Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent*Pseudomonas* sp.

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Isolation and characterization of drought tolerant ACC deaminase and exopolysaccharide producing fluorescent *Pseudomonas* sp.

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Abstract

The enzyme 1-aminocyclopropane-1-carboxylate deaminase catalyzes the degradation of 1-aminocyclopropane-1-carobylic acid (ACC), the immediate precursor of the plant hormone ethylene into α-ketobutyrate and ammonia. The enzyme has been detected in limited number of bacteria and plays a significant role in sustaining plant growth and development under biotic and abiotic stress conditions by reducing stress induced ethylene production in plants. In the present study thirty two fluorescent *Pseudomonas* sp. isolated from rhizosphere and non rhizosphere soils of different crop production systems were screened for drought tolerance using poly ethylene glycol 6000 (PEG 6000). Nine of these isolates were tolerant to metric potential of -0.30 MPa (15% PEG 6000). All the drought tolerant isolates were screened for ACC deaminase activity using ACC as the sole nitrogen source. Among the nine drought tolerant isolates one (SorgP4) was found to be positive for ACC and showed 3.71 ± 0.025 µM/mg protein/ h of α-ketobutyrate under non-stress and 1.42 ± 0.039 µM/mg protein/ h of α-ketobutyrate under drought stress condition respectively. The isolate SorgP4 also showed other plant growth promoting traits (PGP) such as indole acetic acid production (IAA), phosphate solubilization, siderophore and hydrogen cyanide production. The ACC deaminase gene (*acdS*) from the isolate SorgP4 was amplified and the nucleotide sequence alignment of the *acdS* gene showed significant homology with *acdS* genes of NCBI Genbank. The 16S rRNA gene sequencing analysis identified the isolate as *Pseudomonas fluorescens*. Both the sequences were submitted to NCBI GenBank under the accession numbers JX885767 and KC192771 respectively.

Key words: *Pseudomonas fluorescens* · ACC deaminase · drought stress · *acdS* gene · PGP traits

Introduction

The gaseous hormone ethylene (C_2H_4) synthesized in plant tissues from the precursor 1-aminocyclopropane- 1-carboxylic acid (ACC) is involved in multiple physiological and developmental processes in plants, such as tissue differentiation, lateral bud development, seedling emergence, leaf and flower senescence, root hair development and elongation, anthocyanin synthesis, fruit ripening and degreening, production of volatile compounds responsible for aroma in fruits (Abeles et al. 1992; Frankenberger and Arshad 1995; Spaink 1997; Bleecker and Kende 2000). Ethylene also regulates plant responses to biotic and abiotic stresses (Abeles et al. 1992; Roman et al. 1995; O'Donnell et al. 1996; Penninckx et al. 1998).Under ambient conditions, plants produce required levels of ethylene, conferring beneficial effects on plant growth and development; however, in response to biotic and abiotic stresses there is often a significant increase in the endogenous ethylene production that has adverse effects on

plant growth and is thought to be responsible for senescence in plants (Abeles et al. 1992; Woltering and Van Doorn 1988; Nayani et al. 1998; Ali et al. 2012).

Plant growth promoting rhizobacteria (PGPR) are a group of free-living saprophytic bacteria that can be found in the rhizosphere in association with root system and enhance the growth and development of plant either directly or indirectly (Kloepper and Beauchamp 1992; Liu et al. 1995). Interestingly these PGPR strains also possess the enzyme ACC deaminase (Jacobson et al. 1994; Glick et al. 1998; Shah et al. 1997) and this enzyme can cleave the plant ethylene precursor ACC to ammonia and a-ketobutyrate thereby lowers the level of ethylene under various biotic and abiotic stresses (Glick et al. 1998) such as salt stress (Cheng et al. 2007, Mayak et al. 2004a; Zahir et al. 2009), flooding stress (Grichko and Glick 2001), drought stress (Mayak et al. 2004b), heavy metal stress (Belimov et al. 2005; Stearns et al. 2005) and pathogen attack (Wang et al. 2000). ACC deaminase-containing plant growth promoting rhizobacteria lowers the level of ACC in the stressed plants, thereby limiting the amount of stress ethylene synthesis and hence the damage to the plant. These bacteria are beneficial to plant growth as plants are often subjected to ethylene producing stresses. Soil borne fluorescent pseudomonads have excellent root colonizing ability, catabolic versatility and produce a wide range of enzymes and metabolites that favour the plant withstand under varied biotic and abiotic stress conditions (Ramamoorthy et al. 2001; Vivekananthan et al. 2004; Mayak et al. 2004a).

Drought stress is one of the major agricultural problems limiting crop productivity in most of the arid and semiarid regions of the world. This form of abiotic stress, affect the plant water relation at cellular and whole plant level causing specific as well as unspecific reactions and damages. Bacteria can survive under stress conditions due to the production of exopolysaccharide (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; Chenu and Roberson 1996). EPS also help the microorganisms to irreversibly attach and colonize the roots due to involvement of a network of fibrillar material that permanently connects the bacteria to the root surface (Bashan et al. 2004). Inoculation of plants with drought tolerant ACC deaminase containing native beneficial microorganisms may increase drought tolerance of plants growing in arid or semiarid areas. Therefore, in the present investigation, an attempt was made to isolate and characterize exopolysaccharide and ACC deaminase-producing drought tolerant *Pseudomonas* strains from cropped soils of different arid and semiarid natural habitat which will provide the best benefit for drought stress plants.

