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Diversity analysis of ACC deaminase producing bacteria associated with rhizosphere of coconut tree (*Cocos nucifera* L.) grown in Lakshadweep islands of India and their ability to promote plant growth under saline conditions

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ABSTRACT

ACC deaminase producing Plant growth promoting rhizobacteria (PGPR) offers a great promise for ameliorating the negative impacts of salinity stress manifested on plants. In this context, 28 rhizospheric bacteria associated with ACC deaminase potential (198−1069 nmol α-ketobutyrate mg protein^{-1} h^{-1}) were isolated from 5 different islands of Lakshadweep, union territory, India- Agatti, Kavaratti, Bangaram, Kadmat, and Thinnakara islands using DF-minimal medium. The diversity of cultivable ACC deaminase producing bacteria was analysed by PCR-RFLP (Restriction Fragment Length Polymorphism) method using three endonucleases *AluI*, *MspI* and *HaeIII* which led to the grouping of these isolates into six clusters at 80 % similarity index. Subsequently, isolates were functionally characterized for various PGP traits such that indole-3-acetic acid (IAA) production (~10−80 µg mL^{-1}); 16 isolates had phosphate solubilizing potential ranging from ~19 to 88 P mg L^{-1} ; siderophore and ammonia production abilities were observed in 5 and 24 isolates, respectively while two strains tolerated up to 8% NaCl. Phylogenetic analysis of 16S rRNA gene sequences of representative strain from each cluster revealed that twenty-eight ACC deaminase producing PGPR belong to eight distinct genera: *Pseudomonas*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Escherichia*, *Paenibacillus*, *Burkholderia*, and *Klebsiella*. Two isolates, CO1 (*Pseudomonas putida*) and CO8 (*Bacillus paramycoides*) were evaluated for plant growth promoting effects on French bean (*Phaseolus vulgaris*) under salinity (100 mM NaCl) stress. Both the selected isolates in consortium form significantly increased the root length, shoot length, root fresh and dry weight, shoot fresh and dry weight of French bean seedlings exposed to salinity stress, compared to non-inoculated control plants. The co-inoculation with selected strains CO1 and CO8 has significantly improved chlorophyll concentration, relative water content, membrane stability index, gas exchange parameters including net photosynthesis rate (P_N), stomatal conductance (g_s), transpiration rate (E) and water use efficiency of French bean plants by ~100 %, ~85 %, ~40 %, ~198 %, ~80 %, ~70 % and ~75 %, respectively under saline conditions in comparison with non-inoculated plants. Moreover, the consortium treated French bean plants showed lower levels of stress-induced ethylene by 38 %, electrolyte leakage and Malondialdehyde (MDA) content by ~15 % under salt stress compared to non-inoculated ones. This study unveiled the potential of halotolerant strains, *Pseudomonas putida* and *Bacillus paramycoides* as French bean biofertilizers in mitigating the adverse effects of salinity in plant growth in sustainable agriculture.

1. Introduction

Biodiversity is an important component of environmental conservation and is key to agricultural production (Scherr and McNeely, 2008). Soil consists of diverse microscopic life forms which are very important in maintaining ecological balance (Dubey et al., 2019). Among all regions of soil, the rhizospheric region of soil is highly rich in microbiological flora due to the secretion of various nutrients by plants which

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attracts microbes towards it (Glick, 2014). The bacteria present in the rhizospheric region are highly useful to plants as they directly or indirectly enhance plant growth by various mechanisms such as nitrogen fixation, inorganic or organic phosphate and zinc solubilization, production of phytohormones such as auxin, cytokinin, gibberellin, dissociation of stress generated ethylene precursor, ACC by ACC deaminase activity, siderophore production, antagonism against phytopathogens, improvement of root architecture thereby, enhancement of water uptake potential of plants and soil fertility (Aeron et al., 2019). Among all mechanisms involved in plant growth promotion, production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase is an important mechanism of plant growth promotion due to its ability to decay the ACC, precursor of abiotic stress-induced ethylene hormone (Glick et al., 2007). The minimal quantity of ethylene is required for proper metabolism of plants, but when its concentration increases beyond a limit it starts hampering plant growth under stress conditions such as salinity, drought, waterlogging, or pathogen attack. Therefore, the role of ACC deaminase is extremely important in mitigating the negative impact of stress on plants induced by various factors (Gamalero and Glick, 2015).

Salinity is the severe problem in today's agricultural system owing to the problem of less rainfall, use of seawater for irrigation purposes and improper application of agrochemicals in the fields due to which most of the agricultural and cultivable land has been transformed into barren, saline and unproductive one (Parihar et al., 2015). A high concentration of salt induces ion toxicity, oxidative stress, nutritional imbalance and disturbs the osmotic regulations in plants (Fahad et al., 2015). Apart from this, it also increases the secretion of ACC as root exudates, leads to the production of stress- mediated ethylene which in turn adversely affect the physiological processes of plants such as photosynthesis, stomatal conductance, seed germination, mineral and water uptake, root nodulation etc. in other words, adversely impact the overall growth and development of plants (Zörb et al., 2019).

The ACC deaminase producing PGPR could be used as promising agents replacing chemical biofertilizers to improve the growth and enhance tolerance of plants to cope up with salinity stress (Gupta et al., 2020; Santoyo et al., 2019). Therefore, it is essential to explore the diversity of ACC deaminase producing bacterial strains from a particular agro-ecological region which could be used as bioinoculant for combating the salinity stress conditions in sustainable agriculture.

Lakshadweep islands of India are one of the unexplored and less disturbed areas as entry to these islands is restricted. The soils of these islands are made of corals and salinity is high. Coconut plants are one of the most prevalent plant species grown naturally without much human interference.

After taking due consideration of these facts, the present research work was designed to isolate ACC deaminase producing bacteria from the Lakshadweep islands of India. Diversity analysis and phylogenetic profiling of these isolates were done based on PCR-RFLP. The isolates were also evaluated for their other plant growth promoting potential. Finally, the strains were tested for their efficiency to reduce the effect of salinity in French bean plants and the ability to promote plant growth in pot trials.

2. Materials and methods

2.1. Geographical profile of selected sites, soil sampling and analysis

The rhizospheric soil sampling of coconut (*Cocos nucifera* L.) plants were carried out in the month of March 2018 from 5 different inhabited islands of Lakshadweep, India – Agatti, Kavaratti, Bangaram, Kadmat and Thinnakara (Fig. 1).

2 gm of rhizospheric soil was collected by carefully excavating the soil around the coconut plant down to about 15–20 cm in depth from five islands of Lakshadweep. A total of twenty-five samples, five from each island were collected and mixed together to form one composite pool of rhizospheric soil. The rhizospheric soil samples were sieved to remove any debris and large particles, brought to the laboratory in the Ziplock bags and stored at 4°C for further analysis.

2.2. Rhizospheric bacteria isolation and counts

The rhizobacteria were isolated from the composite soil sample by standard serial dilution plating technique on 5 differential medium-Ashby's mannitol agar, Nitrogen free bromothymol blue agar, Pikovaskya's Agar, King's B media. 0.1 mL of appropriate dilution of soil suspension in normal saline was plated on respective medium for isolation of desired rhizobacteria- *Azotobacter*, *Azospirillum*, *Pseudomonas*, and phosphate solubilizing bacteria (PSB). The other non-specific root associated bacteria were isolated on non-selective Luria-Bertani medium. The plates were incubated for 24 h at 28°C and growth was monitored. The total number of colonies were counted and morphologically distinct colonies were purified by sub culturing the

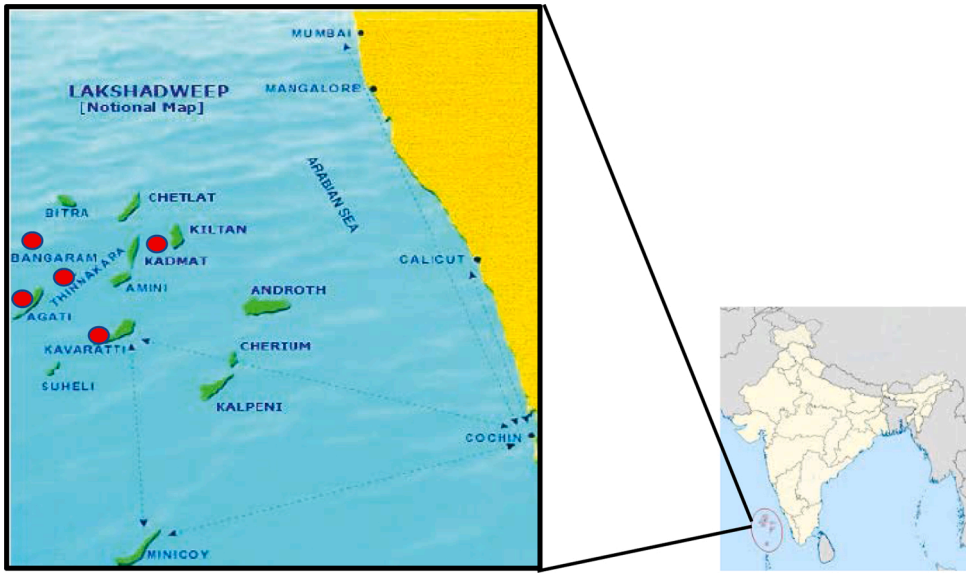

Fig. 1. Map of Lakshadweep territory, India with red dot indicating the soil sampling site (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

isolates.

2.3. Qualitative Screening of isolates for ACC deaminase activity

The morphologically distinct colonies were further characterized for their potential to utilize 1-Aminocyclopropane-1-carboxylic acid (ACC, Merck) as their sole nitrogen source. This was carried out by spot inoculating the bacterial colonies on the sterile minimal DF (Dworkin and Foster, 1958) salts media (DF salts per litre: 4.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 2.0 g Glucose, 2.0 g Gluconic acid and 2.0 g Citric acid with trace elements: 1 mg $FeSO_4.7H_2O$, 10 mg H_3BO_3, 11.19 mg $MnSO_4.H_2O$, 124.6 mg $ZnSO_4.7H_2O$, 78.22 mg $CuSO_4.5H_2O$, 10 mg MoO_3, pH 7.2) amended with 3 mM ACC instead of $(NH_4)_2SO_4$ in accordance with Penrose and Glick (2003). The plates were incubated at 28℃ for 3 days and growth was monitored on daily basis. Colonies developing on the plates were inferred as ACC deaminase producing microbes. Furthermore, the quantitative assessment of ACC deaminase activity was done spectrophotometrically in terms of a-ketobutyrate production at 540 nm by comparing with the standard curve of a-ketobutyrate, which ranged from 0.1 to 1.0 mmol (Honma and Shimomura, 1978). The protein estimation of bacterial cells was done as per Bradford methodology (Bradford, 1976) using bovine serum albumin (BSA) as the standard protein to establish a standard curve. One unit of ACC deaminase activity was expressed as the amount of a-ketobutyrate liberated in nmol per milligram of cellular protein per hour.

2.4. Functional characterization of isolates for plant growth promoting attributes

The ACC deaminase producing isolates were analysed for production of indole acetic acid for which they were grown in LB medium supplemented with 5 mM Tryptophan for seven days. The amount of IAA was visualized by adding Salkowski reagent (35 % perchloric acid + 0.5 M $FeCl_3$) to culture supernatant and calculated spectrophotometrically at 530 nm against standard curve of IAA (Hi Media) in the range of $0-100\ \mu g\ ml^{-1}$ (Ahmad et al., 2008). The isolates were also assayed for siderophore production on Chrome Azurol S (CAS) agar medium in accordance with Schwyn and Neilands (1987). Furthermore, the ACC deaminase producing isolates were also screened for the ability to solubilize inorganic phosphate complex, on Pikovaskya's agar medium supplemented with tricalcium phosphate ($Ca_3(PO_4)_2$). The isolates producing clear halo zone around their colony on medium were considered phosphate solubilizing bacteria (Nautiyal, 1999). The positive strains were furthermore subjected for quantative assessment of phosphate solubilization using NBRIP (National Botanical Research Institute's Phosphate) medium. The solubilized phosphate (Soluble P mg/L) was quantified spectrophotometrically at 420 nm against standard curve of KH_2PO_4 (HI-MEDIA) as per Fiske and Subbarow (1925). The development of yellow to slight brown colour on addition of Nessler's reagent to bacterial culture grown in peptone water was the indication for ammonia production by the respective isolates (Kavamura et al., 2013). The bacterial isolates were characterized for HCN production on King's B medium supplemented with 0.4 % (w/v) glycine. A Whatman filter paper saturated with alkaline picric acid solution (2% Na_2CO_3 in 0.5 % picric acid) was placed on the upper lids of petri plates for 4 days and monitored for the development of red-brown color of filter paper was designated as HCN producing isolates (Miller and Higgins, 1970). Furthermore, the ACC deaminase isolates were screened for salinity tolerance by observing their growth at 28℃ on LB medium amended with different concentration of 2 %–8 % NaCl.

