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Isolation and characterization of plant growth promoting rhizobacteria (pgpr) from the rhizosphere of *coleus forskohlii* grown soil

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ABSTRACT

Among the 30 bacterial isolates were obtained from *Coleus forskohlii* rhizospheric soil of Perambalur and Salem districts in Tamil Nadu. All the isolates were identified as *Azospirillum* spp., *Bacillus* spp., *Pseudomonas* spp., and *Azotobacter* spp. These bacterial strains were tested on morphological, biochemical and screened for their direct growth promoting activities (IAA production, production of Ammonia and Phosphate solubilization) and indirect growth promoting activities (HCN production, Siderophore production). The results obtained showed that among the 30 isolates of Perambalur and Salem districts (P1-P15) and (S1-S15) of ranged from (4.00-9.22x10^6 and 4.66-10.00x10^6) of *Azospirillum* spp., (3.00-7.66x10^6 and 3.88-8.00x10^6) of *Bacillus* spp., (4.66-12.00 and 4.88-13.00) of *Pseudomonas* spp., and (2.22-8.00 and 3.66-9.00) of *Azotobacter* spp. The IAA production of *Pseudomonas* spp. (83%), *Azospirillum* spp (75%), *Azotobacter* spp. (60%) and *Bacillus* spp. (30%). Ammonium production of the isolates, *Bacillus* spp. (96%), *Pseudomonas* spp. (92%), *Azospirillum* spp. (65%) and *Azotobacter* spp. (50%). The highest IAA production of *Pseudomonas fluorescens* (PPf-1) 7.40 µg/ml and *Pseudomonas fluorescens*(SPf-1) 7.60 µg/ml followed by other isolates produced from Perambalur and Salem district. Phosphate solubilization of the isolates, *Bacillus* spp.(83%), *Azotobacter* spp. (68.47), *Pseudomonas* spp. (60.56%) and *Azospirillum* spp. (55%). The siderophore and HCN production produced by all the isolates make it suitable for further investigation of pot and field trials by *Coleus forskohlii* cultivation.

INTRODUCTION

Coleus forkohlii is an important traditional ayurvadic herb that has been a part of Indian medicine for centuries. In the 1970s, researcher isolated a chemically active ingredient in the herb and called it forskolin. It is mostly cultivated in Tamil Nadu and Karnataka. Chemically it is a plant rich in alkaloids which are considered to a have probability of influence on the biological systems. *Coleus* is part of the mint family of plants and has long been cultivated in India, Thailand and parts of south East Asia as a spice and as a condiment for heart ailments and stomach crops. In India, the major medicinal species of *Coleus* is the tuberous *Coleus forskohlii, Coleus amboinicus, Coleus blumei, Coleus malabaricus* and *Coleus scutellaroides* are other species and are mainly used to treat dysentery and digestive disorders (De Souza, 2002).

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and or indirectly. In last few decades a large array of bacteria including species of *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcalisens, Arthobacter, Burkholderia, Bacillus* and *Serratia* have reported to enhance plant growth (Kloepper *et al.*, 1989; Okon and Laban-dera-Gonzalez, 1994; Glick, 1995). The direct promotion by PGPR either providing the plant with a plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment.

The exact mechanisms by which PGPR promote 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, cytokinines and ethylene (Arshad and Frankenberger, 1993; Glick, 1995), (ii) asymbiotic N_2 fixation (Boddey and Dobereiner, 1995), (iii) antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan *et al.*, 1992) and cyanide (Flaishman *et al.*,

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1996), (iv) solubilization of mineral phosphates and other nutrients (De Freitas *et al.*, 1997; Gaur, 1990). Most popular bacteria studied and exploited as biocontrol agent includes the species of fluorescent *Pseudomonas* and *Bacillus*.

In addition to these traits, plant growth promoting bacterial strains must be rhizospheric competent, able to survive and colonize in the rhizospheric soil (Cattelan *et al.,* 1999). 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 (Bent *et al.,* 2001).

Therefore, it is necessary to develop efficient strains in field conditions. One possible approach is to explore soil microbial diversity for PGPR having combination of PGP activities and well adapted to particular soil environment. So keeping in view the above constrains, the present study was designed to isolation and characterization of plant growth promoting rhizobacteria were isolated (*Azospirillum, Bacillus, Pseudomonas* and *Azotobacter*) from commercially grown areas of *Coleus forskohlii*.

