Original Research Article

IN VITRO ASSESSMENT OF *PSEUDOMONAS* sp. STRAIN FCBB-2 FOR EFFECTIVE PLANT GROWTH PROMOTION AND ANTIFUNGAL ACTIVITY UNDER DROUGHT STRESS

ABSTRACT

The role of plant growth-promoting rhizobacteria (PGPR) in adaptation of plants in extreme environments is not completely understood. In the present study, native bacteria were isolated from rhizospheric soils and evaluated for both growth-promoting abilities and antagonistic potential against phytopathogenic fungi. All the *Pseudomonas* spp. strains were characterized for *in vitro* drought tolerance in trypticase soy broth supplemented with different concentrations of polyethylene glycol (PEG6000). Out of nine isolates, only one strain was able to tolerate maximum level of stress (−1.03 MPa) and is further screened for plant growth promoting (PGP) properties under non-stress and drought stress conditions. Strain FCBB-2 isolated from cluster bean plant rhizosphere soil, showed multiple PGP activities such as indole-3-acetic acid (IAA) production, siderophore, hydrogen cyanide (HCN) production and exopolysaccharide (EPS) production and antifungal activity under non-stress and drought stress conditions against various plant fungal pathogens like *Fusarium oxysporum*, *Macrophomina phaseolina*, *Alternaria alternata*, *Sclerotium hydrophilium*, *Pythium aphanidermatum* and *Rhizoctonia solani*. The strain FCBB-2 was identified as *Pseudomonas aeruginosa* based on morphological and 16S rRNA gene sequence analysis and the sequence were submitted to NCBI GenBank under the accession number KT311003.

Keywords: Plant growth promotion, drought stress, Biocontrol and rhizosphere.

INTRODUCTION

Drought stress is one of the major agricultural problems reducing crop yield in arid and semiarid regions of the world. Changes in mean global air temperature and precipitation patterns are leading to longer drought periods and more extremely dry years, and more severe drought conditions will hinder food production in some countries **(Lau and Lennon, 2012)**. At present, strategies to increase the ability of plants to tolerate drought stress involves the use of water-saving irrigation, traditional breeding, and genetic engineering of drought-tolerant transgenic plants. These methods very time taking and laborious due to which these techniques whatsoever not recommended highly. **(Niu *et al.*, 2018)**. Microbes function as biofertilizers, biopesticides, and plant growth promoters and have been utilized to enhance crop growth in numerous countries around the world, but especially in developing and emerging nations **(Bashan *et al.*, 2014)**. For decades, companies worldwide have supplied farmers with nitrogen-fixing inoculants and formulations of plant-growth-promoting (PGP) microbes, both fungi and bacteria, to enhance crop production. Many microbial products are also used by home gardeners and for organic agriculture, and large-scale commercial farms in China, the United States, and Europe are beginning to adopt biological materials as substitutes for chemical fertilizers and pesticides **(Parnell *et al.*, 2016)**. Replacing chemical fertilizers and pesticides is critical for agricultural sustainability **(Kecskés *et al.*, 2016; Menendez and Garcia-Fraile, 2017)**, but there is a

huge gap in information about the effectiveness of PGP microbes based on laboratory studies versus their performance in the field. It was not evidenced that, most of the efficient microbial products or its derivates tested in the field was not analyzed for their harmful effects on other living organisms, which includes human beings **(Martinez-Hidalgo *et al.*, 2019)**.

Rhizosphere associated fluorescent pseudomonads are key players in plant growth-promoting rhizobacteria (PGPR) due to their catabolic versatility, abundant root colonizing and capacity to produce a wide range of metabolites that favor the plant to withstand under varied biotic and abiotic stresses **(Kragelund *et al.*, 1997; Sakshi and Naveen, 2014)**. In addition to abiotic stresses, plants become weak and vulnerable to infection by pathogens (including bacteria, fungi, viruses, and nematodes) and attack by herbivore pests. These wide range of abundant pest and other harmful bacteria and fungi population may differ based on the climatic conditions **(Nicol *et al.*, 2011)**. For example, increasing temperatures are known to facilitate pathogen spread. Mechanisms of plant defense against biotic stresses are complex and consist of several layers of defense. Plants respond to pathogen attack by synthesizing pathogenesis-related (PR) proteins. They encode enzymes like chitinases and glucanases that can hydrolyze the cell walls of fungal pathogens **(Vacheron *et al.*, 2015; Mauch *et al.*, 1988)**. There are several mechanisms by which PGPR bring about control of plant diseases. The most commonly used methods are competition and production of metabolites. The metabolites include antibiotics, siderophores, HCN (Hydrogen Cyanide), cell wall-degrading enzymes, etc., **(Enebak *et al.*, 1998; Kloepper, 1993)**. Rhizobacteria, including PGPR, dispose of a wide range of beneficial functions that may increase plant growth under stress condition **(Dimkpa *et al.*, 2009)**. A broad taxonomic and functional diversity occurs in the plant rhizosphere **(Bouffaud *et al.*, 2014)** and may affect plant fitness under abiotic stress condition (drought, salinity, pollutions, temperature etc.,). PGPR are found in all clades of Proteobacteria especially in Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria, and in Firmicutes such as in Actinobacteria.

