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REGULAR ARTICLE

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

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Abstract
Background and aims Many plant growth-promoting endophytes (PGPE) possessing 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity can reduce the level of stress ethylene and assist their host plants cope with various biotic and abiotic stresses. However, information about the endophytic bacteria colonizing in the coastal halophytes is still very scarce. This study aims at isolating efficient ACC deaminase-producing plant growth-promoting (PGP) bacterial strains from the inner tissues of a traditional Chinese folk medicine *Limonium sinense* (Girard) Kuntze, a halophyte which has high economic and medicinal values grown in the coastal saline soils. Their PGP activity and effects on host seed germination and seedling growth under salinity stress were also evaluated.
Methods A total of 126 isolates were obtained from the surface sterilized roots, stems and leaves of *L. sinense* (Girard) Kuntze. They were initially selected for their ability to produce ACC deaminase as well as other PGP properties such as production of indole-3-acetic acid (IAA), N_2-fixation, and phosphate-solubilizing activities and subsequently identified by the 16S rRNA gene sequencing. For selected strains, seed germination, seedling growth, and flavonoids production in axenically growth *L. sinense* (Girard) Kuntze seedlings at different NaCl concentrations (0–500 mM) were quantified.
Results Thirteen isolates possessing ACC deaminase activity were obtained. The 16S rRNA gene sequencing analysis showed them to belong to eight genera: *Bacillus*, *Pseudomonas*, *Klebsiella*, *Serratia*, *Arthrobacter*, *Streptomyces*, *Isoptericola*, and *Microbacterium*. Inoculation with four of the selected ACC deaminase-producing strains not only stimulated the growth of the host plant but also influenced the flavonoids accumulation. All four strains could colonize and can be re-isolated from the host plant interior tissues.
Conclusions These results demonstrate that ACC deaminase-producing habitat-adapted symbiotic bacteria isolated from halophyte could enhance plant growth under saline stress conditions and the PGPE strains could be appropriate as bioinoculants to enhance soil fertility and protect the plants against salt stress.

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Introduction

Saline soils and saline irrigation constitute a serious problem in agriculture suppressing plant growth and yield worldwide (Hu and Schmidhalter 2005; Karthikeyan et al. 2012). Increased salinization of arable land is expected to have devastating global effects, resulting in 30 % land loss within the next 25 years and up to 50 % by the middle of twenty-first century (Wang et al. 2003). At present, strategies for alleviation of salt stress involve developing salt-resistant cultivars, leaching excess soluble salts from upper to lower soil depths, flushing soils that contain soil crusts at the surface, reducing salt by harvesting salt-accumulating aerial plant parts in areas with negligible irrigation water or rainfall for leaching, and amelioration of saline soils under cropping and leaching (Bacilio et al. 2004; Ramadoss et al. 2013). Halophytes are adapted to saline soils and occur naturally in different salt environments. Understanding the salt-tolerance mechanisms of halophytes and subsequently developing salinity-tolerant crops and plants are now essential for solving the current problem of crop yield reduction. Although there are numerous reports on the physiological and molecular mechanisms of how plants respond to salt stress, the mechanism of salinity tolerance is a very complex phenomenon and the nature remains unresolved (Munns and Tester 2008; Türkan and Demiral 2009). It is noteworthy that most studies fail to consider the fact that all plants in natural ecosystems are thought to be symbiotic with endophytes and these habitat-adapted symbiotic endophytes can have profound effects on host plant stress tolerance and fitness (Rodriguez et al. 2008; Redman et al. 2011).

Plant growth promoting endophytic (PGPE) bacteria are a group of microorganisms associated symbiotically with the terrestrial plants and are known for their beneficial effects on growth and health of the host (Qin et al. 2011b; Reinhold-Hurek and Hurek 2011; Grönemeyer et al. 2012). The known mechanisms used by PGPE can be either direct or indirect, similar to the rhizosphere bacterium, such as nitrogen fixation for plant use, ammonia production, solubilization of mineral phosphate, sequestration of iron for plants by siderophores, production of plant hormones like auxins, cytokinins and gibberellins, and biological control of plant pathogens and deleterious microbes through the production of antibiotics and cell wall degrading enzymes (Lucy et al. 2004; Patel et al. 2012). In addition to these commonly studied mechanisms of plant growth promotion, many PGPE have also been shown to stimulate plant growth through the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which reduces plant ethylene level under unfavorable conditions and confers resistance to stress, by catalyzing the conversion of ACC into ammonia and α-ketobutyrate (Glick et al. 1998). Recently, the use of ACC deaminase possessing endophytes has become a promising alternative for plant growth promotion and to alleviate plant stress caused by salinity (Sun et al. 2009; Khan et al. 2011a; Jha et al. 2011; Karthikeyan et al. 2012; Jha et al. 2012; Rashid et al. 2012). Therefore, production of ACC deaminase is likely an important and efficient way for endophytes to manipulate their plant hosts. However, little is known at present about the bacterial community associated with coastal halophytes, and it is not known whether the native habitat-adapted symbiotic bacteria could be, at least potentially, strong candidates in promoting halophytes growth and contribute to their host salt-tolerance ability.

Halophyte *Limonium sinense* (Girard) Kuntze, the "pioneering plant" in salina land, is a traditional Chinese folk medicine used for the treatment of fever, hemorrhage, hepatitis, and other disorders and mainly distributed along coastal salts marshes in southern China. Recently, *L. sinense* extract was shown to possess anti-virus, antitumor, and hepatoprotective activities, and the major constituents in its leaves and roots were flavonoids (Lin and Chou 2000; Tang et al. 2012). This herb has been regarded as a commercially important and rare endemic perennial herb in Chinese medicine and now being excessive exploitation and the wild resource is critically decreased. Therefore, it is necessary to rescue *L. sinense* and boost its production to meet the human health demands. Thus, a better understanding of the presence of the plant growth promoting endophytic bacteria in *L. sinense* is important. These bacteria may have been adapted to the salt stress condition and could provide significant benefit to the host plants. We may speculate that habitat-adapted symbiotic ACC deaminase-producing PGP bacteria from the halophyte *L. sinense* could be

beneficial for mitigating the salt stress to the plants growing in such salt-affected habitat.

In order to test this hypotheses, the present study focuses on the isolation of putative endophytic bacteria associated with halophyte *L. sinense* collected from coastal salts marshes in Jiangsu Province, east China, and evaluation the plant growth promoting activities using the efficient ACC deaminase-producing isolates under salinity stress. Furthermore, pertaining effects of such stressful situation on the total flavonoids biosynthesis in *L. sinense* were also investigated in plants with and without bacteria inoculation.

