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Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil

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KEYWORDS

Plant growth promoting rhizobacteria (PGPR), Indole acetic acid (IAA), Ammonia, Catalase and Heavy metals

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ABSTRACT

Plant growth promoting rhizobacteria have been identified in influencing the growth and yield of many plants. The effects of PGPR on plant growth can be mediated by direct or indirect mechanisms. In search of efficient PGPR strains with multiple activities, microbial isolates belonging to *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Azospirillium* spp, *Phosphobacteria* spp, *Glucanacetobacter* spp *Aspergillus niger* and *Penicillium* spp were isolated from different sources (Rice field, Mangroves and Effluent soil) of Cuddalore district. These test isolates were biochemically characterized and screened *in vitro* for their plant growth promoting traits like production of Indole acetic acid (IAA), ammonia, HCN and catalase production. All the isolates were able to produce IAA. Production of ammonia was commonly detected in all the isolates. All the test isolates were positive for catalase but none of the isolates produced HCN. On the basis of multiple plant growth promoting activities, the isolates were evaluated for their heavy metal tolerance. Among the isolates used on the heavy metals (Iron, Zinc, Lead, Magnesium and Copper), the effluent isolates were more tolerant to heavy metal and more tolerance were seen on iron metal. Tolerance to heavy metals was observed less frequently in *Azospirillium* spp, *Phosphobacteria* spp and *Glucanacetobacter* spp. The isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. Further rhizobacteria tolerant to multiple heavy metals exhibited a couple of PGP activities.

Introduction

In last few decades a large array of bacteria including species of *Pseudomonas, Azospirillium , Arthrobacteria, Klebsiella, Enterobacter, Alcaligenes, Arthrobacteria, Bacillus and Serratia* have been reported to enhance plant growth (Okon and Labandera – Gonzalez – 2000). The direct promotion by PGPR entails either providing the plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. The effects of PGPR on plant growth can be mediated by direct or indirect mechanisms (Glick, 2003). The direct effects have been most commonly attributed to the production of plant hormones such as auxins, gibberellins fixed nitrogen. These PGPR also affect growth by indirect mechanisms such as suppression of bacterial, fungal and nematode pathogens by production of sideophores, HCN, ammonia, antibiotics, and volatile metabolites etc by competition with the pathogen for nutrients or colonization space.

The exact mechanism by which PGPR plant growth are not fully understood but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 2005) (ii) asymbiotic N_2 fixation (Boddey and Dobereiner, 2000) (iii) antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 2006), antibiotics and cyanide (Flaishman *et al.*, 2001) (iv) solubilization of mineral phosphates and other nutrients (De Freitas *et al.*, 2007). Most popular bacteria studied and exploited as biocontrol agent includes the species of *Pseudomonas flurosence and Bacillus* spp. Some PGPR may promote plant growth indirectly by affecting symbiotic N_2 fixation, nodulation or nodule occupancy (Fuhrmann and Wollum, 2007).

The variability in the performance of PGPR may be due to various environment factors that mat affect their growth and exert their effects on plant. The environmental factors include climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil. To achieve the maximum growth promoting interaction between PGPR and nursery seedlings it is important to discover how the rhizobacteria exerting their effects on plant and whether the effects are altered by various environmental factors, including the presence of other micro organisms. Therefore, it is necessary to develop efficient strains in field conditions. One possible approach is to explore soil microbial diversity for PGPR having PGP activities and well adapted to particular soil environment (Joseph *et al.*, 2007).

Materials and Methods

Isolation of rhizobacteria

The rhizospheric soils were collected from Rice field, Mangrove soil and Effluent contaminated soil in Cuddalore district. All the microbial strains were isolated on their respective media; *Bacillus* spp on Nutrient agar, *Pseudomonas* spp on King's B agar, *Azotobacter* on Jensen's medium, *Azospirillium* spp on Nitrogen free base medium, *Phosphobacteria* spp on Pikvoskys medium, *Glucanacetobacter* spp on LGI medium, *Aspergillus niger and Penicillium* spp on Rose Bengal agar. Bacterial and fungal cultures were maintained on respective slants.