Drought stress due to lack of moisture content can able make drastic changes in physico-chemical and biological nature of soil and makes it harsh for soil microbiota and its activity on crop yield. Water availability controls the production and consumption of protein and polysaccharides by the bacteria **(Roberson and Firestone, 1992)** and thus indirectly influences soil structure. Among various plant growth promoting bacteria, *Pseudomonas* spp. have ability to survive under stress conditions due to its characteristic production of exopolysaccharides (EPS), which protects microorganisms from water stress by enhancing water retention and by regulating the diffusion of organic carbon sources **(Hepper, 1975; Wilkinson, 1958; Roberson and Firestone, 1992; Chenu, 1993;1996)**. The production of EPS helps bacteria to attach and colonize the roots by a network of fibrillar material that makes bacteria adhere to the root surface **(Bashan and Holguin, 1997)**. **Bashan *et al.* (2004)** reported the role of polysaccharides producing *Azospirillum* in soil aggregation **(Sandhya *et al.*, 2009)**. In drought stress conditions the plant becomes most vulnerable to pathogen attack which is very effective and limiting the crop production. In view of this hypothesis, the present research is an attempt to isolate drought tolerant *Pseudomonas* spp. strains from different drought prone areas of India.

MATERIALS AND METHODS

Isolation of *Pseudomonas* spp.

Rhizobacteria were isolated from different rhizosphere soil samples of maize, okra, sorghum, tomato, green gram, and red gram plants collected from arid regions in India. For rhizosphere soil samples, the plants were uprooted, and the bulk soil was removed by gently shaking the plants; rhizosphere soil samples were collected by dipping the roots in containers with sterile normal saline followed by shaking for 30 min. The soil suspensions were serially diluted, and the appropriate

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dilutions were spread plated on King's B agar medium (g/L: Proteose peptone-20.00; dipotassium hydrogen phosphate-1.5; Magnesium Sulphate Heptahydrabad-1.5; Glycerol-10; Agar-15) **(King *et al.*, 1954)**. Different colonies based on their shape, size and morphology and also fluorescent colonies under UV light were picked and purified on respective King's B agar media. The pure cultures were maintained on nutrient agar slants under refrigerated conditions. A fresh broth culture of each isolate was prepared for further experiments in nutrient broth media.

Screening for drought stress tolerance

Trypticase soya broth (TSB) with different water potentials (−0.05, −0.15, −0.30, −0.49, −0.73, −1.03 MPa) was prepared by adding the appropriate concentrations of PEG 6000 **(Michel and Kaufmann, 1973; Sandhya *et al.*, 2009)** and then inoculated with 1% of bacterial cultures cultivated overnight in TSB. Three replicates of each isolate at each concentration were prepared. After incubation at 28 °C under shaking conditions for 24 h, growth was estimated by measuring the optical density at 600 nm using a UV method by spectrophotometrically against TSB broth media as a blank (Shimadzu, UV1800 240V, JAPAN). The growth of the isolates at various stress levels was recorded.

Screening for plant growth promoting activities

IAA estimation

Isolates which were able to grow at maximum water potential level were screened for plant growth promoting. To determine Indole-3-acetic acid production, Luria Bertani broth (LB) (non-stress and drought stress) amended with 5 mM tryptophan was inoculated in replicates with bacterial cultures cultivated overnight (0.5 OD at 600 nm) and incubated at 28 °C for 48 h on incubator shaker. Cells were harvested by centrifugation at 3000 *g* for 10 min and the supernatant was mixed with Salkowsky reagent, followed by incubation for 1h at room temperature under dark conditions. Absorbance of the pink color was read at 530 nm **(Gordon and Weber, 1951)**.

Siderophore production

To determine siderophore production by the isolates, we first spot inoculated 10 μl of bacterial cultures raised over night (0.5 OD at 600 nm) on Chrome Azurol S (CAS) agar plates and incubated at 28 ^{o}C for five days. Development of orange halo around the colony was considered as positive for siderophore production. **(Schwyan and Neilands, 1987)**. In order to screen siderophore production under non-stress and drought stress PEG 6000 broth cultures were prepared, inoculated with 1 % bacterial cultures incubated at 28 ^{o}C for 5 days and checked for development of orange color.

Production of HCN

HCN production under non-stress and drought stress was tested in King's B broth amended with 0.4 % glycine and Whatmann No.1 filter paper strips soaked in 0.5 % picric acid in 2 % sodium carbonate hanged in test tubes, sealed with parafilm and incubated at 28 ^{o}C for four days. Color change of strips from yellow to orange color considered as positive for HCN production **(Bakker and Schipper, 1987; Ali and Sandhya, 2013).** Total cyanogens content was estimated by the method described by Bradbury **(Bradbury *et al.*, 1999)**. After incubation, picrate papers were carefully removed and immersed in 5 ml of distilled water for 30 min by gentle shaking. A blank picrate paper immersed in water was used as a blank. The absorbance of solution thus obtained was measured at 510 nm against blank. Total cyanogens content (ppm) = 396 X absorbance.

