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ORIGINAL ARTICLE

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-carboxylic acid (ACC), the immediate precursor of the plant hormone ethylene, into α-ketobutyrate and ammonia. The enzyme has been detected in a 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. We have screened 32 fluorescent *Pseudomonas* sp. isolated from rhizosphere and non-rhizosphere soils of different crop production systems for drought tolerance using polyethylene glycol 6000 (PEG 6000). Nine of these isolates were tolerant to a substrate metric potential of −0.30 MPa (15 % PEG 6000) and therefore considered to be drought-tolerant. All of these drought-tolerant isolates were screened for ACC deaminase activity using ACC as the sole nitrogen source, and one (SorgP4) was found to be positive for ACC, producing 3.71±0.025 and 1.42±0.039 μM/mg protein/h of α-ketobutyrate under the non-stress and drought stress condition, respectively. The isolate SorgP4 also showed other plant growth-promoting traits, such as indole acetic acid production, 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 sequences have been submitted to the NCBI GenBank under the accession numbers JX885767 and KC192771 respectively.

Keywords *Pseudomonas fluorescens* · ACC deaminase · Drought stress · *acdS* gene · PGP traits

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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, and the 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 the required levels of ethylene, conferring beneficial effects on plant growth and development; however, in response to biotic and abiotic stresses the plant often significantly increases endogenous ethylene production, which 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

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rhizosphere in association with the root system and which enhance the growth and development of the 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) which can cleave the plant ethylene precursor ACC to ammonia and α-ketobutyrate, thereby lowering 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 PGPR lower the level of ACC in the stressed plants, thereby limiting the amount of stress ethylene synthesis and, consequently, damage to the plant. These bacteria are beneficial to plant growth as plants are often subjected to stresses that induce the production of ethylene. Soil-borne fluorescent pseudomonads have excellent root colonizing ability and catabolic versatility, and they produce a wide range of enzymes and metabolites which favor plant resistance to various biotic and abiotic stresses (Ramamoorthy et al. 2001; Mayak et al. 2004; Vivekananthan et al. 2004).

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 affects the plant–water relations at both the cellular and whole-plant level, causing both specific and non-specific reactions and damage. Bacteria can survive under stress conditions due to the production of exopolysaccharide (EPS), which protects microorganisms from water stress by enhancing water retention and regulating the diffusion of organic carbon sources (Wilkinson 1958; Hepper 1975; Roberson and Firestone 1992; Chenu 1993; Chenu and Roberson 1996). EPS also helps 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 native beneficial microorganisms with drought-tolerant ACC deaminase may increase the drought tolerance of plants growing in arid or semiarid areas. Therefore, we made an attempt to isolate and characterize EPS and ACC deaminase from drought-tolerant *Pseudomonas* strains from cropped soils of different arid and semiarid natural habitat as a means to provide the best benefit to drought-stressed plants.

Materials and methods

Isolation of fluorescent *Pseudomonas* sp.

A total of 16 soil samples (non-rhizosphere and rhizosphere) were collected from different ecosystems covering arid, semi-arid and sub-humid zones. Non-rhizosphere soil samples were collected from a depth of 0–15 cm into flasks containing sterile normal saline and kept on a shaker for 30 min. For rhizosphere soil samples, the plants were uprooted and the bulk soil removed by gently shaking the plants; rhizosphere soil samples were collected by dipping the roots in containers containing sterile normal saline followed by shaking for 30 min. The soil suspension samples (non-rhizosphere and rhizosphere) were serially diluted, and the 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 EPS production

Trypticase soya broth with different water potentials (−0.05, −0.15, −0.30, −0.49, and −0.73 MPa) was prepared by adding the appropriate concentrations of polyethylene glycol (PEG 6000) (Michel and Kaufmann 1973; Sandhya et al. 2009) and then inoculated with 1 % of bacterial cultures cultivated overnight in TSB. Six replicates of each isolate at 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 (Thermo Spectronic model 336002; Thermo Fisher Scientific, Waltham, MA). The growth of the isolates at various stress levels was recorded.

