Annals of Microbiology (2019) 69:797–808
https://doi.org/10.1007/s13213-019-01470-x

ORIGINAL ARTICLE

Alleviation of salt stress response in soybean plants with the endophytic bacterial isolate *Curtobacterium* sp. SAK1

Muhammad Aaqil Khan[1] · Sajjad Asaf[2] · Abdul Latif Khan[2] · Ihsan Ullah[3] · Sajid Ali[1] · Sang-Mo Kang[1] · In-Jung Lee[1,4]

Received: 8 September 2018 / Accepted: 26 March 2019 / Published online: 12 April 2019

Abstract

Background Salinity has been a major abiotic stressor that reduce the productivity. Previous studies reported that endophytic bacteria produce plant stress response hormones, antioxidants, and enzymes such as ACC deaminase. Augmentation of these metabolites and enzymes by endophytes mitigates the stress effects of salinity and improves plant growth and productivity.

Methods Bacterial endophytes were isolated from *Artemisia princeps* Pamp, and evaluated for indole-3-acetic acid (IAA), abscisic acid (ABA), siderophore, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase production and the ability to solubilize phosphate in the presence of NaCl (100–400 mM). SAK1 was applied to *Glycine max* cv. Pungsannamul to investigate salinity stress.

Results Our results revealed that with an increase in NaCl concentration, the amount of ABA production in SAK1 increased, whereas IAA levels decreased. Bacterial ABA and JA degrade the reactive oxygen species and protect plants against stressors. Gas chromatography-mass spectrometry (GC-MS) analysis detected different gibberellins (GAs) and organic acids in SAK1. Interestingly, SAK1 inoculation significantly increased plant growth attributes under normal and salinity stress conditions, whereas a decrease in endogenous jasmonic acid and ABA content in the plants was recorded under salinity stress. IAA and GAs enhance number of root tips and hence improve nutrients uptake in plants. Polyphenolic oxidase and peroxidase were alleviated by elevated SAK1 in *G. max* plants under stress. ACC deaminase of SAK1 resulted deamination of ACC, up to 330 nmol α-ketobutyrate mg^{-1} h^{-1} which could be a major reason of ethylene reduction promoting plant growth.

Conclusion SAK1 relieved salinity stress in plants by producing different phytohormones, antioxidants, and ACC deaminase enzyme. SAK1 could be a new addition in batch of plant stress hormone-regulating endophytic bacteria that mitigates the effects of salt stress and promotes plant growth in *G. max*.

Keywords Endophytic bacteria · ACC deaminase · Salt stress · Phytohormones · Antioxidant

Introduction

Various environmental stressors, including drought, high temperature, salinity, flood, insecticides, and soil pH, limit agricultural production, because they influence the yield of agricultural crops (Sgroy et al. 2009; Shrivastava and Kumar 2015). Among these, soil salinization is one of the most severe problems that has a negative impact on productivity and quality and leads to reduction of the cultivated area. Salinity affects almost all aspects of plant growth; it imposes ionic toxicity, osmotic stress, nutrient deficiency, and oxidative stress in plants, and therefore, limits the absorption of soil water (Shrivastava and Kumar 2015). Around 20% of arable land and 33% of irrigated land worldwide are severely affected by salinity stress (Ali et al. 2014; Pitman and Lauchli 2002; Sgroy et al. 2009). Owing to poor agricultural practices, erosion of rocks, and irrigation with salt water, there has been an annual increase of 10% in salinized areas (Shrivastava and Kumar 2015). Salinity affects irrigated land, which produces 40% of the world's food (Pimentel et al. 2004). Moreover,

✉ In-Jung Lee
ijlee@knu.ac.kr

[1] School of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea

[2] Natural and Medical Science Research Center, University of Nizwa, 616 Nizwa, Oman

[3] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

[4] Research Institute for Dok-do and Ulleung-do Island, Kyungpook National University, Daegu, Republic of Korea

Jamil et al. (2011) reported that more than 50% of the arable land would be salinized by 2050.

Extensive efforts and strategies have been used to reduce the toxic effects of salinity stress on crop production; these include plant genetic engineering, the use of salt-resistant varieties, salt stress-mitigating chemicals, and organic matter conditioners (Zhang et al. 2000). However, salinity is a challenging problem for scientists to develop less expensive and viable approaches that are easy to adopt. The use of salt-tolerant microbes is an alternative method to improve crop productivity. Recently, it has been shown that plant growth-promoting endophytic (PGPE) bacteria can help their host plants cope with various biotic and abiotic stresses (El-Awady et al. 2015; Mayak et al. 2004; Sziderics et al. 2007; Yaish et al. 2015; Zhao et al. 2016). Besides the ability to produce phytohormones and secondary metabolites, several 1-aminocyclopropane-1-carboxylate (ACC) deaminase-producing bacteria have been reported to promote the growth of plants under salt stress conditions (Cheng et al. 2007; Jalili et al. 2009). Such plant growth-promoting bacteria (PGPB) like *Alcaligenes*, *Variovorax*, *Rhodococcus*, *Ochrobactrum*, and *Bacillus* have been reported to produce ACC deaminase (Ahmad et al. 2011; Saravanakumar and Samiyappan 2007).

Under salinity stress, the plant hormone ethylene is accumulated in plants; however, bacteria containing ACC deaminase mitigate the production of stress ethylene by degrading the higher amount of ACC produced in response to the stressful conditions. This is because ACC deaminase cleaves ACC to form ammonium and α-ketobutyrate, thereby decreasing ACC levels in plant tissues (Barnawal et al. 2016; Maxton et al. 2018). Previous reports have shown that ACC deaminase-producing PGPB can enhance growth in rice (Jaemsaeng et al. 2018), tomato (Ali et al. 2014; Palaniyandi et al. 2014), canola (Cheng et al. 2007; Jalili et al. 2009; Sergeeva et al. 2006), groundnut (Saravanakumar and Samiyappan 2007), mung bean (Ahmad et al. 2011), musli (Barnawal et al. 2016), and piper (Maxton et al. 2018), under salt stress conditions.

Soybean (*Glycine max*) is considered an important agricultural crop that is widely used as animal and human food, because of its high oil (38%) and protein (18%) content (Guan et al. 2014). It is also known for its partial salt-sensitivity (Guan et al. 2014), causing a 20–40% reduction in yield with increasing salinity stress (Papiernik et al. 2005). High salt stress has negative impacts on growth, seed quality and quantity, and nodulation (Khan et al. 2018). In particular, the symbiotic relationship of rhizobia with legumes is disturbed, causing cell dehydration and ion accumulation. Recent studies on the positive effects of microbes on plants strongly recommend the use of microorganisms for the promotion of plant growth and salt stress mitigation (El-Awady et al. 2015; Sgroy et al. 2009; Szymanska et al. 2016; Yaish et al. 2015; Zhao et al. 2016).

In the present study, the endophytic bacterial strain SAK1, isolated from *Artemisia princeps* Pamp, was analyzed for the production of ACC deaminase, as well as phytohormones that promoted growth parameters of soybean plants under salinity stress.

Materials and methods

Isolation of bacterial endophytes

The endophytic bacteria were isolated using protocols described by Khan et al. (2014) from different plants growing at Pohang beach (latitude 367′56.2″N, longitude 12923′55.1″ E, and an elevation of 9.9 m), South Korea. The isolated bacterial endophytes were screened on LB agar media. The screened endophytic bacteria were incubated for 24 h at 28 °C, and pure colonies were observed for different morphological features that included size, color, shape, and growth pattern, in order to identify the bacteria.