MATERIAL AND METHODS

Isolation of Rhizobacteria

The thirty rhizospheric soil samples were collected from commercially grown *Coleus forskohlii* from Perambalur and Salem districts of Tamil Nadu. All the bacterial strains were isolated on their respective media; *Azospirillum* was on Nitrogen free malic acid medium (Nfb) Dobereiner and Day (1957), *Bacillus* (phosphobacteria) on Pikovskaya's agar medium (Gaur,1990), *Pseudomonas* on King's B medium (King's *et al.*, 1954) and *Azotobacter* on Waksman base No.77 medium (Allen, 1953). The bacterial cultures were maintained on the respective slants. The bacterial isolates were designated as Perambalur (P1-P15) and Salem districts (S1-S15) and the species level identification of all rhizobacteria of Perambalur, *Azospirillum* (PAzs 1 to PAzs 15), *Bacillus* (PB 1 to PB 15), *Pseudomonas* (PPf 1 to PPf 15) and *Azotobacter* (PAzt 1 to PAzt 15) and Salem distict, *Azospirillum* (SAzs 1 to SAzs 15), *Bacillus* (SB 1 to SB 15), *Pseudomonas* (SPf 1 to SPf 15) and *Azotobacter* (SAzt 1 to SAzt 15).

Biochemical characterization of PGPR strains

Selected thirty isolates of *Azospirillum, Bacillus, Pseudomonas* and *Azotobacter* were biochemically carried out. The following biochemical test were carried out separately for *Azospirillum* (pellicle formation, cell shape, motility, gram reaction, acid production from glucose, different carbon sources- malate,succinate, lactose, mannitol, α-ketoglutarate, biotin requirement, nitrate reductase, nitrite reductase activity), *Bacillus* (gram reaction, motility, spore staining, acid production, hydrolysis of starch, hydrolysis of gelatin, casein hydrolysis, catalase test, oxidase test, indole test, methyl test, urease test, VP test, utilization of citrate), *Pseudomonas* (gram reaction, motility, starch hydrolysis, hydrolysis of gelatin, egg yolk reaction, pigment production, casein hydrolysis, catalase test, oxidase test, indole test, methyl red, citrate utilization test, H_2S production), and *Azotobacter* (gram reaction, motility, pigmentation, catalase test, oxidase test, indole test, utilization of citrate, utilization of carbon sources, etc.,) as per the standard methods (Cappuccino and Sherman, 1992).

In vitro screening of bacterial isolates for their plant growth promoting (PGP) activities

Assay for indoleacetic acid (IAA) production

IAA production was detected by the modified method as described by Brick *et al.* (1991). Quantitative analysis of IAA was performed using the method of Loper and Scroth (1986) at 100% concentration of tryptophan (100 µg/ml). Bacterial cultures were grown for 72 h (*Azotobacter* and *Azospirillum*) and 48 h (*Pseudomonas* and *Bacillus*) on their respective media at 28±2°C. Fully grown cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski reagent (50 ml, 35% of perchloric acid, 1ml 0.5 M $FeCl_3$ solution). Development of pink colour indicates IAA production. Optical density was taken at 530 nm with the help of spectrophotometer Spectronic 20 D^+. Concentration of IAA produced by cultures was measured with the help of standard graph of lAA (Hi-media) obtained in the range of 10-100 µg/ml.

Production of NH_3

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 48-72 h at 28±2 °C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Production of HCN

All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lorck (1948). Briefly, nutrient broth was amended with 4.4 g glycine/l and bacteria 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 in the top of the plate. Plates were sealed with parafilm and incubated at 28 ± 2°C for 4 days. Development of orange to red colour indicated HCN production.

Siderophore production

Bacterial isolates were assayed for siderophores production on the Chrome azurol S agar medium (Sigma, Ltd.) described by Schwyn and Neilands (1987). Chrome azurol S agar plates were prepared and divided into equal sectors and spot inoculated with test organism (10µl of 10^6CFU/ml) and incubated at 28±2°C for 48-72 h. Development of yellow-orange halo around the growth was considered as positive for siderophore production.