The cultures able to grow at the maximum stress level were analyzed for their ability to produce EPS (Fett et al. 1986, 1989) under no stress and at the maximum stress level (−0.30 MPa). EPS 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 collected. Highly viscous cultures were diluted with 0.85 % KCl before centrifugation. The pellet was washed twice with 0.85 % KCl to completely extract the EPS. The possible extraction of intracellular polysaccharides was ruled out by testing for the presence of DNA in the supernatant by DPA reagent (Burton 1956). The concentration of protein in the supernatant was estimated by Bradford's reagent (Bradford 1976). The supernatant was then 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 then mixed with 3 volumes of ice-cold absolute alcohol and kept overnight at 4 °C. The precipitated EPS obtained by centrifugation (10,000 *g*, 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

The ACC deaminase activity of drought-tolerant *Pseudomonas* isolates was screened based on the ability of the respective isolate to use ACC as a sole nitrogen source. All nine drought-tolerant *Pseudomonas* isolates were grown in 5 ml of TSB medium at 28 °C for 24 h with shaking (120 rpm). The cells were harvested by centrifugation at 3,000 *g* for 5 min, washed twice with sterile 0.1 M Tris–HCl (pH 7.5), 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 1958) minimal salts medium [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 distilled water, 1,000 ml), 10 ml; distilled water, 990 ml] supplemented with 3 mM ACC as the sole nitrogen source. Plates containing only DF minimal salts medium without ACC were used as the negative control and those with $(NH_4)_2SO_4$ (0.2 % w/v) were used as the positive control. The plates were incubated at 28 °C for 72 h. Growth of isolates on ACC-supplemented plates was compared to the negative and positive controls and was selected based on growth by utilizing ACC as the nitrogen source.

ACC deaminase activity assay

To measure ACC deaminase activity, *Pseudomonas* isolates were grown in 5 ml of TSB medium at 28 °C until they reached the 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 either supplemented with 3 mM final concentration of ACC without PEG (non-stress condition) or with PEG 6000 (−0.30 MPa; drought stress condition) and incubated at 28 °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. Each sample of labilized cell suspension (50 μl) was incubated with 5 μl of 0.3 M ACC in an Eppendorf tube at 28 °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 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 0.56 N HCl and 150 μl of DNF solution (0.1 g 2,4-dinitrophenylhydrazine in 100 ml of 2 N HCl); the mixture was then incubated at 28 °C for 30 min. One milliliter of 2 N 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. A 500-μl aliquot of different α-ketobutyrate solutions (concentration 0, 0.01, 0.05, 0.1, 0.2, 0.5, 0.75, 1 mM) was mixed with 400 μl of 0.56 N HCl and 150 μl DNF solution. One milliliter of 2 N NaOH was added to the mixture and the absorbance read at 540 nm 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 first diluted with 173.5 μl of 0.1 M Tris–HCl (pH 8.0) and then 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 was used to establish a standard curve.

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

The ACC deaminase-positive drought-tolerant isolates were tested in vitro for their multiple PGP traits. The method of

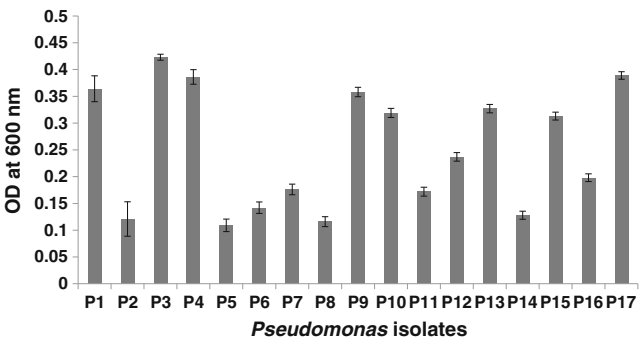


Fig. 1 Growth pattern of fluorescent *Pseudomonas* isolates under drought stress (−0.30 MPa) conditions. *Error bars* Mean ± standard deviation (SD) (n=6)

Table 1 Exopolysaccharide production by *Pseudomonas* isolates under non-stress and drought stress conditions

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

Values are presented as the mean of six replicates ± standard deviation (SD). Values followed by different lowercase letters are significantly different at $P<0.05$ in all treatments