2.5. 16s rRNA gene amplification and restriction fragment length polymorphism analysis

The genomic DNA of potent ACC deaminase producing bacterial isolates was isolated as per Pandey et al., 2013. The amplification of 16S rRNA (~1500 bp) from bacterial genomic DNA was carried out in a polymerase chain reaction (PCR) using universal bacterial primers forward (5′AGAGTTTGATCCTGGCTCAG3′) and reverse (5′AAGGAGGTGATCCAGCCGCA3′) as per standard conditions. The 25 µL standard reaction mixture comprised PCR Buffer 2.5 µL; $MgCl_2$ 2 µL; dNTPs (2 mM) 1 µL; Primers 0.5 µL each; Taq DNA polymerase 0.5 µL; Template DNA 2 µL; Sterile deionized water 16 µL. The PCR amplified product was analysed on 1.2 % agarose gel with 1X TAE buffer and further, purified with Qiaquick PCR purification kit (Qiagen, Valencia, CA).

The purified PCR products were subjected to digestion with three restriction endonucleases *AluI*, *MspI* and *HaeIII* in a 25 µl reaction mixture using recommended buffer at 37 ℃. The restriction fragments were visualized on 3.0 % agarose gel by electrophoresis. Strong and clear bands were scored in binary form (0 for absence; 1 for presence) for similarity and clustering analysis undertaken by software DARwin 6.0.14 software (Dissimilarity Analysis and Representation for Windows) in order to construct dendrogram by UPGMA (unweighted pair grouping with mathematic averages) method. Isolates were grouped on the basis of the restriction patterns obtained through 16S rRNA -RFLP analysis.

2.6. Molecular identification by 16 s rRNA Sequencing and Phylogenetic Analysis

The amplified 16S rRNA amplicon of the representative isolates from each cluster was partially sequenced at Eurofins Genomics India Pvt. Ltd. (Bengaluru, India) by Sanger's di-deoxy nucleotide sequencing method. Resulting 16S rRNA sequences were subjected to similarity search program against closely related bacterial species available at GenBank database through BLAST program provided in the website of National Centre for Biotechnology Information (NCBI). The partial 16 rRNA sequences were submitted to NCBI GenBank database under the accession number MN318320- MN318326. The 16S rRNA partial sequences were aligned using Clustal W program and the phylogenetic tree was constructed by neighbour joining method with 1000 bootstrap replicates in MEGA X.

2.7. Diversity analysis

The diversity profile of culturable and indigenous ACC deaminase producing bacteria from coconut rhizosphere was characterized by using plethora of indices as shown in Table 1 to estimate their richness, diversity and distribution. Species richness (Margalef's index and Menhinick's index), Species evenness (Shannon equitability (E_H), Plieou's evenness index (J) Sheldon index) and Species diversity (Shannon Wiener index, Simpson's index) were calculated based on the standard

Table 1
Different diversity, richness, evenness and similarity indices with their formulas.

Diversity Index	Formula Used
Margalef's Diversity Index	$S-1/\log_e(N)$
Menhinick's index (D_m)	$S/\sqrt{N}$
Simpson's Index of Diversity (D)	$1-[\{\sum n_i (n_i-1)\}/ N (N-1)]$
Shannon Wiener Diversity index (H')	$-\sum_{i=1}^{s} p_i * \log_e (p_i)$
Plieou's evenness (J')/ Shannon's equitability/evenness (E_H) index	$H'/H_{max} = H'/\log_e (S)$
Sheldon index	$e^{H'}/S$

S, total number of species group; N, total number of individuals in a sample; n_i, number of individuals in particular i_{th} species group; p_i, proportion of i_{th} species = ni/N; a = number of common species between two regions; b and c, number of species specific to each region; H_{max}, maximum value of Shannon Wiener Diversity index representing maximum diversity (or, maximum evenness).

formula as per Staddon et al. (1997).

2.8. Pot experiments: ethylene estimation and plant growth promotion under salt stressed and normal conditions

2.8.1. Experiment 1: Estimation of stress induced ethylene by Gas chromatography

The pure cultures of bacterial strains were grown in DF-3 mM ACC minimal medium and incubated for three days at 28 ℃. The cells were harvested and diluted with 0.03 M $MgSO_4$ to the final concentration of 10^8 and 10^5 cfu mL^{-1}. The French bean seeds were surface sterilized by immersing in 70 % ethanol followed by in 1% sodium hypochlorite which were subsequently washed with sterile distilled water for three to five times. The surface sterilized seeds were then incubated with bacterial suspension of respective dilutions for 1 h and three seeds were then sown in each plastic pot filled with autoclaved soil. The pots were arranged in randomized block design with three replicates in growth chamber. They were maintained under optimum light and temperature condition, i.e. 80 % relative humidity, 16:8 light: dark photoperiod at 25 ℃ and regularly irrigated with either sterile distilled water or saline water (100 mM) for 10 days. The estimation of ethylene was evaluated by uprooting and placing ~3 gm treated and non-treated French bean seedlings inside 60 mL glass tubes. The test tubes were kept open for 30 min and then sealed with rubber septum and incubated for 4 h at room temperature. 1 mL of headspace gas was taken out using gas-tight syringe with needle of diameter 0.5 mm from each glass tube and injected into the Gas Chromatograph (Bruker 450-GC, Bruker Corporation, United States) system equipped with Poropak-Q column (3 m x3.175 mm OD) at 70 ℃ and flame ionization detector (FID). The operating parameters of Gas Chromatograph was adjusted to 45 ℃, 150 ℃, and 250 ℃ as oven, injection and detection temperature, respectively while flow rates of N_2, H_2 and air were fixed as 35, 30 and 300 ml min^{-1}, respectively. The amount of ethylene evolved was expressed as μM ethylene g $F.W^{-1}$ $h^{-1}$by comparing with the standard curve of pure ethylene (Siddikee et al., 2011).

2.8.2. Experiment 2: plant growth promotion assay by pots trials

The surface sterilized French bean seeds were incubated with 0.03 M $MgSO_4$ (as control) and bacterial suspension in 0.03 M $MgSO_4$ of respective treatment followed as: CO1, CO8 and CO1 + CO8. The bacterial consortia were prepared by inoculating CO1 and CO8 in nutrient medium in the ratio of 1:1 and incubated overnight (Gupta and Pandey, 2019a).

The plant growth promoting assay under control and saline stressed conditions was carried out in randomized block design with four treatments in triplicates as shown in Table 2. The pot experiment study was done in plastic pots of size 30cm × 30cm filled with autoclaved soil (3 kg soil pot^{-1}). The soil was characterized as sandy loam with pH 4.5, EC 0.0354 dS m^{-1}, 66 % sand, 9% slit, and 26 % clay. Three seeds of French bean were sowed in pots and placed in the greenhouse chamber maintaining optimum light and temperature conditions, i.e. 80 % relative humidity, 16:8 light: dark photoperiod at 25 ℃. For control, French bean seedlings were irrigated with sterile distilled water and for stressed conditions, French bean seedlings were irrigated with saline NaCl salt solution (100 mM), twice a day for 30 days. After 30 days of salt exposure, French bean plants were harvested.

Table 2
Treatment for evaluating the effects of CO1 and CO8 inoculation on French bean plants under control and stressed conditions.

Growth Conditions	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Normal	Uninoculated	CO1 strain inoculated	CO8 strain inoculated	Consortia (CO1 + CO8) inoculated
Saline	Uninoculated	CO1 strain inoculated	CO8 strain inoculated	Consortia (CO1 + CO8) inoculated

2.9. Evaluation of morphological, physiological and biochemical parameters of plants in response to salt stress and bacterial inoculation

2.9.1. Growth parameters and Relative water content

The French bean plants were harvested after 30 days and growth parameters such as root length, shoot length, root fresh and dry weight, shoot fresh and dry weight were recorded and analysed. The electrical conductivity of soils was measured to test initial salinity and that of post-harvest soil. Additionally, the relative water content of leaves was measured in accordance with following equation to assess the water status of normal, salt stressed and ACC deaminase producing bacteria treated French bean plants (Jaemsaeng et al., 2018).

RWC= (Fresh Weight-Dry weight)/ (Turgid (saturated)weight-Dry weight) *100

2.9.2. Parameters of photosynthesis

The Chl a and Chl b as well as total leaf chlorophyll content was determined in accordance with Hiscox and Israelstam (1979) and calculated in terms of mg g^{-1} of Fresh weight (FW) as per Arnon (1949). The net photosynthesis rate (P_N), stomatal conductance (g_s) and transpiration rate (E) were evaluated on the fully expanded leaves of each treatment from the top (adaxial side) by an infrared gas analyser (IRGA) portable photosynthesis measurement system (LI-6400, LI-COR, Lincoln, NE, USA) in the morning from 9:00AM to 11:00AM of Indian Standard Time. The optimum conditions of leaf chamber equipped with LED light source were as follows: 1000 μmol m^{-2} s^{-1} PPFD, 80 % Relative humidity, 28 ℃ leaf temperature and 400 μmol CO_2 mol^{-1}. The water use efficiency was calculated as P_N/E (Silva et al., 2018).

2.9.3. Oxidative stress markers: Malondialdehyde content and Membrane stability index (MSI)

The lipid peroxidation in terms of Malondialdehyde (MDA), was quantified in common bean leaves in terms of thiobarbituric acid reactive substances (TBARS) as per Hodges et al. (1999). The amount of MDA-TBAR complex was calculated at 532 nm, 660 nm, and 440 nm as nmol g^{-1} FW using the molar extinction coefficient of 155 mM^{-1} cm^{-1}. The plant cell membrane permeability due to salinity stress can be assessed through electrolyte leakage measurement using electrical conductivity meter and calculated as per (Singh and Jha, 2016).

2.10. Data analysis

All quantitative data of PGP traits as well as plant morphological related data were analysed with one-way ANOVA followed by Tukey's test. All the statistical analyses were carried out with help of SPSS software. The experiments were performed in three replicates and the mean, as well as standard deviation, were calculated using Microsoft Excel 2016.

3. Results

3.1. Soil analysis and enumeration of rhizobacteria

The soil from five islands: Agatti, Kavaratti, Bangaram, Kadmat and Thinnakara has derived from the disintegration of mainly coral limestone including coral sands, lagoonal sands and muds. Hence, the sampling soil was characterized as sandy loam having pH in the range from 6.958 to 7.300 and electrical conductivity (4.7 mS cm^{-1}).

In order to obtain diverse rhizospheric bacteria, 5 different media were used in this study named as Ashby's mannitol agar, Nitrogen free bromothymol blue agar, Pikovaskya's Agar, King's B media and LB media. The isolates were entitled according the medium from which they were isolated. The population density (cfu gm^{-1} of rhizospheric

soil) of culturable bacteria on Ashby's mannitol agar (Mannitol utilizing bacteria), Nitrogen free bromothymol blue agar (N_2 fixing bacteria), Pikovaskya's Agar (Phosphate solubilizing bacteria), King's B media (*Pseudomonas*) and LB media were found to be $1.27 \times 10^8 \pm 0.02$, $1.12 \times 10^7 \pm 0.02$, $1.35 \times 10^{10} \pm 0.05$, $1.71 \times 10^{10} \pm 0.03$ and $1.89 \times 10^{10} \pm 0.08$ cfu gm^{-1}, respectively. Majority of rhizospheric bacterial population was observed on non-specific LB medium followed by Kings' B medium, indicating the presence of putative *Pseudomonas* species. While low population of N_2 fixing bacteria was found on NfB medium from rhizosphere of coconut plants.

3.2. Screening of rhizospheric isolates for ACC deaminase activity and estimation of ACC deaminase activity

A total of 75 morphologically distinct rhizobacterial isolates were randomly selected from 5 differential medium for further characterization for ACC deaminase activity. Total 75 isolates include: 8 isolates from Ashby's mannitol agar, 10 from Nitrogen free bromothymol medium, 12 from Pikovaskya's agar, 20 isolates from King's B medium and 25 isolates from LB agar medium.