Phosphate solubilization by test bacteria

All isolates were first screened on Pikovskaya's agar plates for phosphate solubilization as described by Gaur (1990). Quantitative analysis of solubilization of tricalcium phosphate in liquid medium was made as described by King (1932). Briefly, the test isolates were inoculated in 25 ml Pikovskaya's broth and incubated for 4 days at 28 ± 2 °C. The bacterial cultures were centrifuged at 15,000 rpm for 30min. Supernatant (1ml) was mixed with 10 ml of chloromolibidic acid and the volume was made up to 45 ml with distilled water. Cholorostannous acid (0.25 ml) was added and the volume was made up to 50 ml with distilled water. The absorbance of the developing blue colour was read at 600 nm. The amount of soluble phosphorus was detected from the standard curve of KH_2PO_4.

RESULTS

Isolation , Biochemical characterization and bacterial population

The plant growth promoting rhizobacterial population in the rhizosphere of *Coleus forskohlii* is given in Table-1. The PGPR population (Cfu g^{-1} of oven dry soil) Perambalur and Salem districts of ranged from (4.00-9.22x10^6 and 4.66 -10.00 x10^6) of *Azospirillum* spp., (3.00-7.66 x10^6 and 3.88-8.00 x10^6) of *Bacillus* spp., (4.66-12.00 and 4.88-13.00 x10^6) of *Pseudomonas* spp., and (2.22-8.00 x10^6 and 3.66-9.00 x10^6) of *Azotobacter* spp.

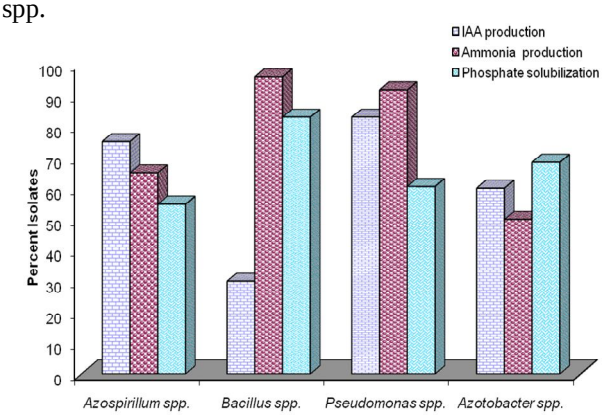

Fig. 1 Direct PGP activities of test isolates

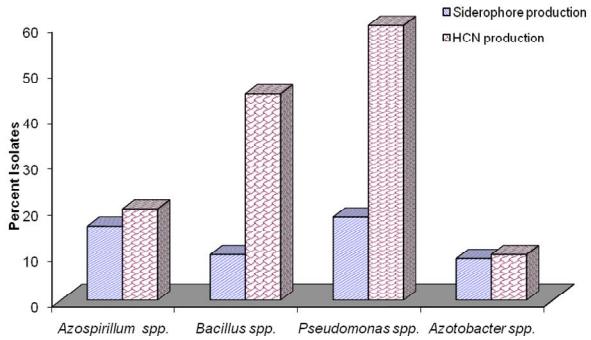

Fig. 2 Indirect PGP activities of test isolates

The population of *Pseudomonas* dominated in the rhizosphere. On the basis of cultural, morphological and biochemical characteristics. The species level identification of thirty isolates were identified into *Azospirillum, Bacillus, Pseudomonas* and *Azotobacter* were described. General characteristics of the isolates are illustrated in (Table-2, 3, 4 and 5). Out of 15 isolates (*Azospirillum* spp.) of Perambalur districts 10 isolates belongs to *Azospirillum lipoferum* and 5 isolates belongs to *Azospirillum brasilense* where as in Salem districts 7 isolates belongs to *Azospirillum lipoferum* and 8 isolates belongs to *Azospirillum brasilense.* The *Bacillus* spp. of Perambalur districts 7 isolates belongs to *Bacillus megaterium*, 5 isolates belongs to *Bacillus polymyxa*, 1 isolate belongs to *Bacillus subtilis* and 2 isolates belongs to *Bacillus cereus* where as in Salem districts 6 isolates belongs to *Bacillus megaterium*, 4 isolates belongs to *Bacillus polymyxa*, 3 isolates belongs to *Bacillus subtilis* and 2 isolates belongs to *Bacillus cereus.* The *Pseudomonas* spp. of Perambalur districts 9 isolates belongs to *Pseudomonas fluorescens*, 3 isolates belongs to *Pseudomonas putida* and 3 isolates of *Pseudomonas striata* where as in Salem districts 8 isolates belongs to *Pseudomonas fluorescens*, 3 isolates belongs to *Pseudomonas putida* and 4 isolates of *Pseudomonas striata*. The *Azotobacter* spp. of Perambalur districts 7 isolates belongs to *Azotobacter chroococcum*, 6 isolates belongs to *Azotobacter beijerinckii* and 2 isolates belongs to *Azotobacter vinelandii* where as in Salem districts 6 isolates belongs to *Azotobacter chroococcum*, 5 isolates belongs to *Azotobacter vinelandii* and 4 isolates belongs to *Azotobacter beijerinckii.*