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Identification and characterization of bacterial isolates

The selected bacterial isolates were subjected to microscopic analysis by gram staining method, morphological, and biochemical characterization by IMViC reactions according to Bergey's manual of determinative bacteriology. For molecular characterization, bacterial genomic DNA was isolated according to **Chen and Kuo (1993)** and the 16S rRNA gene was amplified by polymerase chain reaction (PCR) using universal forward 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse 1425R (5′-AAGGAGGTGATCCAGCCGCA-3′) primers under standard conditions (initial denaturation, 94 °C for 5 min; 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 40 s, extension at 72 °C for 90 s; and final extension at 72 °C for 7 min). The PCR product (~1500 bp) was purified and sequenced (SciGenom Labs, India). The sequence obtained was compared with the existing database of 16S rRNA gene using Blast tool on NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antifungal activity

Spread plate method

For antifungal activity under non-stress conditions the fungal strains used were collected from microbial type culture collection (MTCC), India. An aliquot of 0.1 mL bacterial culture (~10^8 cfu/ml) was spread on the surface of PDA plate (9 cm in diameter) and 0.1 mL sterilized water instead of bacterial culture was spread as control. A disc of agar (diameter of 5 mm) with the pathogenic fungi was placed in the centre of plate and incubated at 28 ^oC for 5 days. After incubation, the size of pathogenic colony was measured. All the treatments and control were set in triplicates. In order to evaluate quantitatively the antagonistic activity of the bacteria, relative inhibition ratio (RIR) was adopted with the following formula.

$$RIR\ (\%) = (D_{CK} - D_T) / D_{CK} \times 100\ \%$$

Where, D_{CK} - Diameter of pathogen colony in control; D_T - Diameter of pathogen colony in treatment. The isolates with RIR more than 50 % were considered to be significant of antagonistic activity **(Tianxing *et al.*, 2013)**.

Plate confrontational culture method

Agar disc with pathogen was inoculated on center of PDA plate under non-stress conditions, test isolates were streaked in triplicates nearby the pathogenic disc with a distance of 1.5 cm and sterile water was streaked in control plate. When the mycelia of the pathogen fully covered the petridish in control, the size of the fungi static zone was examined in the treatments plates in order to measure the antifungal activity. Therefore, the size of inhibition zone calculated by substracting the diameter of pathogenic fungi colony in the test plate from diameter of pathogenic fungi control plate **(Tianxing *et al.*, 2013)**.

Broth method under stress conditions

To screen antifungal activity in broth under drought stress condition, 50 mL of potato dextrose broth was prepared by adding required amounts of PEG 6000 to induce drought stress. Three flasks each of 50 mL broth media were inoculated separately with (i) 0.5 cm agar disc of fungi (ii) 500 µl of overnight bacterial culture and (iii) 0.5 cm agar disc of fungi and 500 µl of overnight bacterial culture. After five days static incubation at 25 ^oC, mycelial dry weight was calculated in the flaks one and three **(Matcham *et al.*, 1985)**.

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Production of lytic enzymes

Strain FCBB-2 was screened for lytic enzyme production (Cellulase, Protease and Chitinase). To test the presence of protease activity (1 % w/v) casein was used as substrate in screening agar medium. Enzyme activity was indicated by the formation of a clear zone around colonies after precipitation with 1 M HCl solution **(Mehraj *et al.*, 2013)**. For screening of chitinase activity, the agar medium amended with colloidal chitin was used. The medium consists of (g/L): Na_2HPO_4-6; KH_2PO_4-3; NH_4Cl-1; NaCl-0.5; yeast extract-0.05; agar-15 and colloidal chitin 1 % (w/v). The colonies showing clear zones on a creamish background were considered as chitinase-producing bacteria **(Saima *et al.*, 2013)**. To screen cellulase activity carboxy methyl cellulose (CMC) (1 % w/v) was used as substrate in screening agar medium. The petri plates were incubated at 37 ˚C for 24 hrs. Plates were flooded with 1 % Congo red solution for 15 minutes then de-stained with 1 M NaCl solution for 15 minutes. Clear zones around the colonies indicated cellulase activity **(Mehraj *et al.*, 2013)**.

Production of HCN and Siderophores under stress conditions

Modified protocol of Schwyan and Neilands **(Schwyan and Neilands, 1987)** for siderophore detection was used to prepare potato dextrose broth of 30 % PEG 6000 concentration to induce drought stress. Four flasks each of 30 ml broth media was inoculated with (i) control without any microorganism, (ii) pathogenic fungi disc, (iii) test bacterial culture and (iv) pathogenic fungi and test bacteria. All flasks were kept for incubation at 28 ^{o}C for three days. Formation of orange to red color was observed after the incubation period. In the same way the modified protocol of **Bakker and Schipper (1987)** was employed to screen HCN production. Experiment was repeated for 3 times to interpret the results.

