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In vitro Assessment of *Pseudomonas sp.* Strain FCBB-2 for Effective Plant Growth Promotion and Antifungal Activity under Drought Stress

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The significance of plant growth-promoting rhizobacteria (PGPR) in plant adaptation to harsh conditions is still unknown. Native bacteria were isolated from rhizospheric soils and tested for growth-promoting qualities as well as antagonistic capabilities against phytopathogenic fungi in this investigation. 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 was submitted to NCBI GenBank under the accession number KT311003.

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1. INTRODUCTION

Drought stress is a serious agricultural issue in arid and semiarid regions of the world, lowering crop productivity. Longer drought durations and more extremely dry years are being caused by changes in global mean air temperature and precipitation patterns, and increasingly severe drought conditions will impede food production in some nations [1]. Water-saving irrigation, traditional breeding, and genetic engineering of drought-tolerant transgenic plants are currently used as ways to improve plants' ability to resist drought stress. These methods very time taking and laborious due to which these techniques whatsoever not recommended highly [2]. Microbes are used as biofertilizers, biopesticides, and plant growth promoters in many countries throughout the world, but mainly in developing and rising countries, to boost crop development [3]. Companies have been providing farmers with nitrogen-fixing inoculants and formulations of plant-growth-promoting (PGP) microorganisms, both fungus and bacteria, for decades in order to boost crop yields. Many microbial substances are also utilised by home gardeners and organic farmers, and large-scale commercial farms in China, the United States, and Europe are starting to use biological materials as insecticides and fertilisers [4]. Replacing chemical fertilizers and pesticides is critical for agricultural sustainability [5,6], 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 [7].

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 [8,9]. 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 [10]. For example, increasing temperatures are known to facilitate pathogen spread. Plant defence mechanisms against biotic stressors are complicated, with multiple layers of defence. Plants produce pathogenesis-related (PR) proteins in response to pathogen attack. They code for enzymes like chitinases and glucanases, which can hydrolyze fungal pathogen cell walls [11,12]. Plant diseases are controlled by PGPR through a number of methods. The most prevalent ways are competition and metabolite production. Antibiotics, siderophores, HCN (Hydrogen Cyanide), cell wall-degrading enzymes, and other metabolites are among them [13,14]. Rhizobacteria, particularly PGPR, have a variety of useful actions that can help plants develop in stressful situations [15]. The plant rhizosphere has a wide range of taxonomic and functional diversity, which can affect plant fitness under abiotic stress conditions [16] (drought, salinity, pollutions, temperature etc.,). All clades of Proteobacteria, including Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria, as well as Firmicutes such as Actinobacteria, have PGPR. 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 [17] 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 [17-21]. 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 [22]. Bashan et al. [23] reported the role of polysaccharides producing *Azospirillum* in soil aggregation [24]. 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.

2. MATERIALS AND METHODS

2.1 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. Plants were uprooted for rhizosphere soil samples, and the bulk soil was removed by gently shaking the plants; rhizosphere soil samples were taken by dipping the roots in sterile normal saline containers and shaking for 30 minutes. The soil suspensions were serially diluted, and the appropriate 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) [25]. 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.

2.2 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 [24,26] 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.

2.3 Screening for Plant Growth Promoting Activities

2.3.1 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 minutes, after which the supernatant was mixed with Salkowsky reagent and incubated for 1 hour at room temperature in the dark. The positive pink color's absorbance was measured at 530 nm [27].

2.3.2 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 [28]. 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 28oC for 5 days and checked for development of orange color.

2.3.3 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 [29,30]. Total cyanogens content was estimated by the method described by Bradbury [31]. 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.

2.4 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 [32] 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).

2.5 Antifungal Activity

2.5.1 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^{o}C 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 [33].

2.5.2 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 [33].

2.6 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 25oC, mycelial dry weight was calculated in the flaks one and three [34].

2.7 Production of Lytic Enzymes

Strain FCBB-2 was screened for lytic enzyme production (Cellulase, Protease and Chitinase). Casein was utilised as a substrate in screening agar medium to test for the existence of protease activity (1 percent w/v). The creation of a clear zone around colonies after precipitation with 1 M HCl solution indicated enzyme activity [35]. 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). Chitinase-producing bacteria were identified in colonies with clear zones on a creamish background [36]. In the screening agar medium, carboxy methyl cellulose (CMC) (1 percent w/v) was utilised as a substrate to test cellulase activity. The petri plates were incubated for 24 hours at 37˚c. Plates were flooded for 15 minutes with a 1 percent Congo red solution, then de-stained for 15 minutes with a 1 M NaCl solution. Cellulase activity was demonstrated by clear zones around the colonies [35].