Plant growth promoting traits of test isolates

In the present investigation 30 isolates of *Azospirillum* spp., *Bacillus* spp., *Pseudomonas* spp., and *Azotobacter* spp. were screened for *in vitro* PGP activities. Screening results of PGP traits are depicted in (Fig.1 & Fig.2). IAA production was shown in all the isolates of *Pseudomonas* (83%), followed by *Azospirillum* (75%), *Azotobacter* (60%) and *Bacillus* (30%). Ammonia production was detected in 96% of isolates of *Bacillus* followed by *Pseudomonas* (92%), *Azospirillum* (65%) and *Azotobacter* (50%). Phosphate solublization was detected in 83% of isolates of *Bacillus* followed by *Azotobacter* (68.47%), *Pseudomonas* (60.56%) and *Azospirillum* (55%). Production of siderophore was detected less frequently than other PGP characteristics. The isolates of *Pseudomonas* spp. were strong siderophore producers (18.22%) followed by *Azospirillum* spp. (16.22%), *Bacillus* spp. (10.00%) and *Azotobacter* spp. (9.00%). The production of HCN was detected for all cultures in less frequently. The *Pseudomonas* spp. were maximum produced (60%), followed by *Bacillus* spp. (45%), *Azospirillum* spp. (20%) and *Azotobacter* spp. (10%).

Quantitative assay of IAA production by PGPR strains

A total of 30 isolates of *Azospirillum* spp, *Bacillus* spp, *Pseudomonas* spp and *Azotobacter* spp. were tested for the quantitative estimation of IAA in the presence of

Table 1 Plant growth promoting rhizobacterial populations of *Coleus forskohlii* from commercially grown area

No. of Isolates	Population ($x10^6$ cfu g^{-1} of oven dry soil)			
	Azospirillum **(PAzs-1 toPAzs-15) and (SAzs-1 to Sazs-15)**	***Bacillus*** **(PB-1 to PB-15) and (SB-1 to SB-15)**	***Pseudomonas*** **(PPf-1 to PPf-15) and (SPf-1 to SPf-15)**	***Azotobacter*** **(PAzt-1toPAzt-15) and (SAzt-1toSAzt-15)**
Perambalore				
P1	9.22	7.66	12.00	8.00
P2	7.00	6.44	10.00	7.22
P3	5.66	6.00	11.22	7.00
P4	8.22	7.22	11.66	7.66
P5	6.00	6.88	9.66	5.44
P6	8.44	7.44	10.44	6.00
P7	7.88	6.22	8.22	6.22
P8	6.22	5.88	8.44	7.88
P9	5.44	5.22	7.66	6.66
P10	7.22	5.44	9.22	7.44
P11	4.88	4.00	9.44	4.66
P12	5.66	3.88	5.00	4.00
P13	8.66	7.00	10.22	6.88
P14	6.22	5.66	4.88	3.44
P15	4.00	3.00	4.66	2.22
Salem				
S1	10.00	8.00	13.00	9.00
S2	9.66	7.66	12.88	8.44
S3	9.44	6.88	10.00	7.22
S4	8.66	6.22	10.44	6.66
S5	8.22	6.44	12.22	8.22
S6	7.00	6.00	8.00	7.88
S7	8.00	5.44	9.66	6.00
S8	7.44	5.22	8.44	7.00
S9	7.22	6.88	4.44	6.88
S10	8.44	7.22	11.66	7.22
S11	6.88	5.88	5.66	6.66
S12	6.00	4.44	7.00	5.44
S13	5.88	5.66	6.22	4.66
S14	8.22	7.22	10.88	8.22
S15	4.66	3.88	4.88	3.66