All 75 isolates were screened for ACC utilizing potential on DF minimal medium amended with 3 mM ACC in place of $(NH_4)_2SO_4$, as sole nitrogen source. Around ~38 % (28 out of 75) of the isolates from the rhizosphere of coconut plants showed growth on DF-ACC medium, inferred as ACC deaminase producers. Out of total 28 isolates, 2 isolates were Mannitol utilizing bacteria, 3 isolates were N_2 fixing bacteria, 4 isolates each were Phosphate solubilizing bacteria and *Pseudomonas* while 15 were other non-specific rhizospheric bacteria isolated on LB medium, comprised bacterial diversity with ACC deaminating potential from coconut rhizosphere. The ACC deaminase activities of all the twenty-eight isolates was quantified ranged from 198 to 1069 nmol α-ketobutyrate mg $protein^{-1}$ h^{-1}.

3.3. Functional characterization for plant growth promoting traits of ACC deaminase producing isolates

All the twenty-eight ACC deaminase producing isolates were examined for multiple direct and indirect plan growth promoting traits such as IAA production, phosphate solubilization, siderophore production, ammonia and HCN production as shown in Table 3.

Rhizospheric bacterial isolates from coconut rhizosphere has shown a very high occurrence of Indole acetic acid (IAA) producers. All the isolates were observed to possessed IAA production trait producing phytohormone in the range of 10.59 to 80.75 μg mL^{-1} in the presence of substrate, Tryptophan. Similarly, all the isolates, except CO4, CO6, CO11, CO16, CO17, CO18, CO19, CO20, CO21, CO22, CO23 and CO28 showed halo zone around the bacterial colonies on Pikovaskya's agar indicating the ability to solubilize inorganic phosphate complex, tricalcium phosphate, both qualitatively as well as quantitively. The phosphate solubilization of all the isolates was ranged from 11.97 to 87.98 P mg L^{-1} with strain CO1 showed highest phosphate solubilization around ~88 P mg L^{-1} in liquid NBRIP medium. The pH of the NBRIP medium after 7 days of incubation with the respective strains was

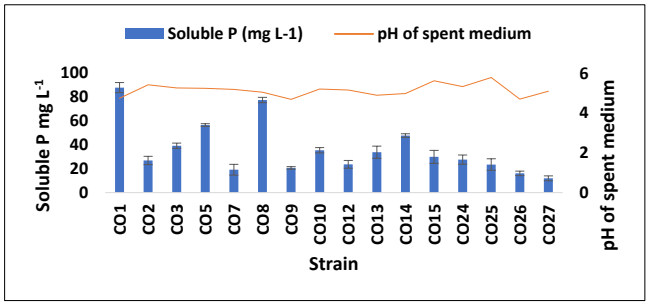

Fig. 2. Phosphate solubilization by ACC deaminase producing isolates in NBRIP medium after 7 days of incubation.

Table 3
Functional characterization of ACC deaminase producing isolates based on direct and indirect plant growth promoting traits.

Isolate	ACC deaminase activity (nmol α-ketobutyrate mg $protein^{-1}$ h^{-1})	IAA production (μg mL^{-1})	Soluble Phosphate (P mg L^{-1})	Siderophore Production	HCN Production	Ammonia Production	Salt tolerance
CO1	965 ± 4.04	80.75 ± 0.96	87.98 ± 4.21	+	–	+	+
CO2	345 ± 4.08	31.93 ± 0.47	26.92 ± 3.47	–	–	+	–
CO3	389 ± 3.07	33.38 ± 0.68	39.25 ± 2.19	–	–	–	–
CO4	631 ± 7.05	24.21 ± 0.89	–	+	–	–	–
CO5	819 ± 8.09	21.31 ± 0.34	56.67 ± 1.12	–	–	+	–
CO6	562 ± 7.04	46.66 ± 0.65	–	–	–	–	–
CO7	462 ± 9.04	15.14 ± 0.88	19.15 ± 4.52	–	–	–	–
CO8	1069 ± 5.02	74.66 ± 0.36	77.58 ± 2.27	+	–	+	+
CO9	601 ± 6.08	17.80 ± 0.07	20.68 ± 1.09	–	–	+	–
CO10	675 ± 8.04	11.49 ± 0.66	35.48 ± 2.23	–	–	+	–
CO11	396 ± 6.07	10.59 ± 0.76	–	–	–	+	–
CO12	825 ± 3.07	25.46 ± 0.86	23.67 ± 3.21	–	–	+	–
CO13	619 ± 1.57	26.99 ± 0.58	33.84 ± 5.09	+	–	+	–
CO14	702 ± 9.04	30.87 ± 0.98	47.77 ± 1.52	–	–	+	–
CO15	559 ± 9.02	21.53 ± 0.56	29.94 ± 5.47	–	–	+	–
CO16	736 ± 2.87	43.17 ± 0.53	–	–	–	+	–
CO17	461 ± 6.42	14.73 ± 0.69	–	–	–	+	–
CO18	304 ± 1.08	39.97 ± 0.81	–	–	–	+	–
CO19	533 ± 8.77	41.59 ± 0.58	–	–	–	+	–
CO20	589 ± 7.79	12.80 ± 0.74	–	–	–	+	–
CO21	584 ± 2.36	25.84 ± 0.69	–	–	–	+	–
CO22	208 ± 5.61	13.97 ± 0.03	–	+	–	+	–
CO23	348 ± 2.95	35.48 ± 0.15	–	–	–	+	–
CO24	584 ± 5.04	21.19 ± 0.89	27.66 ± 3.84	–	–	+	–
CO25	613 ± 7.04	27.61 ± 0.09	23.51 ± 4.82	–	–	+	–
CO26	245 ± 2.00	19.46 ± 0.43	16.11 ± 1.89	–	–	+	–
CO27	198 ± 9.04	25.64 ± 0.93	11.97 ± 2.04	–	–	+	–
CO28	301 ± 1.04	38.73 ± 0.85	–	–	–	+	–

Values ae mean ± SD of three replicates. +, presence, - absence of plant growth promoting trait.

found to be acidic as shown in Fig. 2. Few isolates possessed the ability to fix atmospheric Nitrogen, showed growth on semi-solid NfB medium, while only five isolates out of 28 had the ability to produced Siderophore on CAS agar medium. All the isolates except CO3, CO4, CO6 and CO7 showed ammonia production with Nessler reagent in peptone water while none of the isolates was able to produce HCN.

3.4. Determination of molecular diversity by ARDRA

The molecular diversity of potent ACC deaminase producing bacteria was determined by amplified ribosomal DNA restriction analysis (ARDRA) using three restriction endonucleases *AluI*, *MspI*, and *HaeIII*, which led to the grouping of 28 ACC deaminase producing isolates into 6 clusters with similarity index of 80 %. Majority of isolates fell in group 5 while remaining isolate constitute separate clusters. The PCR-RFLP banding pattern based on binary data (0/1) was analysed by DARwin 6.0.14 software (Dissimilarity Analysis and Representation for Windows) and dendrogram was generated based on UPGMA (unweighted pair grouping with mathematic averages) method depicting the diversity among ACC deaminase rhizospheric isolates (Fig. 3). Eight isolates were selected as representative bacterial strain from each PCR-RFLP pattern for molecular identification through 16S rRNA gene sequencing.

The phylogenetic tree based on partial sequence of 16S rRNA gene sequencing analysis revealed that 28 bacterial strains belonged to 2 phyla: proteobacteria and firmicutes of 8 distinct genera followed as *Pseudomonas, Bacillus, Azospirillum, Azotobacter, Escherichia, Paenibacillus, Burkholderia*, and *Klebsiella* based on BLAST search analysis. The γ-proteobacteria group of bacteria dominated the coconut rhizosphere.

Table 4
16S rRNA partial gene sequence identification of eight representative isolates of ACC deaminase producing rhizospheric bacteria from each PCR- RFLP clusters.

Strain	Homology Species	Class of Bacteria	Gram Reaction	% identity	Accession No.
CO1	*Pseudomonas putida*	γ-proteobacteria	Positive	100 %	MN318320
CO2	*Azospirillum sp.*	α-proteobacteria	Negative	100 %	MN318324
CO3	*Burkholderia cenocepacia*	β-proteobacteria	Negative	100 %	MN318325
CO4	*Escherichia coli*	γ-proteobacteria	Negative	100 %	MN318323
CO8	*Bacillus paramycoides*	Firmicutes	Positive	100 %	MN945369
CO10	*Paenibacillus macerans*	Firmicutes	Negative	100 %	MN318321
CO21	*Klebsiella oxytoca*	γ-proteobacteria	Negative	100 %	MN318326
CO24	*Azospirillum sp.*	α-proteobacteria	Negative	100 %	MN318324

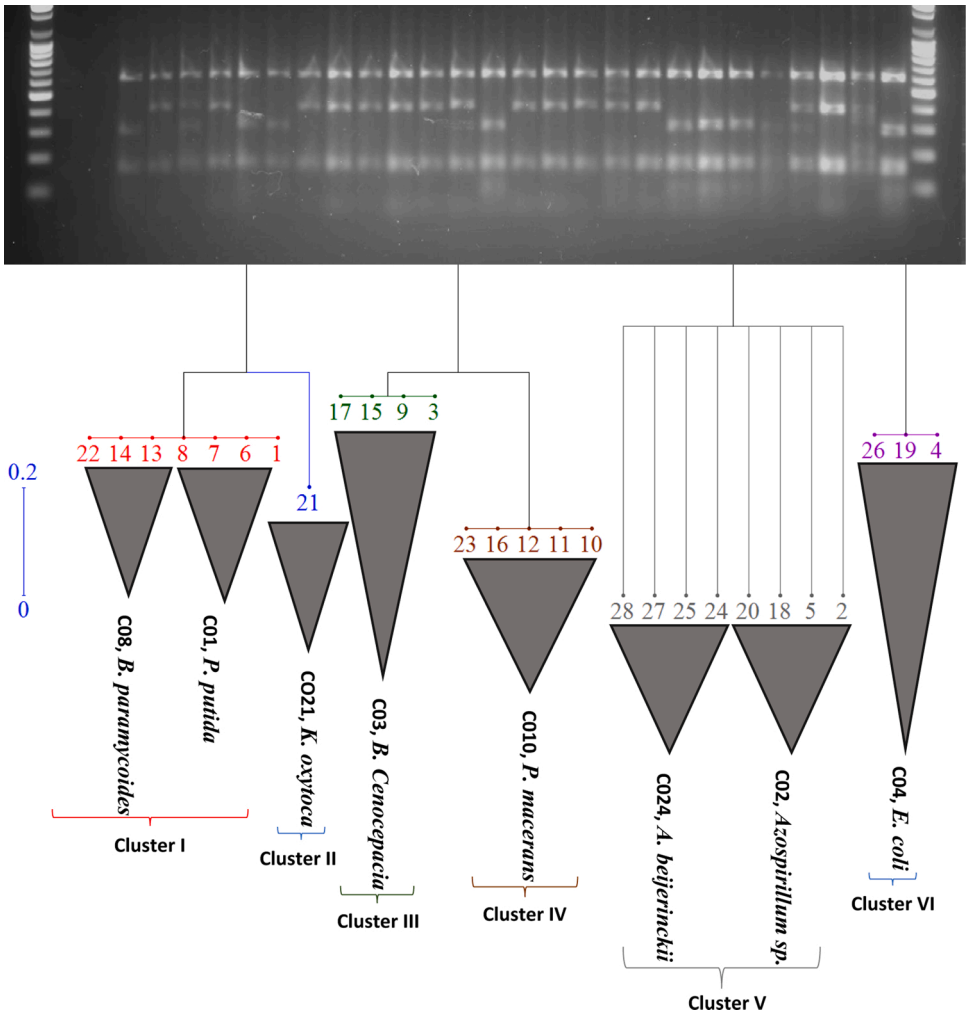

Fig. 3. Dendrogram based on UPGMA method using DARwin program was constructed with PCR-RFLP profiles of twenty-eight ACC deaminase producing bacterial strains isolated from the rhizosphere of coconut plants. The PCR-RFLP profiles was obtained with restriction endonucleases AluI, *MspI*, and *HaeIII*. The scale at left side of the figure represents similarity coefficient.