2.8 Production of HCN and Siderophores under Stress Conditions

Modified protocol of Schwyan and Neilands [28] 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 28oC 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 [29] was employed to screen HCN production. Experiment was repeated for 3 times to interpret the results.

2.9 Production of Exopolysaccharides

The efficient isolate able to grow at maximum stress level was analyzed for its ability to produce EPS [37,38] 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 [39]. Concentration of protein in the supernatant was estimated by Bradford method [40]. The supernatant was filtered over a 0.45 µm nitrocellulose membrane and dialyzed against water at 4°C for many hours. To eliminate any insoluble material, the dialysate was centrifuged at 20,000 g for 25 minutes before being combined with three litres of ice-cold 100% alcohol and refrigerated overnight at 4°C. The precipitated EPS was suspended in water after centrifugation at 10,000 g for 15 minutes and purified further by repeating the dialysis and precipitation processes. The total carbohydrate content of the precipitated EPS was calculated according to Dubois et al. [41].

2.10 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.

3. RESULTS

3.1 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).

3.2 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 (Table 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 (Table 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 (Table 1). Ammonia production was observed in all the isolates under both non-stress and drought stress.

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) (Table 1).

3.3 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).

3.4 Identification of Strain FCBB-2

Microscopic, morphological, and biochemical tests were used to describe the prospective isolate chosen based on drought stress resistance and PGP trait production under drought challenged conditions. The isolate FCBB-2 was found to be Gram negative, motile, rod-shaped bacteria under microscopic examination. Colonies with greenish pigmentation developed on King's B medium isolation as creamy, smooth, shiny, round, convex colonies. Citrate, xylose, melibiose, lactose, and arabinose were all used by the isolate FCBB-2, which was also positive for catalase, oxidase, and malonate usage. Isolate FCBB-2 was identified as Pseudomonas aeruginosa based on NCBI 16s rRNA gene sequence blast analysis, and the nucleotide sequence was submitted to NCBI GenBank under accession number KT311003.1.

Table 1. Plant growth promoting traits of drought tolerant *Pseudomonas* spp. strains 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	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

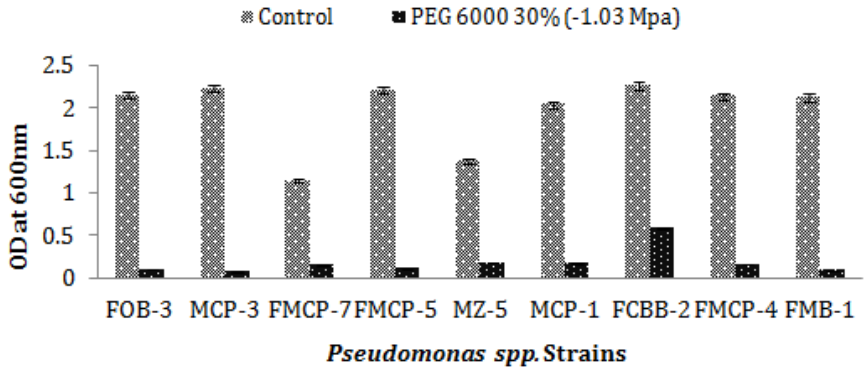

Fig. 1. Drought stress tolerance of *Pseudomonas* spp. strains at -1.03 MPa. Error bars Mean of ± SD (n=3)