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 bacterial cultures cultivated overnight (0.5 OD at 600 nm) and incubated at 28 °C for 48 h. A 1-ml sample of each culture was centrifuged (3,000 *g* for 20 min), and the supernatant was collected for further analysis which involved the addition of 4 ml of Salkowsky reagent, followed by incubation for 1 h at room temperature under dark conditions. Absorbance of the pink color that developed was read at 530 nm. The concentration of proteins in the pellet was determined (Bradford 1976), and the amount of IAA produced was expressed as micrograms per milligrams cell protein. To determine phosphate solubilization, we first spotted 5 μl of bacterial culture raised overnight on Pikovskaya's agar plates containing 2 % tri-calcium phosphate. The plates were then incubated at 28 °C for 24– 72 h and observed for the development of a solubilization zone around the bacterial colonies. For the 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 °C on an incubator shaker (120 rpm). The cells were harvested by centrifugation at 3,000 *g* for 10 min and the supernatant thus obtained was used for the quantitative estimation of phosphate (Fiske and Subbarow 1925). For siderophore production, 1 μl of bacterial culture raised overnight in Luria broth was spotted on Chrome Azurol S agar plates and incubated at 28 °C for 48 h. The plates were observed for the development of an orange halo around the bacterial colony (Schwyn and Neilands 1987). For hydrogen cyanide (HCN) production,

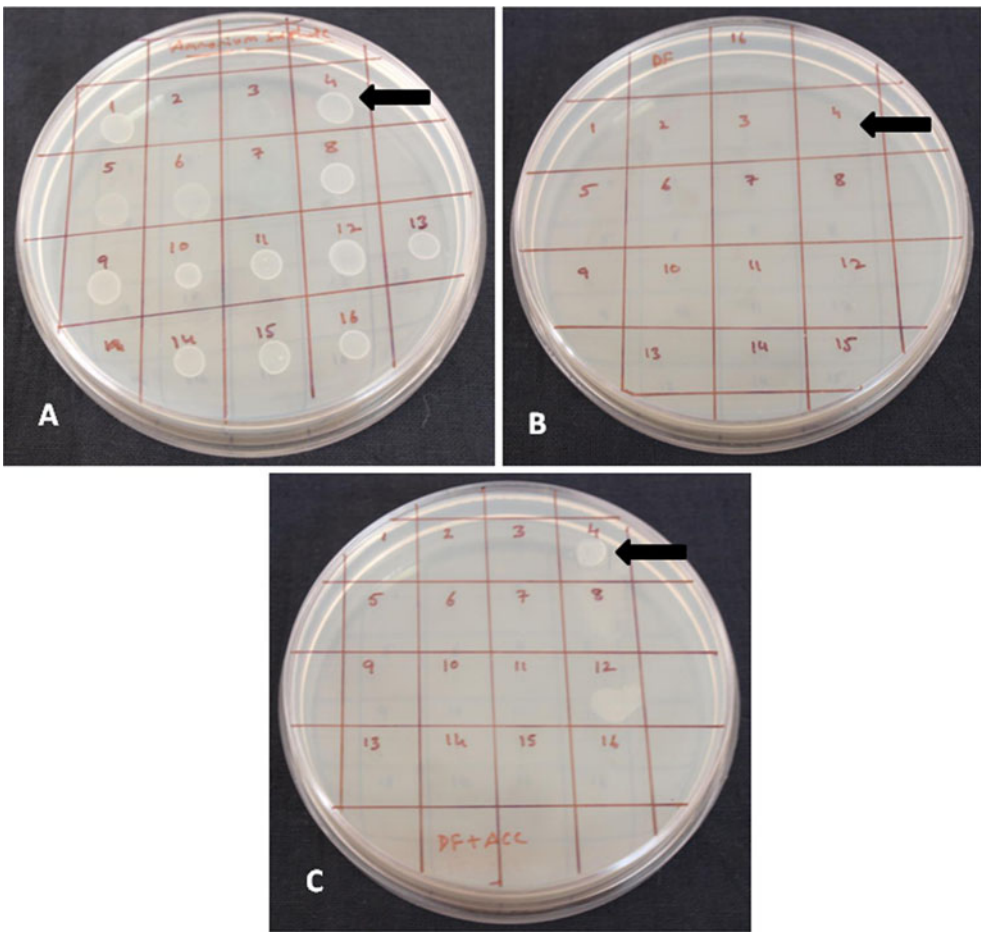
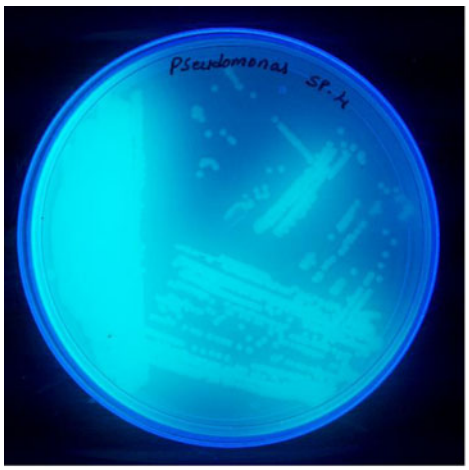


