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Characterization of multiple plant growth promotion traits of *Pseudomonas aeruginosa* FP6, a potential stress tolerant biocontrol agent

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. Thus the present study focuses on the screening of effective PGPR isolate with multiple traits related to biocontrol of phytopathogenic fungi. In our study, a total of 51 bacterial isolates from the rhizosphere soil samples were isolated and screened for their antagonistic activity against wide range of phytopathogens. Bacterial antagonist showing highest percent and broad spectrum antagonism against fungal phytopathogens was selected and further identified as Pseudomonas aeruginosa FP6 on the basis of 16S rDNA gene sequence analysis. P. aeruginosa FP6 was screened for other plant growth promoting factors like phosphate solubilization, production of IAA, ammonia, siderophore and cell wall degrading enzyme activities- cellulase, chitinase and protease. The isolate was able to solubilize phosphate, produce IAA, siderophore, HCN, ammonia and biosurfactant. Study on effects of volatile and non-volatile antibiotic compounds on fungal phytopathogens inhibition showed volatile and diffusible metabolites as the major mechanism. The intrinsic antibiotic test showed that FP6 isolate was resistant to tetracycline, erythromycin, cotrimoxazole, rifampicin, ampicillin, cefepime and cefoxitin. Part of this study focused on the effect of NaCl, temperature, and pH on Pseudomonas aeruginosa FP6. Strain Pseudomonas aeruginosa FP6 was able to grow on up to 4.5 M NaCl, between 20 and 60°C and at pH 5–10. Inoculation of cowpea seeds with the P. aeruginosa FP6 significantly ($P < 0.05$) enhanced seed germination, seedling vigor index, plant height, and also fresh and dry weight in comparison with the control. Results from this study show the multifarious plant growth promoting activities of P. aeruginosa and suggests its potential use in developing a cost-effective eco-friendly multifunctional biofertilizer.

Key words: *Pseudomonas aeruginosa*, PGPR, IAA, volatile metabolites, diffusible metabolites.

INTRODUCTION

Plant pathogens affecting plant health are a major and chronic threat to food production worldwide. As agricultural production intensified over the past few decades, producers became more and more dependent on agrochemicals for crop protection. However, increasing use of chemicals has led to negative effects such as pathogen resistance to the antimicrobial agents and non target environmental impacts [1]. At present, the use of biological agents is becoming more popular as an alternative to chemical pesticides for improving crop yield in an integrated crop management system. In this regard, the use of Plant Growth Promoting Rhizobacteria (PGPR) has found a potential role in developing sustainable systems in crop production [2]. In the rhizosphere 2-5% of bacterial population is PGPR, they stimulate plant growth directly by nitrogen fixation, solubilization of nutrients, production of growth

hormones, 1-amino-cyclopropane-1- carboxylate (ACC) deaminase and indirectly by antagonizing pathogenic fungi by production of siderophores, chitinase, β-1,3-glucanase, antibiotics, fluorescent pigments, and cyanide [3].

Cowpea (*Vigna unguiculata*) is one of the important kharif pulse crops grown in India. It is a warm season crop, well adapted to many areas of the humid tropics and subtropical zones. It is grown throughout India for its long, green vegetable pods, seeds, and foliage for fodder [4]. Cowpea constitutes the cheapest source of dietary protein and energy in tropical regions.

The productivity of crops is greatly affected by various stress factors. Highly alkaline (pH greater than 8.0) soils tending to be high in sodium chloride, bicarbonate and borate, are often associated with high salinity. This reduces nitrogen fixation [5]. Stress factors reduce the ability of plants to absorb water, induce many metabolic changes causing rapid reduction in growth rate, similar to those caused by water stress [6]. In such soils, microorganisms stress tolerating organism will be of great importance.

Several researchers have reported that the influence of PGPR is sometimes crop-specific or niche-specific, or their benefits are limited due to the climatic variability and inconsistency of soil [7,8]. Further, understanding the mechanisms of biocontrol process is critical to the wider use for biocontrol methods. Therefore, the present study was directed towards the selection of a rhizospheric isolate exhibiting maximum PGPR traits and its efficacy for plant growth promotion and control of pathogens.

MATERIALS AND METHODS

Fungal strains

The Plant pathogenic fungal strains *Alternaria alternata* (OTA36), *A. brassicicola* (OCA1), *A. brassicae* (OCA3), *Collectotrichum gleosporioides* (OGC1) were kindly provided by Indian Institute of Horticultural Research Institute, Bangalore. *Phytopthora capisci* (MTCC98-01), *Rhizoctonia solani* (MTCC4633), *Fusarium oxysporum* (MTCC1755), *F. solani* (MTCC 1756) were procured from Microbial Type Culture Collection Centre (MTCC), IMTECH, Chandigarh. These plant phytopathogenic fungi were grown on potato dextrose agar at 28°C and stored on the same medium at 4°C.