The overall molecular characterization of ACC deaminase containing 8 isolates was shown in Table 4 with closet relative species, percent identity and GenBank accession number. The phylogenetic tree was constructed using MEGA X showing the relationship of 8 ACC deaminase producing bacterial species with related bacterial strains (Fig. 4).

3.5. *Plant growth promotion assay to evaluate the effect of bacterial strains (CO1 and CO8) on growth attributes, relative water content and ethylene level of French beans plants under normal and saline conditions*

According to quantative evaluation of ACC deaminase activity and plant growth promoting traits as well as ability to grow in the presence of higher concentration of NaCl, the two most promising strains with high ACC deaminase activity were selected to investigate in vivo growth promoting effects on French bean seedlings using pots experiments under non-saline and saline stressed conditions (Fig. 5).

The higher concentration of ethylene ~56 % was observed in untreated plants irrigated with saline solution in comparison to uninoculated plants grown in normal conditions. Therefore, the effect of ACC deaminase producing strains CO1 and CO8 in reducing the ethylene under salinity stress conditions was evaluated at two different inoculum concentration of 10^5 and 10^8 cfu mL^{-1} (Table 5). However, the strains CO1 and CO8 either alone or in combined form do not exhibited significant ($P \leq 0.05$) reduction in ethylene concentration under salinity stressed conditions in comparison to control (uninoculated) French bean plants when the inoculum concentration was 10^5 cfu mL^{-1}. While,

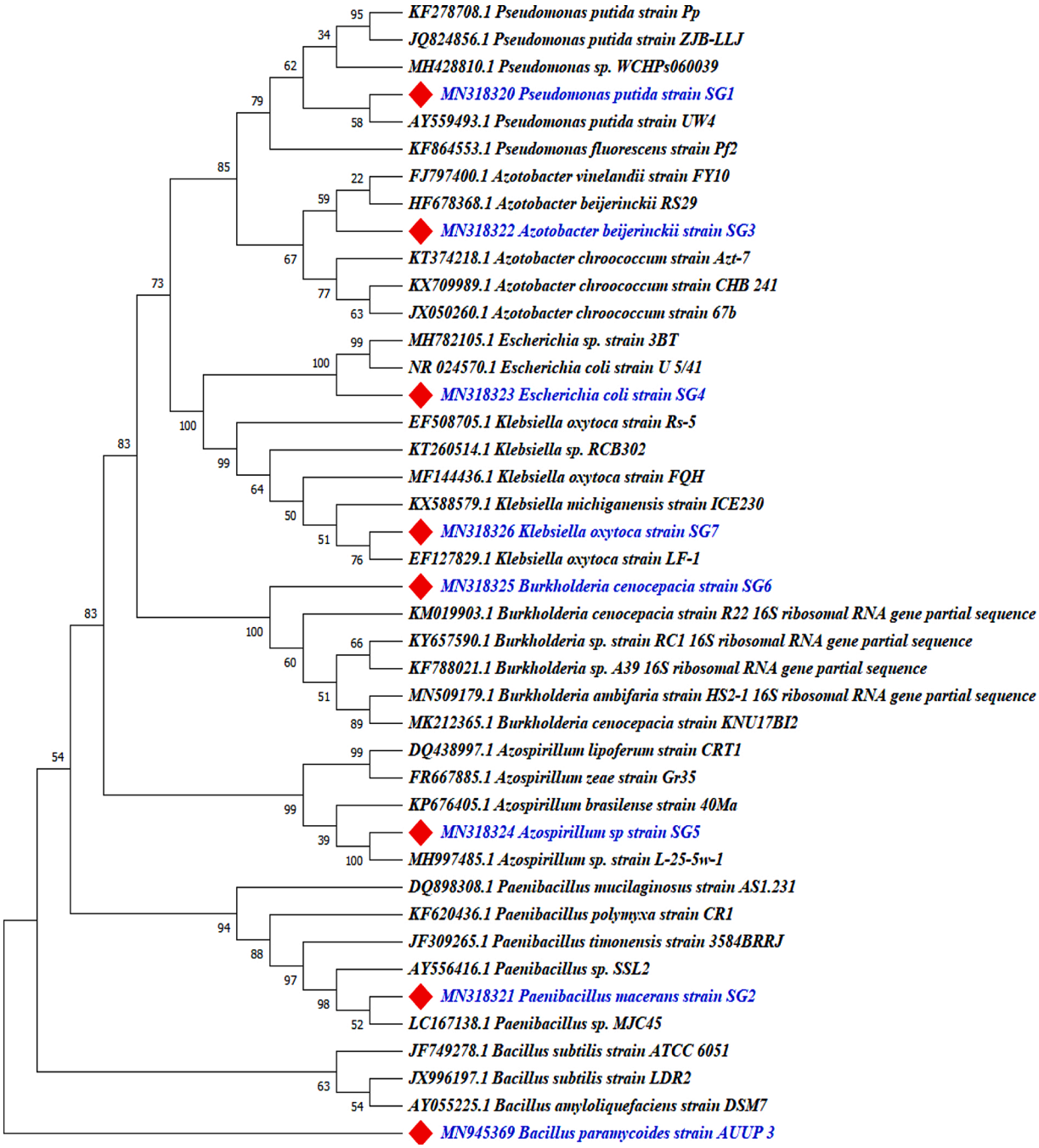

Fig. 4. Neighbour joining phylogenetic dendrogram based on 16 s rDNA sequence alignment of 8 ACC deaminase producing isolates from Coconut rhizosphere with closely related bacteria, retrieved from NCBI GenBank databases. The numbers at branching points refers to bootstrap values based on 1000 replications; Bar 0.20 substitutions per nucleotide position.

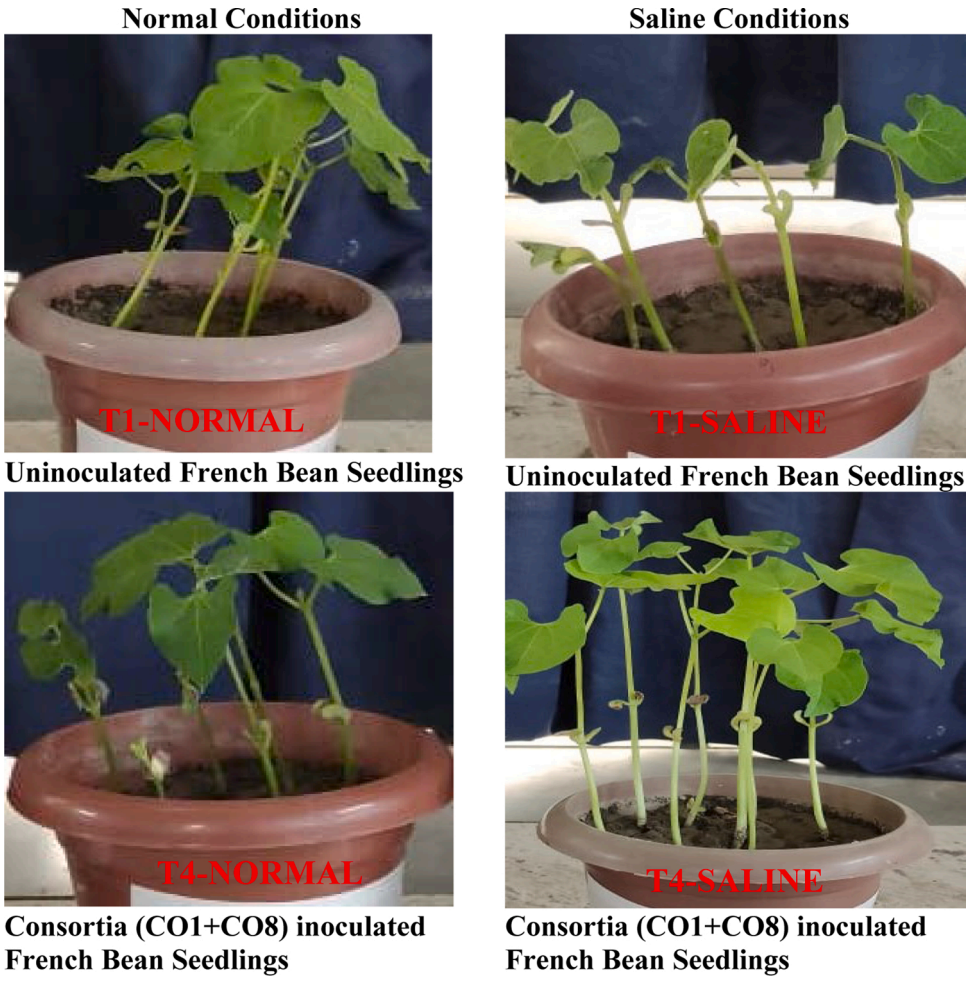

Fig. 5. Effect of inoculation with two ACC deaminase producing PGPR bacterial strains, CO1 and CO8 on the growth of French bean under Normal and Saline stress condtions.

Table 5
Effect of bacterial inoculum on stress induced ethylene from French bean seedlings at two concentration under normal and saline conditions.

Growth Conditions	Treatment	Ethylene (μmol g FW^{-1})	
		10^5 CFU mL^{-1}	10^8 CFU mL^{-1}
Normal	Uninoculated	0.54 ± 0.02^a	0.54 ± 0.02^a
	CO1 strain inoculated	0.52 ± 0.04^a	0.52 ± 0.04^a
	CO8 strain inoculated	0.56 ± 0.02^b	0.56 ± 0.02^b
	Consortia (CO1 + CO8) inoculated	0.50 ± 0.04^a	0.50 ± 0.04^a
Saline (100 mM NaCl)	Uninoculated	0.84 ± 0.07^b	0.84 ± 0.07^b
	CO1 strain inoculated	0.79 ± 0.05^b	0.68 ± 0.01^c
	CO8 strain inoculated	0.77 ± 0.04^b	0.65 ± 0.01^c
	Consortia (CO1 + CO8) inoculated	0.74 ± 0.14^c	0.52 ± 0.04^d

Data represent Mean values ± Standard deviation (n = 3 replicates per treatment); Different letters indicate statistical difference between treatments (Tukey's Test, $P < 0.05$) under normal and saline conditions.

individual and consortium application of CO1 and CO8 strains with ACC deaminase activity showed notable decrease in ethylene emission by 19 % and 38 %, respectively in comparison to uninoculated seedling under saline conditions.

The inoculation with two bacterial strains CO1 and CO8 either in consortia or in individual form at inoculum concentration 10^8 cfu mL^{-1}, had significantly ($P \leq 0.05$) enhance the growth and morphological attributes of plants both under control and saline stressed conditions (Fig. 6). It was observed the application of both the strains in the consortium form has increased the root length (16.7 cm) which was ~49 % higher than the control in normal conditions. Likewise, other parameters such as shoot length (32 %), fresh weight of roots and shoots (79 %, 110 %) and dry weight of roots and shoots (65 %, 30 %) were also increased with the co-inoculation of CO1 and CO8 strains under normal conditions. No significant ($P \leq 0.05$) difference was observed between treatments of French bean seeds with individual bacterial suspension of strains CO1 and CO8. The pot study showed that the impositions of saline stress had retarded the growth of French bean plants. Under saline conditions, the root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight was drastically retarded by 47 %, 51 %, 34 %, 26 %, 35 % and 29 %, respectively in comparison to control plants grown without NaCl stress. However, the combined treatment of PGP strains with ACC deaminase activity has increased the root length by 118 %, shoot length by 105 %, root fresh weight by 93 %, shoot fresh weight by 75 %, root dry weight 124 % and shoot dry weight by 49 %. Under normal conditions i.e. absence of NaCl stress, the RWC content of inoculated French bean plants are numerically higher than that of uninoculated plants but statistically similar to that of uninoculated ones. The individual inoculation of CO1 and CO8 as well as their consortium in French bean plants has showed significant increase relative water content under salt stressed conditions as compared to control. Under saline stress conditions the RWC (~85 %) was markedly increased in CO1 + CO8 consortium inoculated French bean plants as