Fig. 2 Screening of bacterial isolates for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity. **a** DF minimal medium with nitrogen source (positive control), **b** DF minimal medium without any nitrogen source (negative control), **c** DF minimal medium with ACC as nitrogen source. *Arrows Pseudomonas* isolate SorgP4

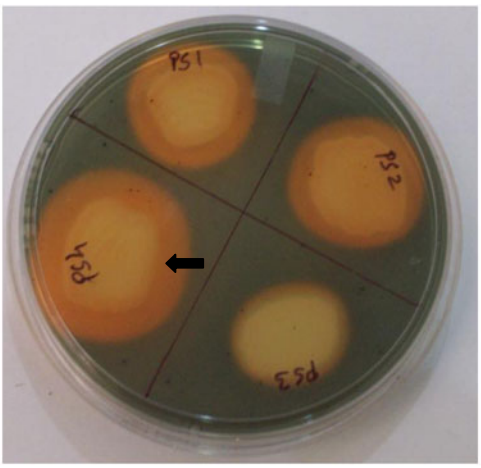
the culture was streaked on King's B medium amended with 0.4 % (w/v) of glycine, and a Whatman no.1 filter paper disc (Whatman, New York, NY) soaked in 0.5 % picric acid (w/v) in 2 % (w/v) sodium carbonate was placed in the lid of petri plate. The plates were sealed with Parafilm and incubated at 28 °C for 4 days for the development of a deep-orange color (Bakker and Schipper 1987).

Characterization of *acdS* and 16S rRNA gene

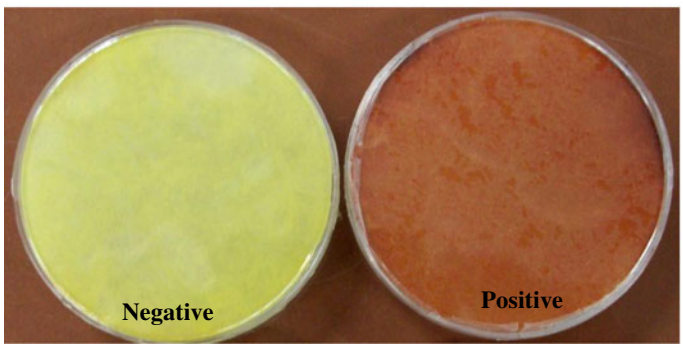
For molecular characterization, bacterial genomic DNA was isolated (Chen and Kuo 1993), and the *acdS* gene was amplified by PCR using the reference primers (Farajzadeh et al. 2010) AccF 5′-ATG AAT CTG AAT CGT TTT GAA C-3′ and 5′-TCA GCC GTT GCG GAA CAG-3′. PCR reactions were carried out in a 25-μl volume of reaction mixture containing 1× reaction buffer, 2.5 mM dNTP mixture, 10 pM of each primer, Taq DNA polymerase (1 U), and 25 ng of template DNA. PCR cycling was performed in a DNA thermal cycler (Eppendorf, Hamburg, Germany) under the following conditions: one cycle of initial denaturation for 5 min at 94 °C, 35 cycles of denaturation for 1 min at 94 °C, annealing for 50 s at 54 °C, and elongation at for 2 min at 72 °C, followed by a final extension at 72 °C for 7 min. The PCR product (approx. 996 bp) was separated by electrophoresis through 1 % agarose gel, purified, and sequenced (Xcelris Genomics Ltd, Ahmedabad, India). The 16S rRNA 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 40 s, extension at 72 °C for 90 s; final extension at 72 °C for 7 min). The PCR product (approx. 1,500 bp) was purified and sequenced (Xcelris Genomics Ltd). The sequences (*acdS* and 16S rRNA genes) obtained were compared with the existing database of *acdS* and 16S rRNA gene and submitted to GenBank.