Indole acetic acid, siderophore production and phosphate solubilization

The pure cultures of all bacterial isolates were screened for indole acetic acid (IAA) and siderophore production, as well as their potential to solubilize phosphate. The method of Patten and Glick (2002) was used for initial confirmation of IAA production by using the Salkowski reagent. The chemical method of Schwyn and Neilands (1987) was used for the determination of siderophore production. Similarly, phosphate solubilization was evaluated following the method of Katznelson and Bose (1959).

Confirmation and quantification of ACC deaminase activity in SAK1

SAK1 was evaluated for the production of ACC deaminase by culturing it in Dworkin and Foster (DF) minimal medium for 20 h at 28 °C, in a shaking incubator at 200 rpm. The method of Penrose and Glick (2003) was used for the confirmation of ACC deaminase enzyme production by SAK1 in DF medium, with slight modifications such as the incubation temperature (28 °C) and centrifugation time (15 min).

For the quantification of ACC deaminase activity, the methods of Shaharoona et al. (2006) and Belimov et al. (2015) were used for the measurement of α-ketobutyrate produced upon the hydrolysis of ACC. The amount of α-ketobutyrate (μmol) produced by this reaction was determined by comparing the absorbance at 540 nm of the sample, to a standard curve of α-ketobutyrate. Here in this reaction, the color of phenyl-hydrazine was developed by the addition of 2 mL of a 2 M NaOH solution,

and after mixing, the absorbance of the mixture was measured at 540 nm by using a spectrophotometer (Eppendorf BioSpectrometer). To determine the ACC deaminase activity of SAK1, the isolate was grown in DF minimal medium for 20 h at 28 °C; after harvesting the cells by centrifugation at 8000×*g* for 10 min, ACC deaminase activity was measured according to the method of Penrose and Glick (2003). In this method, the cell suspension without ACC was used as a negative control, and $(NH_4)_2SO_4$ was used as a positive control.

Bioassay assessment and molecular identification of isolates

The bacterial isolates with diverse plant growth-promoting traits were selected for screening on rice. The sterilized seeds of the rice cultivar 'Waito-C' (dwarf rice variety which is gibberellin-deficient mutant) were treated for 6 h with SAK1 (10^9 cfu mL^{-1}) in a shaking incubator. Similar conditions were used for the untreated seeds as a control. Hoagland solution was used to grow the test seeds for 14 days under these controlled environmental conditions: 14/10 h light/dark (light intensity of 250 μmol m^{-2} s^{-1}), 28/24 °C, and 70% relative humidity.

The standard protocol of Sambrook and Russell (2001) was used for the isolation of genomic DNA. Specific primers 1492R (5′-CGG(T/C)TACCTTGTTACGACTT-3′) and 27F (5′-AGAGTTTGATC(C/A)TGGCTCAG-3′) were used for the amplification of 16S rDNA, as suggested by (Khan et al. 2014). Furthermore, primers used for the amplification of *acdS* genes were as follows: *acdS*f3, 5′-ATCGGCGGCATCCAGWSNAAYCANAC-3′ and *acdSr3*, 5′-GTGCATCGACTTGCCCTCRTANACNGGRT-3′ (Li et al. 2015). The BLAST program of NCBI, GenBank database/EzTaxon, was used to determine the nucleotide sequence homology of the endophytic bacterial isolates. The Neighbor Joining (NJ) method was used for phylogenetic analysis through MEGA v. 6.1 (Tamura et al. 2013).

GC-MS-SIM analysis of isolates for in vitro IAA and ABA production

SAK1 was grown in LB media with different NaCl concentrations (100 to 400 mM) for four consecutive days. LB media, containing 10 g tryptone, 5 g yeast extract, pH 7.0 ± 0.2, was prepared and autoclaved. As the purpose was to examine IAA and ABA production dynamics, upon successful growth, the culture was centrifuged (5000×*g* for 15 min). Culture broth was analyzed for IAA and ABA, following previously published methods (Khan et al. 2016; Qi et al. 1998). The concentration of IAA and ABA in the broths was calculated compared to known standards, by using gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring mode (SIM).

GC-MS-SIM analysis for the quantification of gibberellins in bacterial isolates

Selected strains of endophytic bacteria were cultured, centrifuged (10,000×*g*), and filtered (45-μm filter paper) for quantification of gibberellins (GAs). The isolated culture filtrate (CF) was analyzed for GAs through GC-MS-SIM. The quantification of GAs was performed according to the modified method of Khan et al. (2012).

Different internal standards were used for GA analysis ([17, 17-$2H_2$]. A C18 column (90–130 μm) was used for all extracts for different fractions. For each type of GA, the injection volume of all aliquots was kept at 1 μL for GC-MS (Table 1). The amount of GAs (GA_1, GA_3, GA_4, GA_7, GA_8, GA_9, GA_{12}, GA_{19}, GA_{20}, GA_{24}, and GA_{36}) in the CF were calculated from the peak area ratios. Similarly, retention times were determined by using the standards of hydrocarbon.

Analysis of organic acids in culture broth of bacterial strains

The bacterial cultures were centrifuged (10,000×*g*) for 10 min followed by filtration of the supernatant through a 0.22-μm

Table 1 Description of plants species and isolation of endophytes along with their number of yielded isolates having individual or multiple plant growth-promoting characteristics

Plants name	No of isolates	Isolates having individual plant growth-promoting characteristics			Isolates with multiple PGP characteristics
		IAA production	Siderophore	Phosphate	
Artemisia princeps Pamp.	24	16	5	6	8
Chenopodium ficifolium Smith.	6	1	0	2	0
Oenothera biennis L.	17	12	1	2	1
Echinochloa crus-galli (L.) Beauv.	12	7	1	3	4

Millipore filter. A total of 10 μL filtrate from each sample was injected into a high performance liquid chromatography system (HPLC; Waters 600E), with the following conditions: column, RSpak KC-811 (8.0 × 300 mm); eluent, 0.1% H_3PO_4/H_2O; flow rate, 1.0 mL min^{-1}; temperature, 40 °C. The organic acid retention times and peak areas of chromatograms were compared with standards from Sigma-Aldrich, USA (Kang et al. 2012). All of the samples were analyzed in triplicate.

Plant growth-promoting characteristics of bacterial isolates

The seeds of soybean cv. Pungsannamul were collected from Kyungpook National University's Soybean Genetic Resource Centre, Republic of Korea. The seeds were tested for viability and used in the present study. Surface sterilization of seeds was performed with 2.5% sodium hypochlorite for 30 min, then rinsing with sterilized water. Upon the 10th day of seed germination in the trays, uniform plants were selected for further processing. The autoclaved horticultural soil consisted of peat moss (10–15%), zeolite (6–8%), coco peat (45–50%), perlite (35–40%), with NH_4^+ ~ 0.09 mg g^{-1}, NO_3^- ~ 0.205 mg g^{-1}, P_2O_5 ~ 0.35 mg g^{-1}, and K_2O ~ 0.1 mg g^{-1}.

Plastic pots (10 cm × 10 cm × 9 cm) were used for the growth of soybean seedlings for 21 days consecutively, up to the V1 stage (vegetative stage of unifoliate node). Our experimental design consisted of the following treatments: (a) control (normal soybean), (b) soybean with SAK1, (c) treatment 1 (100 mM NaCl) with or without SAK1, (d) treatment 2 (200 mM NaCl) with or without SAK1, and (e) treatment 3 (300 mM NaCl) with or without SAK1, in a growth chamber (24 h cycle: 28 °C for 14 h and 25 °C for 10 h with a relative humidity of 60 to 70%; Khan et al. 2018). For washing of harvested cells, a 0.8% NaCl solution was used and the optical density was adjusted to 0.5. Different growth parameters were analyzed, such as chlorophyll content, root and shoot length, and fresh biomass of all seedlings.

