

EFFECT OF INOCULATION OF STRAINS WITH ACC DEAMINASE ISOLATED FROM VERMICOMPOST ON SEED GERMINATION AND SOME PHYSIOLOGICAL ATTRIBUTES IN MAIZE (*ZEA MAYS* L.) EXPOSED TO SALT STRESS

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Abstract

Bacterial inoculation containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity can induce stress tolerance in maize. Vermicompost is a rich source of growth promoting microbes, probably with a unique source for bacteria having ACC deaminase activity. The present investigation aimed to isolate strains with ACC deaminase activity from vermicompost and quantify their effects on seed germination and seedling growth of maize under salt stress. The ACC deaminase-producing strains were isolated from the vermicompost by the directional enrichment screening method. The 16S rDNA gene sequence homology analysis combined with physio-biochemical characteristics were used to identify the taxonomic status of. SB was identified as *Stenotropho monas* sp., RC and RF were *Raoultella* sp., and KE was *Klebsiellas* sp. Maize seeds were germinated under varying level of salinity stress (level of salt stress in 0, 50, 100, 150 and 200 mM NaCl solution). Salinity stress reduced seed germination rate, embryo length and root lengths of seedlings of maize. The results showed that inoculation with four isolated bacterial strains improved seed germination and the growth of maize seedlings under the level of salinity stress in 150 mM NaCl solution. Growth improvement in maize seedlings was associated with increase in proline, chlorophyll content. Therefore, strains with ACC deaminase activity can effectively alleviate the damage to corn seeds and seedlings under salt stress.

Key word: Vermicompost; ACC deaminase strain; Salinity stress; Maize (*Zea mays* L.).

Introduction

Plant growth promoting endophytic bacteria (PGPR) bacteria are a group of microorganisms associated with rhizosphere of terrestrial plants and are known for their beneficial effects on plant growth and productivity (Qin *et al.*, 2011b; Reinhold-Hurek & Hurek, 2011; Grönemeyer *et al.*, 2012; Han *et al.*, 2017; Kataoka *et al.*, 2017). They can promote plant growth by fixing nitrogen, improving nutrient uptake such as iron and phosphate, or specifically by altering plant hormone levels such as auxins, cytokinins and ethylene to facilitate growth of plants (Glick, 2012). Ethylene is not only an important plant growth regulator, but also a key feature of plant responses to stress (Glick, 2014). When high concentration of ethylene exists within the plant body, it can cause plant growth inhibition or even death. For the regulation of intrinsic ethylene levels, the bacteria possess 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Glick, 2014). It is believed that active bacterial strains reduce ACC levels in roots, leaves and seeds by secreting ACC deaminase to reduce plant ethylene levels (Glick *et al.*, 2007a; Glick, 2014; Dweipayan *et al.*, 2016). ACC deaminase can hydrolyze ACC to alpha-ketobutyrate and ammonia, thereby reducing the ethylene content in plants (Nadeem *et al.*, 2010; Deepti *et al.*, 2014; Singh *et al.*, 2015; Hansol *et al.*, 2018). Therefore, the level of ethylene in the plant depends on the ratio of ACC oxidase to ACC deaminase activities of these bacteria (Glick, 2014). In addition, activity of ACC deaminase in bacteria is of great importance to effectively reduce plant ethylene levels (Glick, 2014; Win *et al.*, 2018). Ali *et al.*, (2014) found that plant growth-promoting bacterial endophytes containing ACC deaminase had the potential to promote

plant growth and can mitigate salt stress damages.

Salt stress is an important abiotic stress that limits crop production by salt induced osmotic and toxic effects. However, extent of growth reduction in plants depends on intensity and duration of salt stress (Ashraf, 2004). The excessive accumulation of Na⁺ affect the uptake of other nutrients including K⁺, Ca²⁺ and Mg²⁺ (Munns & Tester, 2008). Various strategies have been proposed to alleviate adverse effects of salt stress on plants. Of these strategies, use of bacterial inoculation with ACC de-aminase activities is of great importance. For example, bacterial inoculation with ACC deaminase activity enhanced the growth of different crop species under salt stress conditions such as in *Brassica napus* (Jalili *et al.*, 2009; Siddikee *et al.*, 2015), *Zea mays* (Shahzad *et al.*, 2013) and red pepper (Siddikee *et al.*, 2011). Soil bacteria containing AAC deaminase improve yield in rice (Bal *et al.*, 2013; Qin *et al.*, 2014; Sarkar *et al.*, 2018) by increasing K⁺/Na⁺ ratio or by lowering proline and malondialdehyde (MDA) such as in wheat (Singh & Jha, 2016). However, extent of growth enhancement and stress tolerance in these crop species depends on source from which they have been isolated.

Vermicompost is a manure produced from organic matter by digestion of earthworm (Lim *et al.*, 2014). Earthworms can increase the number of microorganisms in soil and improve the community structure of microorganisms (Maji *et al.*, 2017; Bhadauria *et al.*, 2014). In addition, earthworm not only improve the soil structure by releasing different nutrients but also add different plant hormones including zeatin, kinetin, cytokinin, auxin and abscisic acid (Zhang *et al.*, 2015). It is assumed that PGPR isolated from vermicompost might have unique features in improving growth and yield of maize plants

under salt stress. The principal objective of the present study was to isolate the growth-promoting bacteria with ACC deaminase activity favorable for maize production from the sputum manure, and explore its effect on the salt tolerance in maize.