Production of Exopolysaccharides

The efficient isolate able to grow at maximum stress level was analyzed for its ability to produce EPS **(Fett *et al.*, 1989; 1986)** under non-stress and drought stress (-1.03 MPa). Exopolysaccharide was extracted from 3-day-old cultures raised in TSB (30 % PEG was added to TSB for inducing stress). The culture was centrifuged at 20,000 g for 25 min and the supernatant was collected. The pellet was washed twice with 0.85 % KCl (potassium chloride) to completely extract EPS. The possible extraction of intracellular polysaccharides was ruled out by testing the presence of DNA in the supernatant by DPA reagent **(Burton *et al.*, 1956)**. Concentration of protein in the supernatant was estimated by Bradford method **(Bradford, 1976)**. The supernatant was filtered through 0.45 µm nitrocellulose membrane and dialysed extensively against water at 4 °C. The dialysate was centrifuged at 20,000 g for 25 min to remove any insoluble material and mixed with three volumes of ice-cold absolute alcohol and kept overnight at 4 °C. The precipitated EPS obtained by centrifugation at 10,000 g for 15 min was suspended in water and further purified by repeating the dialysis and precipitation steps. Total carbohydrate content in the precipitated EPS was determined according to **(Dubois *et al.*, 1956)**.

Statistical analysis

Data were statistically tested by analysis of variance (ANOVA) followed by Tukey's multiple comparison test using Instat+ version 3.36. Each treatment was analyzed with at least three replicates and the standard deviation calculated and data expressed as the mean ± SD of three replicates.

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RESULTS

Isolation and drought stress tolerance

A total of nine fluorescent *Pseudomonas* spp. strains were isolated from rhizosphere soil of different crops on King's B Medium. All the isolates were screened for drought stress tolerance using PEG 6000, among nine isolates only one isolate FCBB-2 was able to grow at maximum water potentials -1.03 MPa (Fig. 1).

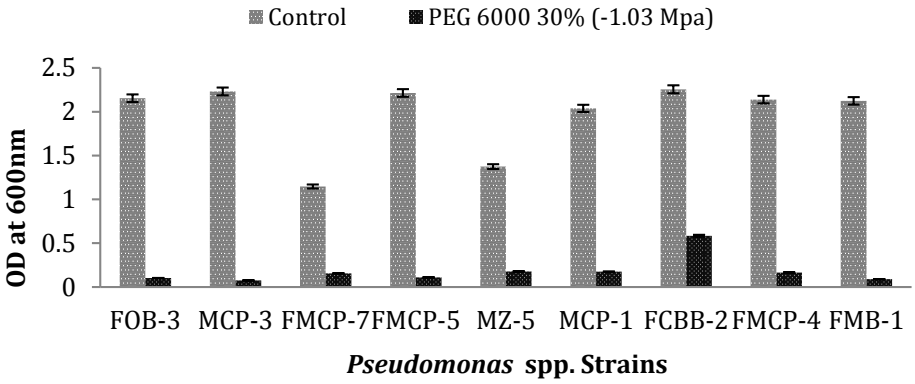

Figure 1 Drought stress tolerance of *Pseudomonas* spp. strains at -1.03 MPa. Error bars Mean of ± SD (n=3).

Screening for PGP traits

Isolates able to grow at maximum drought stress were screened for PGP traits under non-stress and drought stress condition. Among all one isolate FCBB-2 was able to produce all the PGP characters under non-stress and drought stress condition. However, remaining isolates were unable to produce PGP characters under drought stress condition, but significant variation was observed under non-stress condition (Tab 1). Isolate FCBB-2 produced maximum amount of IAA under non-stress (5.89±0.18 mg/mL) followed by isolate FMCP-7 (3.84±0.15 mg/mL) and FMCP-4 (3.77±0.19 mg/mL). Similarly, under drought stress, isolate FCBB -2 was the best to produce IAA (4.90±0.21 mg/mL) followed by FMCP-7 and FMCP-4 (Tab 1). Siderophore production was observed in four isolates under non-stress. Whereas, under drought stress siderophore production was observed only in FCBB-2 (Tab 1, Fig. 2). Hydrogen cyanide production was also observed in FCBB-2 under non-stress and drought stress condition (Fig. 2). Total cyanogen estimated was 257.04±0.21 ppm under non-stress and 318.38±0.29 ppm under drought stress respectively (Tab 1). Ammonia production was observed in all the isolates under both non-stress and drought stress.