100% of Tryptophan concentration. With no addition of tryptophan, production of IAA was not observed. With the addition of tryptophan 100 µg/ml the production of IAA was highest in Perambalur and Salem district sample isolates of florescent *Pseudomonas* spp. ranged from (5.00-7.00 µg/ml and 5.10-7.60 µg/ml) followed by *Azospirillum* spp. of (3.10-6.66 µg/ml and 3.25-6.80 µg/ml), *Azotobacter* spp. of (3.00-6.00 µg/ml and 3.30-6.30 µg/ml) and *Bacillus* spp. of (2.40-5.73 µg/ml and 2.50-5.80 µg/ml). The highest IAA production of *Pseudomonas fluorescens* (PPf-1) 7.40 µg/ml and *Pseudomonas fluorescens* (SPf-1) 7.60 µg/ml followed by other isolates produced from Perambalur and Salem district samples as depicted in Table-6.

DISCUSSION

Plant rhizosphere is known to be preferred ecological niche for various types of soil microorganisms due to rich nutrient availability. It has been assumed that inoculation with diazotrophic bacteria like *Rhizobium, Azotobacter* and *Azospirillum* enhanced the plant growth as a result of their ability to fix nitrogen. Growth promotion may be attributed to other mechanisms such as production of plant growth promoting hormones in the rhizosphere and other PGP activities (Kloepper *et al.,* 1988; Arshad and Frankenberger,1993; Glick,1995; Bhasan and Bashan, 2005).

In the present investigation revealed that the ubiquitous nature of bacteria with inconsistent population load as influenced by soil and environmental factors in the rhizosphere of *Coleus forskohlii* rhizosphere soils collected from 30 different locations of Perambalur and Salem districts of Tamil Nadu, were determined. The populations of *Azospirillum* ranged from (4.00-9.22x10^6 and 4.66-10.00 x10^6), *Bacillus* population of ranged from (3.00-7.66 x10^6 and 3.88-8.00 x10^6), *Pseudomonas* population of ranged from (4.66-12.00 x10^6 and 4.88-13.00 x10^6) and *Azotobacter* population of ranged from (2.22-8.00 x10^6 and 3.66-9.00 x10^6) of soil followed by others. The similar report done by Govinda Rao *et al.* (1987). Geetha (2003) and Karthikeyan *et al.* (2008) reported about the microbial population from various medicinal plants.

Out of 30 isolates of Perambalur and Salem districts belonging to 17 isolates of *Azospirillum lipoferum,* 13 isolates of *Bacillus megatreium,* and 17 isolates of *Pseudomonas fluorescens,* and 13 isolates of *Azotobacter chroococcum,* were screened *in vitro* for PGP activities. The potential of *Pseudomonas* strains to produce indole

Table 2 General characteristics of *Azospirillum* isolates obtained from the rhizosphere soil of *Coleus forskohlii* grown area

No.	Name of the Isolate	Subsurface pellicle formation in Nfb Semi-solid medium	Cell shape	Motility	Gram reaction	Acid production from glucose	Utilization of different carbon sources					Biotin requirement	Nitrite reductase activity	Nitrate reductase activity	Species identification
							Malate	**Succinate**	**Lactose**	**Mannitol**	**α-Keto lutarate**				
Perambalur															
1.	PAzs- 1	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
2.	PAzs- 2	+	Rod	+	- ve		+	+	+	+	+	+	+	+	*A. lipoferum*
3.	PAzs- 3	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
4.	PAzs- 4	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
5.	PAzs- 5	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
6.	PAzs- 6	+	Rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
7.	PAzs- 7	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
8.	PAzs- 8	+	Rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
9.	PAzs- 9	+	Curved rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
10.	PAzs- 10	+	Curved rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
11.	PAzs- 11	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
12.	PAzs- 12	+	Curved rod	+	- ve	-	+	+	+	+	+	+	+	-	*A. brasilense*
13.	PAzs- 13	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
14.	PAzs- 14	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
15.	PAzs- 15	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
Salem															
16.	SAzs- 1	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
17.	SAzs- 2	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
18.	SAzs- 3	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
19.	SAzs- 4	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
20.	SAzs- 5	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
21.	SAzs- 6	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
22.	SAzs- 7	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
23.	SAzs- 8	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
24.	SAzs- 9	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
25.	SAzs- 10	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
26.	SAzs- 11	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
27.	SAzs- 12	+	Rod	+	- ve	+	+	+	+	+	+	+	+	+	*A. lipoferum*
28.	SAzs- 13	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
29.	SAzs- 14	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*
30.	SAzs- 15	+	Curved rod	+	- ve	-	+	+	+	+	+	-	+	-	*A. brasilense*