Materials and methods

Isolation and activity identification of ACC Deaminase:

The sampling sites were situated within Bio-healthy Agricultural Micro-demonstration manor of Northwest A&F University, Yangling, Shaanxi Province. The variety of maize used for the present study was Zhengdan 958. One g of the collected sputum sample was mixed with 50 ml of sterile water and shaken to obtain a vermicompost suspension, which was placed in a 50 ml NA-vermicompost extract complex medium, and cultured at 200 rpm for 2 days at 30°C with shaking. It was then inoculated into a flat containing 50 ml of DF (Chen *et al.*, 2017) at 200 rpm for 1 day at 30°C. Finally, the same volume (1 mL) of the suspension was transferred to the ADF (Chen *et al.*, 2017) culture solution, and then 1 mL of the bacterial solution was taken up and applied to the ADF solid medium, cultured at 30°C until a single colony appeared, then purified and stored in a freezer at 4°C.

The ACC deaminase activity was calorimetrically monitored by measuring the amount of α -ketobutyrate produced from hydrolytic cleavage of ACC following the protocol of Penrose & Glick (2003) with some modifications. The ACC deaminase activity was induced by growing the bacterial cells in a minimal medium containing ACC as the sole nitrogen source. α -ketobutyrate produced by the reaction was determined by comparing the absorbance at 540 nm of the sample to a standard curve of α -ketobutyrate ranging between 0.1 and 1.0 μ mol. The activity of ACC deaminase was the activity of forming 1 μ mol of α -butyric acid per minute in the enzyme system. The protein was measured using the Solarbio's BCA protein concentration assay kit, and the specific activity (U/mg) was the enzyme activity divided by the enzyme protein concentration.

Salt tolerance test of ACC Deaminase: The isolated strains that have been cultured overnight are inoculated under aseptic conditions in a beef paste peptone liquid containing 0.5%, 1.5%, 2.5%, 3.5%, 4.5%, 5.5%, 6.5%, 7.5%, and 8.5% of NaCl. The medium was shaken at 30°C at 200 rpm. After 24 h of shaking the clarified medium was used as a blank control, and the OD value at 600 nm was measured using a spectrophotometer.

Structural morphology and biochemical characterization of ACC Deaminase Strain:

Morpho-physiological and biochemical characteristics of the strains with ACC deaminase were examined according to the Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Individual cultures grown on NA (Nutrient Agar) medium at 30°C were examined for the colony morphological features by using ordinary light microscope. Gram staining was performed as per standard procedures with exponentially growing cultures.

Characterization based on 16S rRNA gene sequencing and phylogenetic analyses:

Most effective strains were identified by partial sequencing of the 16S rRNA gene. A toothpick was used to scrape a small amount of lawn

moss into a centrifuge tube containing 1.5 mL of fresh beef extract protein liquid, and sent it to the Yangling Boning Biotechnology Co., Ltd. for sequencing, and compare the obtained sequence with the Blast sequence in the Genbank database. The strain sequence with high homology to the 16S rDNA sequence of the isolated strain was selected, and multiple sequence alignments were performed using the Clustal W in Mega 7.0 software, and the phylogenetic tree constructed by the maximum likelihood method.

Preparation of bacterial suspensions: The test strains were inoculated into 50 mL of NA medium, shaken for 24 h at 30°C, and then centrifuged at 12,000 rpm for 5 min. The supernatant was discarded and the cells were enriched. The cells were re-suspended in sterile distilled water, and the OD value of each bacterial suspension was adjusted to maintain substantially with same consistency.

Effect of selected isolates on seed germination under saline stress:

Uniform size maize seeds were disinfected with 3% NaClO for 20 min, 75% alcohol for 2 min and rinsed repeatedly with sterile water for 4-5 times. The surface water was dried with a sterile filter paper and use sterile water as a control, immersed the seeds in the bacterial suspension of each strain for 3 h at room temperature, placed the maize seeds on Petri dishes containing sterile filter papers, and added 8 mL of different concentrations of NaCl to each Petri dish. The solutions (0, 50, 100, 150 and 200 mM NaCl) were incubated at 28°C in the dark, and the germination rate of the seeds was recorded after three days. Shoot length and root length of the seedlings were measured.

Biochemical analysis of plant:

The maize seeds were cultured in dark at 28°C until germination, and the seedlings bearing almost uniform shoot length were transplanted into a plastic box for hydroponic culture, cultured with water for 1 day, hydroponically cultured with 1/2 Hoagland's nutrient solution under natural light and temperature. The solution was replaced after every three days. 1/2 Hoagland nutrient solution was aerated for 15 min every day. When the maize seedlings were grown to the three-leaf stage, they were stressed with 150 mM NaCl (Control 1 without salt) while the fermentation broths of each strain having the same optical density was cultured for 24 h. Physiological indicators of all treated samples were measured after 2 days of salt stress.