A

B

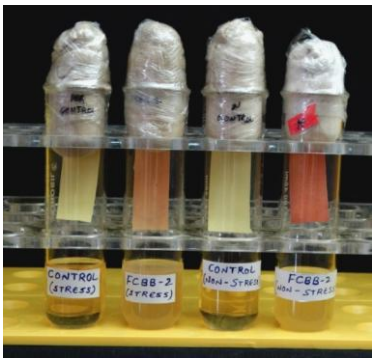

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Figure 2 Screening of *Pseudomonas* sp. strain FCBB-2 for siderophore and HCN (Hydrogen Cyanide) activity under non-stress and drought stress conditions. (A) & (B); siderophore, (C); HCN.

Table 1 Plant growth promoting traits of drought tolerant *Pseudomonas* spp. under non-stressed and drought stressed condition

Isolates	Ammonia Production		IAA (mg/mL)		Siderophore		HCN Total cyanogens (ppm)		Exopolysaccharides, mg/mL	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
MCP-3	+	+	2.42±0.11[a]	1.48±0.12[a]	+	-	-	-	8.64±0.02[a]	19.21±0.01[a]
FMCP-7	+	+	3.84±0.15[b]	2.78±0.19[b]	+	-	-	-	9.01±0.04[b]	21.61±0.01[b]
FCBB-2	+	+	5.89±0.18[c]	4.90±0.21[c]	+	+	257.04±0.21	318.38±0.29	10.56±0.02[c]	41.62±0.02[c]
FMCP-4	+	+	3.77±0.19[d]	2.21±0.14[d]	+	-	-	-	9.87±0.12[d]	18.24±0.11[d]

Legend: NS, non-stressed; DS, drought-stressed; IAA, Indole acetic acid; HCN, hydrogen cyanide; + positive; - negative. Data were analyzed by ANOVA analysis followed by Tukey's multiple comparison test. Values are means of ± SD, n=3. Values with different letters are statistically significantly different at P=0.05.

EPS production:

A significant increase in the concentration of EPS was observed under drought stress condition as compared to non-stress conditions, among the four isolates FCBB-2 producing more amount of EPS under drought stress (41.62±0.02) (Tab 1).

Production of siderophore and HCN under stress

Under multiple stress conditions (biotic & abiotic) *Pseudomonas* spp. strain FCBB-2 has not lost its efficiency to produce siderophore and HCN activity (Fig. 5).

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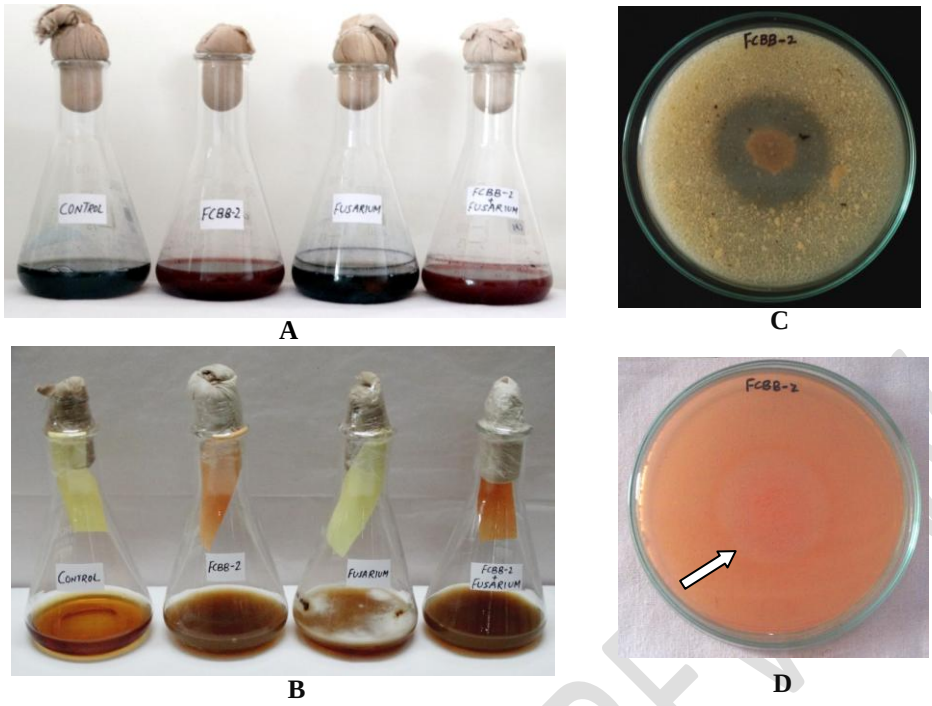

Figure 5 PGP and enzymatic activity of strain FCBB-2. A); Siderophore activity, B); Hydrogen Cyanide (HCN) production under multiple stress conditions (biotic & abiotic), C) protease activity, D); cellulase activity of strain FCBB-2, arrow indicates the halo zone.