Isolation of bacterial antagonist

One gram of each rhizospheric soil sample, collected from different locations in Bangalore, Karnataka was suspended in 10 ml of sterile water and vortexed for 45s. The sample was serially diluted and 100 µl of each dilution was plated onto glucose peptone agar medium and pure cultures were obtained by streaking three or four times in the fresh medium. Bacterial colonies showing prolific growth and having different morphological appearance were selected and stored at 4°C.

Screening for the antagonistic activity *in vitro*

The antagonistic activity of each selected bacterial isolate against the fungal pathogens was studied by dual culture test. A loopful of 48 hrs old culture was spotted in the centre of the potato dextrose agar plate and 6 mm disc of pre grown phytopathogenic fungi inoculated on both sides of the plate. The plates with only fungal disc without bacterial streaks served as control. All *in vitro* antagonism assays were done in triplicate. The percent inhibition was determined after incubating for 3-5 days at 28°C [9]. The percentage growth inhibition was calculated using the following calculation:

$$I = \frac{C\text{-}T}{C} \times 100$$

Where, I= Per cent inhibition, C= Growth in control, T= Growth in treatment

Further to confirm the antagonist activity of the isolated bacterial isolates, fungal pathogens were inoculated (10^6 conidia) and grown in 50 ml of YEG medium (glucose 10 gl^{-1}, yeast extract 2 gl^{-1}) for 2 days at 28°C, 200 rev min^{-1} in the presence or absence of the culture supernatant (1% v/v) of antagonist bacteria, grown in LB for 48 h at 28°C. The biomass was separated, dried at 105°C for 14 hrs and weighed to obtain the dry weight. The values were expressed in percentage in order to obtain reduction in biomass of the aforementioned fungal pathogens.

$$\text{Inhibition \%} = 1 - \left(\frac{\text{Mycelial growth in YEG supplemented with supernatant (mg)}}{\text{Mycelial growth in control (mg)}} \right) \text{x } 100$$

Identification of bacterial antagonists
The bacterial antagonist showing highest antifungal activity was subjected to biochemical characterization according to Bergey's Manual of Determinative Bacteriology [10] and 16S rDNA gene sequence analysis.

Detection of plant growth promoting traits
The antagonistic isolate was analyzed for its ability to solubilize phosphate, produce indole -3- acetic acid (IAA), biosurfactant and salicylic acid.

Phosphate solubilization
Phosphate solubilizing ability of the isolate was checked on Pikovskaya (PVK) medium [11](Pikovskaya, 1948) incorporated with tricalcium phosphate (TCP) [$Ca_3(PO_4)_5$]. Phosphate solubilization index was evaluated according to the ratio of the total diameter (colony diameter + halo zone) and the colony diameter [12]. Quantitative phosphate solubilization was estimated by Fiske and Subbarow method [13].

IAA production
The production of IAA was determined using Van Urk Salkowski reagent [14]. IAA concentration was measured by spectroscopic absorbance measurements at a wavelength of 530 nm according to the standard curve.

Ammonia and salicylic acid production
P. aeruginosa was tested for salicylic acid, which plays an important role in signaling pathway leading to induced systemic resistance (ISR) [15]. Detection of ammonia production was done by adding 1 ml Nessler's reagent to a 72-h-old culture grown in peptone broth and recording the presence of the yellowish brown color.

Screening for antifungal characters
Siderophore production
Siderophore activity of the isolate was determined on Chromo- azurol S (CAS) medium [16]. The production of siderophore in cell free culture supernatant was determined using spectrophotometric method as described by Meyer and Abdallah [17]. Concentration was calculated using absorption maximum and the molar absorption coefficient (λ_{max}= 400 nm and ε= 20 000 $M^{-1}cm^{-1}$).

HCN production
HCN production was tested according the method described by Kremer and Souissi [18]. Discoloration of the filter paper from yellow to orange/ light brown after incubation was considered as microbial production of cyanide.

Antagonism assays
To determine whether siderophore or antibiotics is involved in antagonism, King's B agar medium supplemented with 1% 100µM $FeCl_3$ was inoculated with *P. aeruginosa* FP6. Inoculated iron free King's B agar medium served as control. An actively growing mycelial disc of 9 mm diameter of fungal pathogens was inoculated in center of the petriplate. A week after inoculation, inhibition of mycelial growth was observed in both iron amended and control King's B agar [19].