Leaf SPAD values of each seedling was recorded with a chlorophyll meter (SPAD-502, Minolta, Japan). Proline content in the leaves was determined following the standard protocol of Gao (2006) with minor modifications. The seedling leaves of different treatments were extracted with 3% sulfosalicylic acid, 2.5% ninhydrin solution, extracted with toluene in boiling water bath and the free proline content was determined with a spectrophotometer at 520 nm (Gao, 2006). POD activity was assayed by the guaiacol calorimetry (Gao, 2006). MDA content was measured by the thiobarbituric acid method (Wang, 2006). The root activity in the root tip of the harvested samples was determined by the Triphenyl Tetrazolium Chloride method (TTC) (Gao, 2006).

Statistical analyses: Statistical significance of the treatments was evaluated by analysis of variance test (ANOVA) followed by mean separation by Duncan's multiple range test (DMRT) using by SPSS 20.0 software. The results represent the average values \pm standard error (\pm SEs) of three replicates for each treatment ($p \leq 0.05$).

Results

Isolation of strains with ACC deaminase from vermicompost and identification of its activity and characterization

Isolation and activity identification of ACC Deaminase:

A total of 4 strains with ACC deaminase activity were isolated from vermicompost containing cow dung as a base. The taxonomic status of the strain identified by homology analysis of the 16S rDNA gene sequence was designated as SB, RC, KE, RF, designated as SB, RC, KE, RF, respectively. The results showed that the ACC deaminase activities of the four strains showed different degrees of efficacy. The isolate KE exhibited the highest ACC deaminase activity of 0.739 U, whereas the SB strain had the lowest enzyme activity of 0.109 U, and the RC and RF ACC deaminase activities were 0.610 U and 0.457 U, respectively. The specific activities of ACC deaminase of SB, RC, KE and RF were 0.0053U/mg, 0.0677 U/mg, 0.0585 U/mg and 0.0308 U/mg, respectively (Fig. 1).

Salt tolerance test of ACC Deaminase: Only strains that can grow in saline-alkali soils can further alleviate the adverse effects of salt on plants. Therefore, improved salt tolerance of strains is very important to improve the resistance of plants against saline stress. The OD values of the isolated four strains in the NA medium with different salt concentrations can be seen after 24 h (Fig. 2). The four strains had some growth at low salt concentration, and that of SB in the saline regime. SB grows slowly when the salt concentration is lower than 4.5%, and the other three plants stop growing significantly at 6.5% salt concentration, indicating that the other three strains have greater salt tolerance mechanism. The growth rate of the RC and KE strains was better at 2.5% of salt level. However, at high salt concentrations, all four strains stopped growing, which indicates that at high salt concentrations, the strain has a strong toxic effect and inhibits the activity of ACC deaminase.

Structural morphology and biochemical characterization of ACC deaminase strains:

The four selected strains were observed for colony morphology and the results were summarized in Fig. 3. On the beef paste peptone plate, the single colony of strain SB was light yellow, round, smooth, opaque, with clear edges and intermediate protrusions. The single colony of strain RC was white, round, smooth, opaque, and the edges clear. The single colony of strain KE was grayish white, round, smooth, opaque, and sharp edges. The single colony of the strain RF was white, round, smooth, and the edges clear. Microscopic examination showed that all four strains were rod-shaped.

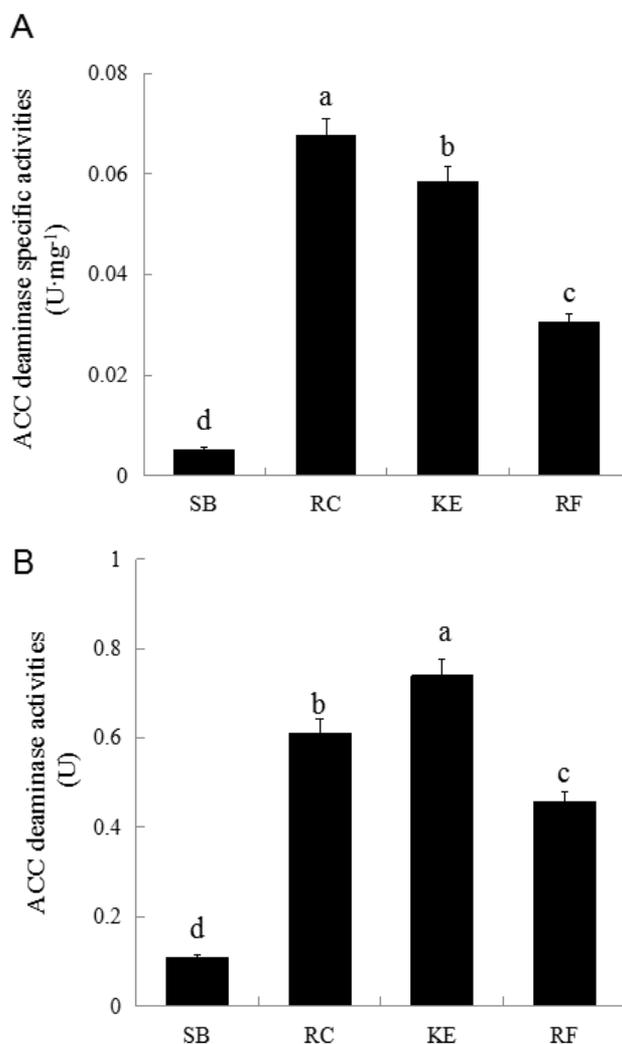


Fig. 1. ACC deaminase activity of four isolated bacteria (A) and ACC deaminase specific activity of isolated strains (B). Values are presented as the mean \pm S.E. (n=3). Different letters accompanying different values indicate statistically significant differences between the values at $p < 0.05$.

