for further study.

**Screening for indole-3-acetic acid production:** The production of indole-3-acetic acid (IAA) was determined for all the 46 strains by following the method of Bric *et al*. (1991). A loop full of each purified bacterial strain was inoculated in to sterile test tubes containing 3 mL of nutrient broth medium (supplemented with 0.01% L-tryphtophan) and were incubated in environmental shaker at 37°C about 180 rpm for overnight. After incubation period about 1.5 mL of each culture was centrifuged at 10,000 rpm for five minutes at 4°C in a micro centrifuge. To this supernatants, 100 µL of 10 mM ortho phosphoric acid was added and they were mixed well by using vortex mixture. Formation of pink color indicates the presence of IAA in the culture filtrate.

**Screening for phosphate solubilization:** The positive strains of IAA producers were selected and they were screened for phosphate solubilization activity. For this, (Pikovskaya, 1948) method was followed. The sterile Pikovskya's medium (Tri calcium phosphate-5 g, D-glucose-10 g, Ammonium sulphate-0.5 g, Potassium chloride-0.2 g, Yeast extract-0.5 g, Manganese sulphate-0.001, Ferrous sulphate-0.001, Magnesium sulphate-0.2 g, Agar-15 g, Distilled water-1000 mL, pH-7.5±0.2) was poured in to petriplates and the selected strains were spot inoculated after solidification and they were kept for incubation for five days at room temperature. After incubation period the halo zone around the colonies indicates the ability of the bacterial strain to solubilise phosphate.

**Effect of PGPR from coastal sand dunes on seed germination of crop plants:** Green gram and black gram were used for the seed germination study. A total of six strains, all have the ability to solubilize phosphate and to produce indole acetic acid, were inoculated into sterile nutrient broth in 6 test tubes, respectively. After incubation period of 24 h at room temperature, the 25 numbers of green gram and black gram seeds were added into the test tubes containing 10 mL of broth culture and 0.3 mL glycerin also added and kept for 2 h for bacterization of seeds. After that, the seeds were placed in tissue paper with respective strain number coded. A control (non-bacterized) for each seed was also placed. Under humid condition, the seed germination was occurred. After germination, the percentages of germination for all the two seeds were recorded by the following formula:

$$\text{Seed germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds}} \times 100$$

**Effect of PGPR from coastal sand dunes on seedling growth of crop plants:** For seedling growth promotion, the green gram and black gram seeds were used. Three types of seed treatments, viz., seed bacterization, soil drenching and Mixed treatments (soil drenching and seed bacterization) were followed by using both sterilized and unsterilized garden red soil. Both sterilized and unsterilized soils were individually filled in plastic cups (150 g each). In seed bacterization treatment, both the seeds (each 25 numbers) were directly soaked in 10 mL of broth culture of respective bacteria for 2 h in the presence of 0.3 mL glycerin as binder. For soil drenching treatment, 10 mL of broth cultures of each of the bacterials strains was directly drenched in the sterilized and unsterilized soil kept in plastic containers and later in each of the cups 5 seeds were placed. In the mixed treatment, bacterized seeds were placed in soils drenched with bacterial

cultures as stated above. Control cups have received 10 mL water plus 0.3 mL glycerin as treatment. In all the treatments, 5 seeds were placed and kept in humid condition and watered daily with sterile distilled water for 7 days. After 7 days, shoot and root length were measured and Seedling vigor was determined using the following formulae:

Seedling vigor = (shoot length+root length)×germination percentage

The potential strain was identified using Bergy's bacteriological manual by biochemical methods.

## RESULTS AND DISCUSSION

The rhizosphere soil of the plant is known to be favored ecological niche for soil microorganisms due to rich nutrient availability so that associative bacteria populated its aerial roots 30 times more densely than its substrate roots (Tsavkelova *et al*., 2004). The accurate mechanism of PGPR to stimulate plant growth promotion is not clearly established, although, several hypotheses such as production of phytohormones, suppression of deleterious orgamisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved (Lalande *et al*., 1989; Liu *et al*., 1992; Glick *et al*., 1995; Bowen and Rovira, 1999) and this beneficial effects of PGPR leads to shifts in the microbial ecology of the rhizosphere also (Kloepper and Schroth, 1981).

In this present study, the abundance of rhizobacterial population of sand dune plant *Ipomoea* sp. in Kanathur, Chennai Coast was ranged from $4.4\times10^{6}$ -$7.5\times10^{7}$ CFU $g^{-1}$ in King's B medium (Fig. 1). On the basis of morphological characteristics, a total of 46 soil isolates were labeled as AMET 1101-AMET 1106 and AMET 1113-AMET 1152. IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. Assessing the IAA production for the 46 strains, 18 showed positive. Regarding IAA productions, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1995) and also can vary among different species and strains and it is also influenced by culture condition, growth stage and substrate availability (Mirza *et al*., 2001).