Quantification of endogenous phytohormones in plants

All plant samples were subjected to endogenous phytohormone analysis and their quantification. The total endogenous ABA content was quantified according to the detailed method of Qi et al. (1998), and each experiment was repeated in triplicate. Similarly, the method of McCloud and Baldwin (1997) was used for quantification of endogenous jasmonic acid (JA) content. All treatment samples (freeze-dried) were used for the extraction and quantification of JA (McCloud and Baldwin 1997). For these analyses, [9, 10-2H2]-9,10-dihydro-JA (20 ng) was used as an internal standard. Furthermore, the peak areas were compared with their respective standards to estimate the amount of endogenous JA. All treatments were repeated in triplicate.

Total proteins, peroxidase, polyphenol oxidase, and glutathione quantification

For quantification of total proteins, the method of Bradford (1976) was used, with slight modifications; the extracts were measured at 595 nm on a SHIMADZU spectrophotometer (Kyoto, Japan). Similarly, the antioxidant enzymes peroxidase (POD) and polyphenol oxidase (PPO) were also analyzed. Briefly, the homogenized leaf samples (400 mg) were centrifuged at 5000×*g* on 4 °C for 15 min. The method of Kar and Mishra (1976) was used for PPO and POD analyses, with slight modifications. The final assay was determined at 420 nm for measuring POD and PPO. The method of Ellman (1959) was used for the determination of glutathione concentration.

Statistical analysis

All experiments were performed in triplicate and results collected were used for further analysis. A two-way ANOVA was used for statistical analysis, by using Bonferroni Post-Hoc test with a significance level ≤ 0.05. For comparing the effect of PGPB on soybean growth and germination, a design that was fully randomized was used. Graphpad Prism 5 (USA) was used for graphical presentation and statistical analysis.

Results

A total of 59 endophytic bacterial strains were isolated from the roots of *Oenothera biennis*, *Chenopodium ficifolium*, *A. princeps*, and *Echinochloa crus-galli* plants, grown predominantly in the Eastern sea coast on sand dunes at Pohang (Table 1). Biochemical and morphological tests were conducted for determination of plant growth-promoting (PGP) traits. All isolates in this study showed one or more PGP traits, like production of IAA, siderophores and solubilization of phosphate. However, only 13 isolates showed two or three traits (Table 1). Based on multiple PGP traits, five isolates were subjected to further analysis on rice. The isolates that promoted the largest increase in the weight of rice plants were selected for additional experiments. Our results on Waito-C rice growth after inoculating selected isolates revealed that SAK1 remarkably increased rice growth (Fig. 1a) as compared to the other selected isolates and controls (Fig. 1a). Similarly, *acdS* gene of the isolated bacterial endophyte was also amplified and confirmed by using specific primers, which revealed that SAK1 has the capability to produce ACC deaminase (Fig. 1b).

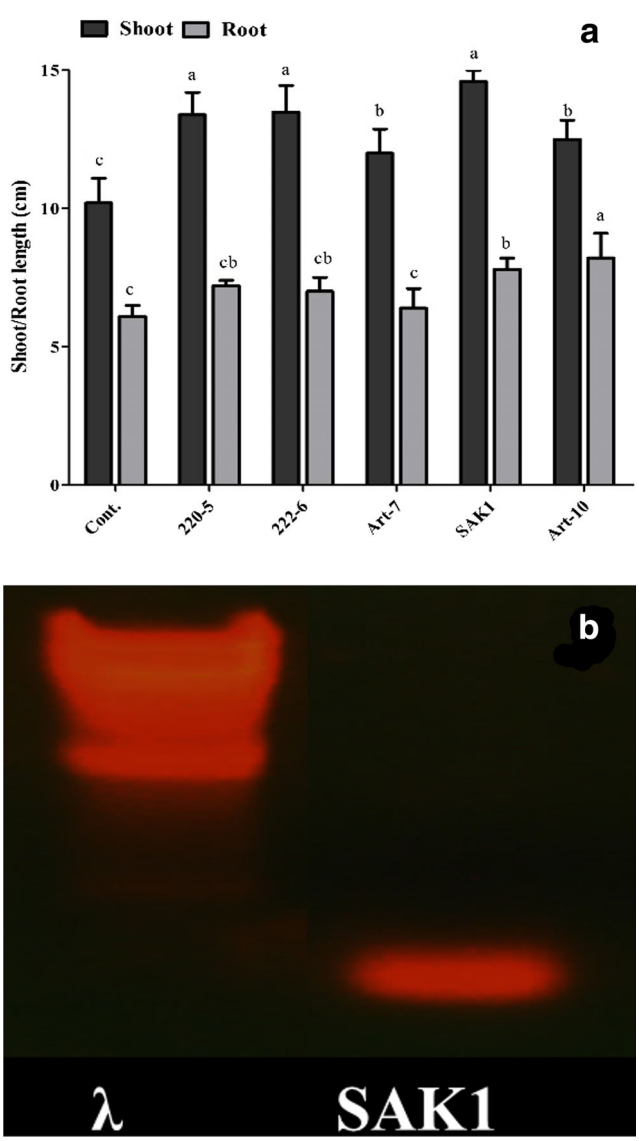

Fig. 1 Growth parameters, siderophores, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. **a** Effect of different bacterial isolates on root and shoot lengths of rice. **b** Amplification of *acdS* gene of SAK1

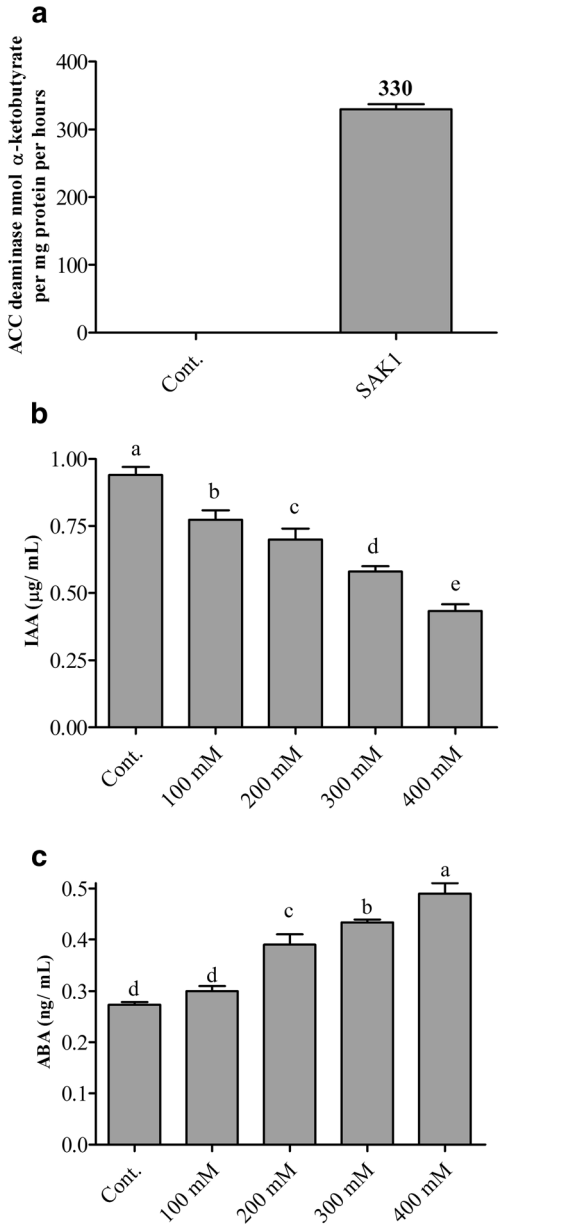

Fig. 2 Characteristics of bacterial isolates. **a** Quantification of ACC (1-aminocyclopropane-1-carboxylate) deaminase production. **b** Indole acetic acid (IAA) content observed in culture broth (CB) of SAK1. SAK1 was grown in culture broth (CB) with high NaCl concentrations (100, 200, 300, and 400 mM). **c** Content of abscisic acid (ABA) in CB of SAK1. SAK1 was grown in CB with high NaCl concentrations (100, 200, 300, and 400 mM). Each data point is the mean of three replicates. Error bars represent standard errors. The bars represented with different letters are significantly different from each other as evaluated by DMRT analysis

Quantification of ACC deaminase and phytohormone production of SAK1

SAK1 was evaluated for the production of ACC deaminase and phytohormones. We estimated the capability of endophytic SAK1 bacteria to produce ACC deaminase on DF minimal media containing its substrate ACC. SAK1 revealed the highest value of deamination of ACC, up to 330 nmol α-ketobutyrate mg^{-1} h^{-1} (Fig. 2a).