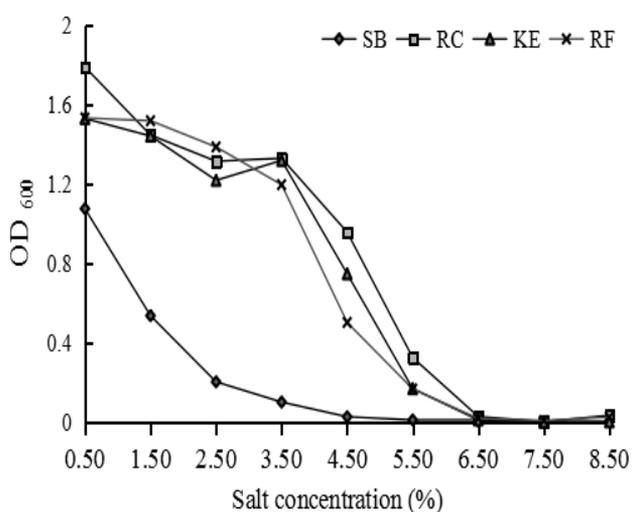


Fig. 2. OD values of four ACC deaminase isolates after 24 hours in NA medium with different salt concentrations. Values are presented as the mean \pm S.E. (n=3). Different letters accompanying different values indicate statistically significant differences between the values at $p < 0.05$.

Table 1. Physiological and biochemical characterization of four isolated strains.

Test index	SB	RC	KE	RF
Gram's staining	-	-	-	-
MR-test	-	-	-	-
VP-test	+	+	+	+
Malonate utilization	+	+	+	+
Citrate salt	+	+	+	+
Nitrate reduction	+	+	+	+
Protease	+	+	-	-
Extracellular amylase	-	-	-	-
Contact enzyme	+	+	+	+
Oxidase	-	-	-	-
Litmus milk	+	+	+	+

Note: “+”: Positive; “-”Negative

Table 2. Salt stress effect on shoot and root growth of three-leaf stage maize seedlings under gnotobiotic conditions.

NaCl concentration (mM)	0	50	100	150	200
Germ(Water, mm)	54.04	64.95	38.25	25.42	13.92
Main root(Water, mm)	147.42	109.77	70.67	40.87	31.05
Germ (SB, mm)	94.99	69.43	50.36	39.54	8.26
Main root (SB, mm)	131.04	97.7	75.26	61.26	30.48
Germ (RC, mm)	80.85	64.13	47.59	30.1	11.02
Main root (RC, mm)	132.05	103.89	70.13	46.77	29.14
Germ (KE, mm)	94.23	66.24	47.49	31.54	14.98
Main root (KE, mm)	106.06	93.06	64.08	32.7	38.98
Germ (RF, mm)	64.55	60.17	39.65	41.19	9.35
Main root (RF, mm)	121.88	106.85	65.75	38.44	32.31

Rojas-Tapias, D., Moreno-Galvan, A., Pardo-Diaz, S., Obando, M., Rivera, D., Bonilla, R., 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). Appl. Soil Ecol. 61, 264e272. <http://dx.doi.org/10.1016/j.apsoil.2012.01.006>

Physiological and biochemical characteristics of the four selected ACC deaminase strains are given in Table 1. The four isolated strains with ACC deaminase activity are Gram-negative strains, all of which can utilize glucose, malonate and citrate; all had the ability to reduce nitrates, which can cause litmus milk to occur. The reduction reaction was different so that the SB strain was alkali-producing, while the all other three strains produced acid. All the four strains showed negative extracellular amylase, negative oxidase and positive contact enzyme. Among the four column strains of SB, RC, KE and RF, only SB and RC had protease activity, and none of the four strains had amylase activity.

Characterization based on 16S rRNA gene sequencing and phylogenetic analyses: The phylogenetic analysis of the isolates was done based on the neighborhood joining method. The isolates SB, RC, RF and KE showed a 99% similarity with the 16S rRNA gene sequence of *Stenotrophomonas maltophilia* strain C3-12, *Raoultella ornithinolytica* strain NEHU.FNSRJ.120, *Raoultella* sp. strain Y4 and *Klebsiella* sp. MS2, respectively (Fig. 4).