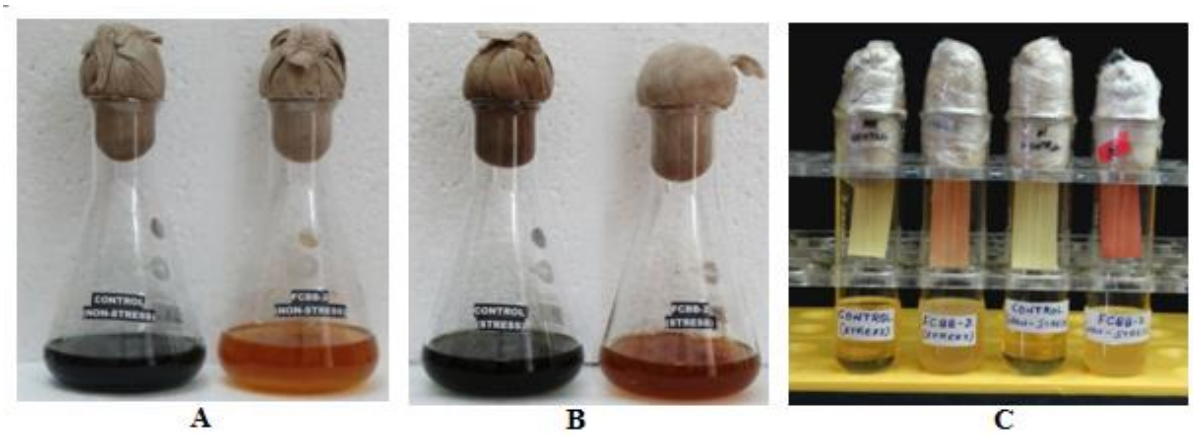

Fig. 2. Screening of *Pseudomonas* sp. strain FCBB-2 for siderophore and HCN (Hydrogen Cyanide) activity under non-stress and drought stress conditions. (A) & (B); siderophore, (C); HCN

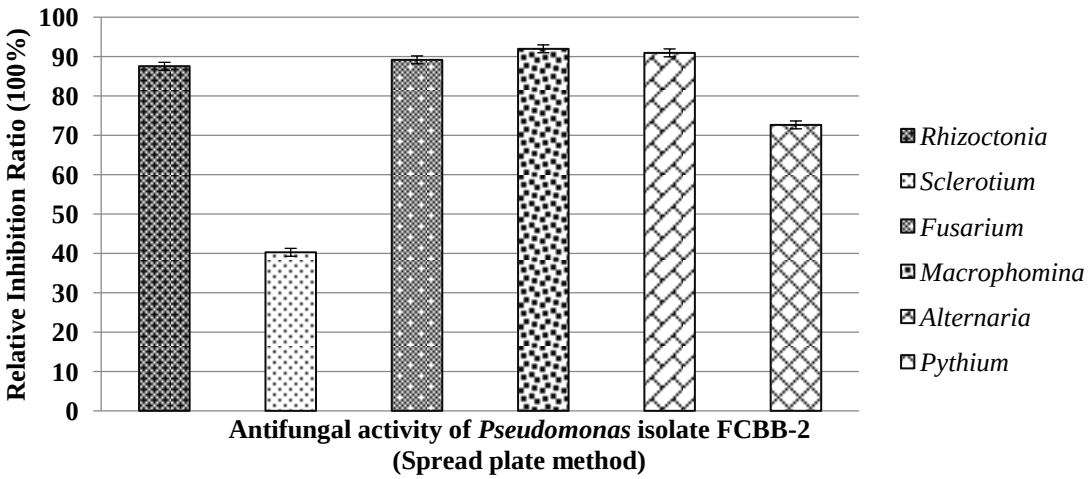

Fig. 3. Relative Inhibition Ratio% (RIR) values of *Pseudomonas* spp. strain FCBB-2 in spread plate method, against plant fungal pathogens. Error bars Mean of ± SD (n=3)

3.5 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 (Table 2).

3.6 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.

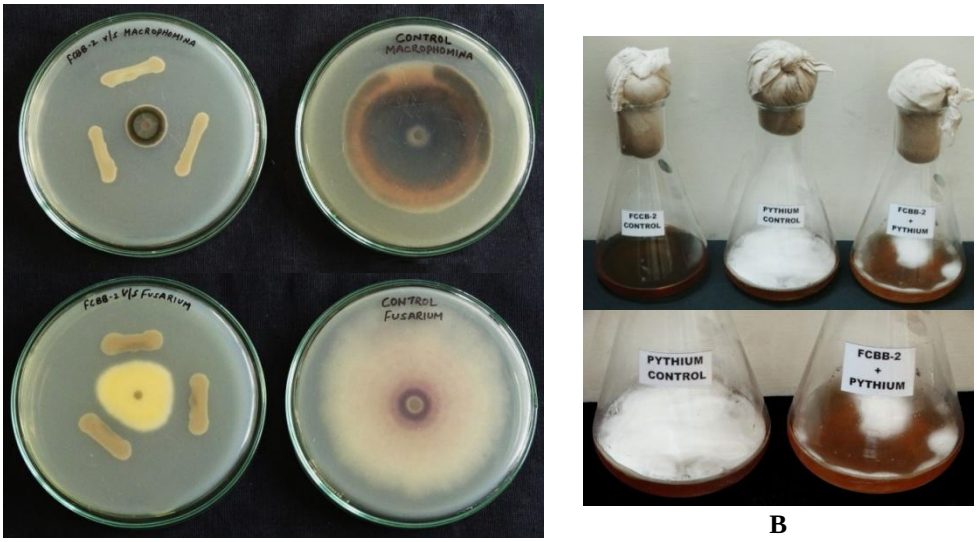

Fig. 4. Biocontrol activity of strain FCBB-2: A; Plate confrontational culture method, B; broth method