Moreover, SAK1 produce ABA and IAA in LB media in the presence of different NaCl concentrations (0, 100, 200, 300, and 400 mM). Here, we noticed that the amount of ABA was rising steadily with the increase in concentration of salt in the media (Fig. 2c). Thus, the increased amount of ABA is produced in response to higher saline stress condition. This also suggests that the production of ABA may be advantageous for the growth of certain species under salinity and drought stress conditions. Conversely, the decreased amount of IAA content was measured under higher salt concentration in the broth, compared to that in normal culture media (Fig. 2b). Hence, the production of ABA is augmented in the

presence of higher NaCl concentration while the amount of IAA is lowered down in the LB medium.

Bioactive GAs detected in culture filtrate of SAK1

The culture filtrate of isolated SAK1 was analyzed for the purpose of GAs. Results of SAK1 analysis showed different components of GAs, e.g., GA_7, GA_8, GA_{24}, and GA_{36}. The quantities of GAs were as follows: GA_7, 0.847 ng mL^{-1}; GA_8, 0.04 ng mL^{-1}; GA_{24}, 0.024 ng mL^{-1}; and GA_{36}, 0.038 ng mL^{-1}, as shown in Fig. 3a.

Detection of organic acids in SAK1

Results of organic acids analysis showed that the cultured filtrate of SAK1 was containing butyric acid, succinic acid, acetic acid, and quinic acid (Fig. 3b). SAK1 revealed the higher amount (8.2 μg mL^{-1}) of acetic acid compare to other organic acids. Malic acid was detected at a concentration of 3.9 μg mL^{-1}, whereas quinic acid, succinic acid, and butyric acid were produced at concentrations of 3.3, 2.5, and 0.66 μg mL^{-1}, respectively.

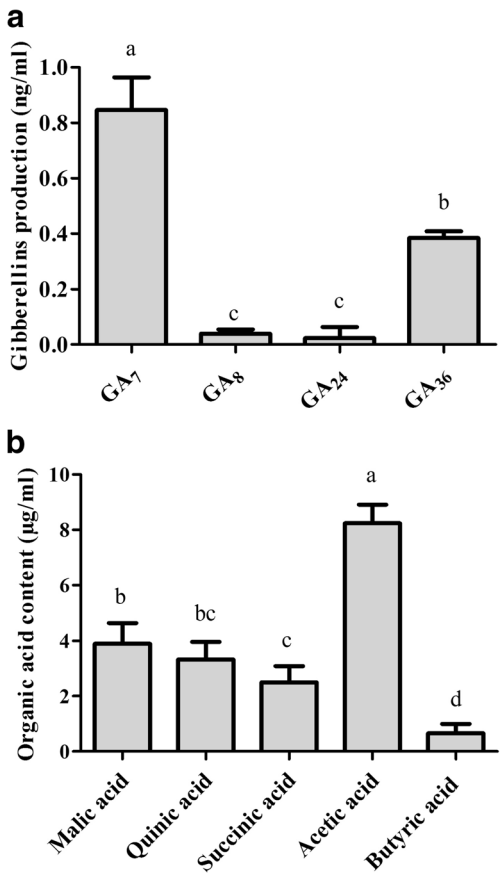

Fig. 3 **a** Gas chromatography-mass spectrometry (GC-MS-SIM) analysis and quantification of different gibberellins (GAs) by comparing them with the internal standard. **b** Organic acids (low molecular weight) produced by SAK1. HPLC analysis and detection of organic acids used, in relation to their respective standards. Given letter(s) are significantly different at the 5% level by DMRT

SAK1 mitigates salinity stress

Soybean plants inoculated with SAK1 revealed interesting results under salinity stress conditions. The plants treated with SAK1 greatly mitigated the adverse effects of salinity stress, and favored plant growth, compared to untreated stressed plants (Table 2). The SAK1-treated plants under salt stress had significantly enhanced length and biomass compared to those of untreated plants. Under normal conditions (Table 2), soybean plants inoculated with the SAK1 had greatly enhanced root/shoot length and weight and chlorophyll content compared to those of control plants. Our results also revealed that the growth of soybean was found to decrease when the salt concentration was increased, but SAK1 inoculation mitigated the negative effects of salt on soybean growth. This showed that the beneficial effects of endophytic SAK1 interaction mitigated salinity stress and promoted root/shoot growth and chlorophyll content at different salt concentrations compared to those in control plants.

Endogenous ABA and JA content of soybean

An increase in ABA levels was observed under different NaCl concentrations for the inoculated and non-inoculated soybean plants (Fig. 4a). However, the amount of endogenous ABA content under different salt concentrations is greatly reduced because of SAK1 inoculation. This demonstrates the stress-mitigating capability of endophytic SAK1. However, there was no significant difference in endogenous ABA levels between control and unstressed SAK1-treated plants (Fig. 4a). Furthermore, our results revealed that higher salt stress enhances endogenous JA content in soybean plants. However, SAK1 treatment lowered the amount of JA in treated plants, when the results were compared to those of controls (Fig. 4b).

Effect of SAK1 on antioxidant activities and total protein content of soybean

The protein level increases proportionally to the increase in salt concentration in soybean (Fig. 5a). However, plants treated with SAK1 showed a reduced amount of total protein at different salt concentrations. The application of SAK1 enhanced total protein content in stressed conditions, compared to control plants (Fig. 5a).

It has already been shown that reactive oxygen species (ROS) are produced during elevated NaCl concentrations that leads to oxidative stress of plants (Habib et al. 2016). Consequently, we also inspected the antioxidant enzyme activities in the present study (Fig. 5). Our results revealed that salt stress significantly increased the activity of PPO in

Table 2 Effect of bacterial isolates on growth attributes of the plant exposed to different concentrations of salts

Treatments	SL (cm)	RL (cm)	SFW (g)	RFW (g)	CC (SPAD)
Control	19.52 ± 2.2ab	16.94 ± 0.8abc	2.11 ± 0.3b	2.17 ± 0.4b	31.83 ± 0.9ab
Isolate SAK1	22.04 ± 2.3a	18.66 ± 0.5a	2.27 ± 0.1a	2.60 ± 0.3a	33.03 ± 1.5a
100 mM NaCl	17.61 ± 2.0bc	16.50 ± 0.5bc	1.92 ± 0.3c	1.94 ± 0.4cb	30.69 ± 1.1bc
100 mM NaCl + SAK1	19.15 ± 2.1ab	18.13 ± 1.4ab	2.09 ± 0.4b	2.15 ± 0.3b	32.03 ± 1.0ab
200 mM NaCl	15.03 ± 4.1 cd	14.83 ± 0.9 dc	1.77 ± 0.3d	1.73 ± 0.5 cd	29.06 ± 1.2c
200 mM NaCl + SAK1	17.84 ± 2.9bc	16.36 ± 1.0bc	1.95 ± 0.5c	1.95 ± 0.4cb	31.46 ± 1.7ab
300 mM NaCl	12.61 ± 2.3d	12.86 ± 1.5d	0.84 ± 0.2f	1.40 ± 0.4e	27.96 ± 1.4d
300 mM NaCl + SAK1	15.60 ± 0.5bcd	13.36 ± 1.6d	1.17 ± 0.4e	1.46 ± 0.5de	28.50 ± 1.5c

uninoculated plants, compared to that in SAK1-treated plants (Fig. 5b). Furthermore, SAK1 greatly reduced PPO activity in soybean plants treated with different salt concentrations (100 to 300 mM). However, no notable difference was observed between inoculated and non-inoculated control plants (Fig. 5b).