Materials and methods

Sampling and isolation of plant-associated bacteria

Healthy plant samples of a coastal halophytic medicinal plant, *L. sinense* (Girard) Kuntze, collected from the coastal region in Jiangsu, east of China (119°26′37.30″ E, 34°40′27.38″N), were used as sources for the isolation of plant-associated bacteria. The samples were firstly surface sterilized using previously described procedures (Qin et al. 2009). The sterilized tissues were also imprinted onto nutrient agar and ISP 2 agar plates (medium see below), incubated at 28 °C for 2 weeks to ensure the effectiveness of surface sterilization. Subsequently, the surface sterilized samples were aseptically crumbled into smaller fragments using a commercial blender and then macerated using a sterile mortar and pestle with sterile distilled water. Then, 100 μl of the tissue extracts and the serial dilutions (10^{-1} to 10^{-3}) were plated onto six different isolation media: nutrient agar (peptone 5 g, beef extract 2 g, agar 18 g, 1 L distilled water, pH 5.0), King's B medium (peptone 20 g, K_2HPO_4 1.5 g, $MgSO_4$-$7H_2O$ 1.5 g, glycerol 20 g, and agar 15 g, pH 7.0), R2A medium (yeast extract 0.5 g, tryptone 0.25 g, peptone 0.25 g, starch 0.5 g, pyruvic acid sodium 0.3 g, K_2HPO_4 0.3 g, $MgSO_4$-$7H_2O$ 0.05 g, agar 15 g, pH 7.0), TWYE medium (Coombs and Franco 2003), trehalose-proline medium (trehalose 5.0 g, proline1.0 g, $(NH_4)_2SO_4$ 1.0 g, NaCl 1.0 g, $CaCl_2$ 2.0 g, K_2HPO_4 1.0 g, $MgSO_4$·$7H_2O$ 1.0 g, agar 15.0 g, pH 7.2) and ISP 5 agar medium (Shirling and Gottlieb 1966). All the plates were supplemented with 3 % NaCl and incubated at 28 °C for 2–4 weeks. A representative of each colony as evident from their colony morphology was picked and transferred to fresh nutrient agar medium or ISP 2 (yeast 4 g, glucose 4 g, malt 5 g, agar 15.0 g, pH 7.2) agar plates to establish pure cultures of bacteria and actinomycetes.

Screening for plant growth promoting activities

ACC deaminase activity assay

To determine the presence of ACC deaminase, the ability of the isolates to use ACC as nitrogen source was checked by growing them onto DF salts minimal agar medium supplemented with 3 mM ACC instead of $(NH4)_2SO_4$ as nitrogen source. The ACC deaminase activity of cell-free extracts was determined by estimating the amount of α-ketobutyrate (α-KB) generated by the enzymatic hydrolysis of ACC (Vessey 2003) and the amount of α-ketobutyrate produced was determined by comparing the absorbance at 540 nm of a sample to a standard curve of α-ketobutyrate ranging between 0.1 and 1.0 nmol. For the detailed quantitative determination of ACC deaminase activity, the protocol described by El-Tarabily (2008a) was carried out. After determining the amount of protein and α-KB, the enzyme activity was expressed as micromoles of α-KB per milligram of protein per hour of the active isolates.

Determination of other plant growth-promoting traits

IAA production was examined using the colorimetric method described by Sheng et al. (2008). The isolates were grown in test tubes containing 3 mL of sucrose-minimal salts medium (sucrose 1 %, $(NH_4)_2SO_4$ 0.1 %, K_2HPO_4 0.2 %, $MgSO_4$ 0.05 %, yeast extract 0.05 %, $CaCO_3$ 0.05 %, NaCl 3.0 %, pH 7.2) supplemented with 0.5 mg mL^{-1} of tryptophan. Log-phase cultures grown at 28 °C were used. The IAA concentration was determined using a calibration curve of pure IAA as a standard following the linear regression analysis. The *nif*H gene was amplified using the primers PolF (5′-TGC GAY CCS AAR GCB GAC TC-3′) and PolR (5-ATS GCC ATC ATY TCR CCG GA-3′) as described by Poly et al. (2001). N-fixation ability was also detected by observing the growth on N-free semi-solid JNFb medium (Döbereiner et al. 1995). Plates were incubated at 28 °C for 7 days and then strains were re-inoculated in the JNFb N-free semi solid medium, and bacterial growth was observed as qualitative evidence of the atmospheric nitrogen fixation. P-solubilization activity was tested on Pikovaskaya's agar medium containing 2 % tricalcium phosphate. The appearance of clear halo zone around

bacterial colonies after incubation for 3–5 days at 28 °C was observed.

Identification and tolerance to salinity of plant-associated bacteria

The selected PGP strains were identified by determination of the 16S rRNA gene sequences. Genomic DNA extraction and PCR amplification of 16S rRNA gene of the strains were carried out according to the procedures described by Qin et al. (2012). The identification of phylogenetic neighbors and the calculation of pairwise 16S rRNA gene sequence identities were achieved using the EzTaxon-e database (Kim et al. 2012). The phylogenetic relationship between the isolates and closely related strains was investigated using the neighbor-joining (Saitou and Nei 1987) algorithm. Phylogenetic tree was generated using molecular evolutionary genetics analysis (MEGA) software version 5 (Tamura et al. 2011). The stability of the clades in the trees was appraised using a bootstrap value with 1,000 repeats (Felsenstein 1985). The tolerance of isolates to salinity was tested by growing the strains on modified mineral-based nutrient agar plates (peptone 1 g, K_2HPO_4 0.2 g, $MgSO_4{\cdot}7H_2O$ 0.2 g, $CaSO_4$ 0.1 g, agar 15 g, 1 L distilled water) supplemented with increasing concentrations of NaCl (0–30 %, *w*/*v*, at intervals of 1 %) at 28 °C.

Effects of isolates on seed germination under saline stress

The strains possessing ACC deaminase activity were selected to study their effects on seed germination under salt stress. *L. sinense* seeds were surface sterilized with 75 % ethanol for 30 s, then 0.1 % HgCl for 7 min, and finally washed with distilled water. A 0.2-ml aliquot of the final washed water was then inoculated onto ISP 2 agar plates, incubated at 28 °C to check the effectiveness of surface sterilization. Bacteria used in the inoculation were grown in nutrient broth and incubated in the absence of light at 28 °C for 24 h; actinomycetes were grown in ISP 2 broth medium and incubated in the absence of light at 28 °C for 72 h. Then, the endophytes suspensions in sterile distilled water ($1.0–2.0\times10^8$ cfu mL^{-1} for bacteria and 10^6 cfu mL^{-1} for actinomycetes) were used for seed inoculation; control seeds were treated with sterile distilled water only. Seeds were soaked at room temperature for 10 h in the bacterial suspensions (1 mL) and placed onto MS medium (with 0, 100, 200, 250, 300, and 500 mM NaCl). Each plate was put 50 seeds, and each treatment was performed in three replications. The plants were maintained and harvested after 8 days at the two-leaf stage.