Biochemical characterization of rhizobacteria

Selected isolates of *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Azospirillium* spp, *Phosphobacteria* spp, *Glucanacetobacter* spp, *Aspergillus niger and Penicillium* spp were biochemically characterized by Gram's reaction and LPCB staining, IMViC test, Oxidase test, H_2S production, NO_2 reduction, Starch and Gelatine hydrolysis as per the standard methods (Cappuccino and Sherman, 2005).

Characterization of rhizobacteria of PGP traits

Production of Indole acetic acid

Indole acetic acid (IAA) production was detected as described by (Brick *et al.*, 2004). Bacterial and fungal cultures were grown for 72 hr (*Azotobacter* spp, *Azospirillium* spp, *Phosphobacteria* spp, *Glucanacetobacter* spp, *Aspergillus niger* and *Penicillium* spp) and 48 hours (*Bacillus* spp and *Pseudomonas* spp) on their respective media at 37^{0}C and 28^{0}C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2ml) was mixed with two drops of orthophosphoric acid and 4ml of the salkowski reagent (50 ml, 35% of perchloric acid, 1ml 0.5 m $FeCl_3$ solution). Development of pink colour indicates IAA production.

Production of ammonia

Bacterial and Fungal isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10ml peptone water in each tube and incubated for 48- 72h at 37^{0}C and 28^{0}C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive for ammonia production (Cappuccino and Sherman, 2005).

Production of HCN and Catalase

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lorck (2004). Briefly, Nutrient broth and RBA broth was amended with 4.4g glycine/l bacteria and fungi were streaked on modified agar plate. A Whatman filter paper no.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated for HCN production. Bacterial and fungal cultures were grown in a Nutrient agar and RBA medium for 24-72 hr at 37^{0}C and 28^{0}C. The cultures were mixed with appropriate amount of H_2O_2 on a glass slide to observe the evolution of oxygen.

Heavy metal tolerance

The selected bacterial and fungal strains were tested for their resistance to heavy metals by agar dilution method (Cervantes *et al.*, 2006). Freshly prepared agar plates were amended with various soluble heavy metals namely Fe, Mg, Cu, Pb and Zn at various concentrations ranging from 50 to 350 µg/ml which was inoculated with overnight grown cultures. Heavy metal tolerance was determined by the appearance of bacterial and fungal growth after incubating the plates at room temperature.

Result and Discussion

In the present investigation *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Azospirillium* spp, *Phosphobacteria* spp, *Glucanacetobacter* spp, *Aspergillus niger* and *Penicillium* spp were screened from different sources like Rice field, Mangrove and Effluent contaminated soil for *in vitro* PGP activites (Table 1). Screening results of PGP traits are depicted in Table 2. IAA production was shown in all the isolates of *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp, *Azospirillium* spp, *Phosphobacteria* spp, *Glucanoacetobacter* spp *Aspergillus niger* and *Penicillium* spp in (100%). Ammonia production was detected in 95% of the isolates. Production of catalase was exhibited by all the isolates of rhizobacteria. However, production of HCN was not detected in rhizobacterial isolates. Catalase activity exhibited by the microbial strains may be potentially very advantageous.

Among all the rhizobacteria studied, *Bacillus* spp, *Pseudomonas* spp, *Aspergillus* spp and *Penicillium* spp were tolerant to all the heavy metals (Fe, Zn, Pb, Mg and Cu). However the other isolates were susceptible to higher level of heavy metals. Majority of the isolates were less tolerant to Iron, *Bacillus* spp (110), *Pseudomonas* spp (200), *Azotobacter* spp (120), *Phosphobacteria* spp(60), *Glucanoacetobacter* spp (60), *Aspergillus* spp (60) and *Penicillium* spp (70) (Table 3). However only few isolates were able to tolerate higher metal concentration. Tolerance to heavy metals was observed less frequently in *Azotobacter* spp, *Azospirillium* spp, *Phosphobacteria* spp and *Glucanacetobacter* spp. None of these isolates were tolerant to high level of metal concentration.