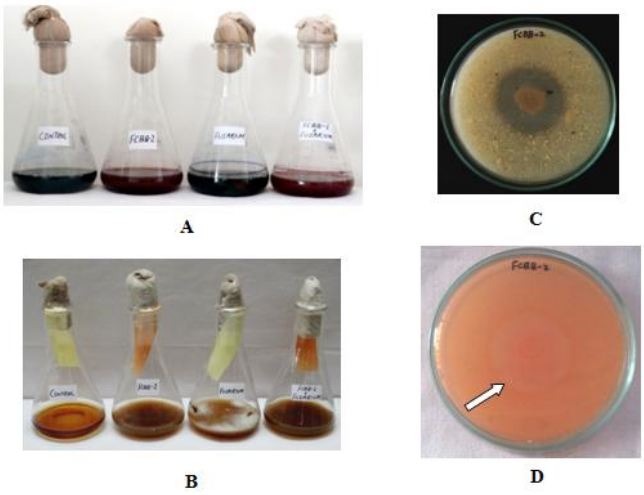

Fig. 5. PGP and enzymatic activity of strain FCBB-2. A); Siderophore activity, B); Hydrogen Cyanide (HCN) production under multiple stress conditions (biotic & abiotic), C) protease activity, D); cellulase activity of strain FCBB-2, arrow indicates the halo zone

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

4. 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 [42]. 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 [43]. 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 [44]. 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. [45] reported levels of 2.13 and 3.6 mg/gm for *Azotobacter* and *Pseudomonas* species, whereas Gravel et al. [46] 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 [47]. Masalha et al. [48] 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 [49]. 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 [50], Roberson and Firestone [17], Ali et al. [51] that EPS production in bacterial species increases 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 [43,52].

Many microbes manufacture and release lytic enzymes that are capable of hydrolyzing a wide range of polymeric materials, including chitin, proteins, cellulose, hemicellulose, and DNA [53]. Different microorganisms' expression and secretion of these enzymes canoCcasionally result in direct reduction of plant disease activity. Serratia marcescens seems to influence Sclerotium rolfsii via chitinase expression, for example [54]. Several components found in the cell walls of fungi and oomycetes can be degraded by lytic enzymes [55]. Cellulases, glucanases, proteases, and chitinases are only a few of the bacterial lytic enzymes that have been discovered. Rhizoctonia solani, Sclerotium rolfsii, and Pseudomonas ultimum illnesses were greatly reduced when a ß-1,3-glucanase-producing Pseudomonas cepacia was utilised [56]. Garbeva et al. [57] investigated the impact of agricultural techniques on the composition and antagonistic activity of Pseudomonas spp. against R. solani. They discovered that disease suppressiveness against R. solani was stronger in grassland than in arable land, which they attributed to an increase of antagonistic Pseudomonas spp. with 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. [58] have demonstrated that biocontrol of *Pythium ultimum* in the rhizosphere of sugar beet was due to the production of extra cellular protease [59].

Because of their dual role in plant growth promotion and disease control, antagonistic bacteria, notably *Pseudomonas spp.* belonging to the plant growth boosting Rhizobacteria, have garnered a lot of interest in biological control of soil-borne diseases [60]. It is an environmentally benign technique to use microorganisms to control diseases, which is a type of biological control [61]. The main indirect way by which rhizobacteria promote plant growth is through functioning as biocontrol agents [44]. The main routes of biocontrol action in PGPR are competition for resources, niche exclusion, induced systemic resistance, and the synthesis of antifungal metabolites [61]. *Pseudomonas spp. was well-known biocontrol agents for soil-borne phytopathogenic fungus control. Their antagonistic activity has been attributed to a variety of methods, including various hydrolytic enzymes, chitinases, HCN, siderophore synthesis, antibiotic production, and so on. Except for S. hydrophilium, Pseudomonas sp. strain FCBB-2 efficiently suppressed the growth of all of the test phytopathogenic fungi in the current investigation. The aforementioned pathways were tested in the current study's strains to determine the various causes of antagonism [62].* 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 [62]. 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. [63], Radhapriya et al. [64] 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.

5. 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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