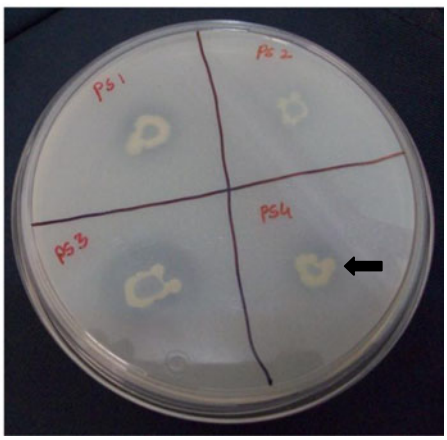
Fluorescens of isolate SorgP4under UV light



Siderophore production by isolate SorgP4



Hydrogen cyanide production by isolate SorgP4



Phosphate solubilization by isolate SorgP4

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

Statistical analysis

Data were statistically tested by analysis of variance using Instat+ver. 3.36. Each treatment was analyzed with at least six replicates and the standard deviation calculated and data expressed as the mean±SD of six replicates.

Results

Isolation and screening for drought tolerance

A total of 17 fluorescent *Pseudomonas* strains were isolated on King′s B medium of which nine isolates (SorgP1, SorgP3, SorgP4, GnP9, RdgP10, SunfP12, SunfP13, BriP15 and BriP17) were able to grow at a minimum water potential (−0.30 MPa) (Fig. 1). Isolates which could tolerate higher levels of drought stress were used to assess EPS production under both the non-stressed and minimum water potential conditions (−0.30 MPa). Strain Rdgp10 produced the maximum amount of EPS (3.22±0.04 mg/mg protein) under the 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) a,nd BriP15 (2.18±0.25 mg/mg protein) (Table 1). Under the drought stress condition, 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 PGP traits

All nine drought-tolerant isolates were screened for ACC deaminase based on the enrichment method, where ACC was used as the sole nitrogen source. Among these nine isolates, isolate SorgP4 grew well on DF minimal salts medium with either ACC or ammonium sulfate serving as the sole nitrogen source compared with growth on DF minimal salts medium without a nitrogen source (Fig. 2). Isolate SorgP4, which tested positive for ACC deaminase, was also screened for other PGP (Fig. 3) and found to produce significant amounts of IAA (48.2±3.0 μg/mg protein) and 41.7 ± 5.0 μg/ml solubilized phosphate. This isolate was also positive for indirect PGP traits such as siderophore and HCN production.

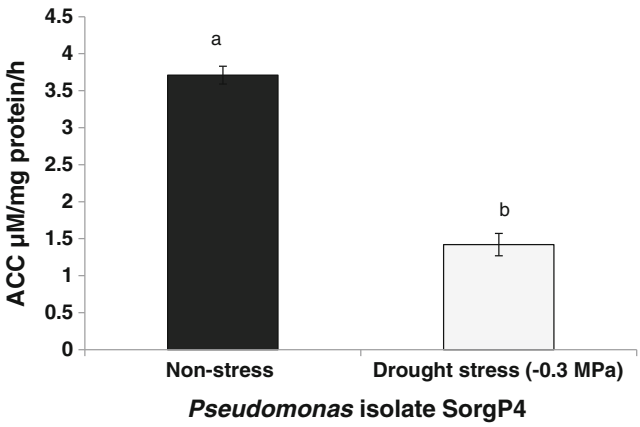


Fig. 4 ACC deaminase activity from *Pseudomonas* isolate SorgP4 under the non-stress and drought stress (−0.3 MPa) conditions. *Error bars* Mean of ± SD (n=6). *Different lowercase letters above columns* indicate a significant difference at $P<0.05$ in all treatments

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 producing ACC deaminase enzyme and showed higher levels of ACC deaminase activity under the non-stress condition than under the drought stress condition (3.71±0.025 vs. μ1.42±0.039-μM/mg protein/h α-ketobutyrate, respectively) (Fig. 4).