(+) Showed positive growth; (-) Showed No growth

acetic acid under *in vitro* condition was reported. All the 30 isolates obtained in the present study were able to produce IAA. The IAA production was detected in all the test isolates of *Pseudomonas fluorescens* (83%), *Azospirillum lipoferum* (75%) and *Azotobacter chroococcum* (60%), followed by *Bacillus megatreium* (30%). High level of IAA production by *Pseudomonas* was recorded by other workers (Xie *et al.,* 1996). Our findings of IAA in *Azotobacter* isolates are in agreement with other workers (Gonzalez-Lopez *et al.*, 1986; Jagnow, 1987; Nieto and Frankenberger, 1989).

Another important trait of PGPR, that may indirectly influence the plant growth, is the production of ammonia. Mostly all the isolates were able to produce ammonia. However, ammonia production was observed less frequently in *Azospirillum* isolates. Phosphate solubilization was most frequently encountered by *Bacillus* isolates (83%), followed by other isolates. The siderophore production was detected among the 30 isolates of *Pseudomonas* spp. (18.22%) followed by *Azospirillum* spp. (16%), *Bacillus* spp. (10%) and *Azotobacter* spp. (9.00%). Siderophore chelates iron and other metals contribute to disease suppression by conferring a competitive advantages to biocontrol agents for the limited supply of essential trace minerals in natural habitats (Hofte *et al.,* 1992; Loper and Henkels, 1997). However, 45% and 60% isolates of *Bacillus* spp. and *Pseudomonas* spp. were detected positive for HCN production. Some of the above PGP traits, which may promote plant growth directly or indirectly or synergistically. Similar to our findings of multiple PGP activities among the PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored (Gupta *et al.,* 1998).

On the basis of preliminary screening, quantitative analysis of IAA production was made on 30 isolates (Perambalur and Salem district) *Azospirillum* spp, *Bacillus* spp, *Pseudomonas* spp and *Azotobacter* spp. There was an increase in the level of IAA with the concentration of tryptophan (100µg/ml). Similar trend of IAA production with increasing concentration of tryptophan was also reported by Barazani and Friedman (2000). The maximum IAA production isolates of *Pseudomonas* spp. of (5.00-7.40 µg/ml and 5.10-7.60 µg/ml) followed by *Azospirillum* spp. of (3.10-6.66 µg/ml and 3.25-6.80 µg/ml), *Azotobacter* spp. of (3.00-6.00 µg/ml and 3.30-6.30 µg/ml) and *Bacillus* spp. of (2.40-5.73 µg/ml and 2.50-5.80 µg/ml). Among the 30 PGPR isolates, *Pseudomonas* spp. recorded higher amount of

Sl. No.	Nmae of Isolate	Gram reaction	Motility	Spore staining	Acid production	Hydrolysis of starch	Hydrolysis of gelatin	Casein hydrolysis	Catalase test	Oxidase test	Indole test	Methyl test	Urease test	Voges-Proskauer test	Utilization of citrate	Species identification
Perambalur																
1.	PB-1	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*Bacillus megaterium*
2.	PB-2	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
3.	PB-3	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
4.	PB-4	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
5.	PB-5	+ve	+	+	+	+	+	-	+	+	-	+	-	+	+	*B. subtilis*
6.	PB-6	+ve	+	+	+	+	+	-	+	+	-	+	-	-	-	*B.cereus*
7.	PB-7	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
8.	PB-8	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
9.	PB-9	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
10.	PB-10	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
11.	PB-11	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
12.	PB-12	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
13.	PB-13	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
14.	PB-14	+ve	+	+	+	+	+	-	+	+	-	+	-	-	-	*B.cereus*
15.	PB-15	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
Salem																
16.	SB-1	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
17.	SB-2	+ve	+	+	+	+	+	-	+	+	-	+	-	-	-	*B.cereus*
18.	SB-3	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
19.	SB-4	+ve	+	+	+	+	+	-	+	+	-	+	-	+	+	*B. subtilis*
20.	SB-5	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
21.	SB-6	+ve	+	+	+	+	+	-	+	+	-	+	-	+	+	*B. subtilis*
22.	SB-7	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
23.	SB-8	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
24.	SB-9	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
25.	SB-10	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
26.	SB-11	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
27.	SB-12	+ve	+	+	+	+	+	-	+	+	-	+	-	-	-	*B.cereus*
28.	SB-13	+ve	+	+	+	+	+	-	+	+	+	+	-	-	+	*B. megaterium*
29.	SB-14	+ve	+	+	+	+	+	-	+	+	-	+	-	+	-	*B. polymyxa*
30.	SB-15	+ve	+	+	+	+	+	-	+	+	-	+	-	+	+	*B. subtilis*