Antagonism due to volatile compounds was evaluated by dual bottom plate method as described by Jayaprakashvel *et al.* [20] and the percentage mycelial growth inhibition was calculated. For examining antagonism due to diffusible compounds, *P. aeruginosa* was grown in King's B medium at 37°C, 120 rpm for 48 hrs. The supernatant was separated by centrifugation at 10,000 rpm for 10 min. Supernatant was concentrated by lyophilization and filtered through a 0.45µm pore size filter (Millipore, India). Test plates were prepared by mixing 9 ml of molten PDA and 1 ml of concentrated supernatant. An actively growing mycelial disc of 9 mm diameter of fungal pathogens was inoculated in center of the petriplate. Plates were incubated for 5 days at 28°C and the results were expressed as mean of percentage inhibition. Plates inoculated with fungal agar plugs alone were used as control.

Lytic enzyme production

Cellulase and chitinase activities were tested on nutrient agar plates, with the respective substrate added (carboxymethyl-cellulose (CMC) 1 %, w/v, chitin 0.5 %, w/v) [21,22]. Protease activity was checked on the skim milk agar plate as described by Sevinc and Demirkan [23]. Lipase production was detected on the Rhodamine B olive agar plate as described by Sheikh Abdul Hamid *et al*. [24]. Aliquots of bacterial culture (10 µl), grown overnight in LB broth, were spot-inoculated onto the above mentioned plates. Plates were incubated for 2-8 days at 30°C and formation of a transparent halo zone around the colony was considered positive for enzyme activity.

Biosurfactant production

Biosurfactant production was evaluated by hemolytic activity and by oil spread method [25].

Detection of Acyl Homoserine Lactones (AHL)

C. violaceum CV026 was streaked on LB plate containing 25 µg/ ml kanamycin. After overnight growth at 28°C, cells were transferred to sterile water using a sterile loop and the OD was adjusted to 0.4 at 600 nm. Five ml of this bacterial suspension was added to 200 ml of cooled King's B agar and poured into plates. A single colony of *P. aeruginosa* FP6 was inoculated into 5 ml of LB medium and grown for 24 hrs. 10 µl of *P. aeruginosa* FP6 was spotted on agar plates containing CV026 and air dried. Plates were incubated for 2-3 days at 28°C. Quorum-sensing was detected by the appearance of a violet halo around the colony due to violacein production as a result of the activation of the reporter gene in *C. violaceum* CV026 [26].

Determination of Intrinsic resistance profile

The sensitivity of *P. aeruginosa* FP6 isolate to six β-lactams- ampicillin (10µg), ceftazidime (30µg), ceftriaxone (30µg), cefepime (30µg),cefoxitin (30 µg), imipenem (10µg) and eight non-β-lactam antibiotics, gentamicin (10µg), erythromycin (15µg) amikacin (30µg), rifampicin (5µg), polymyxinB (300U) ciprofloxacin (5µg), tetracycline (30µg), chloramphenicol (30µg) and cotrimoxazole (1.25/23.75µg) was determined on Muller-Hinton agar plates by disc diffusion method [27].

Bacterial growth under stress conditions

Bacterial growth study under stress condition with respect to growth temperature, pH and salt (NaCl) was studied by growing the isolate for 5 days in Erlenmeyer flasks (250ml) containing 50ml of nutrient broth inoculated with 100 µl of 24 hrs bacterial broth (10^8 cfu ml^{-1}). The effect of temperature was studied by incubating the culture over a range of 20- 60°C. Influence of pH on isolate growth was studied by growing the bacteria at 30°C in the medium with different pH range (citrate buffer for pH 5–6; phosphate buffer for pH 7 and Tris-HCl buffer pH 8–10). In addition strain was grown over different salt concentration ranging from 0.5M to 5M at pH 7.0 and 30°C. After incubation, the bacterial cells were then pelleted by centrifugation at 10,000g for 15 min at 4°C and the pelleted cells were resuspended in 1 ml of distilled water to determine the turbidity of the bacterial suspension at 600 nm by spectrophotometer.

Heavy metal tolerance

Strain was also tested for its resistance to heavy metals by agar dilution method. Freshly prepared agar plates were amended with various soluble heavy metal salts namely Hg, Co, Cd, Pb, Zn and Cr, at various concentrations ranging from 0.1 to 50mM were inoculated with overnight grown culture. Heavy metal tolerance was determined by the appearance of bacterial growth after incubating the plates at room temperature for 24-48hrs.