Amplification of ACC deaminase

The 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

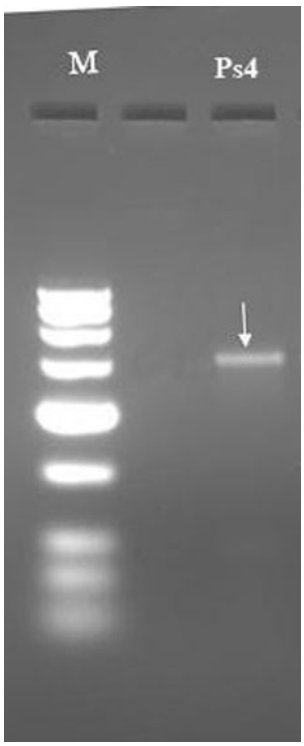


Fig. 5 Amplification of ACC deaminase (*acdS*) gene. *M* DNA ladder, *Ps4 Pseudomonas* isolate SorgP4

the results of the ACC deaminase assay. A BLASTN search was performed for the nucleotide sequence of partial length of the SorgP4 *acdS* gene; the sequence showed a 100 % homology with the *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 the outgroup (Fig. 6). The generated phylogenetic tree showed that ACC deaminase sequences of *Pseudomonas* isolate SorgP4, *Pseudomonas* strain 6G5, *P. fluorescens* strain FPG3 fell into the same clade with a high bootstrap value of 100 %. By 16S rDNA gene sequencing, we identified isolate SorGP4 as *P. fluorescens* which showed a 100 % homology with that of *P. 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 stresses, such as salt and drought, with the latter being one of the most serious problems associated with plant growth and development affecting agricultural demands. The introduction of drought-tolerant ACC deaminase-producing microorganisms in drought-stressed soils can alleviate this stress in crop plants by lowering stress-induced ethylene production. Drought-tolerant microorganisms can survive in these habitats and bind to the seed coat or root of developing seedlings, resulting in the deamination of ACC, which is the immediate precursor of ethylene, in plant cells through the production of ACC deaminase. This in turn leads to a lowering of the plant ethylene level and thereby facilitates the growth and development of plants (Glick et al. 1998). EPS material possesses unique water-retention and cementing properties and thus play a vital role in the formation and stabilization of soil aggregates and the regulation of nutrients and water flow across plant roots through biofilm formation (Tisdall and Oades 1982; Roberson and Firestone 1992).