(+) Showed positive growth; (-) Showed No growth

Table 4 Characterization of *Pseudomonas* isolates obtained from the rhizosphere soil of *Coleus forskohlii* grown area

Sl. No.	Nmae of Isolate	Gram reaction	Motility	Starch hydrolysis	Hydrolysis of gelatin	Egg yolk reaction	Pigment production	Casein hydrolysis	Catalase test	Oxidase test	Indole test	Methyl test	Citrate utilization test	H_2S Production	Species identification
	Perambalur														
1.	PPf-1	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*Pseudomonas fluorescens*
2.	PPf-2	- ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
3.	PPf-3	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
4.	PPf-4	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P.fluorescens*
5.	PPf-5	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
6.	PPf-6	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. putida*
7.	PPf-7	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. striata*
8.	PPf-8	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. striata*
9.	PPf-9	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
10.	PPf-10	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. putida*
11.	PPf-11	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
12.	PPf-12	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P.putida*
13.	PPf-13	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
14.	PPf-14	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
15.	PPf-15	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. striata*
	Salem														
16.	SPf-1	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
17.	SPf-2	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. striata*
18.	SPf-3	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
19.	SPf-4	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
20.	SPf-5	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
21.	SPf-6	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. striata*
22.	SPf-7	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. putida*
23.	SPf-8	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
24.	SPf-9	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
25.	SPf-10	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
26.	SPf-11	-ve	+	-	-	-	+	+	+	+	-	-	+	-	*P. striata*
27.	SPf-12	-ve	+	-	+	-	+	+	+	+	-	-	+	-	*P.fluorescens*
28.	SPf-13	-ve	+	-	+	+	+	+	+	+	-	-	+	-	*P. putida*
29.	SPf-14	-ve	+	-	+	+	+	+	+	+	-	-	+	-	*P. putida*
30.	**SPf-15**	**-ve**	**+**	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	**-**	**+**	**-**	***P. striata***

(+) Showed positive growth; (-) Showed No growth

Table 5 Characterization of *Azotobacter* isolates obtained from the rhizosphere soil of *Coleus forskohlii* grown area

Sl. No.	Nmae of Isolate	Gram reaction	Motility	Pigments		Catalase	Oxidase test	Indole test	Methyl test	Citrate utilization test	Utilization of different Carbon source		Species identification
				Water soluble	Water insoluble						Starch	Raffinose	
	Perambalur												
1.	PAzt-1	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*Azotobacter chroococcum*
2.	PAzt-2	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
3.	PAzt-3	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
4.	PAzt-4	-ve	+	+	Pale color	+	+	+	+	+	+	+	*A. vinelandii*
5.	PAzt-5	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
6.	PAzt-6	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
7.	PAzt-7	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
8.	PAzt-8	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
9.	PAzt-9	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
10.	PAzt-10	-ve	+	+	Pale color	+	+	+	+	+	+	+	*A. vinelandii*
11.	PAzt-11	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
12.	PAzt-12	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
13.	PAzt-13	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
14.	PAzt-14	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
15.	PAzt-15	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
	Salem												
16.	SAzt-1	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
17.	SAzt-2	-ve	+	+	Pale color	+	+	+	+	+	+	+	*A. vinelandii*
18.	SAzt-3	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
19.	SAzt-4	-ve	+	+	Pale color	+	+	+	+	+	+	+	*A. vinelandii*
20.	SAzt-5	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
21.	SAzt-6	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
22.	SAzt-7	-ve	+	+	Brown to black	+	-	+	+	+	-	+	*A. chroococcum*
23.	SAzt-8	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
24.	SAzt-9	-ve	+	+	Pale color	+	+	+	+	+	+	+	*A. vinelandii*
25.	SAzt-10	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
26.	SAzt-11	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
27.	SAzt-12	-ve	+	+	Pale color	+	+	+	+	+	+	+	*A. vinelandii*
28.	SAzt-13	-ve	-	+	Yellowish	-	+	+	+	+	-	-	*A. beijerinckii*
29.	SAzt-14	-ve	+	+	Brown to black	+	+	+	+	+	+	+	*A. chroococcum*
30.	SAzt-15	-ve	+	+	Pale color	+	+	+	+	+	+	+	*A. vinelandii*