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Identification of strain FCBB-2

The prospective isolate selected on the basis of drought stress tolerance and PGP traits production under drought stressed condition was characterized based on microscopic, morphological, and biochemical studies. Microscopic studies revealed that the isolate FCBB-2 was Gram negative, motile, rod-shaped bacteria. On King's B medium isolate appeared as creamy, smooth, shiny, circular, convex colonies with greenish pigmentation. The isolate FCBB-2 utilized citrate, xylose, melibiose, lactose, arabinose and positive for catalase, oxidase, and malonate utilization. On the basis of 16s rRNA gene sequence blast analysis on NCBI, isolate FCBB-2 was identified as *Pseudomonas aeruginosa*, and the nucleotide sequence was submitted to NCBI GenBank under accession No. KT311003.1.

Biocontrol Activity

Strain FCBB-2 was effective in inhibiting fungal plant pathogens like *F. oxysporium, M. phaseolina, A. alternata, P. aphanidermatum* and *R. solani*. Interestingly in all the methods described here, strain FCBB-2 was unable to inhibit the pathogen *S. hydrophilium* (Fig. 3). In non-stress and drought stress conditions mycelial dry weight in control flasks were effectively higher than in the treated flasks, the results were analysed to check whether strain FCBB-2 inhibiting the fungal pathogen or not (Tab 2).

Lytic enzymes activity

Production of lytic enzymes by the isolate FCBB-2 was an added advantage to show effective biocontrol activity, isolate FCBB-2 was effectively producing lytic enzymes like cellulases, proteases

(Fig. 5) and chitinases. These enzymes were qualitatively estimated by the presence of halozone around the bacteria colony.

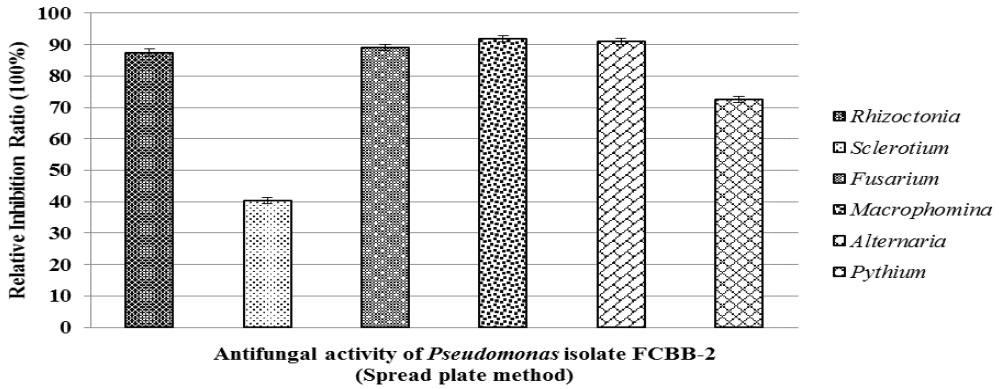
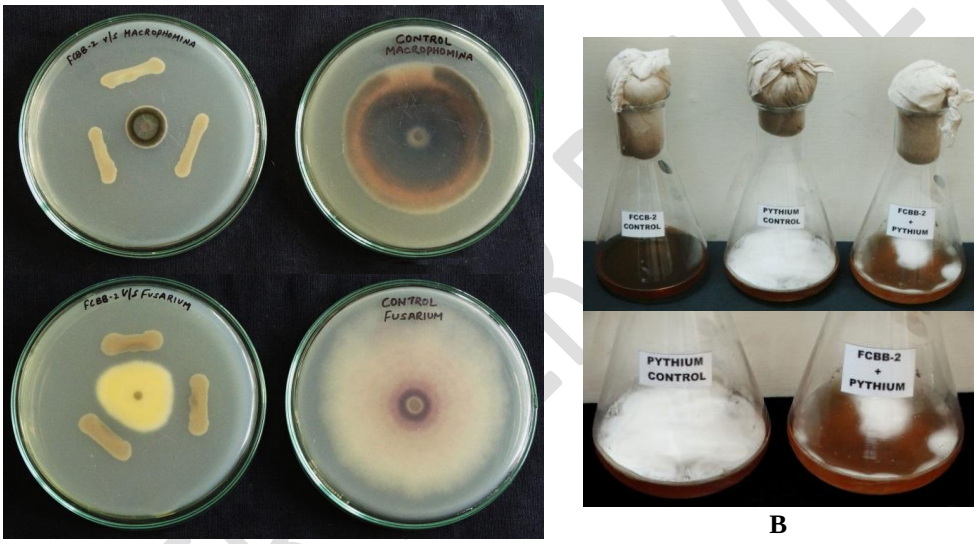


Figure 3 Relative Inhibition Ratio % (RIR) values of *Pseudomonas* spp. strain FCBB-2 in spread plate method, against plant fungal pathogens. Error bars Mean of ± SD (n=3).



A **B**

Figure 4 Biocontrol activity of strain FCBB-2: A; Plate confrontational culture method, B; broth method.