Materials and methods

Isolation of fluorescent *Pseudomonas* sp.

A total of sixteen soil samples (non-rhizosphere and rhizosphere) were collected from different ecosystems covering arid, semi-arid and sub-humid zones. The non-rhizosphere soil was collected from a depth of 0-15 cm in sterile normal saline and kept on shaker for 30 min and for rhizosphere soil samples the plants were uprooted, bulk soil was removed by gently shaking the plants and the rhizosphere soil was collected by dipping the roots in sterile normal saline followed by shaking for 30 min. The soil suspension (non-rhizosphere and rhizosphere) was serially diluted and appropriate dilutions were spread plated on solid King's B medium. The plates were incubated at 28±2°C and fluorescent colonies were selected for further studies.

Screening for drought tolerance and exopolysaccharide production

Trypticase soya broth (TSB) with different water potentials (−0.05, −0.15, −0.30, −0.49, and −0.73 MPa) was prepared by adding appropriate concentrations of polyethylene glycol (PEG 6000) (Michel and Kaufmann 1973; Sandhya et al. 2009) and was inoculated with 1% of overnight raised bacterial cultures in TSB. Six replicates of each isolate with each concentration were prepared. After incubation at 28°C under shaking conditions (120 rpm) for 24 h, growth was estimated by measuring the optical density at 600 nm using a spectrophotometer (Thermospectronic, 336002, USA). The growth of the isolates at various stress levels was recorded.

The cultures able to grow at maximum stress level were analyzed for their ability to produce EPS (Fett et al. 1986, 1989) under no stress and maximum stress level (−0.30 MPa). Exopolysaccharide was extracted from 3-day-old cultures raised in TSB (15% PEG 6000 was added to TSB for inducing stress). The culture was centrifuged at 20,000 *g* for 25 min and the supernatant was collected. Highly viscous cultures were diluted with 0.85% KCl before centrifugation. The pellet was washed twice with 0.85% KCl 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 1956). Concentration of protein in the supernatant was estimated by Bradford's reagent (Bradford 1976). Then, the supernatant was filtered through 0.45 μm nitrocellulose membrane and dialysed extensively against

water at 4°C. The dialysate was centrifuged (20,000 *g*) for 25 min to remove any insoluble material and mixed with 3 volumes of ice-cold absolute alcohol and kept overnight at 4°C. The precipitated EPS obtained by centrifugation (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).

Screening for ACC deaminase activity

Screening for ACC deaminase activity of drought tolerant *Pseudomonas* isolates was done based on their ability to use ACC as a sole nitrogen source. All the nine drought tolerant *Pseudomonas* isolates were grown in 5 ml of TSB medium incubated at 28^{o}C at120 rpm for 24 h. The cells were harvested by centrifugation at 3000 *g* for 5 min and washed twice with sterile 0.1 M Tris-HCl (pH 7.5) and resuspended in 1 ml of 0.1 M Tris-HCl (pH 7.5) and spot inoculated on petri plates containing modified DF (Dworkin and Foster) salts minimal medium (Dworkin and Foster, 1958), glucose, 2.0 g; gluconic acid, 2.0 g; citric acid, 2.0 g; KH_2PO_4, 4.0 g; Na_2HPO_4, 6.0 g; $MgSO_4{\cdot}7H_2O$, 0.2 g; micro nutrient solution ($CaCl_2$, 200 mg; $FeSO_4{\cdot}7H_2O$, 200 mg; H_3BO_3, 15 mg; $ZnSO_4{\cdot}7H_2O$, 20 mg; Na_2MoO_4, 10 mg; KI, 10 mg, NaBr, 10 mg; $MnCl_2$, 10 mg; $COCl_2$, 5 mg; $CuCl_2$, 5 mg; $AlCl_3$, 2 mg; $NiSO_4$, 2 mg; distill water, 1000 ml), 10 ml and distill water, 990 ml; supplemented with 3 mM ACC as sole nitrogen source. Plates containing only DF salts minimal medium without ACC as negative control and with $(NH_4)_2SO_4$ (0.2% w/v) as positive control. The plates were incubated at 28^{o}C for 72 h. Growth of isolates on ACC supplemented plates was compared to negative and positive controls and was selected based on growth by utilizing ACC as nitrogen source.

ACC deaminase activity assay

To measure ACC deaminase activity, *Pseudomonas* isolates were grown in 5 ml of TSB medium at 28^{o}C until they reached stationary phase. To induce ACC deaminase activity under non-stress and drought stress conditions, the cells were collected by centrifugation, washed twice with 0.1 M Tris-HCl (pH 7.5), suspended in 2 ml of modified DF minimal medium supplemented with 3 mM final concentration of ACC without PEG for non-stress condition and with PEG 6000 (-0.30 MPa) for drought stress respectively, and incubated at 28^{o}C with shaking for another 36-72 h.