As shown in Fig. 6c, the enzymatic activity of POD was enhanced in the plants, similar to that of total protein content, under high salinity stress. SAK1 greatly arrested the activity of POD in soybean plants under salt stress, compared to that in uninoculated plants (Fig. 5c). Contrary to this, the initial application of SAK1 induced POD activity to a large extent compared to that in control unstressed plants (Fig. 5c).

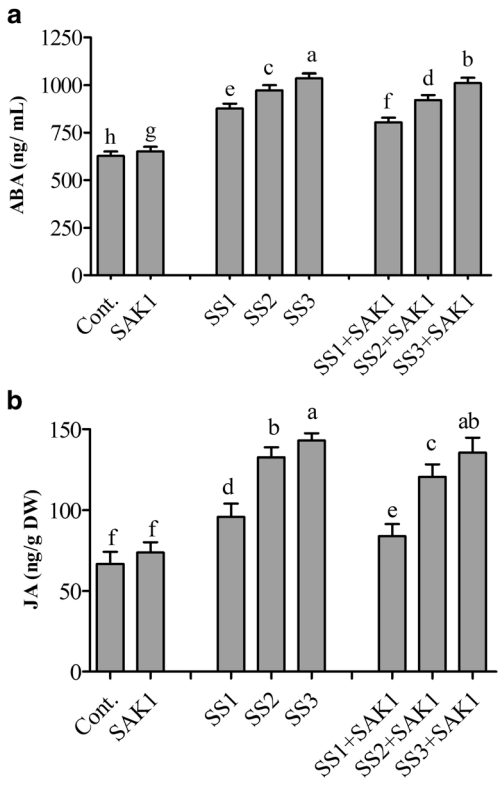

Fig. 4 Jasmonic acid (JA) and abscisic acid (ABA) content quantification in soybean. **a** Effect of SAK1 on the ABA content in soybean growing in high NaCl concentrations (100, 200, and 300 mM). **b** Effect of SAK1 on the content of JA in soybean growing in high NaCl concentrations (100, 200, and 300 mM). Each data point is the mean of three replicates. Error bars represent standard errors. The columns represented with different letters are significantly different from each other as evaluated by DMRT analysis

Reduced glutathione is a central cellular antioxidant and signaling compound that stimulates many vital cellular processes. Exposure to different concentration of salt stress caused an increased formation of ROS and leads to oxidative stress. Our results demonstrated that glutathione concentrations were slightly higher in SAK1-inoculated soybean plants under salinity stress (Fig. 5d). In addition, a decrease in glutathione concentration was observed in soybean at all NaCl concentrations tested (100, 200, and 300 mM). It appears that no notable differences were observed in glutathione concentrations between SAK1-inoculated and control soybean plants (Fig. 5d). The increase in glutathione content implicates a response to oxidative stress, to enhance plant growth and ameliorate salt stress.

Identification and phylogenetic analysis of SAK1

Molecular identification and phylogenetic analysis of SAK1 were performed by amplifying and sequencing the 16S rRNA gene and comparing it to a database of known 16S rRNA sequences. The results revealed that SAK1 exhibited a high level of 16S rRNA sequence identity (99.93%) with *Curtobacterium oceanosedimentum* ATCC31317^{T} (T:superscript) (Fig. 6). The sequence was submitted to NCBI with the accession number MF949056.

Discussion

SAK1 was employed in the present study as salinity stress relevant to enhance the plant tolerance against high salt concentration. Biochemical analysis of SAK1 showed the bacterial strain produced different phytohormones such as ABA, JA, IAA, GAs, and antioxidants including polyphenolic oxidase, peroxidase, and reduced glutathione. The SAK1 also produced different organic acids and ACC

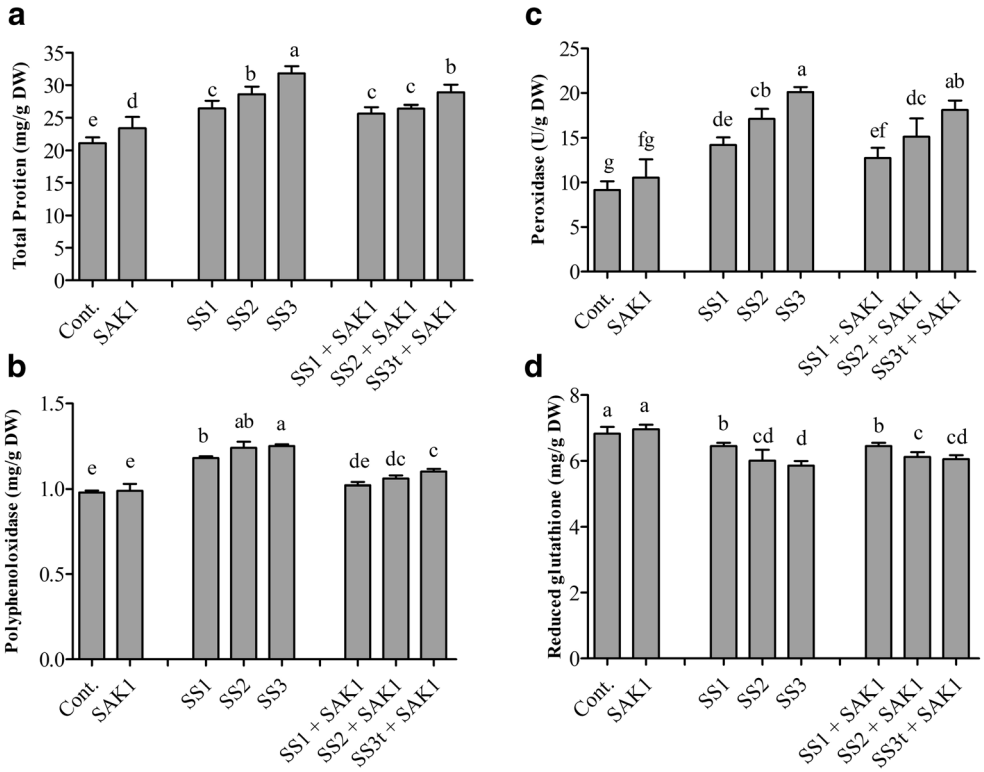

Fig. 5 Assessment of peroxidase, polyphenol oxidase, and total proteins in soybean. Effect of SAK1 on **a** total protein content, **b** polyphenol oxidase (PPO), **c** peroxidase, and **d** reduced glutathione activities in plants exposed to high concentrations of NaCl (SS1 = salt stress 1, SS2 = salt stress 2, SS3 = salt stress 3). Data are expressed as the mean of three replicates. Error bars represent standard errors, with different letters indicating statistically significant differences

deaminase enzyme. Phytohormones, e.g., IAA and GAs, have been known growth hormones which enhance shoot length, root length, and number of root tips, leading to improve nutrients uptake and hence improve plant health under stress conditions (Ullah et al. 2013). ABA and JA are common stress response phytohormones which degrade the reactive oxygen species and protect plants from the hazardous effects of stressors. In addition, antioxidants produced by SAK1 are linked with oxidative stress tolerance. Ethylene is a gaseous plant growth regulator which regulates plant homoeostasis and results in reduced root and shoot growth. Bacterial ACC deaminase degrades ACC and reduces the deleterious effect of ethylene, ameliorating plant stress and promoting plant growth (Cheng et al. 2007). Inoculation bacteria like SAK1 producing ACC deaminase induce longer roots which might be helpful in the uptake of relatively more water soil under drought/salinity stress conditions, thus increasing water use efficiency of the plants under salinity (Shaharoona et al. 2006).