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants (Pradhan and Sukla, 2006). In this present study, totally 18 strains, those showed positive results for IAA, they were screened for phosphate solubilization assay, six

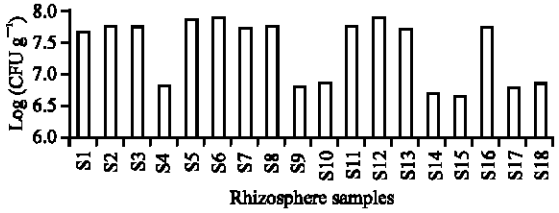


Fig. 1: Abundance of rhizobacterial population in sand dunes of Chennai coast

Table 1: Effect of PGPR from coastal sand dunes on seed germination of crop plants

| | Seed germination (%) | |
|---|---|---|
| Strain No. | Black gram | Green gram |
| AMET1101 | 86.67 | 93.33 |
| AMET1104 | 80.00 | 86.67 |
| AMET1124 | 73.33 | 86.67 |
| AMET1135 | 86.67 | 66.67 |
| AMET1136 | 86.67 | 100 |
| AMET1148 | 93.33 | 100 |

strains named AMET1101, AMET1104, AMET1124, AMET1135, AMET1136 and AMET1148. In comparison to non-rhizospheric soil, a considerably higher concentration of phosphate-solubilizing bacteria is commonly found in the Rhizosphere (Raghu and MacRae, 1966).

These six strains were also screened for their effect on seed germination. Of these two strains AMET1136, AMET1148 showed remarkable increase in the seed germination of black gram and green gram (Table 1). Plant growth promotion of Black gram and Green gram by these two effective strains, AMET1136 and AMET1148 was checked using three types of seed treatments such as seed bacterization, soil drenching and mixed (seed+soil) treatment in two types of soil such as sterilized and unsterilized. After 7 days of plant growth in both type of sterilized and unsterilized soil, shoot and root length was measured for both black gram and green gram from which the seedling vigor was determined (Table 2). It was shown that both black gram and green gram showed better growth in all the three types of treatments than the control and further mixed treatment had better growth promotion followed by seed bacterization and soil drenching. This result was observed in both sterilized and unsterilized soil and AMET1148 showed better results than the AMET1136. Dominated and the potential strain AMET1148 also identified as *Pseudomonas* sp. the families like Pseudomonadaceae, Enterobacteriaceae, lavobacteriaceae, Burkholderiaceae, Xanthomonadaceae and Bacillaceae are well known plant-associated bacteria

Table 2: Effect of PGPR from coastal sand dunes on seedling growth of crop plants

| | Green gram | | | | Black gram | | | |
|---|---|---|---|---|---|---|---|---|
| Treatments | Shoot length (cm) | Root length (cm) | Germination (%) | Seedling vigor | Shoot length (cm) | Root length (cm) | Germination (%) | Seedling vigor |
| **Unsterlized soil samples** | | | | | | | | |
| Control | 14.06 | 3.5 | 93.33 | 1638.87 | 7.5 | 1.53 | 93.33 | 842.76 |
| AMET1136 seed bacterization | 16.5 | 3.46 | 93.33 | 1862.86 | 12.46 | 4.53 | 93.33 | 1585.67 |
| AMET1136 soil drenching | 14.2 | 3 | 93.33 | 1605.27 | 10.13 | 4.16 | 93.33 | 1333.68 |
| AMET1136 mixed treatment | 18.6 | 3.5 | 93.33 | 2062.59 | 14.56 | 4.6 | 93.33 | 1788.20 |
| AMET1148 seed bacterization | 18.3 | 5.03 | 93.33 | 2177.38 | 15.43 | 5.06 | 93.33 | 1912.33 |
| AMET1148 soil drenching | 16.9 | 4.1 | 93.33 | 1959.93 | 14.1 | 4.56 | 93.33 | 1741.53 |
| AMET1148 mixed treatment | 19.4 | 4.96 | 93.33 | 2273.52 | 17.23 | 4.03 | 93.33 | 1984.19 |
| **Sterilized soil samples** | | | | | | | | |
| Control | 7.5 | 3.46 | 93.33 | 1022.89 | 9.53 | 5.53 | 93.33 | 1405.54 |
| AMET1136 seed bacterization | 18 | 4.53 | 93.33 | 2102.72 | 12.96 | 7.03 | 93.33 | 1865.66 |
| AMET1136 soil drenching | 15.96 | 4.26 | 93.33 | 1887.13 | 12.16 | 5.1 | 93.33 | 1610.87 |
| AMET1136 mixed treatment | 19.03 | 5.5 | 93.33 | 2289.38 | 17.06 | 5.2 | 93.33 | 2077.52 |
| AMET1148 seed bacterization | 19.5 | 5.03 | 93.33 | 2289.38 | 16.46 | 5.26 | 93.33 | 2027.12 |
| AMET1148 soil drenching | 17.6 | 6.9 | 93.33 | 2286.58 | 15.13 | 4.16 | 93.33 | 1800.33 |
| AMET1148 mixed treatment | 20.46 | 5.13 | 93.33 | 2388.31 | 18.23 | 5.73 | 93.33 | 2236.18 |

(Garbeva *et al*., 2001; Halda-Alija, 2003; Loiret *et al*., 2004; Park *et al*., 2005). Fluorescent pseudomonads occur commonly in the rhizosphere of plants and they are an important functional group of beneficial bacteria for the control of soil borne plant pathogens (Ellis *et al*., 2000).

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