ACC deaminase activity was determined by measuring the production of α-ketobutyrate and ammonia generated by the cleavage of ACC by ACC deaminase (Honma and Shimomura 1978; Penrose and Glick 2003). The induced bacterial cells were harvested by centrifugation at 3,000 *g* for 5 min, washed twice with 0.1 M Tris-HCl (pH 7.5), and resuspended in 200 µl of 0.1 M Tris-HCl (pH 8.5). The cells were labilized by adding 5% toluene (v/v) and

then vortexed at the highest speed for 30 s. Fifty µl of labilized cell suspension was incubated with 5 µl of 0.3M ACC in an eppendorf tube at 28^{o} C for 30 min. The negative control for this assay included 50 µl of labilized cell suspension without ACC, while the blank included 50 µl of 0.1 M Tris- HCl (pH 8.5) with 5 µl of 0.3 M ACC. The samples were then mixed thoroughly with 500 µl of 0.56 N HCl by vortexing and the cell debris was removed by centrifugation at 12, 000 *g* for 5 min. A 500 µl aliquot of the supernatant was transferred to a glass test tube and mixed with 400 µl of 0.56N HCl and 150 µl of DNF solution (0.1 g 2,4-dinitrophenylhydrazine in 100 ml of 2N HCl); and the mixture was incubated at 28^{o} C for 30 minutes. One ml of 2N NaOH was added to the sample before the absorbance at 540 nm was measured.

The concentration of α -ketobutyrate in each sample was determined by comparison with a standard curve generated as follows: 500 µl α-ketobutyrate solutions of 0, 0.01, 0.05, 0.1, 0.2, 0.5, 0.75 and 1 mM were mixed respectively with 400 µl of 0.56 N HCl and 150 µl DNF solution. One ml of 2N NaOH was added and the absorbance at 540 nm was determined as described above. The values for absorbance versus α-ketobutyrate concentration (mM) were used to construct a standard curve.

Protein concentrations determination

The protein concentration of toluenized cells was determined by the method of Bradford (1976). A 26.5 µl aliquot of the toluene-labilized bacterial cell sample used for the ACC deaminase enzyme assay was diluted with 173.5 µl of 0.1 M Tris-HCl (pH 8.0), and boiled with 200 µl of 0.1 N NaOH for 10 min. After the cell sample was cooled to room temperature, the protein concentration was determined by measuring the absorbance at 595 nm immediately after mixing the solution with 200 µl of Bradford's reagent. Bovine serum albumin (BSA) was used to establish a standard curve.

Screening of drought tolerant *Pseudomonas* sp. for their plant growth promoting traits

ACC deaminase positive drought tolerant isolate were tested *in vitro* for their multiple PGP traits. The method of Gordon and Weber (1951) was followed for the estimation of indole acetic acid (IAA). Luria Bertani broth (LB) amended with 5-mmol tryptophan was inoculated with overnight raised bacterial cultures (0.5 OD at 600 nm) and incubated at 28^{o} C for 48 h. One ml of culture was centrifuged (3,000 *g* for 20 min) and supernatant separated. To the supernatant, 4 ml of Salkowsky reagent was added followed by incubation for 1 h at room temperature under dark conditions. Absorbance of the pink colour developed was read at 530 nm. Concentration of the proteins in the pellet was determined (Bradford 1976) and the amount of IAA produced was expressed µg/mg

cell protein. For studying phosphate solubilization, 5 µl of overnight raised culture was spotted on Pikovskaya's agar plates containing 2% tri-calcium phosphate. The plates were incubated at 28^{o} C for 24 to 72 h, and observed for appearance of zone of solubilization around the bacterial colonies. For quantitative analysis, 5 ml of NBRI-BBP medium (Mehta and Nautiyal 2001) was inoculated in replicates with 50 µl of bacterial culture (0.5 OD at 600 nm) followed by incubation for 7 days at 28^{o} C incubator shaker at 120 rpm. The cells were harvested by centrifugation at 3,000 *g* for 10 min and the supernatant thus obtained were used for quantitative estimation of phosphate (Fiske and Subbarow 1925). For siderophore production, 1µl of overnight raised culture in Luria broth was spotted on Chrome Azurol S (CAS) agar plates and incubated at 28^{o} C for 48 h. Plates were observed for the appearance of orange halo around the bacterial colony (Schwyn and Neilands 1987). For HCN production, the culture were streaked on King's B medium amended with 0.4% (w/v) of glycine and Whatman no.1 filter paper disc soaked in 0.5% picric acid (w/v) in 2% (w/v) sodium carbonate was placed in the lid of petri plate. The plates were sealed with parafilm and incubated at 28^{o} C for 4 days for development of deep orange colour (Bakker and Schipper 1987).