PGPB that produce ACC deaminase and phytohormones belong to various bacterial genera, including

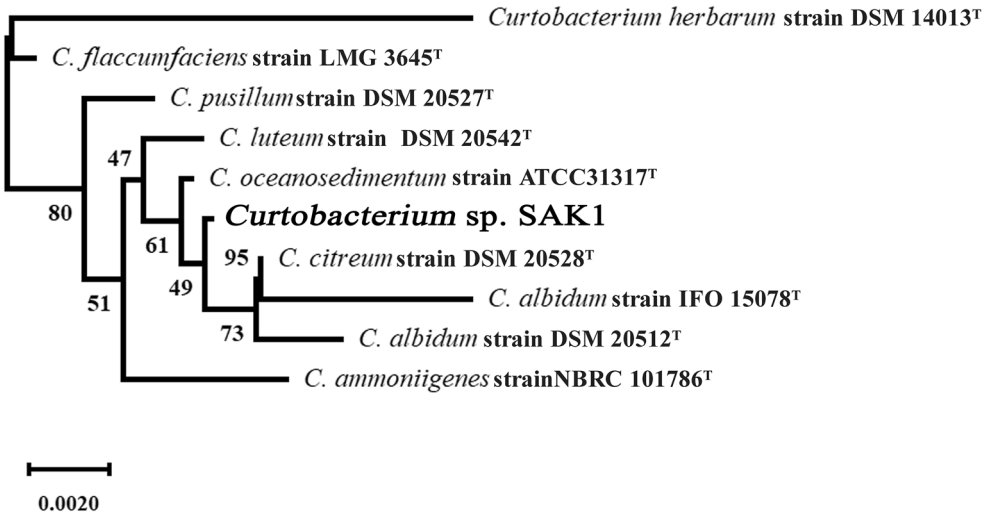

Fig. 6 Phylogenetic tree of SAK1 was constructed 16S rRNA sequences using neighbor joining (NJ) and maximum likelihood methods. Numbers above the branches represent the bootstrap values. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree

Arthrobacter, *Microbacterium*, *Bacillus*, *Burkholderia*, *Rhizobium*, *Klebsiella*, and *Pseudomonas*, that are known to improve crop growth and enhance plant tolerance to various abiotic and biotic stresses (Ali et al. 2018; Ali and Kim 2018; Egamberdieva et al. 2015; Gamalero et al. 2010; Grover et al. 2011; Yue et al. 2007) including salinity. Plant ACC is a main component of ethylene biosynthesis which is a stress hormone produced during stress and damages the plant tissues. The strain SAK1 ACC deaminase degrades ACC and reduces the deleterious effect of ethylene, ameliorating plant stress and promoting plant growth (Sergeeva et al. 2006).

Previous studies reported that salinity stress affects biochemical, morphological, and physiological functions in crop plants (Shrivastava and Kumar 2015).

In this study, NaCl suppressed plant growth, and with increasing salt concentrations, a higher decline in plant growth was observed (Table 2). However, with the help of SAK1, the adverse influence of NaCl stress on plant growth could be alleviated, by increasing biomass and chlorophyll content, compared to control plants (Table 2). This clearly demonstrates that SAK1 alleviates the debilitating effects of salt stress. Irizarry and White (2017) reported that *Curtobacterium oceanosedimentum* produce IAA and enhanced the growth of cotton plant under salinity stress. Similarly, Bianco and Defez (2009, 2010) reported that in saline environment IAA-producing bacteria also increase root and shoot length of chickpea and *Medicago* plants, compare to uninoculated plants.

Abscisic acid (ABA), a phytohormone important for the growth and development of plants, plays a vital role in many stress signaling pathways, and is a notable plant stress marker. Plants adjust ABA levels constantly through stomatal closure, minimizing water loss and activating many stress-responsive genes, in response to changing physiological and environmental conditions (Zhang et al. 2006). Salinity stress leads to an increase in ABA content (Wang et al. 2001). Our findings show that plants inoculated with SAK1 significantly produce lowers endogenous ABA production compared to non-inoculated plants (Fig. 4a). The production of ABA by PGPB improves growth of the plant and mitigates salt stress (Cohen et al. 2008). Suppressive influence of salinity stress on plant germination has been associated with a reduced level of endogenous hormones. Endophytic originated plant growth regulators have been found to have same effects in plant as of exogenous phytohormones (Ullah et al. 2019). In this regard, the SAK1, used in present study, could be a new addition in batch of plant stress hormone-regulating endophytic bacteria (PSHEB).

Salt stress leads to higher levels of ROS such as hydroxyl radicals, hydrogen peroxide, and superoxide and causes cellular toxicity in plants (Hasegawa et al. 2000; Mittova et al. 2004). To counteract adverse effects of ROS and oxidative stress, plants activate their antioxidant defense machinery, such as superoxide dismutase (SOD), POD, catalase (CAT), and glutathione reductase (GR), that protect the plant against cellular stress and scavenge excess ROS (Kim et al. 2005). In the present study, SAK1-inoculated soybean plants showed lower ROS levels, and the antioxidant enzyme activities (TP, PPO, and POD) were considerably reduced compared to those of control soybean plants growing under different salinity levels. Similar results were reported for lettuce, potato, and okra, where PGPB decreased the activities of ROS under increasing salinity stress (Gururani et al. 2013; Habib et al. 2016; Hyo Shim Han 2005).

Glutathione is an important antioxidant that regulates stress responses by reacting with ROS and maintaining an intracellular redox state; it also enhances growth and salt tolerance in plants (Chen et al. 2012; Csiszár et al. 2014). When intracellular GSH concentrations are reduced, plants repeatedly demonstrate a decrease in oxidative stress sensitivity (Grant et al. 1997; Kushnir et al. 1995). In the present study, concentrations of glutathione were found to be slightly higher in SAK1-inoculated soybean plants than in non-inoculated plants. It has been previously reported that glutathione regulates biotic and abiotic stress responses, and plays a pivotal role in the photosynthetic regulation of plants (Diaz-Vivancos et al. 2015; Gill et al. 2013). In SAK1-inoculated soybean plants, increases in glutathione levels were observed compared to those in non-inoculated plants, which indicated a comparatively high potential of ROS scavenging that increased photosynthesis, growth, and shoot biomass. Our findings support the results of Fatma et al. (2014), who also found that an increase in glutathione levels during salt stress could improve growth and photosynthesis in plants.

Together, our results lead to the conclusion that the application of *Curtobacterium* sp. SAK1 greatly mitigates the effects of salt stress and promotes plant growth in *G. max*.

Authors' contributions MAK, SA, SMK, and SA conducted the experiments. ALK and IU helped in writing of the manuscript. IJL designed, supervised, and financed the research. All authors have read and agreed to its content and also that the manuscript conforms to the journal's policies.