Effects of selected strains inoculation on plant growth promotion and total flavonoids production under saline stress

The most efficient four strains screened from the seed germination experiment were selected for continue inoculation. The surface sterilized seeds were inoculated with the endophytic bacterial suspensions and placed onto MS medium (with 0, 100, 200, and 250 mM NaCl). Seeds were soaked in sterile water as control. Each treatment was performed in nine replicas. The plants were grown in a glasshouse at 25 °C and a 16/8 day/night regime. After 12 weeks, plants were removed from the growth container, roots were rinsed with water to remove attached MS media, and growth promoting effects were evaluated by measuring the root length, shoot length, leaf number, and area. The total flavonoids compounds were extracted from the whole plant using 70 % ethanol solution. The total flavonoids content was performed by a modification of the $AlCl_3$ complexation method as described before (Tavares et al. 2010). The contents of total flavonoids were measured and then expressed as milligrams of rutin equivalent (milligrams of rutin per kilogram of dry weight), through the calibration cure of Rutin. The reference substance was bought from Shanghai YuanYe Biotechnology Co., Ltd.

Inocula recovery

The inoculated *L. sinense* seedlings after 12 weeks were sterilized and cut into 0.2×0.5 cm small sections, respectively. Then, the samples were plated onto nutrient agar media and incubated for 2–5 days at 28 °C. The colonies were identified for their morphological characteristics and compared with the original inoculated strains.

Statistical analysis

Statistical analysis was conducted by using analysis of variance statistical package for social sciences software 18.0, and means were compared using the least significant difference (LSD) method; $P \leq 0.05$ was considered significant. The results were expressed as mean ± SD.

Results

ACC-deaminase activity of isolated plant-associated bacteria

In the present study, a total of 126 plant-associated bacterial strains were isolated from the inner tissues of *L. sinense*. Out of these, only 13 isolates were able to grow on DF agar medium amended with ACC, indicating that they have the ACC deaminase activities, while the rest isolates grew well only on the medium supplemented with $(NH_4)_2SO_4$. Eleven of them were isolated from leaves, and two strains were obtained from roots. Based on the quantitative assays, 13 isolates showed different level of ACC deaminase activity (Table 1). Highest ACC deaminase activity was exhibited by the isolate KLBMP 4941 (177.22 μmol α-KB/mg Pr·h), followed by strains KLBMP 5180 (147.59 μmol α-KB/mg Pr·h), KLBMP 4905 (133.83 μmol α-KB/mg Pr·h), and KLBMP 4942 (114.38 μmol α-KB/mg Pr·h).

Characterization of other PGP properties of the isolates

All of the ACC deaminase-producing strains were tested for other PGP properties. Biosynthesis of IAA showed differences among strains and was summarized in Table 1. Ten strains could produce IAA and the determined levels ranged from significant 0.15 to 8.24 mg/L. The PCR reaction showed six isolates contained an amplifiable product of the expected size (~360 bp) on agarose gel electrophoresis, and they could also grow on N-free semi-solid JNFb medium, suggesting that they have nitrogen fixation activity. In the phosphate solubilization test, only four strains showed the appearance of well-developed clearing zones after incubation for 5 days. The isolate KLBMP 5045 displayed all the PGP characteristics. The NaCl tolerance of all the isolates was tested, and the results showed that all of them were able to tolerate 7 % NaCl, five of them could tolerate more than 10 % NaCl and one strain KLBMP 5045 was able to tolerate 23 % NaCl. All the 13 strains were selected to study their effects on seed germination and seedling growth under saline stress.

Identification of plant-associated bacteria

After DNA extraction and PCR amplification, phylogenetic analysis was carried out. The 13 ACC deaminase-producing strains were identified by partial and full-length sequencing of the 16S rRNA gene sequences. The percentage of 16S rRNA gene sequence similarities (95.7–100 %) of these isolates to the closest type strains was

Table 1 Plant growth promoting traits of isolated endophytic bacteria associated with *L. sinense*

No. of isolates and origin	Nitrogen fixation	Phosphate solubilization	IAA (mg/L)	ACC[a] μmol α-KB/ (mg Pr·h)	Salt tolerance range %
KLBMP 4905 (leaf)	−	−	–[b]	133.83c±0.48	0–13
KLBMP 4920 (leaf)	−	−	3.26a±0.04[a]	8.65a±0.81	0–8
KLBMP 4941 (leaf)	+	−	0.15a±0.02[a]	177.22 cd±0.19	0–8
KLBMP 4942 (leaf)	+	−	–	114.38d±0.38	0–7
KLBMP 4960 (leaf)	−	+	5.97a±0.05[a]	8.20a±0.39	0–8
KLBMP 4968 (leaf)	−	−	–[b]	9.39a±0.90	0–16
KLBMP 5037 (leaf)	+	−	–[b]	10.32a±0.42	0–10
KLBMP 5045 (leaf)	+	+	–[b]	10.90a±0.41	0–23
KLBMP 5066 (root)	+	−	–	6.62a±0.03	0–7
KLBMP 5084 (leaf)	−	+	8.24a±0.05[a]	8.79a±0.37	0–7
KLBMP 5180 (root)	−	−	–	147.59cd±0.52	0–13
KLBMP 5219 (leaf)	−	+	–[b]	10.62a±0.59	0–8
KLBMP 5229 (root)	+	−	6.08a±0.04[a]	7.45a±0.67	0–9

Values sharing the same letter(s) in column do not differ significantly according to Duncan's multiple range test (P=0.05)

[a] Mean of three replicate observations ± SD (Standard deviation)

[b] Mean of lower than 0.1 mg/L

presented in Table 2. Based on the sequence of the 16S rRNA gene, these strains distributed under eight different genera: *Bacillus* (three isolates), *Pseudomonas* (two isolates), *Klebsiella* (three isolates), *Serratia* (one isolate), *Arthrobacter* (one isolate), *Streptomyces* (one isolate), *Isoptericola* (one isolate), and *Microbacterium* (one isolate).