Evaluation of plant growth promotion ability of the isolate on cowpea

The seeds of cowpea, *Vigna unguiculata* C-152, were used throughout the study. Cowpea seeds used for the experiments were obtained from University of Agricultural Sciences, GKVK, Bangalore. The cowpea seeds were surface sterilized in 70% ethanol for 2 min and in 2% sodium hypochlorite for 5 min and followed ten times washing in sterile distilled water. The seeds were then soaked in 10^8 cfu/ml bacterial suspension using sterilized carboxymethylcellulose (CMC;1%, w/v) as an adhesive to facilitate attachment of bacterial cells to the seed coat, incubated at 28°C in an incubator rotary shaker at 150 rpm for 6 hrs and shade dried before use. Seeds treated with sterile distilled water amended with CMC served as control. Seeds were sown in plastic pots containing sterile sand. Seed germination, seedling vigor and the measurements of root length and shoot length of cowpea was analyzed 10 days after germination. The experiment was carried out with three replicates of 50 seeds each**.**

Statistical analysis

Analysis of variance was performed using SPSS version 18 statistical package and mean comparison were carried out using Duncan's multiple range test and by students T test.

RESULTS AND DISCUSSION

Screening of bacterial antagonist

Of the 51 bacterial isolates from soil screened for their ability to inhibit a wide range of fungal pathogens. Of which 15 isolates showed significant antagonistic activity against a broad range of phytopathogens used. Interestingly, bacterial isolate FP6 showed the highest inhibitory activity against all the fungal pathogens (Table 1; figure1). The degree of inhibition ranged from 26.34 % to 94.6 % by dual culture test with 99% mycelia growth reduction.

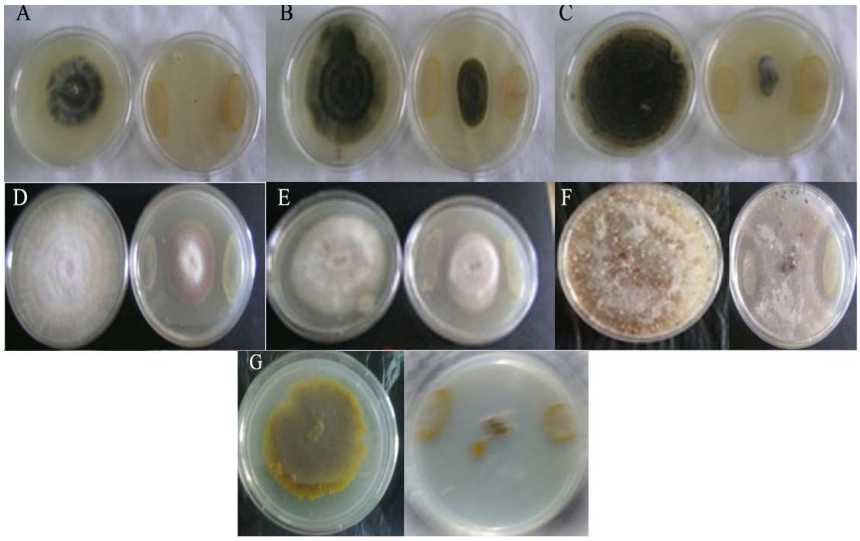

Figure1. Antagonism by dual culture plate.(A) *A.alternate*,(B)*A. brassicae*,(C)*C. gloeosporioides*,(D) *F. oxysporum*, (E) *F. solani*,(F) *Rhizoctonia solani*(G) *A. brassicicola*

Table1. Broad spectrum antifungal activity of *Pseudomonas aerugonisa* FP6 isolate towards fungal pathogen.

Fungal phytopathogens	Mean diameter zone of inhibition (mm)	Reduction in growth (%)*
Alternaria brassicae	65.30± 1.61^{e,f}	90.03± 0.03^{c}
Alternaria brassicicola	76.0 ± 3.0^{f,g}	99.0 ± 0.36^{c}
Alternaria alternate	94.6 ±2.9^{h}	99.42 ± 0.02^{c}
Collectrichum gleosporoides	48.63 ± 5.3^{c}	98.73 ± 0.37^{c}
Fuarium oxysporum	36.85 ± 1.58^{b}	99.2 ± 0.08^{c}
Fusarium solani	26.34 ± 5.3^{b}	99.43 ± 0.06^{c}
Phytophthora capsici	83.0 ± 1.7^{g}	98.77 ± 0.03^{c}
Rhizoctonia solani	51.52 ± 1.9^{c,d}	99.43 ± 0.06^{c}

Results are the mean of triplicates ±SE. In columns, values with the same letters are not significantly different (P < 0.05 Duncan test).