Effect of inoculated strains on seed germination and some physiological attributes in maize under salt stress

Effect of inoculated strains on seed germination:

Statistical analysis of the data recorded three days after seed germination is summarized in Table 2. Data on germ length indicated that inoculation with all the isolates enhanced germ length significantly in the absence of salt. In the case of low salt, the germ treated by the bacterial solution had significantly greater length than that in the non-inoculated treatment. At a salt concentration of 150 mM, the seeds inoculated with the broth had a germ length of at least 5 mm compared to that of the non-inoculation, while at 200 mM NaCl, the length of the germ and the main root did not increase significantly. With the increase of salt concentration, the germ and main root length were significantly shortened, and the growth of maize was significantly inhibited. Thus, inoculation with ACC deaminase effectively alleviated the damage of maize seeds under salt stress.

Under high salt treatment, seed germination is generally inhibited, but the four strains with ACC deaminase isolated from inoculation significantly improved the germination rate of Zhengdan 958 maize seed under high salt stress. Compared with the normal condition (no salt), the germination of maize seeds was found to be promoted under low salt conditions, and the germination rate of water-treated maize seeds reached the highest at 50 mM NaCl (0.7% over without salt). The bioavailability was not obvious, and the isolate RF strain was significantly higher than that in the water treatment, but it was still lower than when it was subjected to saline regime. When the concentration reached 150 mM, the maximum maize seed germination rate was observed by the isolate KE (58% over control), and at 200 mM, the corn seed inoculated with the isolate RC had the highest germination rate (116% over control) (Fig. 5).

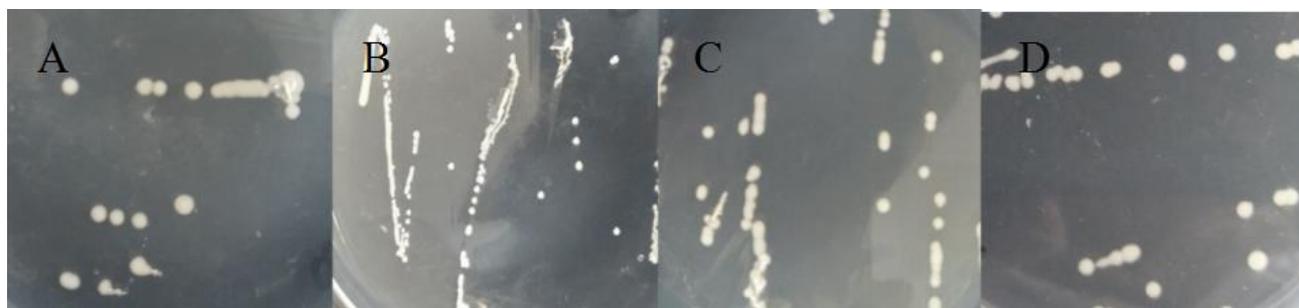


Fig. 3. Colony morphology of four isolates with ACC deaminase on NA medium. (A) SB (B) RC (C) KE (D) RF.