Effects of PGP strains on the seed germination and seedling growth under saline stress

Only four strains namely KLBMP 4941, KLBMP 4942, KLBMP 5180, and KLBMP 5084 among the thirteen ACC deaminase producing isolates could significantly enhance the seed germination percentage under salt stress condition (Table 3). Generally, seed germination percentage (both inoculated and control groups) was gradually reduced along with the increasing of the concentration of NaCl. At the low NaCl concentration (0, 100, and 200 mM), the greatest effect was found from strain KLBMP 4941, of which the germination percentage enhanced by 16.9, 150, and 81.8 %, respectively. At the moderate NaCl concentration (250 mM), all strains enhanced germination percentage by 25–60.7 % except strain KLBMP 4942. For the high NaCl concentration (300, 500 mM), all the four strains showed excellent seed germination promoting effects, enhanced by at least 100 %. However, at the high NaCl concentration, the seedling growth was inhibited and the growth became slowly. Thus, their roles were further assessed under low and moderate salinity stress (100, 200, and 250 mM).

After 12 weeks of bacteria and host–plant association, the results indicated that plant growth index were similar in bacteria inoculated and non-inoculated seedlings grown without salt stress (Table 4). Generally, the increased salt concentration resulted in a gradual reduction in plant growth especially over 100 mM NaCl (Table 4, Fig. 1). However, bacteria-inoculated *L. sinense* seedlings show significant increases in plant root length, shoot length, leaf number, and area as compared to the non-inoculated control under salinity stress (100, 200, and 250 mM). At 200 mM salt concentration, they significantly ($P \leq 0.05$) increased root length by 79–159 %, shoot length by 50–131 %, and total number of leaves up by 38 % as compared to the control plants. At 250 mM salinity, the percentage increase in root length in PGP strains inoculated plants was 52–127 %, in plant shoot length it was 17–68 %, and in leaf area was 87–702 %, indicating the effect of PGPE in alleviating the effects of salt stress of *L. sinense* seedlings was prominent.

Effects of PGP strains on the host flavonoids production under saline stress

Changes in total flavonoids content in *L. sinense* seedlings were shown as response to both NaCl and PGP

Table 2 Identification of the ACC deaminase producing putative endophytic bacteria by 16S rRNA gene sequences

No. of isolates	Genera	Nearest type strain	Similarity (%) of 16S rRNA gene	Accession number
KLBMP 5180	*Arthrobacter*	*A. soli* (EF660748)	98.4	JN638413
KLBMP 4905	*Bacillus*	*B. megaterium* (D16273)	99.7	JN638417
KLBMP 4941		*B. flexus* (AB021185)	100	JN638419
KLBMP 5066		*B. aerophilus* (AJ831844)	100	JX993775
KLBMP 4920	*Pseudomonas*	*P. brassicacearum* subsp. *brassicacearum* (AF100321)	95.7	JN638415
KLBMP 4968		*P. brassicacearum* subsp. *brassicacearum* (AF100321)	99.7	JN638422
KLBMP 5045	*Klebsiella*	*Klebsiella pneumoniae* subsp. *rhinoscleromatis* (ACZD01000038)	99.5	JN638428
KLBMP 5219		*Klebsiella pneumoniae* subsp. *rhinoscleromatis* (ACZD01000038)	99.8	JN638423
KLBMP 5229		*Klebsiella pneumoniae* subsp. *rhinoscleromatis* (ACZD01000038)	99.8	JN638421
KLBMP 5084	*Streptomyces*	*S. pactum* (AB184398)	100	JX993791
KLBMP 4942	*Isoptericola*	*I. dokdonensis* (DQ387860)	100	JX993798
KLBMP 4960	*Serratia*	*S. rubidaea* (AB004751)	100	JN638418
KLBMP 5037	*Microbacterium*	*M. paraoxydans* (AJ491806)	99.7	JN638426

Table 3 Effects of inoculation of four ACC deaminase producing putative PGPE strains on *L. sinense* seed germination after 8 days

No. of isolates	0 mM NaCl	100 mM NaCl	200 mM NaCl	250 mM NaCl	300 mM NaCl	500 mM NaCl
control	83 %(±0.57)ab	30 %(±1.00)a	33 %(±2.31)a	28 %(±1.53)a	15 %(±1.00)a	12 %(±2.08)a
KLBMP 4942	85 %(±1.73)ab	43 %(±0.58)ab	53 %(±0.57)ab	28 %(±0.58)a	37 %(±2.52)b	28 %(±1.53)ab
KLBMP 5180	77 %(±1.15)a	51 %(±0.58)bc	42 %(±1.52)ab	45 %(±1.00)a	35 %(±1.00)b	25 %(±1.73)ab
KLBMP 5084	78 %(±1.53)a	58 %(±1.16)bc	55 %(±1.52)ab	42 %(±1.53)a	38 %(±2.08)b	27 %(±1.53)ab
KLBMP 4941	97 %(±1.16)b	75 %(±3.61)c	60 %(±2.64)b	35 %(±2.00)a	35 %(±1.00)b	40 %(±0.57)b

Data (%-value means: germination rates of seeds) are represented as average of three replicates. Values sharing the same letter(s) in column do not differ significantly according Duncan's multiple range test (P=0.05)

strains inoculation (Fig. 2). The amount of total flavonoids was enhanced with increasing concentrations of salt. Similarly, when endophytic bacteria were present, an increase in content of flavonoids was also observed regardless of salt concentration. In 100 mM NaCl stress, a significant increase (17.03±0.22, 17.31±0.73, 21.74±0.29, and 24.38±0.25 mg/kg) in total flavonoids content was observed under endophytic association plants compared with control (7.30±0.08 mg/kg). In 200 mM salt stress treatments, plants with bacteria association had 1.8- to 3.6-fold increase in flavonoids quantity compared to non-inoculated control plants. At highest salinity level of 250 mM of NaCl, accumulation of total flavonoids was enhanced up to 2.5- to 3.6-fold in the seedlings inoculated with PGPEs compared to control plant. In total, effect of strains KLBMP 4942, KLBMP 5084, and KLBMP 5180 on accumulation of the total flavonoids was better than strain KLBMP 4941 in every salinity treatment. After 12 weeks growth, all of the four PGP strains were successfully re-isolated from the inner tissues of *L. sinense* seedlings, demonstrating that the four strains could be putative endophytes (Supplementary Fig. 1).

Discussion

Endophytic bacteria have been found within different organs, such as roots, stems, leaves, and seeds of studied plants. In comparison with rhizosphere and phyllosphere bacteria, endophytic bacteria are likely to interact more closely with their host. In these very close plant–endophyte interactions, plants provide nutrients and residency for bacteria, which in exchange can directly or indirectly improve plant growth and health, especially under various biotic and abiotic stresses (Weyens et al. 2009). The understanding of the role of these bacteria on promoting plant growth may allow some practical applications for a more sustainable crops production.