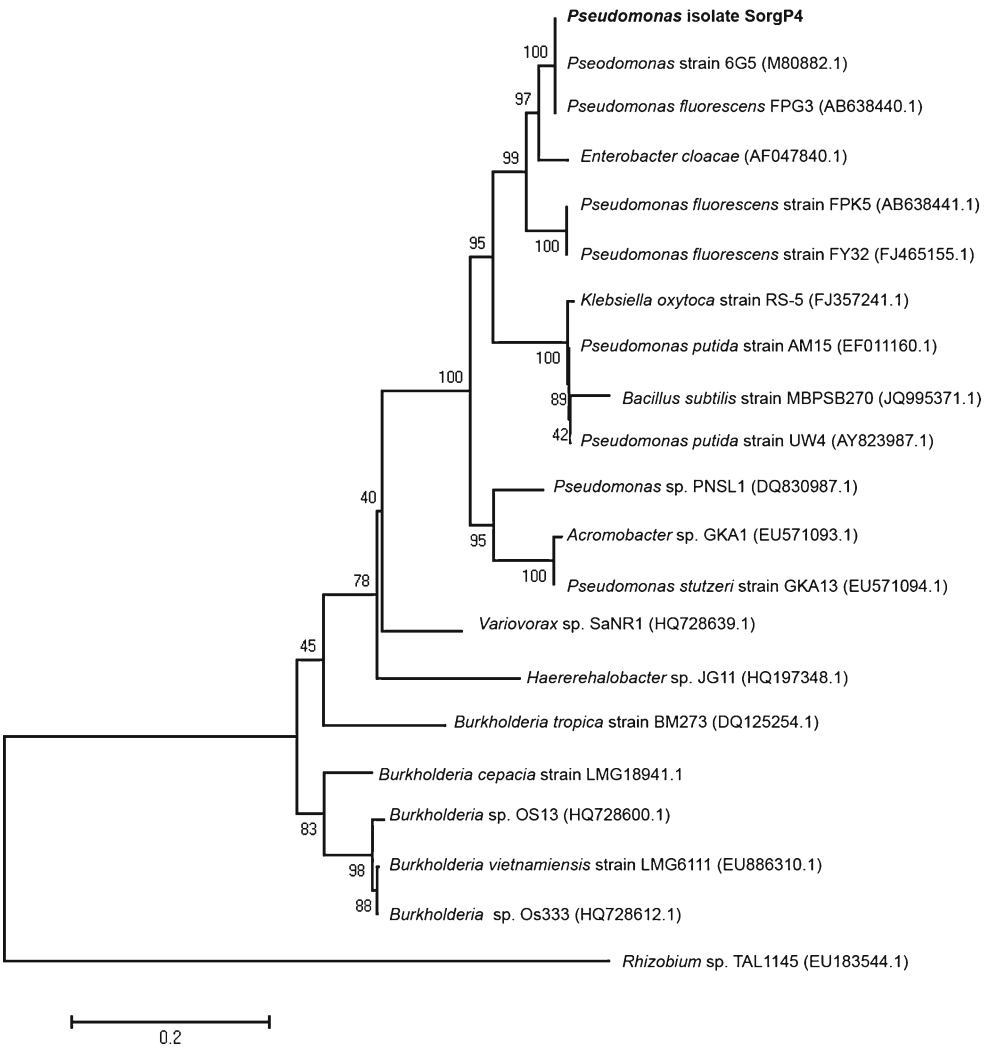


Fig. 6 Phylogenetic analysis of *Pseudomonas* sp. based on *acdS* gene sequences available from the NCBI Genbank database. Distances and clustering analysis with the neighbor-joining method was performed by using the software packages Mega ver. 4.0. Bootstrap values (n=500) are listed as percentages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

In our study we isolated and characterized the drought-tolerant and ACC deaminase- and EPS-producing *Pseudomonas* isolate SorgP4 from a stressed ecosystem. A total of 17 fluorescent *Pseudomonas* sp. grown under arid and semi-arid conditions were isolated and characterized for drought tolerance. Of these 17 isolates, nine were able to tolerate maximum level of drought stress (−0.30 MPa). These selected isolates produced higher levels of EPS under stressed conditions than under non-stressed ones, indicating that EPS production in bacteria occurs as a response to the stress (Roberson and Firestone 1992). A role for EPS material in the protection of *A. brasilense* Sp245 cells against desiccation was suggested by Konnova et al. (2001). Hartel and Alexander (1986) observed a significant correlation between the amount of EPS produced by cowpea *Bradyrhizobium* strains and desiccation tolerance. It is likely that EPS can provide a microenvironment that holds water and dries out more slowly than the surrounding microenvironment, thus protecting bacteria from dessication and fluctuations in water potential (Wilkinson 1958; Hepper 1975).

PGPR that have ACC deaminase activity help plants to withstand stress (biotic or abiotic) by reducing the level of the stress hormone ethylene through the activity of enzyme ACC deaminase, which hydrolyzes ACC into α-ketobutyrate and ammonia instead of ethylene (Glick et al. 1998; Arshad et al. 2007). In our study, we screened drought-tolerant bacteria having ACC deaminase activity and multiple PGP traits and found that one of the 17 strains screened showed ACC deaminase activity. Variations in the levels of ACC deaminase activity in the strains were noted under non-stress and drought-stress conditions, respectively. Biochemical assay of ACC deaminase revealed the secretion of this enzyme by *Pseudomonas* isolate SorgP4, which was confirmed with the PCR amplification of *acdS* gene using the same primer as reported earlier (Farajzadeh et al. 2010), resulting in a partial *acds* gene from *Pseudomonas* isolate SorgP4. The ACC deaminase gene encoding the ACC deaminase enzyme has been isolated from different soil bacteria under both non-stress and abiotic stress conditions (Klee et al. 1991; Campbell and Thomson 1996; Hontzeas et al. 2005; Rodríguez-Díaz 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. Therefore, we suggest that 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 and 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 in order to uncover their efficiency as PGP 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 None.

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