Characterization of *acdS* and 16S rRNA gene

For molecular characterization, bacterial genomic DNA was isolated (Chen and Kuo 1993) and the *acdS* gene was amplified by polymerase chain reaction (PCR) using the reference primers (Farajzadeh et al. 2010) AccF 5'-ATG AAT CTG AAT CGT TTT GAA C-3' and 5'-TCA GCC GTT GCG GAA CAG-3'. PCR reactions were carried out in 25 µl reaction mixture contained 1x reaction buffer, 2.5 mM dNTP mixture, 10 pM of each primers, Taq DNA polymerase (1U) ; 25 ng of template DNA. PCR was conducted in a DNA Thermal Cycler (appendroff) under the following conditions: 5 min initial denaturation at 94^{o} C, 35 cycles of 1 min denaturation at 94^{o} C, annealing at 54^{o} C for 50s and 2 min of elongation at 72^{o} C, followed by final extension at 72^{o} C for 7 min. PCR product (~996 bp) were separated by electrophoresis through 1% agarose gel, purified and sequenced (Xcelris Genomics Ltd., Ahmedabad, India).The 16SrRNA gene was amplified by PCR using universal forward (5′ AGAGTTTGATCCTGGCTCAG 3′) and reverse (5′AAG GAGGTGATCCAGCCGCA 3′) primers under standard conditions (initial denaturation 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 40s, extension at 72°C for 90s, and final extension at 72°C for 7 min). The PCR product (~1,500 bp) was purified and sequenced (Xcelris Genomics Ltd., Ahmedabad, India). The sequences (*acdS* and 16S rRNA genes) obtained was compared with the existing database of *acdS* and 16S rRNA gene and submitted to GenBank.

Statistical analysis

Data were statistically tested by analysis of variance (ANOVA) using Instat + version 3.36. Each treatment was analyzed with at least six replicates and a standard deviation (SD) was calculated and data are expressed in mean ± SD of six replicates.

Results

Isolation and screening for drought tolerance

A total of 17 fluorescent *Pseudomonas* were isolated on King's B medium of which nine isolates (SorgP1, SorgP3, SorgP4, GnP9, RdgP10, SunfP12, SunfP13, BriP15 and BriP17) could grow at a minimum water potential (-0.30MPa) (Fig. 1). Isolates, which could tolerate higher levels of drought stress, were used for EPS production under both no stressed conditions as well as under minimum water potential (−0.30 MPa). The strain Rdgp10 produced maximum amount of EPS (3.22 ± 0.04 mg/mg protein) under non-stressed condition, closely followed by isolate SorgP3 (2.85 ± 0.07 mg/mg protein), sorgP4 (2.75 ± 0.06 mg/mg protein), SunfP12 (2.71 ± 0.10 mg/mg protein) and BriP15 (2.18 ± 0.25 mg/mg protein) (Table 1). Under drought stress isolate SunfP12 was the best producer of EPS (4.63 ± 0.10 mg/mg protein) followed by Rdgp10 (4.33 ± 0.05 mg/mg protein), SorgP4 (4.16 ± 0.05 mg/mg protein) and SorP3 (3.73 ± 0.07 mg/mg protein) (Table 1).

Screening for ACC deaminase and plant growth promoting traits

All the nine drought tolerant isolates were screened for ACC deaminase based on the enrichment method, where ACC was used as the sole nitrogen source. Among nine isolates, one isolates (SorgP4) grew well on DF salt minimal medium with either ACC or ammonium sulfate serving as the sole nitrogen source which was compared with DF salt minimal medium without nitrogen source (Fig. 2). Isolate SorgP4 which was positive for ACC deaminase was screened for other plant growth promoting traits (Fig. 3). The isolate SorgP4 produced significant amount of indole acetic acid (IAA) (48.2 ± 3.0 µg/mg protein) and 41.7 ± 5.0 µg/ml phosphate solubilization. Isolate SorgP4 was also positive for indirect plant growth promoting traits such as siderophore and hydrogen cyanide (HCN) production.

ACC deaminase activity

The ACC deaminase enzyme activity was assayed under both non-stress and drought stress conditions by quantifying the amount of α-ketobutyrate produced during the deamination of ACC by the enzyme ACC deaminase. Isolate SorgP4 utilized ACC as a sole source of nitrogen by the production of ACC deaminase enzyme and it

showed the greater amount of ACC deaminase activity (3.71 ± 0.025 μM/mg protein/h of α-ketobutyrate) under non-stress and 1.42 ± 0.039 μM /mg protein/h of α-ketobutyrate under drought stress condition respectively (Fig. 4).

Amplification of 1-aminocyclopropane-1-carboxylic acid deaminase

ACC deaminase (*acdS*) gene from the drought tolerant isolate SorgP4 was amplified using PCR with the reference primers. The expected amplification product of approximately 996 bp was observed in the isolate SorgP4 (Fig. 5), confirming the results of ACC deaminase assay. A BLASTN search was performed for the nucleotide sequence of partial length of SorgP4 *acdS* gene , which showed a 100% homology with *acdS* gene of *Pseudomonas fluorescens* strain FPG3 (AB638440) and *Pseudomonas* strain 6G5 (M80882) respectively. The sequence was submitted to GenBank under the accession no. KC192771.