Salinity is one major limiting factor to plant growth and crop productivity (Allakhverdiev et al. 2000). In the coastal regions of eastern China, there are plant species successfully adapted to saline environments and differentiated in the evolutionary strategy for this tolerance. The Chinese folk medicine *L. sinense* is mainly distributed along salts marshes along Chinese seashore, in which the wetland of Jiangsu province is the broadest. This salt-secreting halophyte has various physiological, biochemical, and molecular level mechanisms that allow optimal growth in saline conditions (Chen et al. 2007), and perhaps part of its adaptive success would depend at least on its ability to establish and maintain effective associations with habitat-adapted symbiotic plant growth promoting endophytic bacteria. However, at present, there are no published works about the isolation and characterization of the endophytic bacteria associated with this halophyte in natural saline conditions. Our work is the first report about the isolation and plant growth promoting potential evaluation of bacteria associated with this important and critically decreased halophyte. However, as the unequivocal microscopic proof of endophytic capacities of the isolated strains has not yet been carried out, we refer to the isolates as putative endophytes.

The ability plant growth promoting bacteria (PGPB) in exhibiting various PGP traits such as nitrogen fixation, production of phytohormones, phosphate solubilization, siderophore production, ACC deaminase activity, and resistance to certain pathogens has been well documented by many workers (Forchetti et al. 2007; Karthikeyan et al. 2012). However, studies on endophytic bacteria with PGP potential from medicinal halophytes are still few. Bacteria that have ACC deaminase activity help

Table 4 Effects of four ACC deaminase producing putative PGPE strains on growth characteristics of *L. sinense* exposed to different salt concentration after growth for 12 weeks

Bacterial inoculation	NaCl (mmol l^{-1})	Root length (cm)	% Increase	Shoot length (cm)	% Increase	Number of leaves	% Increase	Leaf area (mm^2)	% Increase
control	0	7.50(±3.74)a	–	8.43(±0.65)a	–	17.00(±1.73)a	–	162.00(±9.00)a	–
control	100	1.80(±0.89)a	–	7.37(±1.73)a	–	16.67(±2.08)a	–	194.00(±15.39)b	–
control	200	2.70(±0.10)a	–	3.27(±0.42)a	–	13.33(±3.79)a	–	21.33(±2.52)a	–
control	250	2.87(±1.56)a	–	3.27(±0.15)a	–	9.33(±2.08)a	–	21.00(±3.61)a	–
KLBMP 4941	0	6.80(±1.00)a	–	9.05(±2.08)a	7	15.33(±1.16)a	–	458.67(±14.84)c	182
KLBMP 4941	100	5.17(±0.91)a	187	8.53(±0.25)a	16	17.00(±2.00)a	2	268.00(±9.17)c	38
KLBMP 4941	200	7.00(±2.52)b	159	6.33(±0.71)bc	94	15.33(±1.53)a	15	134.67(±3.06)b	531
KLBMP 4941	250	5.07(±2.06)a	77	5.50(±0.10)b	68	15.00(±1.00)b	61	132.00(±6.56)c	529
KLBMP 4942	0	6.83(±3.09)a	–	8.57(±1.35)a	2	17.33(±1.53)a	2	273.33(±14.29)b	69
KLBMP 4942	100	5.27(±0.90)a	193	7.00(±1.56)a	–	16.67(±3.06)a	–	127.67(±11.59)a	–
KLBMP 4942	200	7.00(±0.30)b	159	7.53(±0.98)c	131	17.67(±2.08)a	33	367.33(±8.39)e	1622
KLBMP 4942	250	4.40(±0.53)a	53	3.83(±0.45)a	17	14.33(±2.31)b	54	39.33(±13.58)a	87
KLBMP 5084	0	5.50(±1.51)a	–	8.67(±0.45)a	3	19.67(±5.69)a	16	432.33(±19.43)c	167
KLBMP 5084	100	7.27(±4.59)a	304	6.63(±0.60)a	–	17.33(±4.04)a	4	313.33(±16.01)d	62
KLBMP 5084	200	4.83(±1.01)ab	79	4.90(±0.46)b	50	13.33(±2.31)a	–	148.00(±7.94)c	594
KLBMP 5084	250	6.50(±2.70)a	127	5.43(±1.12)b	66	14.67(±2.08)b	57	168.33(±14.22)d	702
KLBMP 5180	0	6.30(±1.90)a	–	10.07(±0.83)a	19	16.33(±2.31)a	–	429.00(±18.36)c	165
KLBMP5180	100	5.43(±0.76)a	202	9.07(±3.43)a	23	20.00(±5.19)a	20	435.67(±6.51)e	125
KLBMP5180	200	5.33(±1.06)ab	98	6.77(±1.40)bc	107	18.33(±5.03)a	38	257.33(±10.60)d	1106
KLBMP5180	250	4.37(±0.42)a	52	5.30(±0.99)b	62	15.67(±1.53)b	68	102.00(±11.79)b	586

The letters after each number represent the results of statistical analysis. The same letter indicates that no significant difference was observed at P=0.05. Values in the bracket refer to mean ± SD. Distilled water was used as control

Fig. 1 ACC deaminase-producing bacteria inoculation effect on shoot and root growth of 12 weeks old *L. sinense* seedlings exposed to different salt concentration (100, 200, and 250 mM) under gnotobiotic conditions. **a** Without NaCl, **b** 100 mM NaCl, **c** 200 mM NaCl, **d** 250 mM NaCl

plants to withstand stress (biotic and abiotic) by reducing the level of stress ethylene. Previous studies also showed that organisms with a level of ACC deaminase activity of 20 nmol α-KB/mg Pr·h or higher is sufficient to promote host plant growth (Penrose and Glick 2003). In this study, we tested thirteen ACC deaminase possessing (6.62–177.22 μmol α-KB/mg Pr·h) putative endophytic bacteria for their growth-promoting activity under salt stress. Our study revealed that seed treatment with four ACC deaminase-possessing PGPE strains improved seed germination and vigor over the non-inoculated seeds of *L. sinense* under salt stress. In total, the PGP effects are consistent with the ACC deaminase activity level. Observing the ACC deaminase activity, the four most promising strains (KLBMP 4941, KLBMP 4942, KLBMP 5180, and KLBMP 5084) nearly displayed highest ACC deaminase levels except for strain KLBMP 5084. Special case was strain KLBMP 4905, which exhibited higher ACC deaminase activity (133.83 μmol α-KB/mg Pr·h) but did not promote the seed germination under salt stress. However, we still need to construct the ACC deaminase-deficient mutants to test the ability of the wild-type strains, and to prove that the growth-promotion activity was solely attributable to the ACC deaminase (Sun et al. 2009). The 13 strains were also screened for their tolerance levels of NaCl. All the strains could tolerate at least 7 % (*w*/*v*) NaCl concentrations on modified mineral-based NA plates. Tolerance of putative endophytic bacterial strains to high salinity levels in the present study was probably because of the naturalization in the endophytic saline habitats. The results of our current study confirmed the findings of earlier works performed by other researchers who demonstrated the increased resistance to various stress (Mayak et al. 2004; Shaharoona et al. 2007; Cheng et al. 2007; Zhang et al. 2011; Ahmad et al. 2011; Siddikee et al. 2011) in plants treated with ACC deaminase containing PGPB. Similar improvement of seed germination has been reported with other halophytes. Jha et al. (2012) reported that