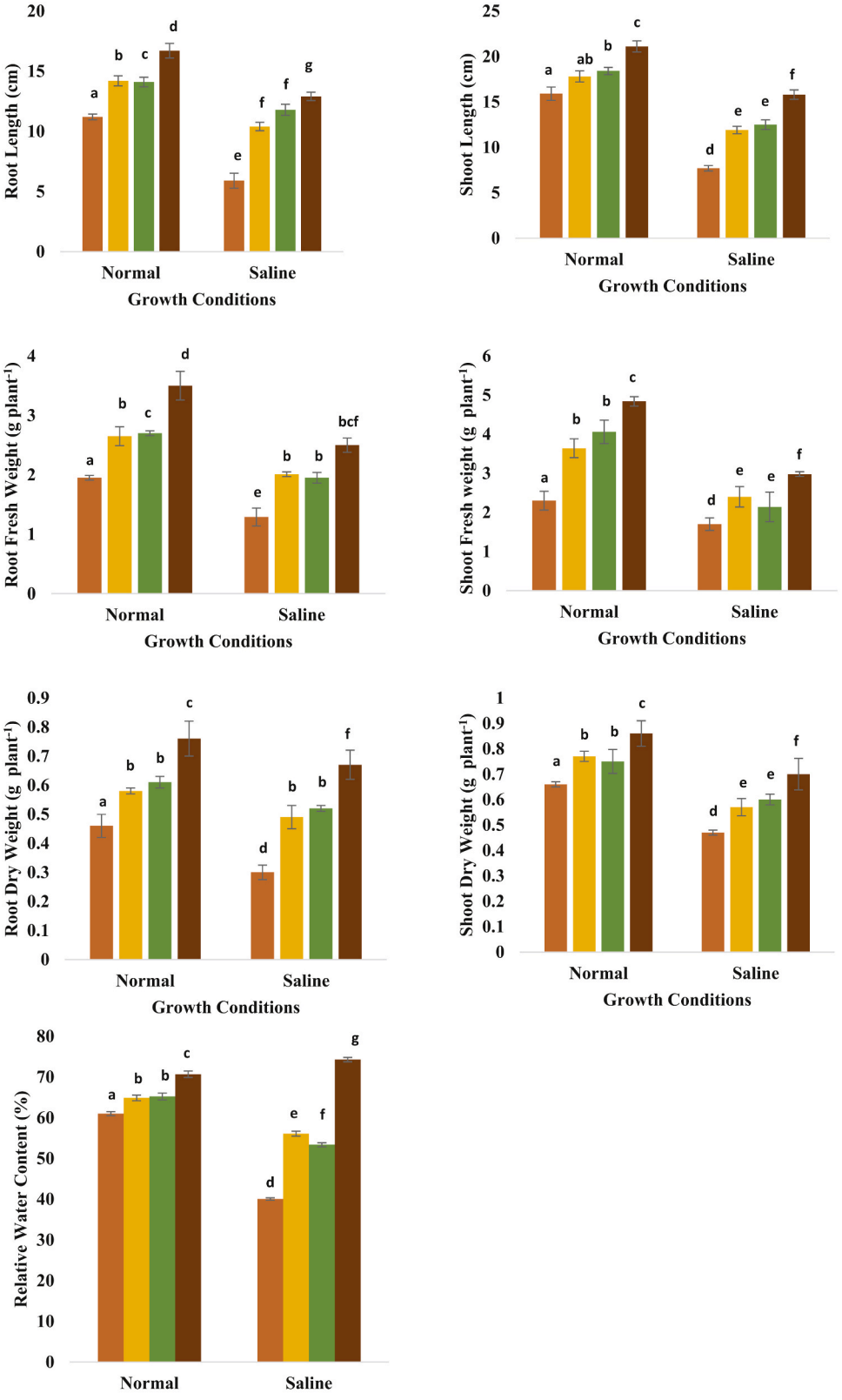

Fig. 6. Different plant growth attributes associated with roots and shoots (length, fresh weight and dry weight) of French bean seedlings with and without bacterial inoculation in control and salt stressed conditions. Data represent Mean values ± Standard deviation (n = 3 replicates per treatment); Different letters indicate statistical difference between treatments (Tukey's Test, $P < 0.05$) under normal and saline conditions. Orange Bar, Treatment 1 (Control); Yellow Bar, Treatment 2 (CO1 strain); Green Bar, Treatment 3 (CO8 strain); Brown Bar, Treatment 4 (Consortia).

compared to uninoculated stressed plants. Additionally, the consortium treatment has also significantly lowered the EC of salt stressed soil to 3.548 dS m^{-1} from 5.8 dS m^{-1} (untreated saline stressed soil) measured through hand held electrical conductivity meter at the harvesting stage.

3.6. *Evaluation of photosynthetic parameters of French bean in response to bacterial inoculation under normal and saline conditions*

Under normal conditions, consortia (CO1 + CO8) inoculated French bean plants displayed increment of chlorophyll *a* (~117 %), chlorophyll *b* (~40 %), and total chlorophyll (~42 %) in comparison to non-

inoculated French bean plants. The 100 mM NaCl treatments lead to ~55 %, ~21 % and ~56 % reduction in contents of chlorophyll *a*, chlorophyll b and total chlorophyll of French bean plants. The bacterial inoculation of CO1 and CO8 significantly ($P < 0.05$) stimulated the production of chlorophyll photosynthetic pigment under salt stressed conditions (Fig. 7). Under saline conditions, individual and consortium inoculation has significantly improved the chlorophyll *a* content by 2.9- to 4.1-fold, chlorophyll b by 0.17- to 0.33- fold and 0.25- to 0.97- fold in relative to control French bean plants. chlorophyll *a*/b ratio was increased significantly ($P < 0.05$) in all CO1 and CO8 inoculated plants as compared to non-inoculated salt exposed French bean plants. A similar pattern in relation to gas exchange characteristics was also

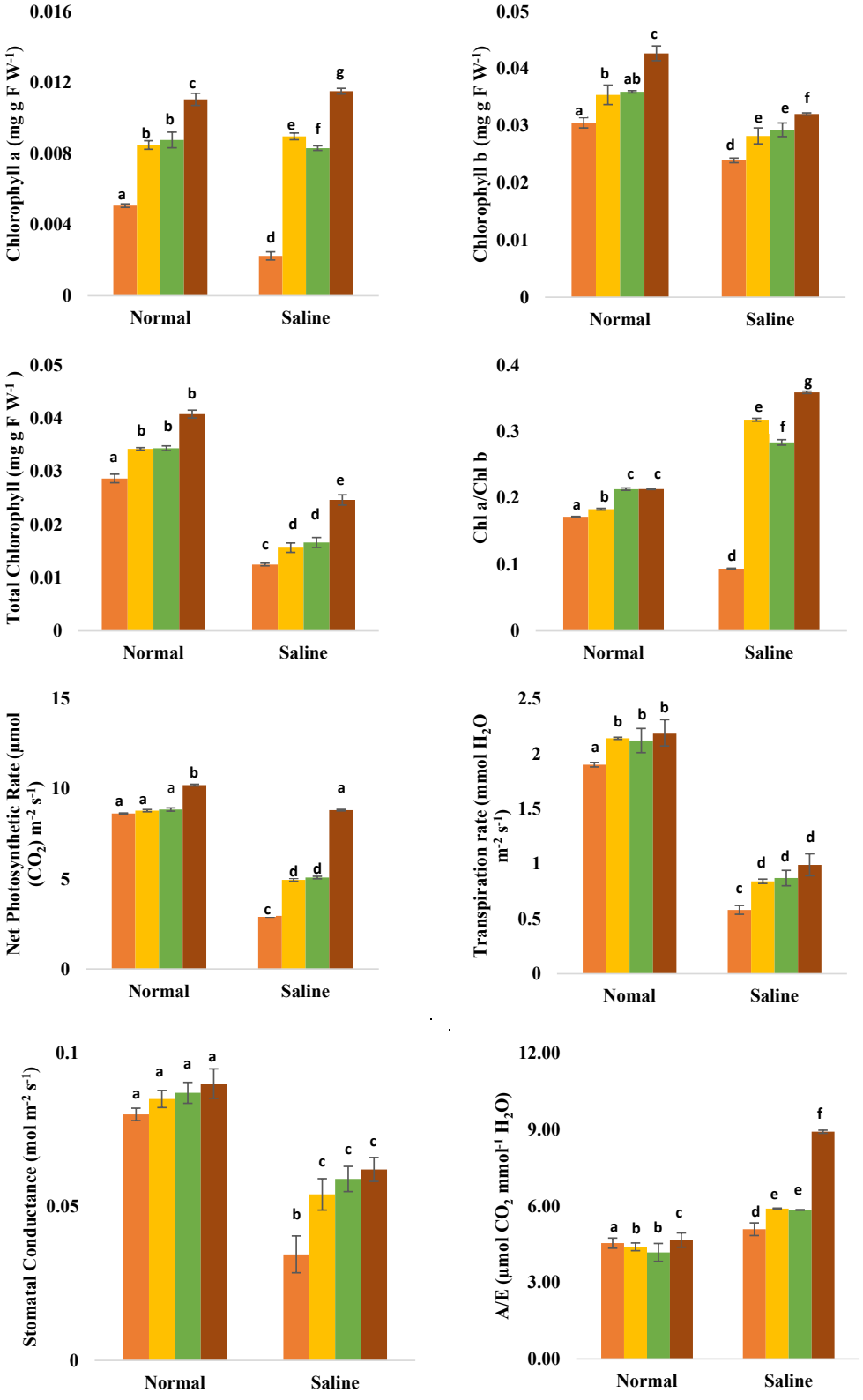

Fig. 7. Photosynthetic parameters of French bean seedlings with and without bacterial inoculation in control and salt stressed conditions.
Data represent Mean values ± Standard deviation (n = 3 replicates per treatment); Different letters indicate statistical difference between treatments (Tukey's Test, $P < 0.05$) under normal and saline conditions. Orange Bar, Treatment 1 (Control); Yellow Bar, Treatment 2 (CO1 strain); Green Bar, Treatment 3 (CO8 strain); Brown Bar, Treatment 4 (Consortia).

observed by bacterial inoculated French bean plants under saline stress conditions. In the absence of salt stressed conditions, the net photosynthetic rate and other gas exchange parameters of individually and consortium inoculated French bean plants was higher in comparison to non-inoculated French bean plants. The net photosynthesis rate (P_N), stomatal conductance (g_s) and transpiration rate (E) of French bean seedlings was significantly reduced by ~65 %, ~54 % and ~70 % because of 100 mM NaCl salinity. The CO1 and CO8 applications either individual or consortium has alleviated the negative impact of salinity on photosynthesis parameters and significantly enhanced P_N in the range ~68−200%, g_s in the range ~56 %-80 % and E in the range of ~ 44 %–70 % at 100 mM NaCl in relative to non-inoculated plants. The imposition of salt stress has increased the water use efficiency (P_N/E) of French bean plants by ~12 % in comparison to normal plants. The consortium application of CO1 and CO8 significantly ($P < 0.05$) enhanced water use efficiency (P_N/E) under salt stressed conditions.

3.7. Evaluation of lipid peroxidation (MDA) levels and membrane stability index of French bean in response to bacterial inoculation under normal and saline conditions

Additionally, in absence of saline stress conditions, the bacterial inoculation has decreased the MDA content in French bean plants but it did not differ significantly between inoculated and uninoculated plants. The salinity stress has significantly ($P < 0.05$) increased MDA content by around ~34 % in comparison to untreated plant. The MDA content in French bean leaves were significantly reduced by 9%, 8% and 15 % at CO1, CO8 individual treatment as well as their consortium treatment, respectively in comparison with non-inoculated treatment plants (Fig. 8).

Furthermore, the consortium of ACC deaminase producing bacterial strains CO1 and CO8 was found to be effective in lowering electrolyte leakage and thus, increasing membrane stability in relation to uninoculated stressed plants under saline stress conditions (Fig. 8).

3.8. Analysis of species diversity

The diversity indices, species richness and species evenness indices were calculated for the ACC deaminase producing PGPR obtained in the present study and values are depicted in Table 6 below. It was observed that higher the values of species richness (3.38, 2.82) and diversity indices such as Simpson's Index of Diversity (1/D), Shannon Wiener Diversity index (H') (1.00,2.07), more is the diversity. The higher the values of evenness index (Plieou's evenness (J', 1.00) more uniform distribution of individual species.

Table 6
Assessment of diversity indices for ACC deaminase producing isolates associated with coconut rhizosphere.