Table 2 Mycelium dry weight under non-stress and drought stress condition against strain FCBB-2

fungal strains	Mycelial dry weight in control flask (gm)		Mycelial dry weight in treated flask (gm)		Resistant or susceptible
	NS	DS	NS	DS	
Fusarium	0.361±0.22	0.120±0.21	0.113±0.10	0.040±0.12	Resistant
Sclerotium	0.499±0.11	0.170±0.10	0.412±0.10	0.187±0.07	Susceptible
Rhizoctonia	0.655±0.11	0.239±0.02	0.291±0.11	0.097±0.07	Resistant
Alternaria	0.811±0.13	0.299±0.11	0.181±0.16	0.057±0.05	Resistant
Macrophomina	0.106±0.14	0.033±0.10	0.014±0.01	0.005±0.17	Resistant
Pythium	0.896±0.12	0.310±0.16	0.392±0.12	0.165±0.17	Resistant

Legend: Numerical values are mean±SD of three independent observations; NS, non-stressed; DS, drought-stressed; gm- gram

DISCUSSION

The beneficial free-living soil bacteria, PGPR, protect plants from damages caused by phytopathogens by a number of different indirect mechanisms such as production of antibiotics, antifungal metabolites and defence enzymes, exhibiting rhizospheric competition with phytopathogens, secretion of iron chelating siderophores and HCN **(Glick, 1995a)**. Plant rhizosphere is a preferential niche for various types of microorganisms in the soil. In the present investigation, a total of 9 *Pseudomonas* spp. was isolated from soils of different crops across India, of which one could grow up to a minimal potential of -1.03 MPa and were screened for PGP traits like production of IAA, siderophore and HCN under non-stress and drought stress conditions. Production of PGP traits under drought stress conditions can be helpful in maintaining better nutritional status of the plant thus influencing plant-microbe interaction under drought conditions **(Sandhya *et al.*, 2010).** Nine bacterial isolates were screened *in vitro* for their plant growth promoting (PGP) abilities, of which only four isolates were selected based on drought stress tolerance for further characterization. In the first all the isolates were tested for ammonia production and more or less all the strains are positive for ammonia production. Ammonia production by PGPR is one the important constituent as it will provide the Nitrogen to the plants for growth. Plants use phytohormones, such as auxins (e.g., indole acetic acid) to influence many cellular functions **(Glick *et al.*, 2012)**. All the isolates used in this study presented IAA production, most of the isolates generating levels were higher to those presented in other reports. **Ahmad *et al.* (2008)** reported levels of 2.13 and 3.6 mg/gm for *Azotobacter* and *Pseudomonas* species, whereas **Gravel *et al.* (2007)** reported levels of 3.3 and 6.2 mg/gm for *P. putida* and *Trichoderma atroviride*. However, isolate FCBB-2 showed much higher IAA production levels of 6.24 and 5.49 mg/gm under non-stress and drought stress conditions, respectively. IAA production by the isolates was positively related with root length elongation, reports suggest that auxins production helps development of lateral roots, which are essential in observing water and nutrients. Exogenous sources of IAA are responsible for changes in the morphology of the root system and influence the uptake of nutrients by the plant **(San Francisco *et al.*, 2005)**. **Masalha *et al.* (2000)** found that plants cultivated under non-sterile conditions showed no iron-deficiency symptoms in contrast to plants grown in a sterile system, reinforcing the role of soil microbial activity in iron acquisition, namely through iron-bacterial siderophore complex generation. Four isolates under non-stressed and only one isolate FCBB-2 under drought stressed condition were showing siderophores production.

It has been reported that overproduction of HCN may control fungal diseases in wheat seedlings **(Flaishman *et al.*, 1996)**. Only one isolate FCBB-2 was positive for HCN production under non-stress and drought stress conditions. Siderophore and HCN production by the isolate FCBB-2 under non-stress and drought stress showed that strain FCBB-2 did not lose its production efficiency. Although the siderophore and HCN production time increased from 48 h to 96 h, which is due to the influence of PEG 6000 on growth rate of isolate. Reduced growth rate resulted in delayed induction of siderophore and HCN by strain FCBB-2. Furthermore, even in drought stress conditions strain FCBB-2 has not lost its efficiency to produce siderophore and HCN (Fig. 5) this relates with the production of EPS by bacteria, which helped the bacteria to sustain even in the stress conditions. *Pseudomonas* sp. strain FCBB-2 which could tolerate minimal water potential tested (-1.03 MPa) showed accumulation of EPS under drought stress condition than under non-stressed condition, indicating the role of EPS in stress tolerance. The EPS production of *Pseudomonas* sp. strain FCBB-2 was significantly higher under drought stress (41.62±0.02 mg/ml protein) compared to non-stress (10.56±0.02 mg/ml protein). The results are similar with the findings of **Sandhya and Ali (2014); Roberson and Firestone (1992); Ali *et al.* (2014)** that EPS production in bacterial species increases

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with increase in drought stress. EPS produced by the bacterial cells form a protective sheath around the cells and help in their survival as water potential declines **(Kibertus *et al.*, 1979; Sandhya *et al.*, 2010)**.

Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA **(Pal and McSpadden, 2006)**. Expression and secretion of these enzymes by different microbes can sometimes result in the suppression of plant pathogen activities directly. For example, control of *Sclerotium rolfsii* by *Serratia marcescens* appeared to be mediated by chitinase expression **(Ordentlich, *et al.*, 1988)**. Lytic enzymes can degrade several components that are present in the cell walls of fungi and oomycetes **(Chet and Inbar, 1994)**. A wide variety of bacterial lytic enzymes are known, including cellulases, glucanases, proteases, and chitinases. A ß-1,3-glucanase-producing *Pseudomonas cepacia* significantly decreased the incidence of diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *P. ultimum* **(Fridlender *et al.*, 1993). Garbeva *et al.* (2004)** studied the effect of agricultural practices on the composition of *Pseudomonas* spp. and their antagonistic activity towards *R. solani*. They observed that disease suppressiveness against *R. solani* was higher in grassland than in arable land and linked this to an increased number of antagonistic *Pseudomonas* spp. possessing chitinolytic activity. In the present study isolate FCBB-2 was producing lytic enzymes like cellulases, proteases and chitinases, hence it will be helpful in the suppression of fungal growth by showing biocontrol activity. **Dunne *et al.* (1997)** have demonstrated that biocontrol of *Pythium ultimum* in the rhizosphere of sugar beet was due to the production of extra cellular protease **(Praveen et al., 2014)**.

The biological control of soil-borne pathogens with antagonistic bacteria, particularly *Pseudomonas* spp. belonging to plant growth promoting Rhizobacteria, has received prominent attention because of the dual role of these bacteria in plant-growth promotion and disease control **(Zehnder *et al.*, 2001)**. The application of microorganisms to control diseases, which is a form of biological control, is an environment-friendly approach **(Lugtenberg and Kamilova, 2009)**. The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents **(Glick, 2012)**. In general, competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity in PGPR **(Lugtenberg and Kamilova, 2009)**. *Pseudomonas* spp. was well known biocontrol agents used for the control of soil-borne phytopathogenic fungi. Various mechanisms have been attributed to their antagonistic activity, namely, different hydrolytic enzymes, chitinases, HCN, and siderophore production and production of antibiotics and so forth. In the current study, *Pseudomonas* sp. strain FCBB-2 inhibited the growth of all the test phytopathogenic fungi effectively other than *S. hydrophilium*. The said mechanisms were evaluated in the strains of the current study to identify the various reasons for antagonism **(Praveen et al., 2012)**. Three methods were used in the present study where the spread plate and plate confrontational method were mainly used to check biocontrol ability of strain under non-stress conditions as for these two method agar media is used and it is difficulty prepare the agar media with drought stress conditions. The third broth method is solely used to check the antagonism under stress conditions using PEG6000. In turn, strain FCBB-2 that inhibited the growth of all four fungi also possessed drought tolerance (Fig. 1). This feature of possessing both characters make the selection an ideal one for their better performance under field conditions **(Praveen et al., 2014)**. On the basis of 16s rDNA gene sequence analysis and biochemical characters strain was identified as *Pseudomonas aeruginosa* and the nucleotide sequence was submitted to NCBI GenBank under accession No. KT311003.1. The present study demonstrates that the isolation of indigenous drought tolerant *Pseudomonas* spp. may be helpful in the development of microbial inoculants as biocontrol agents to mitigate abiotic stresses in plants, as we know in abiotic stress

conditions especially drought stress plant becomes weaker and there are several chances of pathogen attack. By formulating these types of strains will be having effective importance in the agriculture. Since strain FCCB-2 found as *P. aeruginosa* which is an opportunistic human pathogen and does not have any agricultural importance, and it is not always strains of *P. aeruginosa* cause disease in humans and have been reported by many authors and some plant growth promoting activities by Adesemoye *et al.* **(2008)** Radhapriya *et al.* **(2015)** for this reason in the present we would like to provide detailed methods to characterize plant growth promoting rhizobacteria. Furthermore, due to these contradictory facts we could not be able to characterize this strain to evaluate its characteristics under *in vivo* conditions.

CONCLUSION

Drought stress and biological control are two inseparables against plant growth and health. In the present study it was more focused on the isolation and preliminary characterization of *Pseudomonas* spp. strains in order to characterize them with multiple PGP traits and antagonistic activity. Among all strains, the most prospective strain FCBB-2 having a potential PGPR features along with biocontrol activity under *in vitro* conditions. Using such potential strains with multiple PGP characteristics will have a prospective opportunity in amelioration of biotic and abiotic stress factors in plants. Current results provides an evident for the importance of plant growth promoting bacteria in the development of bioinoculants for multiple uses. The experimental procedures and findings used here can be applied to characterize other beneficial microorganisms for sustainable agriculture and develop microbial products.

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