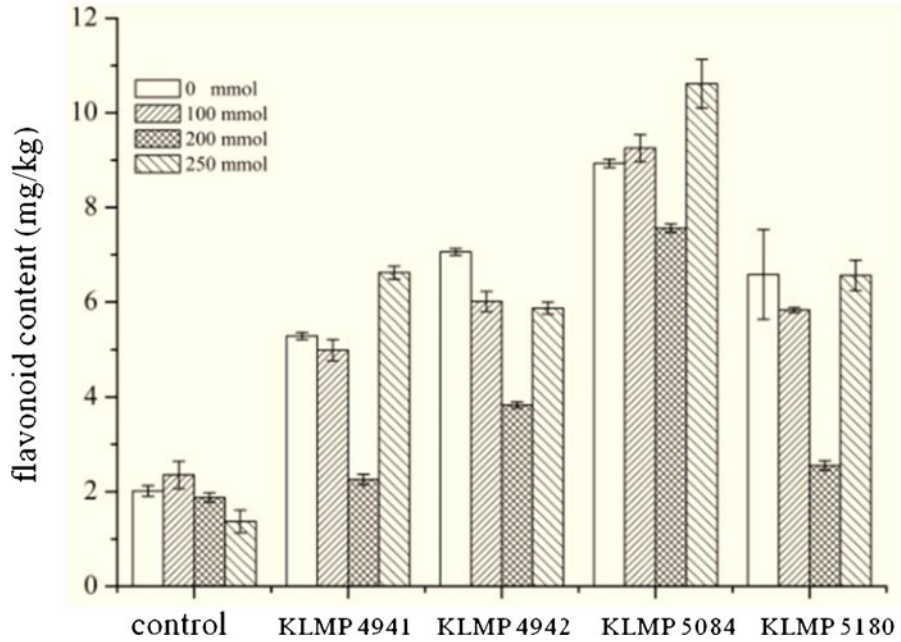

Fig. 2 Total flavonoids profile of whole plant *L. sinense* treated with salt stress under putative endophytic interaction (control means no endophytes inoculation). *Bars* represent means plus standard error

different halotolerant diazotrophic bacteria from the halophyte *Salicornia brachiata* were able to withstand high salt concentration and were able to facilitate plant growth promotion in the presence of growth inhibitory levels of salt. Our present study agrees with previous findings that halophytes are useful sources of halotolerant bacteria with plant growth-promoting potential. The results presented here also support the hypothesis that PGPE can contribute to the salt habitats adaptation of halophytes.

The ACC deaminase activity isolates were also screened multiple plant growth promoting traits, including IAA production, phosphate solubilization, and nitrogen fixation. Results summarized in Table 1 showed that some of them exhibited capacity to grow in nitrogen-free conditions and produce IAA. Maybe the applied *nif*H PCR reaction using the primers PolF and PolR was not suitable for all the *nif*H-sequences in the present study. Some isolates may still be characterized as nitrogen fixers, if alternative *nif*H-primers systems would have been applied. Thus, acetylene reduction is still quite valid as general method and recommended to test the nitrogen fixation ability of the isolates. Notably, the three most promising strains (KLBMP 4941, KLBMP 4942, and KLBMP 5180) had no or very low similar levels of IAA but had significant different levels of ACC deaminase (Table 1), suggesting that the ability of the three strains to promote plant growth under salt condition may be related to their capacity to produce ACC deaminase. One of the most promising strain KLBMP 5084 exhibited lower ACC deaminase activity (only 8.79 μmol α-KB/mg Pr·h) but produced maximum 8.24 mg/L IAA. Since IAA secreted by bacteria may promote root growth directly, by stimulating cell elongation or cell division (Pattern and Glick 2002), the observed positive effect of strain KLBMP 5084 on seed germination and seedling growth could be due to this mechanism. This result was consistent with a salt tolerant isolate of *Azospirillum brasilense* NH producing high IAA under salt stress, which was suggested as the mechanism to improve salt tolerant growth of wheat plants (Nabti et al. 2010). Obviously, there could be more than one mechanism that PGPE employed for protection against salt stresses.

Phylogenetic analysis of 16S rRNA gene sequence revealed that diverse assemblage of cultivable PGP strains belonging to eight different genera exist as endophytes associated with halophyte *L. sinense* (Table 2). The 16S rRNA gene sequence of isolate KLBMP 4920 showed 95.7 % similarity with the type strain *Pseudonomas brassicacearum* subsp. *brassicacearum*, which has been described as the major root-associated bacteria of *Arabidopsis thaliana* and *Brassica napus* plants and has the ability to suppress plant pathogens by producing

antifungal compounds (Ortet et al. 2011). Thus, it is quite possible that this strain represents a new species of the genus *Pseudonomas*. *Pseudomonas* sp. is widespread bacteria in agricultural soils and has many traits that make them well-matched as plant growth-promoting rhizobacteria (Noori and Saud 2012). The 16S rRNA gene sequences revealed that the four PGPE distributed onto four different genera and corresponded to the bacterial species *Bacillus flexus* and actinobacterial species *Arthrobacter soli*, *Streptomyces pactum*, and *Isoptericola dokdonensis*. The genus *Bacillus* is very abundant and occupies diverse ecological niches. Plant growth promoting strains of *Pseudomonas* and *Bacillus* have been widely studied for enhancement of plant growth (Choudhary and Johri 2008; Hol et al. 2013) and have been isolated from other halophytes (Ozawa et al. 2007; Sgroy et al. 2009; Jha et al. 2012).