** The dry weight of treated biomass (fungus grown in the presence of antagonist) was subtracted from the control biomass (fungal biomass grown in the absence of antagonist) and expressed in percentage in order to obtain reduction in biomass. The data represents the average of three replicates.*

Morphological, biochemical and molecular characterization of the selected isolate

The isolate was gram-negative, motile rod, oxidase positive, liquefied gelatin and produced diffusible pigments. By biochemical characterization, the isolate was identified as belonging to the genus *Pseudomonas*. 16S rDNA sequencing confirmed the isolate as *Pseudomonas aeruginosa*. In the phylogenetic tree, FP6 strain and other closest *Pseudomonas aeruginosa* strains were grouped together at a 99 % similarity (Figure 1), and the strain was designated as *P. aerugonisa* FP6. The nucleotide sequence data of *Pseudomonas aerugonisa* FP6 has been deposited in the GenBank nucleotide sequence database under the accession number JN861778.

Figure.1. Neighbour- joining phylogenetic tree showing relationships between strain FP6 and several other strains of *Pseudomonas*, based on their 16S rDNA sequences.

Growth promotion traits

P. aeruginosa FP6 showed clear visible halos around the colonies on Pikovskaya agar medium after 3 days of incubation. Solubilization index was observed to be 2.26. The maximum amount of soluble phosphates released was 270 µg/ml. pH of the culture medium dropped significantly from 7.2 to 4.9 as compared to control where it remained constant at 7.2 (data not shown), which may be due to microbial production of organic acids as reported earlier [28,29].

One of the traits of plant growth promoting microorganisms is their ability to produce IAA. The isolate *P. aeruginosa* FP6 showed positive for the production of IAA (Figure 2). A significant increase in IAA production was observed in the presence of the precursor, L-tryptophan i.e., 80 µg/ml when compared to its absence (16 µg/ml), these results are in line with that of earlier studies [30]. IAA production was increasing up to 96 hrs when bacteria reached stationary phase of growth, and then decreased slowly which may be due to release of IAA degrading enzymes. Increased amount of IAA production in the presence of the precursor, L-tryptophan shows that the isolate is dependent on the L-tryptophan precursor and probably synthesized IAA through Trp pathways.

PGPR also activate plant defense resulting in systemic protection against plant pathogens, a phenomenon termed induced systemic resistance (ISR). ISR is regulated by salicylic acids, jasmonic acid and by ethylene- dependent signaling pathway [31]. Our isolate did not produce salicyclic acid. The isolate exhibited strong production of ammonia, which is usually taken up by plants as a source of nitrogen for their growth [32].

Siderophore production

Change in the color of the CAS agar from blue to orange red by the isolate confirmed the ability of *P. aeruginosa* FP6 to produce siderophore (figure 2). The maximum production of siderophore (85.7 µM) was recorded after 36 hrs of incubation. Bellis and Ercolani [33] have reported rootlet elongation on cucumbers grown under gnotobiotic conditions due to siderophore production by *Pseudomonas*.

Hydrogen cyanide (HCN)

Microbial production of HCN has been reported as an important antifungal trait to control root infecting fungi [34]. Strong HCN production was recorded by our isolate *P. aerugonisa* FP6, as evidenced by change in color of filter paper from yellow to reddish-brown after 2-3 days of incubation (Figure 2). Higher amount of HCN was produced

by the isolate (0.09± 0.01) at 625 nm absorbance. HCN production by fluorescent *Pseudomonas* isolated form potato and wheat rhizosphere was reported by Bakker and Schippers [35].

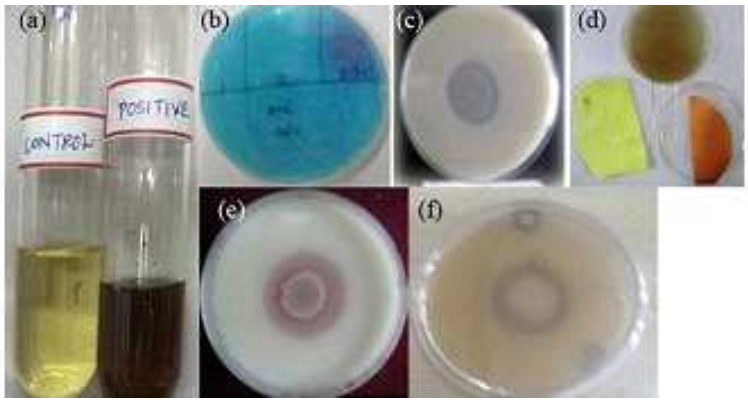

Figure 2. PGPR traits of *P.aeruginosa* FP6. (a) IAA production (b) siderophore production on CAS agar (c) phytase production (d) production of HCN on KB agar supplemented with glycine (e) proteolytic activity on skim milk agar (f) production of acyl homoserine lactones.