Phylogenetic analysis of the partial sequence of the isolate SorgP4 with existing sequences in the database revealed a significant polymorphism between the sequences. The bootstrap value for this dendogram ranged from 40% to 100% with *Rhizobium* sp. as out group (Fig. 6). The generated phylogenetic tree showed that ACC deaminase sequences of *Pseudomonas* isolate SorgP4, *Pseudomonas* strain 6G5, *Pseudomonas fluorescens* strain FPG3 fell into the same clade with a high bootstrap value of 100%. By 16S rDNA gene sequencing, isolate SorGP4 was identified as *Pseudomonas fluorescens* which showed a 100% homology with that of *Pseudomonas fluorescens* strain WS32 (JN210910) in the existing GenBank database. The sequence was submitted to GenBank under the accession no. JX885767.

Discussion

Plants are constantly exposed to abiotic stress, such as drought, which is one of the most serious problems associated with plant growth and development affecting agricultural demands. Introduction of drought tolerant ACC deaminase-producing microorganisms in the drought-stressed soils can alleviate stress in the crop plants by lowering stress induced ethylene production. Drought tolerant microorganisms could survive in these habitats and bound to seed coat or root of developing seedlings, and cause deamination of ACC the immediate precursor of ethylene in plants by ACC deaminase leading to lowering of plant ethylene level and thereby facilitating the growth and development of plants (Glick et al. 1998). Exopolysaccharides possess unique water holding and cementing properties, thus play a vital role in the formation and stabilization of soil aggregates and regulation of nutrients and water flow across plant roots through biofilm formation (Roberson and Firestone 1992; Tisdall and Oadea 1982).

In the present study we have isolated and characterized drought tolerant ACC deaminase and EPS producing *Pseudomonas* isolate SorgP4 from stressed ecosystem. A total of seventeen fluorescent *Pseudomonas* sp. grown under arid and semi arid conditions were isolated and characterized for drought tolerance. Of these 17 isolates, nine could tolerate maximum level of drought stress (-0.30 MPa). The EPS production of these selected isolates was higher under stressed than under no stress conditions, indicating that EPS production in bacteria occurs as a response to the stress (Roberson and Firestone 1992). Role of exopolysaccharide material has been suggested in the protection of *A. brasilense* Sp245 cells against desiccation (Konnova et al. 2001). Hartel and Alexandre (1986) observed a significant correlation between the amount of EPS produced by cowpea *Bradyrhizobium* strains and their desiccation tolerance. Probably EPS can provide a microenvironment that holds water and dries more slowly than the surrounding microenvironment, thus protecting bacteria from drying and fluctuations in water potential (Hepper 1975; Wilkinson 1958).

PGPR that have ACC deaminase activity help plants to withstand stress (biotic or abiotic) by reducing the level of stress ethylene through the activity of enzyme ACC-deaminase that hydrolyzes ACC into α-ketobutyrate and ammonia, instead of ethylene (Glick et al. 1998; Arshad et al. 2007). In the present study, we screened drought tolerant bacteria having ACC deaminase activity with multiple PGP traits, and found that one out of the screened 17 strains showed ACC deaminase activity. Variations in levels of ACC deaminase activity of the strains were noted under non-stress and drought stress. Biochemical assay of ACC deaminase revealed the secretion of enzyme by *Pseudomonas* isolate SorgP4 as confirmed with the PCR amplification of *acdS* gene using the same primer as reported earlier (Farajzadeh et al. 2010) obtained a partial *acds* gene from *Pseudomonas* isolate SorgP4. ACC deaminase gene encoding ACC deaminase enzyme has been isolated from different soil bacteria under non-stress and under abiotic stresses (Klee et al. 1991; Campbell and Thompson 1996; Hontzeas et al. 2005; Rodriguez-Diaz et al. 2008; Jha et al. 2009; Onofre-Lemus et al. 2009). ACC deaminase producing bacteria are known to facilitate the growth of a variety of plants especially under stressful conditions such as flooding, heavy metals, high salt and drought, so, the *acdS* gene coding for enzyme ACC deaminase can be a very useful candidate gene for the development of bio-inoculants for abiotic stress management in plants.

Conclusion

Our results suggest that, the selection and use of ACC deaminase-producing drought tolerant PGPR, with multiple PGP activities for the facilitation of plant growth in drought environments, will be a highly important area for future

research. Hence, further evaluation of these drought tolerant bacterial strains is needed to uncover their efficiency as plant growth promoting bacteria in soil plant systems.