Species Richness	
• Margalef's Diversity Index	3.38
• Menhinick's index (Dm)	2.82
• Species Diversity	
• Simpson's Index of Diversity (1/D)	1.00
• Shannon Wiener Diversity index (H')	2.07
• $e^{H'}$	7.92
• Species evenness	
• Plieou's evenness (J')	1.00
• Sheldon index	0.99

4. Discussions

The 1-Aminocyclopropane-1-Carboxylate (ACC) deaminase is an inducive enzyme responsible for degrading the ACC, immediate precursor of ethylene, into α-ketobutyrate and ammonia. Under stress conditions, the sudden rise in ethylene level confers deleterious effects on plants such as inhibition of root development and formation of root nodules, impairs the nutrient and water uptake efficiency, reduce chlorophyll content and photosynthesis process leads to senescence and abscission process in plants (Gupta and Pandey, 2019a, 2019b). Therefore, ACC deaminase is the most significant and beneficial trait of plant growth promoting soil bacteria that act as a sink for ACC released as root exudate in response to stress conditions and subsequently, regulated the ethylene level and its associated growth inhibition manifested on growth and development of plants (Goswami and Deka, 2020).

The present study deciphers the diversity and plant growth promoting traits of ACC deaminase containing rhizobacteria associated with the rhizosphere of coconut plants, widely cultivated in the south-western coastal region, especially Lakshadweep archipelago. The isolation of plant growth promoting bacteria from the rhizosphere of coconut plant and their functional characterization has been studied in previous studies (Gupta and Pandey, 2019a; 2019b; George et al., 2012) but the population dynamics of particularly, ACC deaminase producing bacteria in coconut rhizosphere is less explored.

In this study, the rhizospheric bacterial population associated with coconut plants were screened for the presence of putative ACC deaminase producing bacterial strains. Out of 75 isolates, 28 rhizobacteria were referred as ACC deaminase producing isolates. PCR-restriction fragment length polymorphism (RFLP)-based analysis is the preliminary molecular genetic profiling tool for the assessment of microbial diversity associated with the sample (Rasmussen, 2012). All the ACC deaminase producing isolates from the rhizosphere of was grouped into

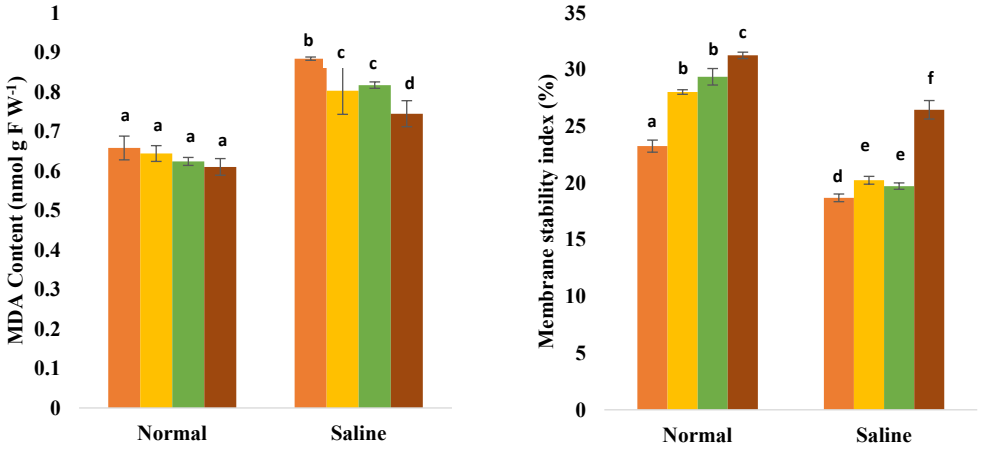

Fig. 8. MDA content and membrane stability index of French bean seedlings with and without bacterial inoculation in control and salt stressed conditions.
Data represent Mean values ± Standard deviation (n = 3 replicates per treatment); Different letters indicate statistical difference between treatments (Tukey's Test, $P < 0.05$) under normal and saline conditions. Orange Bar, Treatment 1 (Control); Yellow Bar, Treatment 2 (CO1 strain); Green Bar, Treatment 3 (CO8 strain); Brown Bar, Treatment 4 (Consortia).

six clusters based on RFLP profiles which indicates the diversity and distinctness of ACC deaminase containing rhizospheric bacteria within coconut plants. This technique was previously used for determining the genetic diversity of nitrogen fixing and other plant growth promoting rhizospheric and endophytic bacterial strains associated with different crop plants such as rice, wheat, sugarcane, chickpea, maize, Faba bean, etc. (Beneduzi et al., 2013, 2008; Upadhyay et al., 2009; Youseif, 2018; Wang et al., 2014; Chen et al., 2018).

Based on 16S rRNA gene sequence analysis of representative strain from each cluster, all the ACC deaminase producing isolates were distributed among two phyla with proteobacteria (75 %) being most dominant followed by firmicutes (25 %) which aligned with the previous report (Kour et al., 2019). The phylum proteobacteria were classified into three classes: Alpha-, Beta- and Gamma-proteobacteria represented by six genera including *Pseudomonas*, *Azotobacter*, *Escherichia*, *Azospirillum*, *Burkholderia*, *Klebsiella*. While other genera *Bacillus*, *Paenibacillus* was identified within phyla Firmicutes. It was also observed that the rhizosphere of coconut was mainly colonized by Gram-negative bacteria community while Gram-positive bacteria were represented by species of *Pseudomonas* and *Bacillus*.

In similar research conducted by thirteen bacterial strains with ACC deaminase activity belonging to eight genera followed *as Bacillus, Pseudomonas, Klebsiella, Serratia, Arthrobacter, Streptomyces, Isoptericola*, and *Microbacterium* were isolated from the coastal halophyte *Limonium sinense* (Girard) Kuntze, China (Qin et al., 2014). The ACC deaminase producing *Burkholderia* species was isolated from rhizospheric soil of rice growing in the coastal region of Odisha, India which modulates the growth and development of rice seedlings by undermining the effects of ethylene produced in response to salt stress (Sarkar et al., 2018).

The ACC deaminase containing soil bacteria is known to possess multifarious PGPR traits such as IAA production, tricalcium phosphate solubilization, siderophore, HCN and ammonia production (Glick, 2014).

The bacterial strains *Pseudomonas putida* and *Bacillus paramycoides* were shown to exhibit the highest potential for enhancement of indigenous pool of phytohormone, IAA (80.75 μg mL^{-1} and 74.66 μg mL^{-1}, respectively) among the 28 ACC deaminase producing strains which are in line with that reported by Manjunatha et al. (2019) and Akhgar et al. (2014). The increased amount of Indole acetic acid will result in an improved root system of plants, in turn, modulate water and nutrient intake efficiency of plants from soil (Etesami et al., 2015; Kang et al., 2019).

Because of phosphate being the limiting nutrient, phosphate solubilization is the most beneficial trait of PGPR making phosphorous available $(PO_4)^{-3}$ to the plants by lowering the pH or producing organic acids such as oxalic acid, gluconic acid etc. (Kalayu, 2019). Most of the ACC deaminase producing isolates of the current study ~57 % exhibited inorganic phosphate (tricalcium phosphate) solubilization by decreasing the pH of spent medium, similar to previous studies by Chauhan et al. (2017), Xu et al. (2014) and Yu et al. (2011).

Siderophores are the iron-chelating agents secreted by bacteria for making iron available to the plants under iron limiting conditions. Moreover, siderophore producing bacteria play an important role in plant growth promotion, biological control against phytopathogens attack, bioremediation (Pahari et al., 2017). The ammonia production was shown by most of the isolates while none of them exhibit HCN production in the present study. Ammonia production promotes the plant growth either directly or indirectly by increasing pH, making unfavourable environment for the growth of phytopathogen (Richard et al., 2018). Apart from PGP traits, the isolates were also screened for *invitro* salinity tolerance up to 8% (w/v) NaCl concentration which indicated two strains CO1(*Pseudomonas putida*) and CO8 (*Bacillus paramycoides*) as halotolerant PGPR strains.

To compute the diversity of cultivable ACC deaminase producing microbes associated with coconut rhizosphere various species richness, species diversity and evenness indices have been used. These indices were used in earlier reports also to calculate the species richness, diversity and evenness of bacterial species associated with different niches (Jha et al., 2010; Azmi and Chatterjee, 2016; Verma et al., 2014).

It is revealed from Margalef's and Menhinick's index (3.38 and 2.82, respectively) that rhizospheric soil sample from coconut plants has the good richness of ACC producing bacterial species. The diversity of ACC deaminase producing bacteria has been deduced by employing non-parametric based heterogenicity indices such as the Shannon Wiener index, Simpson's index of diversity. The major difference between both the indices is that the Shannon-Wiener index focused more on abundance, rare species are equally represented provide information about the actual distribution of species in the specific ecological niche while Simpson's diversity index emphasized more on dominant species with a higher value indicating more dominance and less diversity. In the present study, the value of the Simpson index (1/D) is 1.00, hence coconut rhizosphere was colonized with less diverse species of ACC deaminase producing rhizobacteria. In most of the cases, the Shannon Wiener index has the limitation that it is difficult to compare the diversity among communities with narrowly constrained Shannon index values. Therefore, the concept of e_H has been postulated which determines the effective number of species found in the particular region, in another way, calculates real biodiversity (Whittaker, 1972). In the present study, the value of the Shannon index is 2.07 which can be converted to eH or $\exp(2.07) = 7.92$ which means the sample with the Shannon index of 2.07 has an equivalent diversity as a sample with 7.92 equally common species. The evenness indices including Plieou's evenness index and Sheldon index are mathematical measures that provide information on how the abundance of individuals is distributed among all the species in the specific locations. Furthermore, through evenness indices including Plieou's evenness index and Sheldon index (1.00 and 0.99), it has been deduced that ACC deaminase producing bacterial species from coconut rhizosphere were evenly distributed.

Furthermore, the plant growth promoting potential of two isolates CO1 and CO8, selected based on PGP traits and salt tolerance assay was evaluated in French bean plants under control and saline stressed conditions. Both the isolates in consortium form were found to be effective in alleviating the salinity stress which can be inferred from the improved plant morphological parameters such as root length, shoot length, fresh and dry weight of roots and shoots under saline stress condition in comparison to the negative control. Because of dual culture assay and pot experiment trials, it was found that both the strains were compatible with each other and work synergistically and efficiently to improve plant growth and development under stressed conditions. This is in congruence with previous findings that demonstrated the positive impact of a consortium of PGPR in comparison to the performance of individual treatment under stressed conditions (Panwar et al., 2014; Xia et al., 2020; Zafar-ul-Hye et al., 2019; Periasamy et al., 2019). Since both the isolates possess sufficient ACC deaminase activity which in turn minimizes the increased ethylene concentration in response to saline stress, responsible for retardation of root development, inhibition of seed germination, promotion of aging, senescence and abscission process in plants. The improved root system consequently enhanced water uptake potential of plants which in turn increased the relative water status of the plants. This could also be attributed by the increased amount of plasma membrane intrinsic protein (PIP) or channel proteins in roots and leaves which in turn enhanced relative water content of plants treated with a consortium of CO1 and CO8 in comparison to control untreated under salt stress conditions (Kapilan et al., 2018; Gond et al., 2015). This is in agreement with previous research demonstrating the role of co-inoculation ACC deaminase producing *Kocuria rhizophila* and *Cronobacter sakazakii* in enhancing the growth and conferring salt tolerance to wheat crop plants (Afridi et al., 2019). The salt stress caused significant reduction in stomatal conductance and CO_2 assimilation rate (Gomes et al., 2017); affect the electron transport system and gas exchange parameters (Huang et al., 2014); inhibit chlorophyll biosynthesis, alter the structures of associated cell organelles like chloroplast

by oxidative peroxidation (Sozharajan and Natarajan, 2016); hinders photosystem (PSII) performance (Percey et al., 2016) which decreased the overall photosynthetic efficiency of green plants. There was an evident from our current study that Chlorophyll pigment content (Chl a, Chl b, total chlorophyll and Chl a/ Chl b), Net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) was decreased in the leaves of salt stressed uninoculated French bean plants. This might be due to the decreased leaf stomatal conductance which contribute to lowering leaf transpiration rate and photosynthetic rate (Muchate et al., 2016). The results are in parallel with those (Bacha et al., 2017) who also found significant reduction of chlorophyll content and gas exchange parameters in salt stressed conditions. In our findings, ACC deaminase producing CO1 and CO8 inoculation has improved the chlorophyll biosynthesis, P_N, g_s and E and alleviated the salt stress induced reduction in photosynthetic performance of French bean plants. This was in agreement with the previous studies that inoculation with ACC deaminase producing PGPR *Kocuria rhizophila* Y1, *Azospirillum lipoferum* FK1, *Enterobacter* sp. UPMR18 enhances the synthesis process of chlorophyll (total chlorophyll, chlorophyll *a*, chlorophyll *b*, and the chlorophyll *a/b* ratio) and other gas exchange attributes thus, improve the photosynthetic potential of maize, chickpea and okra plants under salt stress conditions (Li et al., 2020; El-Esawi et al., 2019; Habib et al., 2016). Similarly, water use efficiency of all the inoculated French bean plants with CO1 and CO8 were higher than that of control plants under normal and saline stressed conditions, however, water-use efficiency was higher under salinity stress conditions as reported by (Ansari et al., 2019; Gandonou et al., 2018).