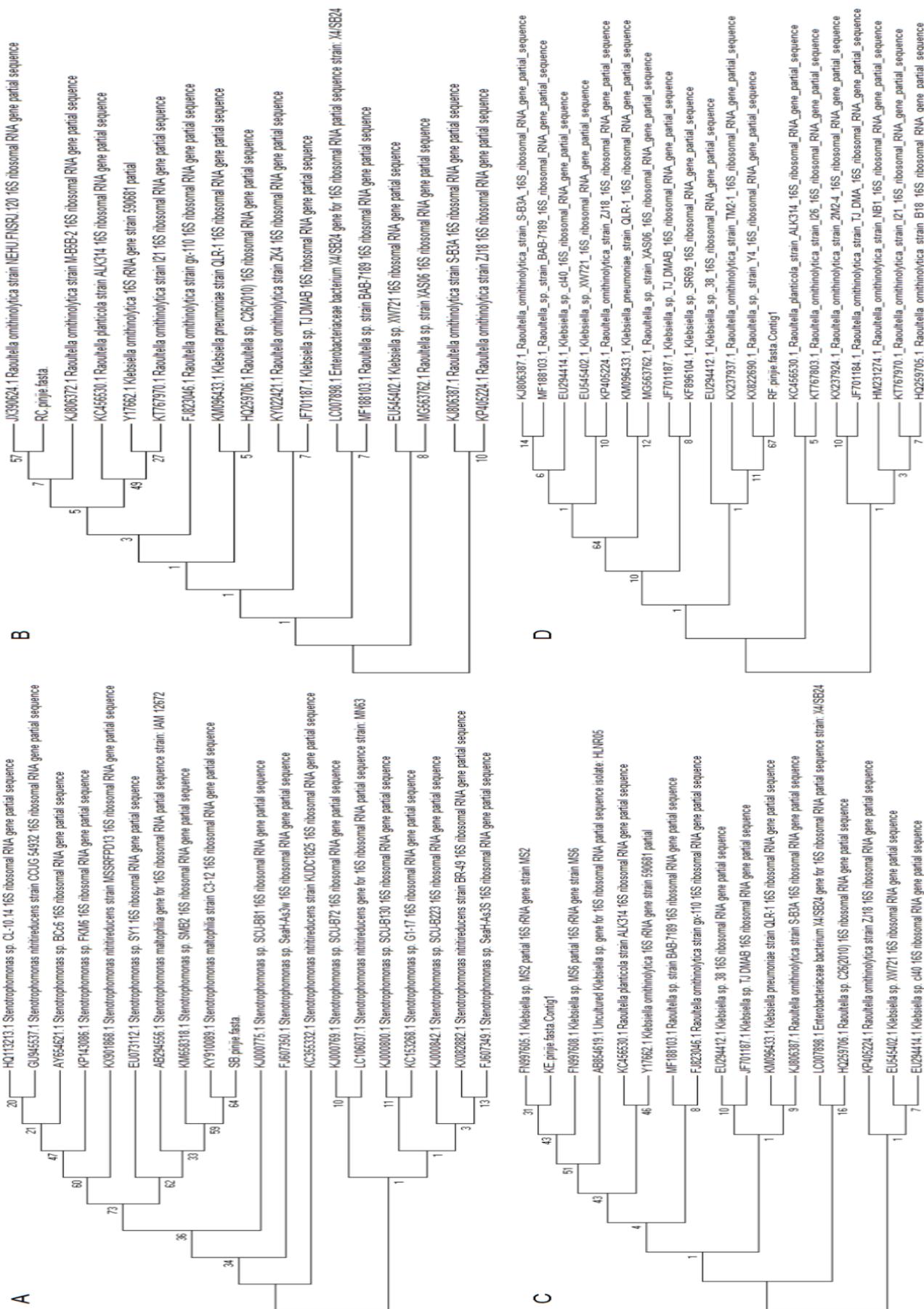


Fig. 4. Neighbor joining tree showing phylogenetic relationship between the selected PGPR from vermicompost and their representative species from GenBank genebase. (a) SB; (b) RC; (c) KE; (d) RF.

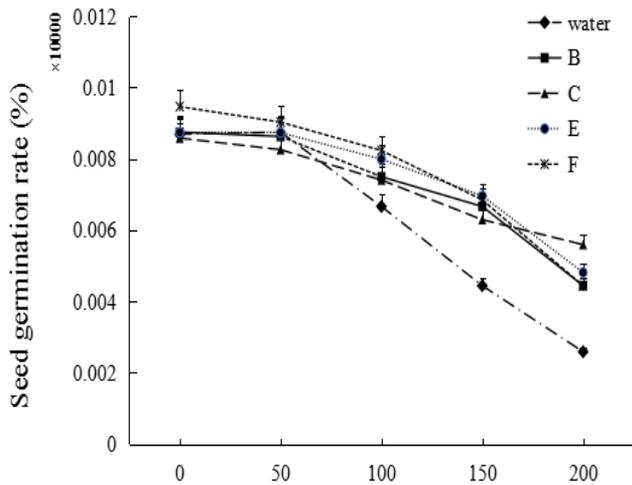


Fig. 5. Effects of inoculation on the seed germination rate of Zhengdan 958 corn under different salt conditions (0 mM, 50 mM, 100 mM, 150 mM, 200 mM).

Effects of inoculated strains on SPAD values in maize leaves: Data for the SPAD values of maize leaves showed that the maize without inoculation was the best in the salt-free condition, and the SPAD values of the leaves reached 32.5 and the uninoculated growth SPAD was 26.8 under salt stress. There were different cases of retardation, but they were all lower than those observed in CK1. The leaf SPAD values of RC, KE, RF and SB strains were 8.10%, 7.35%, 5.36% and 1.50% higher than that of non-inoculated salt stress. At the $p < 0.05$ level, there was no significant difference between the inoculated strains and CK2 in leaf SPAD values (Fig. 6A).

Effect of inoculated strains on proline content in maize leaves: The proline content of corn leaves is shown in Fig. 6B. Under normal conditions (no salt), the proline content in the plant was relatively low. Under 150 mM NaCl, the proline content of the maize plants treated with bacteria was significantly higher than that of the non-bacterial treatment. The inoculated SB strain had 1.23% growth rate, the inoculated RC strain had 1.86%, inoculated KE 4.92%, and that of inoculated RF strain was 18.51%. Except for the significant difference between the inoculated RF strain and the non-inoculated one, the rest were not significant with the uninoculated treatment under salt stress.

POD accumulation: The results of POD activity in maize leaves showed that the POD activity in plants was $484 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ under salt-free conditions. Under salt stress, the POD activity in the maize leaves without inoculation had been $583 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. The POD activity of the maize plants inoculated with the strains was significantly different from that of the non-bacterial treatment. The strain SB was the most effective isolate which enhanced POD activity in maize leaves by 10.99% compared with that in the uninoculated control (Fig. 6C).

MDA accumulation: The results of MDA content in maize leaves indicated that the MDA content in maize

leaves was $0.8 \mu\text{mol}\cdot\text{g}^{-1}$ under salt-free conditions. Under salt stress, the MDA content of the maize leaves was $1.35 \mu\text{mol}\cdot\text{g}^{-1}$. The MDA content of maize leaves inoculated with four strains was lower than that of CK2 but higher than that in CK1. The MDA content of the maize plants treated with bacteria significantly varied from that of the non-bacterial treatment. The MDA content in the maize leaves inoculated with strains SB, RC, KE and RF was decreased by 8.56%, 14.26%, 14.74% and 13.76%, respectively, compared with that of the uninoculated controls (Fig. 6D).