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Ethical standards

The authors declare that all the experiments were conducted according to the current laws of the country in which they were performed

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Legends

Fig. 1 Growth pattern of fluorescent *Pseudomonas* isolates under drought stress (-0.30 MPa) conditions. Error bars are mean of ± standard deviation, n = 6

Fig. 2 Screening of bacterial isolates for ACC deaminase activity. A- DF minimal medium with nitrogen source (positive control); B- DF minimal medium without any nitrogen source (negative control); C-DF minimal medium with ACC as nitrogen source. Arrows indicates the *Pseudomonas* isolate SorgP4

Fig. 3 Plant growth promoting traits of *Pseudomonas* isolate SorgP4

Fig. 4 ACC deaminase activity from *Pseudomonas* isolate SorgP4 under non-stress and drought stress (-0.3MPa). Error bars are mean of ± standard deviation, n = 6. Values with different letters are significantly different at $P<0.05$ in all the treatments

Fig. 5 Amplification of ACC deaminase (*acdS*) gene. (M) DNA Ladder, (Ps4) *Pseudomonas* isolate SorgP4

Fig. 6 Phylogenetic analysis of *Pseudomonas* sp. based on *acdS* gene sequences available from the NCBI Genbank data base. Distances and clustering with the neighbor-joining method was performed by using the software packages Mega version 4.0. Bootstrap values (n = 500) are listed as percentages at the branching points.

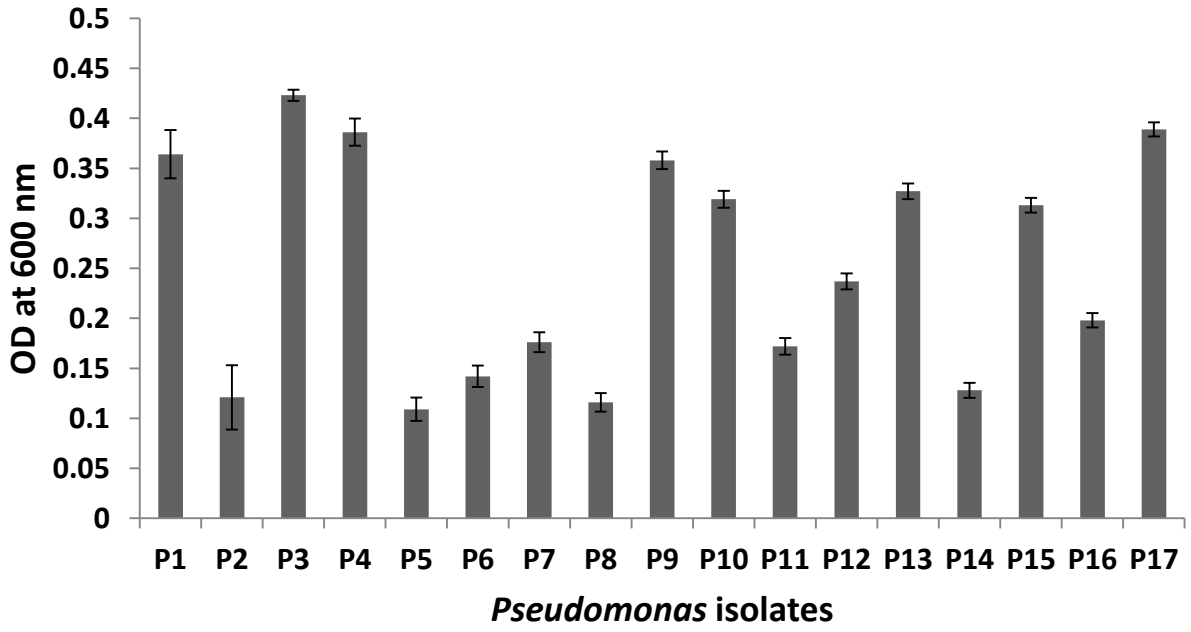

0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
OD at 600 nm
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15
P16
P17
Pseudomonas isolates