Funding This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016R1A6A1A05011910).

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals N/A

Informed consent state N/A

References

Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 57:578–589. https://doi.org/10.1139/w11-044

Ali S, Kim W-C (2018) Plant growth promotion under water: decrease of waterlogging-induced acc and ethylene levels by ACC deaminase-producing bacteria. Front Microbiol 9:1096–1096. https://doi.org/10.3389/fmicb.2018.01096

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–167. https://doi.org/10.1016/j.plaphy.2014.04.003

Ali S, Khan MA, Kim W-C (2018) *Pseudomonas veronii* KJ mitigates flood stress-associated damage in *Sesamum indicum* L. Appl Biol Chem 61:575–585. https://doi.org/10.1007/s13765-018-0392-2

Barnawal D, Bharti N, Tripathi A, Pandey SS, Chanotiya CS, Kalra A (2016) ACC-deaminase-producing endophyte brachybacterium paraconglomeratum strain smr20 ameliorates chlorophytum salinity stress via altering phytohormone generation. J Plant Growth Regul 35:553–564. https://doi.org/10.1007/s00344-015-9560-3

Belimov AA, Dodd IC, Safronova VI, Shaposhnikov AI, Azarova TS, Makarova NM, Davies WJ, Tikhonovich IA (2015) Rhizobacteria that produce auxins and contain 1-amino-cyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). Ann Appl Biol 167:11–25. https://doi.org/10.1111/aab.12203

Bianco C, Defez R (2009) *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. J Exp Bot 60:3097–3107. https://doi.org/10.1093/jxb/erp140

Bianco C, Defez R (2010) Improvement of phosphate solubilization and Medicago plant yield by an indole-3-acetic acid-overproducing strain of *Sinorhizobium meliloti*. Appl Environ Microbiol 76:4626–4632. https://doi.org/10.1128/AEM.02756-09

Bradford MM (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

Chen J-H, Jiang H-W, Hsieh E-J, Chen H-Y, Chien C-T, Hsieh H-L, Lin T-P (2012) Drought and salt stress tolerance of an Arabidopsis glutathione *S*-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. Plant Physiol 158:340–351. https://doi.org/10.1104/pp.111.181875

Cheng Z, Park E, Glick BR (2007) 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53:912–918. https://doi.org/10.1139/W07-050

Cohen AC, Bottini R, Piccoli PN (2008) *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Growth Regul 54:97–103. https://doi.org/10.1007/s10725-007-9232-9

Csiszár J, Horvath E, Vary Z, Galle A, Bela K, Brunner S, Tari I (2014) Glutathione transferase supergene family in tomato: salt stress-regulated expression of representative genes from distinct GST classes in plants primed with salicylic acid. Plant Physiol Biochem 78:15–26. https://doi.org/10.1016/j.plaphy.2014.02.010

Diaz-Vivancos P, de Simone A, Kiddle G, Foyer CH (2015) Glutathione linking cell proliferation to oxidative stress. Free Radic Biol Med 89:1154–1164. https://doi.org/10.1016/j.freeradbiomed.2015.09.023

Egamberdieva D, Jabborova D, Hashem A (2015) *Pseudomonas* induces salinity tolerance in cotton (*Gossypium hirsutum*) and resistance to Fusarium root rot through the modulation of indole-3-acetic acid. Saudi J Biol Sci 22:773–779. https://doi.org/10.1016/j.sjbs.2015.04.019

El-Awady MAM, Hassan MM, Al-Sodany YM (2015) Isolation and characterization of salt tolerant endophytic and rhizospheric plant growth-promoting bacteria (PGPB) associated with the halophyte plant (*Sesuvium verrucosum*) grown in KSA. Int J Appl Sci Biotechnol 3:9. https://doi.org/10.3126/ijasbt.v3i3.13440

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77. https://doi.org/10.1016/0003-9861(59)90090-6

Fatma M, Asgher M, Masood A, Khan NA (2014) Excess sulfur supplementation improves photosynthesis and growth in mustard under salt stress through increased production of glutathione. Environ Exp Bot 107:55–63. https://doi.org/10.1016/j.envexpbot.2014.05.008

Gamalero E, Berta G, Massa N, Glick BR, Lingua G (2010) Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences for the growth of cucumber under salt-stress conditions. J Appl Microbiol 108:236–245. https://doi.org/10.1111/j.1365-2672.2009.04414.x

Gill SS, Anjum NA, Hasanuzzaman M, Gill R, Trivedi DK, Ahmad I, Pereira E, Tuteja N (2013) Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. Plant Physiol Biochem 70:204–212. https://doi.org/10.1016/j.plaphy.2013.05.032

Grant CM, MacIver FH, Dawes IW (1997) Glutathione synthetase is dispensable for growth under both normal and oxidative stress conditions in the yeast *Saccharomyces cerevisiae* due to an accumulation of the dipeptide gamma-glutamylcysteine. Mol Biol Cell 8:1699–1707

Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231–1240. https://doi.org/10.1007/s11274-010-0572-7

Guan R, Qu Y, Guo Y, Yu L, Liu Y, Jiang J, Chen J, Ren Y, Liu G, Tian L, Jin L, Liu Z, Hong H, Chang R, Gilliham M, Qiu L (2014) Salinity tolerance in soybean is modulated by natural variation in GmSALT3. Plant J 80:937–950. https://doi.org/10.1111/tpj.12695

Gururani MA, Upadhyaya CP, Strasser RJ, Yu JW, Park SW (2013) Evaluation of abiotic stress tolerance in transgenic potato plants with reduced expression of PSII manganese stabilizing protein. Plant Sci 198:7–16. https://doi.org/10.1016/j.plantsci.2012.09.014

Habib SH, Kausar H, Saud HM (2016) Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. Biomed Res Int 2016:10. https://doi.org/10.1155/2016/6284547

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463–499. https://doi.org/10.1146/annurev.arplant.51.1.463

Hyo Shim Han KDL (2005) Physiological responses of soybean—inoculation of *Bradyrhizobium japonicum* with PGPR in saline soil conditions research. J Agric Biol Sci 1:216–221

Irizarry I, White JF (2017) Application of bacteria from non-cultivated plants to promote growth, alter root architecture and alleviate salt stress of cotton. J Appl Microbiol 122(4):1110–1120. https://doi.org/10.1111/jam.13414

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

Jalili F, Khavazi K, Pazira E, Nejati A, Rahmani HA, Sadaghiani HR, Miransari M (2009) Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate

salinity stress on canola (*Brassica napus* L.) growth. J Plant Physiol 166:667–674. https://doi.org/10.1016/j.jplph.2008.08.004

Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. Crit Rev Plant Sci 30:435–458. https://doi.org/10.1080/07352689.2011.605739

Kang SM, Khan AL, Hamayun M, Shinwari ZKS, Kim YH, Joo GJ, Lee IJ (2012) *Acinetobacter calcoaceticus* ameliorated plant growth and influenced gibberellins and functional biochemical. Pak J Bot 44: 365–372

Kar M, Mishra D (1976) Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. Plant Physiol 57:315–319

Katznelson H, Bose B (1959) Metabolic activity and phosphate-dissolving capability of bacterial isolates from wheat roots, rhizosphere, and non-rhizosphere soil. Can J Microbiol 5:79–85

Khan AL, Hamayun M, Kang S-M, Kim Y-H, Jung H-Y, Lee J-H, and Lee I-J (2012) Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of Paecilomyces formosus LHL10. BMC Microbiology 12:3. https://doi.org/10.1186/1471-2180-12-3

Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, Al-Khiziri S, Ullah I, Ali L, Jung HY, Lee IJ (2014) Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. J Microbiol 52:689–695. https://doi.org/10.1007/s12275-014-4002-7