Antagonism assays

Table 2 shows the effect of siderophore, diffusible and volatile compounds produced by *P. aeruginosa* FP6, evaluated in terms of radial growth of the test fungus. Siderophore mediated inhibition of fungal growth ranged from of 20 to 88.4% in the absence of $FeCl_3$ whereas the inhibition percentage ranged from 12.2 to 66.7% in presence of $FeCl_3$. The complete inhibition of *A. alternata* and *F. oxysporum* mycelia growth by *P. aeruginosa* FP6 both in the presence and in the absence of ferric chloride indicates that the antagonism mechanism is by both siderophore and antimetabolite production. Production of volatile compound (HCN) inhibited the mycelial growth to the extent of 68.62 to 99.93 %. The *P. aeruginosa* FP6 culture filtrate also showed the production of diffusible metabolites (non-volatile), where the inhibition percentage ranged from 12.47 to 53.3%. Haas *et al.* [36] have reported that HCN production by strains of *P. fluorescens*, helped in suppression of *Thielaviopsis basicola* causing black root rot of tobacco.

Table 2. Percentage inhibition of phytopathogenic fungi by *Pseudomonas aerugonisa* FP6 through different antagonistic mechanism

Phytopathogenic fungi	Culture filtrate	HCN	Siderophore	
			With $FeCl_3$	Without $FeCl_3$
C.gloeosporoides	55.30±0.02^{g}	99.93±0.06^{d}	61.40±0.06^{e}	72.12±0.09^{d}
A.brassicae	34.50±0.03^{c}	99.75±0.24^{d}	47.66±0.09^{d}	53.56±0.02^{b}
A.brassicicola	45.06±0.07^{e}	99.92±0.08^{d}	66.71±0.02^{g}	88.45±0.05^{g}
A. alternata	40.82±0.04^{d}	99.85±0.15^{d}	62.80±0.00^{f}	65.07±0.05^{c}
R.solani	27.40±0.05^{b}	68.62±0.15^{a}	12.22±0.01^{a}	72.25±0.07^{f}
F.oxysporum	19.46±0.03^{a}	88.61±0.28^{c}	19.95±0.08^{c}	20.09±0.07^{a}
F.solani	48.54±0.05^{f}	85.04±0.55^{b}	12.47±0.04^{b}	71.78±0.05^{e}

Results are the mean of triplicates ± SE. Mean with the same letter are not significantly different of ANOVA test ($P < 0.05$).

Several studies have reported the involvement of antibiosis in biocontrol of plant pathogens, which might be Phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (2,4-DAPG), Pyoluteorin (Plt) or Pyrrolnitrin (Prn) [37-39].Antagonism is known to be mediated by a variety of compounds of microbial origin, e.g., bacteriocins, enzymes, toxic substances and volatiles. In the present study, siderophore, diffusible as well as volatile compounds were found to produce significant quantitative effects. Volatile substances showed maximum inhibition against all phytopathogens. Diffusible (non-volatile) metabolite activity of the antagonist was more potent against *C. gloeosporoides*. Siderophore mediated activity was more potent against *A. brassicicola* followed by *R. solani*.

Detection of Lytic enzymes and Biosurfactant

The isolate produced protease and lipase enzyme showing zone of clearance around the colony on skim milk agar and orange fluorescence colonies on Rhodamine B olive agar plate. Dunne *et al*. [40] have demonstrated that biocontrol of *Pythium ultimum* in the rhizosphere of sugar beet was due to the production of extra cellular protease. The isolate showed positive for biosurfactant production in the haemolytic assay and by oil spread method

indicating biosurfactant production. Several studies have reported the zoosporicidal activity of biosurfactant which play a role in the control of oomycete diseases [41,42].

Quorum sensing

Quorum sensing is the major mechanism by which many bacteria regulate production of antifungal factors. Our isolate showed positive for quorum sensing, *C. violaceum* CV026 produced violacein in the presence of AHLs molecule from *P. aeruginosa*FP6 (Figure2). Chin-A-Woeng *et al*. [43] have shown that quorum sensing is the most important regulation mechanism for PCA production in the closely related species to *P. aurantiaca* B-162, namely *P. aeruginosa*.

Intrinsic antibiotic resistance

The isolate was highly susceptible to ciprofloxacin, gentamicin, amikacin, chloramphenicol, polymyxin B, imipenem, ceftazidime, ceftriaxone and showed moderate resistance against tetracycline, erythromycin, cotrimoxazole, rifampicin, ampicillin, cefepime and cefoxitin.

Bacterial growth under stress conditions

The bacterial growth under stress condition by selected isolates was found to increase linearly until 3days at 20–60°C, pH 5–10 and salt concentration up to 4.5 M but at 5M NaCl concentration growth of the isolate was decreased. Results revealed the potential of the strain to survive under stressful conditions (high salt and pH). Acid and salt tolerance is important for the growth and survival of microorganisms in rice soil. The ability of the strain to adapt to temperature stress may be important for the survival of the microorganisms during drought.