Furthermore, the combined inoculation of CO1 (*Pseudomonas putida*) and CO8 (*Bacillus paramycoides*) has reduced the levels of malondialdehyde (MDA) in comparison to uninoculated plants which is in consistent with the previous study that consortium treatment by *Ochrobactrum anthropic*, *Pseudomonas palleroniana, Pseudomonas fluorescens* and *Pseudomonas palleroniana* has enhanced the growth and reduced the levels of malondialdehyde and its associated oxidative stress in finger millet under water stressed conditions (Chandra et al., 2020). Additionally, the Membrane stability index (MSI) of consortium treated French bean plants was increased which suggested that the strains have the potential to decrease the electrolyte leakage thus restoring the membrane integrity and permeability (Tahir et al., 2019). Apart from ACC deaminase activity, other direct PGP traits such as IAA production, Phosphate solubilization as well as Indirect PGP traits such as siderophore, Ammonia and HCN production, has also contributed in an overall enhancement of plant growth under stressed conditions. The findings of the current study were in congruence with previous studies which demonstrated the plant growth promotion of Barley, mustard, Arabidopsis crops with ACC deaminase producing *Pseudomonas fluorescens, Pseudomonas argentinensis, P. azotoformans, Bacillus subtilis* when subjected to saline stress conditions (Azadikhah et al., 2019; Phour and Sindhu, 2020).

5. Conclusion

In conclusion, the present investigation carried out the enumeration and diversity analysis of ACC deaminase producing rhizobacteria from sandy loamy soil of the coconut rhizosphere of the Lakshadweep islands. Plant growth promoting characterization of isolates revealed that all ACC deaminase producers were capable of producing phytohormone, indole acetic acid, some could solubilize inorganic phosphate, produce siderophore and Ammonia. In comparison to control treatments, the application of consortium of (CO1 + CO8) has significantly enhanced the French bean growth under salt stress. This showed the potential of ACC deaminase producing CO1 and CO8 strain with multiple PGP traits for the facilitation of growth enhancement in the stress environment. However, further evaluation of these strains is needed to validate their performance in curtailing the salinity effects with minimum use of chemical fertilizers in actual field conditions.

CRediT authorship contribution statement

Sangeeta Pandey: Conceptualization, Methodology, Data curation, Writing - review & editing, Funding acquisition. **Shikha Gupta:** Writing - original draft.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jbiotec.2020.10.024.

References

Aeron, A., Khare, E., Jha, C.K., Meena, V.S., Aziz, S.M.A., Islam, M.T., Kim, K., Meena, S. K., Pattanayak, A., Rajashekara, H., 2019. Revisiting the plant growth-promoting rhizobacteria: lessons from the past and objectives for the future. Arch. Microbiol. 1–12.

Afridi, M.S., Amna, Sumaira, Mahmood, T., Salam, A., Mukhtar, T., Mehmood, S., Ali, J., Khatoon, Z., Bibi, M., Javed, M.T., Sultan, T., Chaudhary, H.J., 2019. Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: involvement of ACC deaminase and antioxidant enzymes. Plant Physiol. Biochem. 139, 569–577. https://doi.org/10.1016/j.plaphy.2019.03.041.

Ahmad, F., Ahmad, I., Khan, M.S., 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res. 163, 173–181. https://doi.org/10.1016/j.micres.2006.04.001.

Akhgar, A.R., Arzanlou, M., Bakker, P.A.H.M., Hamidpour, M., 2014. Characterization of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing Pseudomonas spp. in the rhizosphere of salt-stressed Canola. Pedosphere 24, 461–468. https://doi.org/10.1016/S1002-0160(14)60032-1.

Ansari, M., Shekari, F., Mohammadi, M., Juhos, K., Végvári, G., Biró, B., 2019. Salt-tolerant plant growth-promoting bacteria enhanced salinity tolerance of salt-tolerant alfalfa (Medicago sativa L.) cultivars at high salinity. Acta Physiol. Plant. 41 https://doi.org/10.1007/s11738-019-2988-5.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24, 1.

Azadikhah, M., Jamali, F., Nooryazdan, H.-R., Bayat, F., 2019. Growth promotion and yield enhancement of barley cultivars using ACC deaminase producing Pseudomonas fluorescens strains under salt stress. Span. J. Agric. Res. 17, e0801. https://doi.org/10.5424/sjar/2019171-13828.

Azmi, S.A., Chatterjee, S., 2016. Population dynamics of soil bacteria in some areas of Midnapore coastal belt, West Bengal, India. 3 Biotech 6, 37. https://doi.org/10.1007/s13205-015-0361-y.

Bacha, H., Tekaya, M., Drine, S., Guasmi, F., Touil, L., Enneb, H., Triki, T., Cheour, F., Ferchichi, A., 2017. Impact of salt stress on morpho-physiological and biochemical parameters of Solanum lycopersicum cv. Microtom leaves. S. Afr. J. Bot. 108, 364–369. https://doi.org/10.1016/j.sajb.2016.08.018.

Beneduzi, A., Peres, D., da Costa, P.B., Zanettini, M.H.B., Passaglia, L.M.P., 2008. Genetic and phenotypic diversity of plant-growth-promoting bacilli isolated from wheat fields in southern Brazil. Res. Microbiol. 159, 244–250. https://doi.org/10.1016/j.resmic.2008.03.003.

Beneduzi, A., Moreira, F., Costa, P.B., Vargas, L.K., Lisboa, B.B., Favreto, R., Baldani, J.I., Passaglia, L.M.P., 2013. Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. Appl. Soil Ecol. 63, 94–104. https://doi.org/10.1016/j.apsoil.2012.08.010.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Chandra, D., Srivastava, R., Glick, B.R., Sharma, A.K., 2020. Rhizobacteria producing ACC deaminase mitigate water-stress response in finger millet (Eleusine coracana (L.) Gaertn.). 3 Biotech 10, 65.

Chauhan, A., Guleria, S., Balgir, P.P., Walia, A., Mahajan, R., Mehta, P., Shirkot, C.K., 2017. Tricalcium phosphate solubilization and nitrogen fixation by newly isolated Aneurinibacillus aneurinilyticus CKMV1 from rhizosphere of *Valeriana jatamansi* and its growth promotional effect. Braz. J. Microbiol. 48, 294–304. https://doi.org/10.1016/j.bjm.2016.12.001.

Chen, Y.X., Zou, L., Penttinen, P., Chen, Q., Li, Q.Q., Wang, C.Q., Xu, K.W., 2018. Faba bean (Vicia faba L.) nodulating rhizobia in Panxi, China, are diverse at species, plant