Interestingly, three PGPE strains are actinobacteria. Endophytic actinobacteria are of special interest since they possess many properties that could benefit to plant growth and the plant growth promoting actinobacterial endophytes possess similar mechanisms like PGPR (Qin et al. 2011b). In the last decades, the endophytic actinobacterial genera, such as *Streptomyces*, *Micrococcus*, *Arthrobacter*, *Micromonospora*, *Pseudonocardia*, and *Amycolatopsis* have been isolated from various plant species and many of them have positive effects on host plants (Qin et al. 2011a; Li et al. 2012; Rungin et al. 2012). For example, several *Streptomyces* species, such as *Streptomyces rimosus* and *Streptomyces viridis*, have the ability to produce IAA and improve plant growth. Treatment of seeds with auxin-producing strains increased the germination capacity and enhanced an intensive seedling growth (El-Tarabily et al. 2008b; Khamna et al. 2010). Although ACC deaminase have been reported to be produced by many bacteria, scarce study has been made to screen endophytic actinobacteria for their potential to produce ACC deaminase and to enhance plant growth. To the best of our knowledge, present study is the first report to show that endophytic actinobacterial strain belonging to the genus *Isoptericola* associated with a halophyte exhibits the PGP activity and the first report of utilization the ACC deaminase endophytic actinobacteria to enhance the seed germination and seedlings growth during salt stresses.

Flavonoids are ubiquitous plant secondary products with a myriad of developmental and physiological roles in plants and have become hot topic due to various human health benefits (Buer et al. 2010). It is known that the content of flavonoids may depend on many aspects such as environment, cultural practices, biotic and abiotic stresses, and genetic factors (Pandino et al. 2010). In general, flavonoids are a remarkably diverse group of secondary products with a vast array of biological functions, including apparent roles in stress protection (Winkel-Shirley 2002). Current study showed decreased growth but an accumulation of total flavonoids contents in *L. sinense* seedlings when exposed to salt stress without bacteria association. However, plants hosting putative endophytic bacteria exhibited better growth and higher total flavonoids contents in seedlings compared to control plants. A positive correlation was found between flavonoids biosynthesis and increased NaCl concentration in plants treated with putative endophytes. It has also been widely accepted that plant–microorganisms symbiotic association can improve plant growth and also enhance nutritional quality and biochemical contents. The same effects that PGPE association correlated with quantitative changes in isoflavones contents has already been mentioned in other reports on different host plants (Sharifi et al. 2007; Khan et al. 2011b). Our results are also in correlation with the previous studies. Maybe the increased levels of total flavonoids were not only the results of increased biomass (root length, shoot length, leaf number, and area) compared to control plants under salt stress but also of other unknown mechanisms that needed to be further elucidated.

Conclusion

Endophytes represent a new area of research and offers wide range of benefits to host plants. The present study suggests that halophyte *L. sinense* is naturally associated with a variety of putative endophytic bacteria, which exhibited higher tolerance to salinity and displayed different plant growth promoting traits. Our results provide strong evidence that the role of habitat-adapted symbiotic ACC deaminase producing putative endophytic bacteria in the performance of *L. sinense* plants in salt stressful condition consists not only in the improved seed germination and seedlings growth, but also in the improvement host flavonoids production. These effects could be due to the bacterial adaptation to the endophytic saline environment where the bacteria were isolated. However, a defined endophytic localization of the bacteria still needs to be determined by microscopic or other molecular methods. Taking into

account the fact that the wild Chinese folk medicine *L. sinense* is critically decreased, from our findings arise the possibility of using this biotechnological approach to obtain habitat-adapted symbiotic PGP bacteria to improve the quality and productivity of *L. sinense* and other important plants in saline soil. However, the favorable role of these putative PGPE still needs to be investigated under field condition.

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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(7):578–589

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N (2000) Ionic and osmotic effects of NaCl-induced inactivation of Photosystems I and II in *Synechococcus* sp. Plant Physiol 123:1047–1056

Bacilio M, Rodriguez H, Moreno M, Hernandez JP, Bashan Y (2004) Mitigation of salt stress in wheat seedlings by a gfp-tagged *Azospirillum lipoferum*. Biol Fertil Soils 40:188–193

Buer CS, Imin N, Djordjevic MA (2010) Flavonoids: new roles for old molecules. J Integr Plant Biol 52:98–111

Chen SH, Guo SL, Wang ZL, Zhao JQ, Zhao YX, Zhang H (2007) Expressed sequence tags from the halophyte *Limonium sinense*. DNA Seq 8(1):61–67

Cheng Z, Park E, Glick BR (2007) 1-Aminocyclopropane-1-carboxylate (ACC) deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53(7):912–918

Choudhary DK, Johri BN (2008) Interactions of *Bacillus* spp. and plants-with special reference to induced systemic resistance (ISR). Microbiol Res 164:493–513

Coombs JT, Franco CMM (2003) Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603–5608

Döbereiner HG, Evans E, Seifert U, Wortis M (1995) Spinodal fluctuations of budding vesicles. Phys Rev Lett 75:3360–3363

El-Tarabily KA (2008) Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. Plant Soil 308:161–174

El-Tarabily KA, Nasser AH, Hardy GESJ, Sivaithamparam K (2008) Plant growth promotion and biological control of *Pythium aphanidermatum*, a pathogen of cucumber, by endophytic actinomycetes. J App Microbiol 106:13–26

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–789

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G (2007) Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. Appl Microbiol Biotechnol 76:1145–1152

Glick BR, Penrose DM, Li JP (1998) A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. J Theor Biol 190(1):63–68

Grönemeyer JL, Burbano CS, Hurek T, Reinhold-Hurek B (2012) Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia. Plant Soil 356:67–82

Hol WH, Bezemer TM, Biere A (2013) Getting the ecology into interactions between plants and the plant growth-promoting bacterium *Pseudomonas fluorescens*. Front Plant Sci 4:81. doi:10.3389/fpls.2013.00081

Hu YC, Schmidhalter UM (2005) Drought and salinity: a comparison of their effects on the mineral nutrition of plants. J Plant Nut Soil Sci 168:541–549

Jha Y, Subramanian RB, Patel S (2011) Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. Acta Physiol Plant 33:797–802

Jha B, Gontia I, Hartmann A (2012) The roots of the halophyte *Salicornia brachiata* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. Plant Soil 356:265–277

Karthikeyan B, Joe MM, Islam MR, Sa T (2012) ACC deaminase containing diazotrophic endophytic bacteria ameliorate salt stress in *Catharanthus roseus* through reduced ethylene levels and induction of antioxidative defense systems. Symbiosis 56:77–86

Khamna S, Yokota A, Peberdy JF, Lumyong S (2010) Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. EurAsia J BioSci 4:23–32

Khan AL, Hamayun M, Kim YH, Kang SM, Lee IJ (2011a) Ameliorative symbiosis of endophyte (*Penicillium funiculosum* LHL06) under salt stress elevated plant growth of *Glycine max* L. Plant Physiol Biochem 49(8):852–861

Khan AL, Hamayun M, Kim YH, Kang SM, Lee JH, Lee IJ (2011b) Gibberellins producing endophytic *Aspergillus fumigatus* sp. LH02 influenced endogenous phytohormonal levels, isoflavonoids production and plant growth in salinity stress. Process Biochem 46:440–447

Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721

Li J, Zhao GZ, Varma A, Qin S, Xiong Z, Huang HY, Zhu WY, Zhao LX, Xu LH, Zhang S, Li WJ (2012) An endophytic *Pseudonocardia* species induces the production of artemisinin in *Artemisia annua*. PLoS One 7(12):e51410

Lin LC, Chou CJ (2000) Flavonoids and phenolics from *Limonium sinense*. Planta Med 66:382–383

Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek 86:1–25

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance in tomato to salt stress. Plant Physiol Biochem 42(6):565–572

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681

Nabti E, Sahnoune M, Ghoul M, Fischer D, Hofmann A, Rothballer M, Schmid M, Hartmann A (2010) Restoration of growth of durum wheat (*Triticum durum* var. waha) under saline conditions due to inoculation with the rhizosphere bacterium *Azospirillum brasilensis* NH and extracts of the marine alga *Ulva lactuca*. J Plant Growth Reg 29(1):6–22

Noori MSS, Saud HM (2012) Potential plant growth-promoting activity of *Pseudomonas* sp. isolated from paddy soil in Malaysia as biocontrol agent. J Plant Pathol Microb 3:120

Ortet P, Barakat M, Lalaouna D, Fochesato S, Barbe V, Vacherie B, Santaella C, Heulin T, Achouak W (2011) Complete genome sequence of a beneficial plant root-associated bacterium, *Pseudomonas brassicacearum*. J Bacteriol 193(12):3146

Ozawa T, Wu J, Fujii S (2007) Effect of inoculation with a strain of *Pseudomonas pseudoalcaligenes* isolated from the endorhizosphere of *Salicornia europea* on salt tolerance of the glasswort. Soil Sci Plant Nutr 53:12–16

Pandino G, Courts FL, Lombardo S, Mauromicale G, Williamson G (2010) Caffeoylquinic acids and flavonoids in the immature inflorescence of globe artichoke, wild cardoon, and cultivated cardoon. J Agric Food Chem 58:1026–1031

Patel D, Jha CK, Tank N, Saraf M (2012) Growth enhancement of Chickpea in saline soils using plant growth-promoting rhizobacteria. J Plant Growth Regul 31:53–62

Pattern CL, Glick BR (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801

Penrose DM, Glick BR (2003) Methods for isolating and characterizing ACC deaminase-containing plant growth promoting rhizobacteria. Physiol Plant 118:10–15

Poly F, Monrozier LJ, Bally R (2001) Improvement in the RFLP procedure for studying the diversity of *nif*H genes in communities of nitrogen fixers in soil. Res Microbiol 152:95–103

Qin S, Li J, Chen HH, Zhao GZ, Zhu WY, Jiang CL, Xu LH, Li WJ (2009) Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol 75:6176–6186

Qin S, Xing K, Fei SM, Lin Q, Chen XM, Cao CL, Sun Y, Wang Y, Li WJ, Jiang JH (2011a) *Pseudonocardia sichuanensis* sp. nov., a novel endophytic actinomycete isolated from the root of *Jatropha curcas* L. Antonie Van Leeuwenhoek 99(2):395–401

Qin S, Xing K, Jiang JH, Xu LH, Li WJ (2011b) Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Appl Microbiol Biotechnol 89(3):457–473

Qin S, Chen HH, Zhao GZ, Li J, Zhu WY, Xu LH, Jiang JH, Li WJ (2012) Abundant and diverse endophytic actinobacteria associated with medicinal plant *Maytenus austroyunnanensis* in Xishuangbanna tropical rainforest revealed by culture-dependent and culture-independent methods. Environ Microbiol Rep 4(5):522–531

Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K (2013) Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springerplus 2(1):6. doi:10.1186/2193-1801-2-6

Rashid S, Charles TC, Glick BR (2012) Isolation and characterization of new plant growth-promoting bacterial endophytes. Appl Soil Ecol 61:217–224

Redman RS, Kim YO, Woodward CJ, Greer C, Espino L, Doty SL, Rodriguez RJ (2011) Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. PLoS One 6(7):e14823

Reinhold-Hurek B, Hurek T (2011) Living inside plants: bacterial endophytes. Curr Opin Plant Biol 14(4):435–443

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim YO, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416

Rungin S, Indananda C, Suttiviriya P, Kruasuwan W, Jaemsaeng R, Thamchaipenet A (2012) Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105). Antonie Van Leeuwenhoek 102(3):463–472

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic tree. Mol Biol Evol 4:406–425

Sgroy V, Cassán 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(2):371–381

Shaharoona B, Jamro GM, Zahir ZA, Arshad M, Memon KS (2007) Effectiveness of various *Pseudomonas* spp. and *Burkholderia caryophylli* containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.). J Microbiol Biotechnol 17(8):1300–1307

Sharifi M, Ghorbanli M, Ebrahimzadeh H (2007) Improved growth of salinity-stressed soybean after inoculation with pre-treated endophytic fungi. J Plant Physiol 164:1144–1151

Sheng XF, Xia JJ, Jiang CY, He LY, Qian M (2008) Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. Environ Pollut 156:1164–1170

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Siddikee MA, Glick BR, Chauhan PS, Wj Y, 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 ACC deaminase activity. Plant Physiol Biochem 49:427–434

Sun Y, Cheng Z, Glick BR (2009) The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium *Burkholderia phytofirmans* PsJN. FEMS Microbiol Lett 296(1):131–136

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Tang XH, Yan LF, Gao J, Yang XL, Xu YX, Ge HY, Yang HD (2012) Antitumor and immunomodulatory activity of polysaccharides from the root of *Limonium sinense* Kuntze. Int J Biol Macromol 51(5):1134–1139

Tavares L, Carrilho D, Tyagi M, Barata D, Serra AT, Duarte CMM, Duarte RO, Feliciano RP, Bronze MR, Chicau P, Espirito-Santo MD, Ferreira RB, dos Santos CN (2010) Antioxidant capacity of Macaronesian traditional medicinal plants. Molecules 15:2576–2592

Türkan I, Demiral T (2009) Recent developments in understanding salinity tolerance. Environ Exp Bot 67:2–9

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Wang WX, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14

Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant-endophyte partnerships take the challenge. Curr Opin Biotechnol 20(2):248–254

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effect of stress. Curr Opin Plant Biol 5:218–223

Zhang YF, He LY, Chen ZJ, Wang QY, Qian M, Sheng XF (2011) Characterization of ACC deaminase-producing endophytic bacteria isolated from copper-tolerant plants and their potential in promoting the growth and copper accumulation of *Brassica napus*. Chemosphere 83:57–62