Table: 1 Isolates from various sources

Isolate	Rice field	Mangrove Soil	Effluent Soil
Bacillus spp	+	+	+
Pseudomonas spp	+	+	+
Azotobacter spp	+	+	+
Azospirillium spp	+	+	-
Phosphobacteria spp	+	+	+
Glucanacetobacter spp	-	-	+
Aspergillus niger	+	+	+
Penicillium spp	+	+	+

(+)- positive (-)- negative

Table: 2 Plant growth promoting characteristics of rhizobacterial isolates

Isolate	No of isolate	IAA production	Ammonia production	Catalase
Bacillus spp	3	100	95.0	100
Pseudomonas spp	3	100	94.2	100
Azotobacter spp	3	100	45.0	100
Azospirillium spp	3	100	40.0	100
Phosphobacteria spp	3	100	55.2	100
Glucanacetobacter spp	3	100	50.6	100
Aspergillus niger	3	100	90.5	100
Penicillium spp	3	100	85.6	100

Table 3: Concentration which inhibited Microbial growth

Isolate	Resistance	Inhibitionof Microbial growth (µg/ml)		
Bacillus spp	Iron	110	180	190
	Zinc	140	150	170
	Lead	150	150	160
	Magnesium	180	180	180

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	Copper	180	180	180
Pseudomonas spp	Iron	200	260	290
	Zinc	250	250	260
	Lead	260	260	260
	Magnesium	280	260	280
	Copper	300	260	260
Azotobacter spp	Iron	120	160	180
	Zinc	130	140	150
	Lead	140	150	150
	Magnesium	140	140	140
	Copper	150	160	170
Glucanoacetobacter spp	Iron	60	-	-
	Zinc	80	-	-
	Lead	90	-	-
	Magnesium	90	-	-
	Copper	110	-	-
Phosphobacteria spp	Iron	60	80	90
	Zinc	60	80	90
	Lead	70	60	70
	Magnesium	80	70	70
	Copper	80	80	70
Azospirillium spp	Iron	-	90	90
	Zinc	-	80	90
	Lead	-	70	70
	Magnesium	-	60	70
	Copper	-	60	60
Penicillium spp	Iron	70	160	170
	Zinc	90	140	160
	Lead	100	110	130
	Magnesium	120	120	140
	Copper	130	140	150
Aspergillus niger	Iron	60	170	170
	Zinc	80	140	150
	Lead	90	130	140
	Magnesium	120	120	130
	Copper	150	140	150

The organic content in soil samples was considered as one of the key determinants driving the microbial community structure (Zhou *et al*., 2002). However, reports on plant-dependent rhizosphere effect on microbial community functions are limited. Plant roots influence soil borne microbial communities *via* several mechanisms, including excretion of organic compounds, competition for nutrients and providing solid surface for attachment. Any microbial utilization in agriculture requires an evaluation of the environmental risks associated with the introduction of indigenous or non- indigenous microorganisms into the plant rhizosphere as well as an assessment of the most suitable conditions for effective and successful establishment of the PGPR inoculation in the rhizosphere of the host plant. Furthermore, it is known that some PGPR strains are able to express multiple beneficial functions (Kloepper and Schrot, 2008).

Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals. Burd *et al* (2004) found that by decreasing the heavy metal toxicity, PGPR increases plant growth. The selection of microorganisms both metal tolerant and efficient in producing PGPR compounds can be useful to speed up the recolonization of the plant rhizosphere in polluted soils. Heavy metals, at higher concentration are toxic to cells and may cause cell death by interacting with nucleic acids and enzyme active site. In addition to these traits, plant growth promoting bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizospheric soil. Unfortunately, the interaction between associative PGPR and plant can be unstable. The good results obtained *in vitro* cannot always be dependably reproduced under field conditions. It is expected that inoculation with rhizobacteria containing PGP characteristics consequently promote root and shoot growth promoting (PGP) traits on soil-plant system is needed to uncover their efficacy as effective PGPR.

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