Effect of inoculated strains on maize root triphenyl tetrazolium chloride (TTC): The reduction intensity of TTC in maize roots was $0.135 \text{ mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ under salt-free conditions. Under salt stress, the reducing strength of TTC of maize roots was $0.055 \text{ mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$. Among the four strains inoculated, KE was the most effective isolate, which increased the TTC reduction strength of maize roots by 126% compared to that in the uninoculated control, but lower than that under salt-free conditions. The root TTC reduction intensity of the other three strains was similar to that of the isolate KE (Fig. 6E).

Discussion

In recent years, it has been reported that inoculation of PGPR containing ACC deaminase activity can alleviate salt stress, and increase crop yield (Zahir *et al.*, 2011; Estevez *et al.*, 2009; Ahmad *et al.*, 2011). Plant growth promoting rhizobacteria (PGPR) develop symbiotic association with host plants and improve plant health (Qin *et al.*, 2011b) through direct (by providing nitrogen, precursors of plant hormones and iron) or indirect mechanisms (preventing plant pathogenic bacteria) (Lucy *et al.*, 2004; Raaijmakers *et al.*, 2009).

We isolated four strains with ACC deaminase activity from the sputum, and the 16S rDNA gene alignment showed that they belonged to three genera: *Stenotrophomonas*, *Lauria*, and *Krebs*, and the 16S rDNA gene sequence of the three genera showed 99% similarity. Salt stress reduced the growth of maize plants and bacterial inoculation with any of the three strains alleviated the adverse effects of salt stress. However, growth promoting effect was different in different bacterial strains. For example, shoot growth was maximally enhanced by inoculation with RF strains under saline conditions. Likewise, root growth was maximal in maize plants when inoculated with SB strain. Such differences in growth promotion under saline conditions were probably due to differences in their abilities to auxin in addition to activity of ACC deaminase (Glick *et al.*, 2007b). Similarly, differential effect on shoot or root growth might have been due to differences in sensitivity to salt stress. For example, inhibitory levels of salinity, root growth is relatively more affected than shoot growth (Lin & Kao, 2001). In addition, plants inoculated with bacteria having different activities of ACC-deaminase might have different quantities of ethylene in their rhizospheric environment which affect roots to greater extent than on shoot.