growth promoting ability, and symbiosis related gene levels. Front. Microbiol. 9, 1338. https://doi.org/10.3389/fmicb.2018.01338.
Dubey, A., Malla, M.A., Khan, F., Chowdhary, K., Yadav, S., Kumar, A., Sharma, S., Khare, P.K., Khan, M.L., 2019. Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers. Conserv. 28, 2405–2429.
Dworkin, M., Foster, J.W., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75, 592–603.
El-Esawi, M.A., Al-Ghamdi, A.A., Ali, H.M., Alayafi, A.A., 2019. Azospirillum lipoferum FK1 confers improved salt tolerance in chickpea (Cicer arietinum L.) by modulating osmolytes, antioxidant machinery and stress-related genes expression. Environ. Exp. Bot. 159, 55–65. https://doi.org/10.1016/j.envexpbot.2018.12.001.
Etesami, H., Alikhani, H.A., Hosseini, H.M., 2015. Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. MethodsX 2, 72–78. https://doi.org/10.1016/j.mex.2015.02.008.
Fahad, S., Hussain, S., Matloob, A., Khan, F.A., Khaliq, A., Saud, S., Hassan, S., Shan, D., Khan, F., Ullah, Najeeb, Faiq, M., Khan, M.R., Tareen, A.K., Khan, A., Ullah, A., Ullah, Nasr, Huang, J., 2015. Phytohormones and plant responses to salinity stress: a review. Plant Growth Regul. 75, 391–404. https://doi.org/10.1007/s10725-014-0013-y.
Fiske, C.H., Subbarow, Y., 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66, 375–400.
Gamalero, E., Glick, B.R., 2015. Bacterial modulation of plant ethylene levels. Plant Physiol. 169, 13–22. https://doi.org/10.1104/pp.15.00284.
Gandonou, C., Hermann, P., Séraphin, A., Agapit, W., David, H., Françoise, A., Mensah, A., 2018. Effects of salinity stress on growth in relation to gas exchanges parameters and water status in amaranth (Amaranthus cruentus). Int. J. Plant Physiol. Biochem. 10, 19–27. https://doi.org/10.5897/IJPPB2018.0280.
George, P., Gupta, A., Gopal, M., Thomas, L., Thomas, G., 2012. Multifarious beneficial traits and plant growth promoting potential of Serratia marcescens KiSII and Enterobacter sp. RNF 267 isolated from the rhizosphere of coconut palms (Cocos nucifera L.). World J. Microbiol. Biotechnol. 29 https://doi.org/10.1007/s11274-012-1163-6.
Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169, 30–39. https://doi.org/10.1016/j.micres.2013.09.009.
Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J., McConkey, B., 2007. Promotion of plant growth by bacterial ACC deaminase. CRC. Crit. Rev. Plant Sci. 26, 227–242. https://doi.org/10.1080/07352680701572966.
Gomes, M.A., da, C., Pestana, I.A., Santa-Catarina, C., Hauser-Davis, R.A., Suzuki, M.S., 2017. Salinity effects on photosynthetic pigments, proline, biomass and nitric oxide in Salvinia auriculata Aubl. Acta Limnol. Bras. 29 https://doi.org/10.1590/s2179-975x4716.
Gond, S.K., Torres, M.S., Bergen, M.S., Helsel, Z., White Jr., J.F., 2015. Induction of salt tolerance and up-regulation of aquaporin genes in tropical corn by rhizobacterium P antoea agglomerans. Lett. Appl. Microbiol. 60, 392–399.
Goswami, M., Deka, S., 2020. Plant growth-promoting rhizobacteria—alleviators of abiotic stresses in soil: a review. Pedosphere 30, 40–61. https://doi.org/10.1016/S1002-0160(19)60839-8.
Gupta, S., Pandey, S., 2019a. ACC deaminase producing Bacteria with multifarious plant growth promoting traits alleviates salinity stress in french bean (Phaseolus vulgaris) plants. Front. Microbiol. 10, 1506. https://doi.org/10.3389/fmicb.2019.01506.
Gupta, S., Pandey, S., 2019b. Unravelling the biochemistry and genetics of ACC deaminase-An enzyme alleviating the biotic and abiotic stress in plants. Plant Gene 18, 100175. https://doi.org/10.1016/j.plgene.2019.100175.
Gupta, A., Singh, S.K., Singh, M.K., Singh, V.K., Modi, A., Singh, P.K., Kumar, A., 2020. Chapter 7 - plant growth–promoting rhizobacteria and their functional role in salinity stress management. In: Singh, P., Kumar, A., Borthakur, A. (Eds.), Abatement of Environmental Pollutants. Elsevier, pp. 151–160. https://doi.org/10.1016/B978-0-12-818095-2.00007-2.
Habib, S.H., Kausar, H., Saud, H.M., 2016. Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-Scavenging enzymes. Biomed Res. Int. 2016, 6284547 https://doi.org/10.1155/2016/6284547.
Hiscox, J., Israelstam, G., 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57, 1332–1334.
Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611. https://doi.org/10.1007/s004250050524.
Honma, M., Shimomura, T., 1978. Metabolism of 1-Aminocyclopropane-1-carboxylic acid. Agric. Biol. Chem. 42, 1825–1831. https://doi.org/10.1271/bbb1961.42.1825.
Huang, C., Wei, G., Jie, Y., Wang, L., Zhou, H., Ran, C., Huang, Z., Jia, H., Anjum, S.A., 2014. Effects of concentrations of sodium chloride on photosynthesis, antioxidative enzymes, growth and fiber yield of hybrid ramie. Plant Physiol. Biochem. 76, 86–93. https://doi.org/10.1016/j.plaphy.2013.12.021.
Jaemsaeng, R., Jantasuriyarat, C., Thamchaipenet, A., 2018. Molecular interaction of 1-aminocyclopropane-1-carboxylate deaminase (ACCD)-producing endophytic Streptomyces sp. GMKU 336 towards salt-stress resistance of Oryza sativa L. cv. KDML105. Sci. Rep. 8, 1950. https://doi.org/10.1038/s41598-018-19799-9.
Kalayu, G., 2019. Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int. J. Agron. 2019, 4917256 https://doi.org/10.1155/2019/4917256.
Kang, S.-M., Shahzad, R., Bilal, S., Khan, A.L., Park, Y.-G., Lee, K.-E., Asaf, S., Khan, M.A., Lee, I.-J., 2019. Indole-3-acetic-acid and ACC deaminase producing Leclercia adecarboxylata MO1 improves Solanum lycopersicum L. Growth and salinity stress tolerance by endogenous secondary metabolites regulation. BMC Microbiol. 19, 80. https://doi.org/10.1186/s12866-019-1450-6.
Kapilan, R., Vaziri, M., Zwiazek, J.J., 2018. Regulation of aquaporins in plants under stress. Biol. Res. 51, 1–11.
Kavamura, V.N., Santos, S.N., da Silva, J.L., Parma, M.M., Ávila, L.A., Visconti, A., Zucchi, T.D., Taketani, R.G., Andreote, F.D., de Melo, I.S., 2013. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiol. Res. 168, 183–191. https://doi.org/10.1016/j.micres.2012.12.002.
Kour, D., Rana, K.L., Yadav, N., Yadav, A.N., Kumar, A., Meena, V.S., Singh, B., Chauhan, V.S., Dhaliwal, H.S., Saxena, A.K., 2019. Rhizospheric microbiomes: biodiversity, mechanisms of plant growth promotion, and biotechnological applications for sustainable agriculture. In: Kumar, A., Meena, V.S. (Eds.), Plant Growth Promoting Rhizobacteria for Agricultural Sustainability: From Theory to Practices. Springer, Singapore, Singapore, pp. 19–65. https://doi.org/10.1007/978-981-13-7553-8_2.
Li, X., Sun, P., Zhang, Y., Jin, C., Guan, C., 2020. A novel PGPR strain Kocuria rhizophila Y1 enhances salt stress tolerance in maize by regulating phytohormone levels, nutrient acquisition, redox potential, ion homeostasis, photosynthetic capacity and stress-responsive genes expression. Environ. Exp. Bot. 174, 104023 https://doi.org/10.1016/j.envexpbot.2020.104023.
Manjunatha, B.S., Paul, S., Aggarwal, C., Bandeppa, S., Govindasamy, V., Dukare, A.S., Rathi, M.S., Satyavathi, C.T., Annapurna, K., 2019. Diversity and tissue preference of osmotolerant bacterial endophytes associated with pearl millet genotypes having differential drought susceptibilities. Microb. Ecol. 77, 676–688. https://doi.org/10.1007/s00248-018-1257-2.
Miller, R.L., Higgins, V.J., 1970. Association of cyanide with infection of birdsfoot trefoil by Stemphylium loti. Phytopathology 60, 104–110.
Muchate, N.S., Nikalje, G.C., Rajurkar, N.S., Suprasanna, P., Nikam, T.D., 2016. Plant salt stress: adaptive responses, tolerance mechanism and bioengineering for salt tolerance. Bot. Rev. 82, 371–406. https://doi.org/10.1007/s12229-016-9173-y.
Pahari, A., Pradhan, A., Nayak, S.K., Mishra, B.B., 2017. Bacterial siderophore as a plant growth promoter. In: Patra, J.K., Vishnuprasad, C.N., Das, G. (Eds.), Microbial Biotechnology: Volume 1. Applications in Agriculture and Environment. Springer, Singapore, Singapore, pp. 163–180. https://doi.org/10.1007/978-981-10-6847-8_7.
Pandey, S., Singh, S., Yadav, A.N., Nain, L., Saxena, A.K., 2013. Phylogenetic diversity and characterization of novel and efficient cellulase producing bacterial isolates from various extreme environments. Biosci. Biotechnol. Biochem. 77, 1474–1480. https://doi.org/10.1271/bbb.130121.
Panwar, M., Tewari, R., Nayyar, H., 2014. Microbial consortium of plant growth-promoting rhizobacteria improves the performance of plants growing in stressed soils: an overview. Phosphate Solubilizing Microorg. Princ. Appl. Microphos Technol. 257–285. https://doi.org/10.1007/978-3-319-08216-5_11.
Parihar, P., Singh, S., Singh, R., Singh, V.P., Prasad, S.M., 2015. Effect of salinity stress on plants and its tolerance strategies: a review. Environ. Sci. Pollut. Res. 22, 4056–4075. https://doi.org/10.1007/s11356-014-3739-1.
Penrose, D.M., Glick, B.R., 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plant. 118, 10–15. https://doi.org/10.1034/j.1399-3054.2003.00086.x.
Percey, W., McMinn, A., Bose, J., Breadmore, M., Guijt, R., Shabala, S., 2016. Salinity effects on chloroplast PSII performance in glycophytes and halophytes1. Funct. Plant Biol. 43 https://doi.org/10.1071/FP16135.
Periasamy, K., Jayashree, R., Poornima, R., 2019. Plant growth promoting Bacillus spp. And Paenibacillus alvei on the growth of Sesuvium portulacastrum for phytoremediation of salt affected soils. Int. J. Curr. Microbiol. Appl. Sci. 8, 2847–2858. https://doi.org/10.20546/ijcmas.2019.804.332.
Phour, M., Sindhu, S.S., 2020. Amelioration of salinity stress and growth stimulation of mustard (Brassica juncea L.) by salt-tolerant Pseudomonas species. Appl. Soil Ecol. 149, 103518 https://doi.org/10.1016/j.apsoil.2020.103518.
Qin, S., Zhang, Y.-J., Yuan, B., Xu, P.-Y., Xing, K., Wang, J., Jiang, J.-H., 2014. Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte Limonium sinense (Girard) Kuntze and evaluating their plant growth-promoting activity under salt stress. Plant Soil 374, 753–766. https://doi.org/10.1007/s11104-013-1918-3.
Rasmussen, H.B., 2012. Restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP) and gel electrophoresis - valuable tool for genotyping and genetic fingerprinting. In: Magdeldin, S. (Ed.), Gel Electrophoresis. IntechOpen, Rijeka. https://doi.org/10.5772/37724.
Santoyo, G., Equihua, A., Flores, A., Sepulveda, E., Valencia-Cantero, E., Sanchez-Yañez, J.M., Morales, L.R., Govindappa, M., de los Santos-Villalobos, S., 2019. Plant growth promotion by ACC deaminase-producing bacilli under Salt stress conditions. In: Islam, M.T., Rahman, M.M., Pandey, P., Boehme, M.H., Haesaert, G. (Eds.), Bacilli and Agrobiotechnology: Phytostimulation and Biocontrol, vol. 2. Springer International Publishing, Cham, pp. 81–95. https://doi.org/10.1007/978-3-030-15175-1_5.
Sarkar, A., Pramanik, K., Mitra, S., Soren, T., Maiti, T.K., 2018. Enhancement of growth and salt tolerance of rice seedlings by ACC deaminase-producing Burkholderia sp. MTCC 12259. J. Plant Physiol. 231, 434–442. https://doi.org/10.1016/j.jplph.2018.10.010.
Scherr, S.J., McNeely, J.A., 2008. Biodiversity conservation and agricultural sustainability: towards a new paradigm of 'ecoagriculture' landscapes. Philos. Trans. R. Soc. B Biol. Sci. 363, 477–494.
Siddikee, M.A., Glick, B.R., Chauhan, P.S., jong Yim, W., Sa, T., 2011. Enhancement of growth and salt tolerance of red pepper seedlings (Capsicum annuum L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-amino-cyclopropane-1-carboxylic acid deaminase activity. Plant Physiol. Biochem. 49, 427–434. https://doi.org/10.1016/j.plaphy.2011.01.015.

Silva, E.N., Silveira, J.A.G., Aragão, R.M., Vieira, C.F., Carvalho, F.E.L., 2018. Photosynthesis impairment and oxidative stress in Jatropha curcas exposed to drought are partially dependent on decreased catalase activity. Acta Physiol. Plant. 41, 4. https://doi.org/10.1007/s11738-018-2794-5.

Singh, R.P., Jha, P.N., 2016. The multifarious PGPR Serratia marcescens CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (Triticum aestivum L.). PLoS One 11, 1–24. https://doi.org/10.1371/journal.pone.0155026.

Sozharajan, R., Natarajan, S., 2016. Influence of NaCl salinity on plant growth and nutrient assimilation of Zea mays L. J. Appl. Adv. Res. 1, 54. https://doi.org/10.21839/jaar.2016.v1i1.16.

Staddon, W.J., Duchesne, L.C., Trevors, J.T., 1997. Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-Source utilization patterns. Microb. Ecol. 34, 125–130. https://doi.org/10.1007/s002489900042.

Tahir, M., Ahmad, I., Shahid, M., Shah, G.M., Farooq, A.B.U., Akram, M., Tabassum, S.A., Naeem, M.A., Khalid, U., Ahmad, S., Zakir, A., 2019. Regulation of antioxidant production, ion uptake and productivity in potato (Solanum tuberosum L.) plant inoculated with growth promoting salt tolerant Bacillus strains. Ecotoxicol. Environ. Saf. 178, 33–42. https://doi.org/10.1016/j.ecoenv.2019.04.027.

Upadhyay, S.K., Singh, D.P., Saikia, R., 2009. Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. Curr. Microbiol. 59, 489–496. https://doi.org/10.1007/s00284-009-9464-1.

Verma, D.P., Yadav, A.N., Kazy, S., Saxena, A., Suman, A., 2014. Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (Triticum aestivum) growing in central zone of India. Int. J. Curr. Microbiol. Appl. Sci. 3, 432–447.

Wang, H., Wang, S.D., Jiang, Y., Zhao, S.J., Chen, W.X., 2014. Diversity of rhizosphere bacteria associated with different soybean cultivars in two soil conditions. Soil Sci. Plant Nutr. 60, 630–639. https://doi.org/10.1080/00380768.2014.942212.

Xia, M., Chakraborty, R., Terry, N., Singh, R.P., Fu, D., 2020. Promotion of saltgrass growth in a saline petroleum hydrocarbons contaminated soil using a plant growth promoting bacterial consortium. Int. Biodeterior. Biodegradation 146, 104808. https://doi.org/10.1016/j.ibiod.2019.104808.

Xu, M., Sheng, J., Chen, L., Men, Y., Gan, L., Guo, S., Shen, L., 2014. Bacterial community compositions of tomato (Lycopersicum esculentum Mill.) seeds and plant growth promoting activity of ACC deaminase producing Bacillus subtilis (HYT-12-1) on tomato seedlings. World J. Microbiol. Biotechnol. 30, 835–845. https://doi.org/10.1007/s11274-013-1486-y.

Youseif, S.H., 2018. Genetic diversity of plant growth promoting rhizobacteria and their effects on the growth of maize plants under greenhouse conditions. Ann. Agric. Sci. 63, 25–35. https://doi.org/10.1016/j.aoas.2018.04.002.

Yu, X., Liu, X., Zhu, T.H., Liu, G.H., Mao, C., 2011. Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. Biol. Fertil. Soils 47, 437–446. https://doi.org/10.1007/s00374-011-0548-2.

Zafar-ul-Hye, D.M., Bhutta, T., Shaaban, M., Hussain, S., Qayyum, M., Aslam, U., Zahir, Z., 2019. Influence of plant growth promoting rhizobacterial inoculation on wheat productivity under soil salinity stress. Phyton (B. Aires) 88, 119–129. https://doi.org/10.32604/phyton.2019.06570.

Zörb, C., Geilfus, C.-M., Dietz, K.-J., 2019. Salinity and crop yield. Plant Biol. 21, 31–38. https://doi.org/10.1111/plb.12884.