Fig. 1

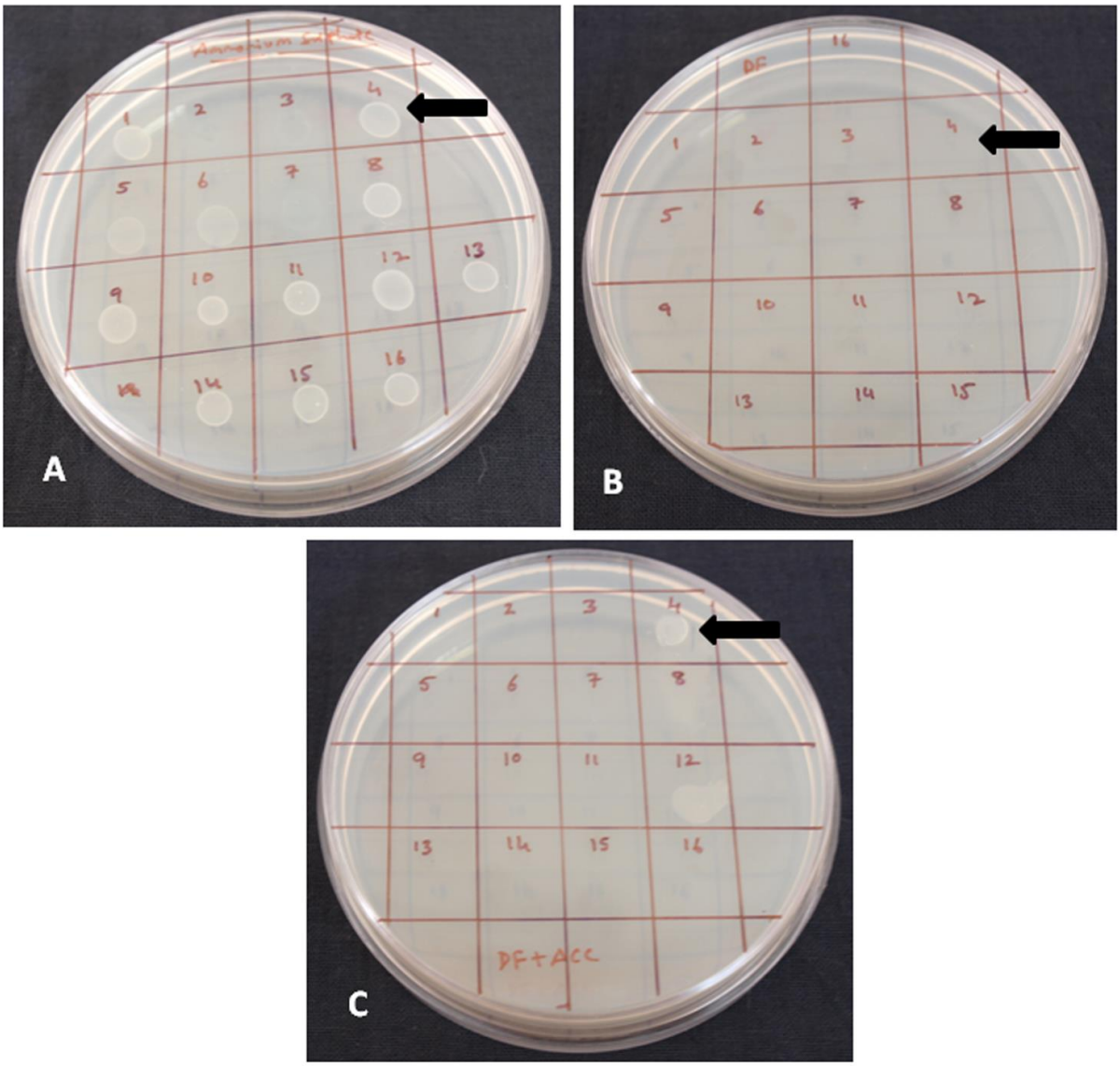
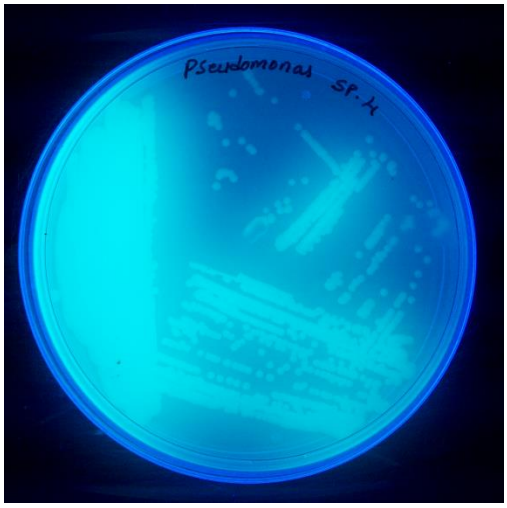
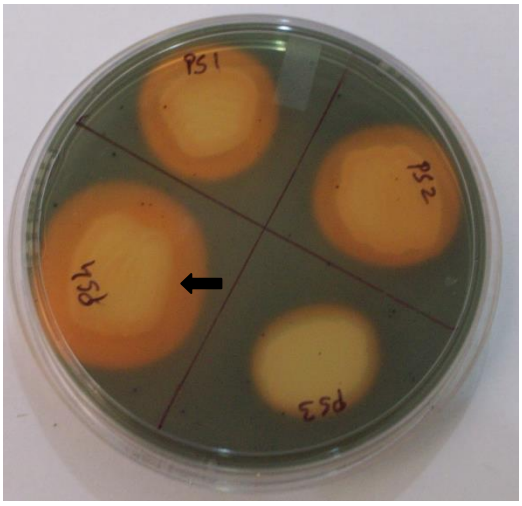


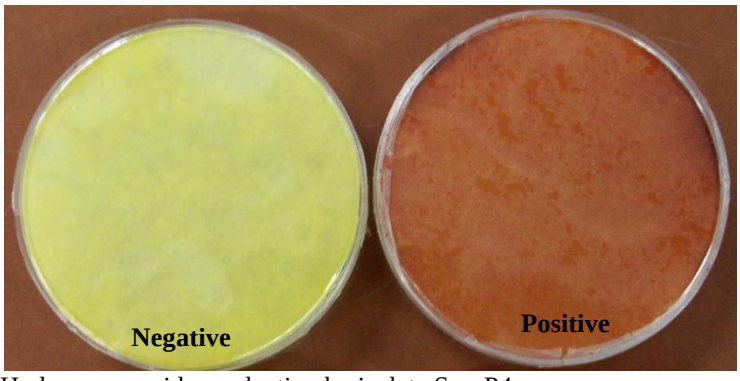
Fig. 2



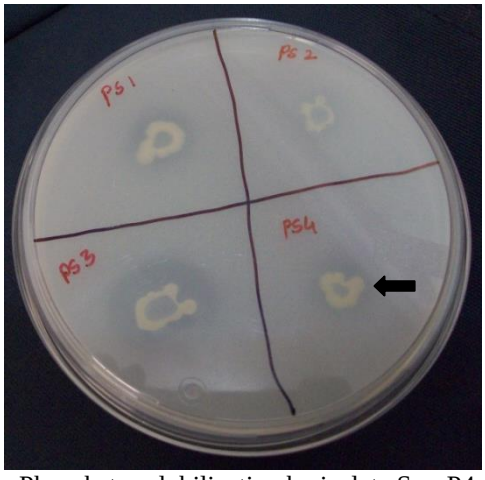
Fluorescens of isolate SorgP4under UV light