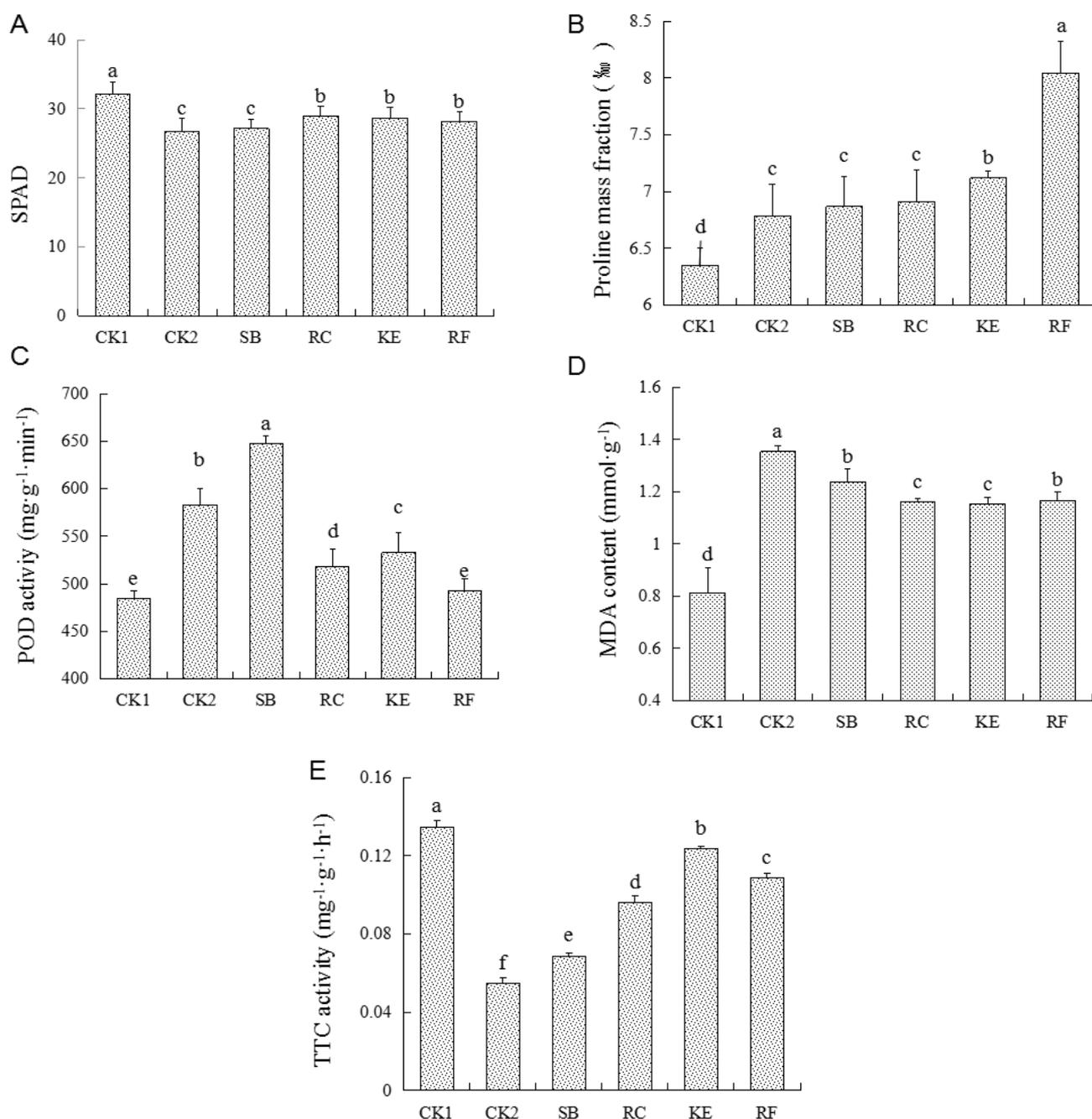


Fig. 6. Effects of inoculation with ACC deaminase on the SPAD content (A), and the proline concentration (Pro, B), and the activities of peroxidase (POD, C), and the malondialdehyde (MDA) content (D), and the activities of root in three-leaf-old maize under salt stress (150 mM) condition. Three-leaf-old seedlings were treated hydroponically and stressed with 150 mM NaCl. Values are presented as the mean \pm S.E. (n=3). Different letters accompanying different values indicate statistically significant differences between the values at $p < 0.05$.

Current research indicates that seed treatment with PGPR strains improves seed germination and seedling vigor of uninoculated seeds (Zahir *et al.*, 2011). These results are similar to some already published reports that bacterial strains with ACC deaminase activity enhanced plant growth under salt stress conditions (Grover *et al.*, 2011; Qin *et al.*, 2014). Bacterial inoculation increased the seed germination and seedling vigor index. This can be explained in view of the bacterial capability to produce indole acetic acid (IAA) in rhizospheric environment, which is adsorbed on the surface of seeds and roots and used by plants (Bharathi *et al.*, 2004). Bacterial

inoculation also promoted the activity of alpha-amylase which enhance the mobilization of food reserves in germinating seeds.

In this study, the effects of inoculation with ACC deaminase strains on plant physiological indices were also studied. The results from the present study showed that bacterial inoculation enhanced the photosynthetic pigments measured as SPAD values. These results could be related to the findings of Rojas-Tapias *et al.*, (2012) who found that bacterial inoculation to maize plants grown under saline conditions enhanced the uptake of Mg and photosynthetic pigments. Similarly, salt induced

osmotic stress cause growth reduction by loss of cell turgor and stomatal closure. In addition, plants with greater ability to osmotically adjust are more tolerant to salt stress (Ashraf *et al.*, 2008). In the present study, it has been found that bacterial inoculation increased the proline content in salt stressed maize plants. How bacterial inoculation enhanced the compatible solutes such as proline and glycinebetaine is somewhat controversial. In some studies, bacterial inoculation caused osmotic adjustment by enhanced accumulation of proline and in some other studies accumulation of glycinebetaine did the same job. For example, inoculation with *Bacillus amyloliquefaciens* NBRISN13 to salt stressed rice plants induces greater biosynthesis of proline (Nautiyal *et al.*, 2013). In contrast, inoculation with *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* reduce proline accumulation but enhance the accumulation of glycinebetaine in rice plants (Jha *et al.*, 2011). It could be associated with type of bacterial inoculation, plant endogenous antioxidant potential and osmotic adjustment capacity. For example, in the present study, activities of antioxidant enzymes in the roots increased except POD enzyme activity, particularly with the inoculation of strain SB. These results could be explained in view of the findings of Nunkaew *et al.*, (2014) who reported that inoculation with ALA producing bacteria reduced the generation of H₂O₂, and increased the activities of battery of antioxidant enzymes in salt stressed rice plants such as CAT, APX, SOD, and glutathione reductase (GR). Similarly, bacterial inoculation reduced MDA accumulation in salt stressed plants (Wu *et al.*, 2014). Nutrient uptake was also increased in maize plants grown under non-saline or saline conditions which was in parallel to what earlier had been reported. The reason may have been that the strain has the ability to produce IAA and promote plant growth by transforming non-absorbable phosphorus and nitrogen for plant use. In other words, the plant growth promotion by the strain could be mainly to provide the plant with nutrients such as N, P and Fe. The bacterial inoculation having ACC deaminase activity increased the root hydraulic conductance (Groppa *et al.*, 2012), transport of K⁺ from root to shoot and maintains sufficient levels of K⁺ to alleviate the Na⁺ toxicity (Wang *et al.*, 2016).

Thus, four bacterial strains of ACC deaminase helped in alleviating the adverse effects of salt stress on maize by reducing oxidative stress, improving antioxidant potential and nutrient uptake, thus, vermicompost proved to be a good source for bacterial isolation. In addition, it is practically feasible to use ACC deaminase-producing strains from sputum to grow maize plants in salt-affected fields. However, further research is needed to test different types of bacterial fertilizers and strain types to assess the effectiveness of these strains in field applications.

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