(+) Showed positive growth; (-) Showed No growth

Table 6 Production of indole acetic acid (IAA) by rhizobacterial isolates grown on their respective medium*

No. of Isolates	IAA production at 100% tryptophan concentration (µg/ml)			
	Azospirillum **(PAzs-1 toPAzs-15) and (SAzs-1 to Sazs-15)**	***Bacillus*** **(PB-1 to PB-15) and (SB-1 to SB-15)**	***Pseudomonas*** **(PPf-1 to PPf-15) and (SPf-1 to SPf-15)**	***Azotobacter*** **(PAzt-1toPAzt-15) and (SAzt-1toSAzt-15)**
Perambalore				
P1	6.66	5.73	7.40	6.00
P2	5.97	4.87	7.10	5.10
P3	6.44	5.36	6.83	5.30
P4	6.00	3.25	6.00	5.93
P5	3.88	3.77	6.13	4.77
P6	6.38	2.83	7.40	4.30
P7	5.00	4.00	7.00	5.96
P8	4.00	3.53	7.93	4.00
P9	4.67	5.20	6.88	4.87
P10	5.28	3.60	6.50	5.25
P11	6.20	4.30	5.97	4.10
P12	3.66	3.00	7.15	5.50
P13	6.30	4.20	6.87	4.40
P14	5.37	3.10	5.55	5.37
P15	3.10	2.40	5.00	3.00
Salem				
S1	6.80	5.80	7.60	6.30
S2	5.93	4.82	6.96	5.47
S3	5.00	5.53	6.76	6.57
S4	4.67	4.97	7.12	5.73
S5	5.90	3.10	6.10	6.33
S6	6.00	5.00	7.20	5.80
S7	4.23	4.20	7.23	6.10
S8	6.77	4.38	7.00	6.00
S9	4.83	3.86	7.21	5.92
S10	6.10	5.66	6.90	5.27
S11	6.57	4.00	6.00	4.80
S12	3.83	3.53	6.77	6.20
S13	4.00	2.87	5.89	5.00
S14	6.20	3.30	6.52	4.00
S15	3.25	2.50	5.10	3.30

***** For *Azospirillum* Nfb media, *Bacillus* Pikovskaya's media, *Pseudomonas* King's B media, *Azotobacter* Waksman base

IAA than that of other isolates, which was followed by *Azospirillum* spp, *Azotobacter* spp. and *Bacillus* spp. The highest IAA production of *Pseudomonas fluorescens* (PPf-1) 7.40 µg/ml and *Pseudomonas fluorescens* (SPf-1) 7.60 µg/ml followed by other isolates produced from Perambalur and Salem district. The variation in the production of IAA by different isolates of PGPR and its related role on the plant growth promoting activity was earlier studied under *in vitro* conditions (Crozier and Arrude, 1988; Gopal, 2004).

In addition to these traits, plant growth promoting rhizobacterial strains must be rhizospheric component, able to survive and colonize in the rhizospheric soil. Unfortunately, the interaction between associative PGPR and plants can be unstable. The good results obtained *in vitro* cannot always be dependably reproduced under pot and field conditions. It is expected that inoculation with rhizobacteria containing PGP characteristics consequently promote root, shoot growth and yield. Further evaluation of the isolates exhibiting multiple plant growth promoting (PGP) traits on soil-plant system under pot and field conditions.

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