Heavy metal tolerance

P. aeruginosa FP6 was tolerant to majority of the heavy metals, Hg (0.1mM), Co (10mM), Cd (0.1mM), Pb (50mM), Zn(1mM) Cr (50mM). Microorganisms have developed the mechanisms to cope with a variety of toxic metals for their survival in the environment enriched with such metals. Our isolate showed tolerant to multiple heavy metals and also exhibited PGPR activities. The selection of microorganisms both metal tolerant and efficient in producing plant growth promoting compounds can be useful to speed up the colonization of the plant rhizosphere in polluted soils.

Effect of *P. aeruginosa* on Plant growth

In our study, cowpea seeds treated with *P. aeruginosa* FP6 showed stimulatory effects on all plant vegetative parameters. A significant difference was observed between *P. aeruginosa* FP6 treated and non-treated plants. Results regarding the effect of *P. aeruginosa* FP6 inoculation on root and shoot of cowpea plant have been summarized in Table 3. Seed germination index was higher in bacterized seeds (92%), which germinated earlier in comparison to control (72%) and good overall seed vigor as compared to control (untreated). Analysis of data obtained from pot experiments reveals that the isolate has significant effect on root and shoot as compared to that of control (Figure 3).

Figure 3. Effect of *P. aeruginosa* FP6 on growth of cowpea plant in treated seeds (a) and untreated seed (B)

Table 3. Effect of inoculation of the *Pseudomonas aeruginosa* on cowpea (*Vigna unguiculata*)

Attributes	control	*P. aeruginosa*	*P* value
Seed germination (%)	72	92	0.0395
Vigour index	1441.16	1766.40	-
Shoot length (cm)	11.57± 0.36	12.85±0.37	0.018
Root length (cm)	9.27± 0.66	13.57± 0.63	0.000
Fresh shoot weight (g)	0.305± 0.01	0.354± 0.01	0.033
Fresh root weight (g)	0.018± 0.001	0.028± 0.002	0.005
Dry shoot weight (g)	0.050± 0.005	0.0725± 0.007	0.029
Dry root weight (g)	0.0072± 0.00	0.0157± 0.003	0.047

Results obtained were of mean of triplicates ±SE. Data was analyzed using Students t- test.

CONCLUSION

The present study was an attempt to demonstrate the multifunctional property of *Pseudomonas aeruginosa* FP6. This isolate has broad spectrum antagonistic activity which would help in establishing resistance against deleterious microorganisms occupying the microbial niche in the rhizosphere. Due to the diverse mechanism established by our isolate in the suppression of phytopathogens, it can be developed as an effective biocontrol agent.