Siderophore production by isolate SorgP4



Hydrogen cyanide production by isolate SorgP4



Phosphate solubilization by isolate SorgP4

Fig.3

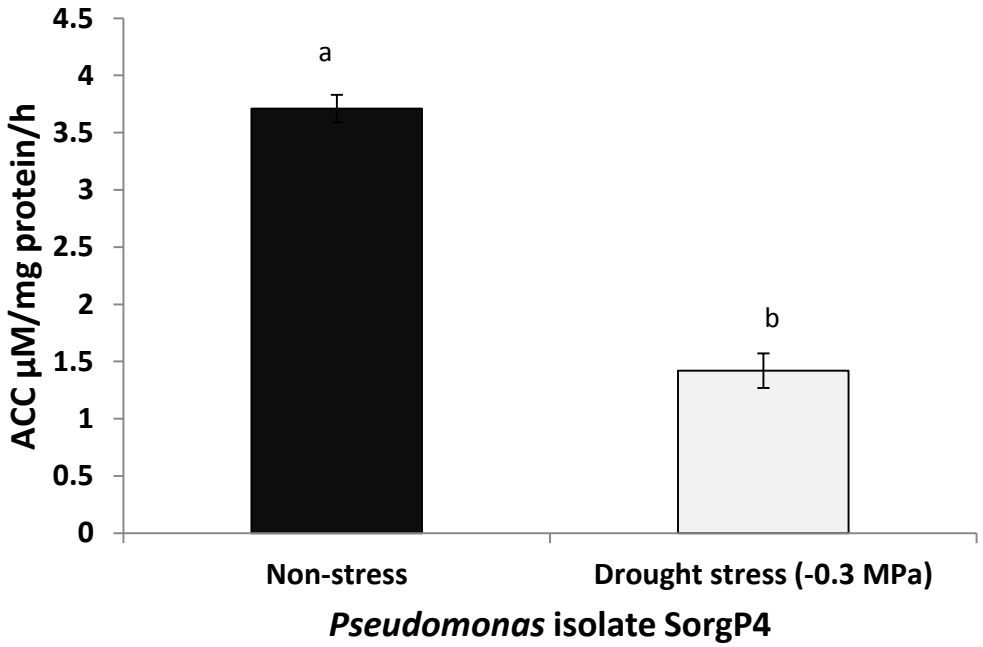

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
ACC µM/mg protein/h
a
b
Non-stress
Drought stress (-0.3 MPa)
Pseudomonas isolate SorgP4

Fig. 4

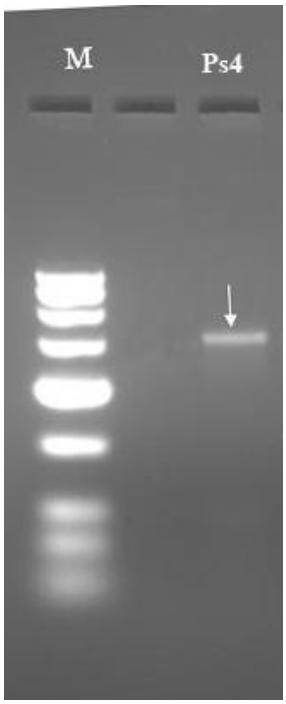

M
Ps4

Fig. 5

Isolates	Non-stress (mg/mg protein)	Drought stress (-0.30 MPa) (mg/mg protein)

Fig. 6

SorP1	0.89 ± 0.07^{a}	1.65 ± 0.11^{a}
SorP3	2.85 ± 0.07^{b}	3.73 ± 0.09^{b}
SorP4	2.75 ± 0.06^{c}	4.16 ± 0.05^{c}
GnP9	1.28 ± 0.04^{d}	2.42 ± 0.11^{d}
Rdgp10	3.22 ± 0.04^{e}	4.33 ± 0.05^{e}
SunfP12	2.71 ± 0.10^{f}	4.63 ± 0.10^{f}
SunfP13	1.62 ± 0.08^{g}	2.76 ± 0.11^{g}
BriP15	2.18 ± 0.25^{h}	3.34 ± 0.07^{h}
BriP17	1.74 ± 0.10^{i}	3.18 ± 0.04^{i}

Table 1 Exopolysaccharide production by *Pseudomonas* isolate under non-stress and drought stress condition

Values are the means of six replicates with ± SD. Values with different letters are significantly different at $P<0.05$ in all the treatments