Khan AL, Al-Harrasi A, Al-Rawahi A, Al-Farsi Z, Al-Mamari A, Waqas M, Asaf S, Elyassi A, Mabood F, Shin JH, Lee IJ (2016) Endophytic fungi from frankincense tree improves host growth and produces extracellular enzymes and indole acetic acid. PLoS One 11: e0158207. https://doi.org/10.1371/journal.pone.0158207

Khan MA, Ullah I, Waqas M, Hamayun M, Khan AL, Asaf S, Kang SM, Kim KM, Jan R, Lee IJ (2018) Halo-tolerant rhizospheric *Arthrobacter woluwensis* AK1 mitigates salt stress and induces physio-hormonal changes and expression of GmST1 and GmLAX3 in soybean. Symbiosis. https://doi.org/10.1007/s13199-018-0562-3

Kim SY, Lim JH, Park MR, Kim YJ, Park TI, Seo YW, Choi KG, Yun SJ (2005) Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. J Biochem Mol Biol 38:218–224

Kushnir S, Babiychuk E, Kampfenkel K, Belles-Boix E, Van Montagu M, Inze D (1995) Characterization of *Arabidopsis thaliana* cDNAs that render yeasts tolerant toward the thiol-oxidizing drug diamide. Proc Natl Acad Sci U S A 92:10580–10584

Li Z, Chang S, Ye S, Chen M, Lin L, Li Y, Li S, An Q (2015) Differentiation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase from its homologs is the key for identifying bacteria containing ACC deaminase. FEMS Microbiol Ecol 91. https://doi.org/10.1093/femsec/fiv112

Maxton A, Singh P, Masih SA (2018) ACC deaminase-producing bacteria mediated drought and salt tolerance in *Capsicum annuum*. J Plant Nutr 41:574–583. https://doi.org/10.1080/01904167.2017.1392574

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572. https://doi.org/10.1016/j.plaphy.2004.05.009

McCloud ES, Baldwin IT (1997) Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. Planta 203:430–435. https://doi.org/10.1007/s004250050210

Mittova V, Guy M, Tal M, Volokita M (2004) Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. J Exp Bot 55:1105–1113. https://doi.org/10.1093/jxb/erh113

Palaniyandi SA, Damodharan K, Yang SH, Suh JW (2014) *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of 'Micro Tom' tomato plants. J Appl Microbiol 117:766–773. https://doi.org/10.1111/jam.12563

Papiernik SK, Grieve CM, Lesch SM, Yates SR (2005) Effects of salinity, imazethapyr, and chlorimuron application on soybean growth and yield. Commun Soil Sci Plant Anal 36:951–967. https://doi.org/10.1081/CSS-200050280

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801. https://doi.org/10.1128/aem.68.8.3795-3801.2002

Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase containing plant growth promoting rhizobacteria. Physiol Plant 118(1):10–15. https://doi.org/10.1034/j.1399-3054.2003.00086.x

Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E, Nandagopal S (2004) Water resources: agricultural and environmental issues. BioScience 54:909–918. https://doi.org/10.1641/0006-3568(2004)054[0909:WRAAEI]2.0.CO;2

Pitman MG, Lauchli A (2002) Global impact of salinity and agricultural ecosystems. Salinity: environment–plants–molecules. pp 3–20. https://doi.org/10.1007/0-306-48155-3_1

Qi Q, Rose PA, Abrams GD, Taylor DC, Abrams SR, Cutler AJ (1998) (+)-Abscisic acid metabolism, 3-ketoacyl-coenzyme A synthase gene expression, and very-long-chain monounsaturated fatty acid biosynthesis in *Brassica napus* embryos. Plant Physiol 117:979–987

Sambrook JF, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, New York, p 19–20

Saravanakumar D, Samiyappan R (2007) ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. J Appl Microbiol 102:1283–1292. https://doi.org/10.1111/j.1365-2672.2006.03179.x

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47–56

Sergeeva E, Shah S, Glick BR (2006) Growth of transgenic canola (*Brassica napus* cv. Westar) expressing a bacterial1-aminocyclopropane-1-carboxylate (ACC) deaminase gene on high concentrations of salt. World J Microbiol Biotechnol 22:277–282. https://doi.org/10.1007/s11274-005-9032-1

Sgroy V, Cassan F, Masciarelli O, Del Papa MF, Lagares A, Luna V (2009) Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. Appl Microbiol Biotechnol 85:371–381. https://doi.org/10.1007/s00253-009-2116-3

Shaharoona B, Bibi R, Arshad M, Ahmed Z, Zahir Z, Hassan Z-U (2006) 1-Aminocylopropane-1-carboxylate (acc)- deaminase rhizobacteria extenuates acc-induced classical triple response in etiolated pea seedlings. Pak J Bot 38:1491–1499

Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123–131. https://doi.org/10.1016/j.sjbs.2014.12.001

Sziderics AH, Rasche F, Trognitz F, Sessitsch A, Wilhelm E (2007) Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.). Can J Microbiol 53:1195–1202. https://doi.org/10.1139/w07-082

Szymanska S, Plociniczak T, Piotrowska-Seget Z, Hrynkiewicz K (2016) Endophytic and rhizosphere bacteria associated with the roots of the halophyte *Salicornia europaea* L. community structure and metabolic potential. Microbiol Res 192:37–51. https://doi.org/10.1016/j.micres.2016.05.012

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197

Ullah I, Khan AR, Park G-S, Lim J-H, Waqas M, Lee I-J, Shin J-H (2013) Analysis of phytohormones and phosphate solubilization in

Photorhabdus spp. Food Sci Biotechnol 22:25–31. https://doi.org/10.1007/s10068-013-0044-6

Ullah I, Al-Johny BO, Al-Ghamdi KMS, Al-Zahrani HAA, Anwar Y, Firoza A, Al-Kenani N, Almatrya MAA (2019) Endophytic bacteria isolated from *Solanum nigrum* L., alleviate cadmium (Cd) stress response by their antioxidant potentials, including SOD synthesis by *sodA* gene. Ecotoxicol Environ Saf 174:197–207. https://doi.org/10.1016/j.ecoenv.2019.02.074

Wang Y, Mopper S, Hasenstein KH (2001) Effects of salinity on endogenous ABA, IAA, JA, and SA in *Iris hexagona*. J Chem Ecol 27:327–342. https://doi.org/10.1023/a:1005632506230

Yaish MW, Antony I, Glick BR (2015) Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their potential role in salinity tolerance. Antonie Van Leeuwenhoek 107:1519–1532. https://doi.org/10.1007/s10482-015-0445-z

Yue H, Mo W, Li C, Zheng Y, Li H (2007) The salt stress relief and growth promotion effect of Rs-5 on cotton. Plant Soil 297:139–145. https://doi.org/10.1007/s11104-007-9327-0

Zhang J, Klueva NY, Wang Z, Wu R, Ho T-HD, Nguyen HT (2000) Genetic engineering for abiotic stress resistance in crop plants in vitro cellular & developmental biology. Plant 36:108–114. https://doi.org/10.1007/s11627-000-0022-6

Zhang J, Jia W, Yang J, Ismail AM (2006) Role of ABA in integrating plant responses to drought and salt stresses. Field Crop Res 97:111–119. https://doi.org/10.1016/j.fcr.2005.08.018

Zhao S, Zhou N, Zhao ZY, Zhang K, Wu GH, Tian CY (2016) Isolation of endophytic plant growth-promoting bacteria associated with the halophyte *Salicornia europaea* and evaluation of their promoting activity under salt stress. Curr Microbiol 73:574–581. https://doi.org/10.1007/s00284-016-1096-7