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REFERENCES

[1]. B. Gerhardson, *Trends Biotechnol*., **2002**, 20, 338.
[2]. M.Shoebitz, C.M Ribaudo, M. A. Pardo, M.L.Cantore, L.Ciamp, J. A.Cura, *Soil Biol. Biochem* **2009**, 41,1768.
[3]. K.K. Pal, K.V.B.R. Tilak, A.K.Saxena, R.Dey, C.S Singh, *Microbiol. Res*., **2001,** 156, 209.
[4]. I.P. Ahlawat, B.G. Shivakumar, Textbook of Field Crops Production. Indian Council of Agricultural Research, New Delhi, India, **2005**.
[5]. L.M. Bordeleau, D. Prevost, *Plant and Soil*, **1994,** 161, 115.
[6]. E. Epstein, Genetic Engineering of Osmoregulation, Plenum Press, New York, **1980**,7-21.
[7]. A.Khalid, M.Arshad, Z.A Zahir, *J.Appl. Microbiol*., **2004**, 96(3), 473.
[8]. S.C.Wu, Z.H.Cao, Z.G.Li, K.C.Cheung, M.H.Wong, *Geoderma*, **2005**, 125,155.
[9]. H.A. Idris, N.Labuschagne, L.Korsten, *Biocontrol*, **2007,** 40, 97.
[10]. J.G.Holt, N.R. Krieg, P.H.A Sneath., J.T.Staley, S.T.Williams (9th edn), Bergey's manual of Determinative Bacteriology,. Williams and Wilkins Co., Baltimore, **1994.**
[11]. R.I. Pikovskyas, *Mikrobiologiya*,**1948**. 17,362.
[12]. M.Edi-Premono, A.M. Moawad, P.L.G.Vlek, *Indones. J. Crop Sci*. **1996**, 11, 13.
[13]. C.H.Fiske, Y.Subbarow, *J. Biol. Chem*., 1925,6,376.
[14]. J.M.Bric, R. M.Bostock, S.E. Silverstone, *Appl. Environ. Microbiol*., **1991**,57, 535.
[15]. J.M.Meyer, P.Azelvandre, D.C. Georges, *Biofactors*, **1992**, 4: 23.
[16]. B. Schwyn, J.B. Neilands, Anal. Biochem., **1987**, 160,47.
[17]. J.M.Meyer, M.A. Abdallah, *J Gen. Microbiol*., **1978**, 107, 319.
[18]. R.J. Kremer, T.Souissi, *Curr. Microbiol.*, **2001**, 43,182.
[19]. K.Radheshyam, A.J.Jayaswal, M.Fernandez, G Ralph, *Appl. Environ. Microbiol.*, **1990**, 56,1053.
[20]. M.Jayaprakashvel, Ph.D, thesis, University of Madras, Chennai, India, **2008**.
[21]. H. Ariffin, N. Abdullah, M.S. Umi Kalsom, Y. Shirai, M.A. Hassan, *International Journal of Engineering and Technology*,**2006,** 3(1) 47.
[22]. M. Dirceu, R.S. Romeiro, A. W.V. Pomella, J.T. deSouza, Biological Control, 2008, 47,309.
[23]. N. Sevinc, E. Demirkan**,** *J. Biol. Environ. Sci.*, **2011,** 5(14), 95.
[24]. N. Sheikh Abdul Hamid, H.B. Zen, Q.B. Tein, M. Yasin, Y.M. Halifah, N.I. Saar, F.A. Bakar, *World J. Microbiol. Biotechnol.*, **2003,** 19, 961.
[25]. J.T. De Souza,M. de Boer,P. de Waard,T.A. Van Beek, J.M. Raaijmakers, *Appl. Environ. Microbiol.*, **2003,** 69, 7161.
[26]. M. Samina, D.N. Baig, F. Jamil, B. Weselowski, G. Lazarovits, *J.Microbiol. Biotechnol*., **2009,** 19(2), 1688.

[27]. A.W.Bauer, W.M. Kirby, J.C. Sherris, M. Turck, *Am. J.Clin. Pathol.*, **1966,** 45,493.
[28].Y.P.Chen, P.D. Rekha, A.B. Arun, F.T. Shen, W.A. Lai, C. Young, *Appl. Soil Ecol.*, **2006,**. 34,33.
[29]. A. Pandey, P. Trivedi, B. Kumar, L.M.S Palani, *Curr. Microbiol.*, **2006**, 53,102.
[30]. Y.J. Megha, M.Sc thesis, University of Agricultural Sciences, Dharwad, **2006.**
[31].M. Maurhofer,C. Reimmann, P. Schmidli-sacherer, S. Heeb, D. Haas, G.Defago, *Phytopathology*,**1998,** 88, 678.
[32]. F. Ahmad,I. Ahmad,M.S. Khan, *Microbiol. Res.*, **2008**, 163, 173.
[33]. P.Bellis, G.L. Ercolani, *Appl. Environ. Microbiol.*, **2001,** 67, 1945.
[34].A.Ramette, M. Frapolli, G. Defago, Y. Monenne Loccoz, *Mol. Plant Microbe In.*, **2003**, 16,525.
[35]. A.W. Bakker,B. Schippers, Soil Biol. Biochem., **1987**, 19, 451.
[36]. D. Haas,T. Dberhansil, G.Defago, *J. Gen. Microbiol*., **1991**, 137, 2273.
[37]. K. Geetha, S. Vikineswary, *J.Ind. Microbiol. Biotechnol.*,**2002,** 17, 51.
[38]. V.O.Stockwell, K.B Johnson, D.Sugar, J. E. Loper, *Phytopathology*, **2002**.92, 1202.
[39]. R.R. Rakh, L.S. Raut, S.M. Dalvi, A.V. Manwar, *Recent Res Sci Technol*., **2011,** 3(3), 26.
[40]. C.Dunne, J.J.Crowley, Y.Moenne - Loccoz, D.N.Dowling, F.J. De Bruijn, F. O'Gara, *Microbiology*, **1997**, 143, 3921.
[41]. C.J.Nielsen, D.M.Ferrin, M.E.Stanghellini, *Can. J. Pl. Pathol.*,**2006,**28,450.
[42]. H.Tran, A.Ficke, T.Asiimwe, M.Hofte, J.M.Raaijmakers, *New Phytologist*, **2007,** 175,731.
[43].T.F.C. Chin-A-Woeng, G.V.Bloomberg, B.J.J. Lugtenberg, *New Phytologist*, **2003**,157,503.