Canadian Science Publishing

Canadian Journal of Microbiology
Revue canadienne de microbiologie

Evaluation of the plant growth promoting abilities of endophytic bacteria from the psammophyte *Ammodendron bifolium*

Journal:	*Canadian Journal of Microbiology*
Manuscript ID	cjm-2017-0529.R2
Manuscript Type:	Article
Date Submitted by the Author:	23-Jan-2018
Complete List of Authors:	Zhu, Yanlei; Shaanxi Normal University, College of Life Sciences; Xinjiang Normal University, College of Life Sciences She, Xiaoping; Shaanxi Normal University, College of Life Sciences
Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	Endophytic bacteria, Plant growth-promoting traits, Seed germination, Ammodendron bifolium

Evaluation of the plant growth promoting abilities of endophytic bacteria from the psammophyte *Ammodendron bifolium*

Running Title: Plant growth promoting abilities of *Ammodendron bifolium* endophytic bacteria

Yanlei Zhu[1,2], Xiaoping She[1*]

1 College of Life Sciences, Shaanxi Normal University, Xi'an 710119, Shaanxi, China

2 College of Life Sciences, Xinjiang Normal University, Urumqi 830054, Xinjiang, China

E-mail addresses: Yanlei Zhu, zhuyanlei1226@163.com; Xiaoping She, shexiaoping550@163.com.

***Corresponding author**

Xiaoping She, College of Life Sciences, Shaanxi Normal University, Xi'an 710119, Shaanxi, China. Telephone number: +86 29 85310266. Fax number: +86 29 8530 3736. E-mail: shexiaoping550@163.com.

Abstract: The objective of this study was to assess the plant growth-promoting abilities of 45 endophytic bacterial isolates from *Ammodendron bifolium* through physiological characteristics detection and endophytic bacteria-plant interaction. Each of these isolates exhibited 1 or more plant growth-promoting traits, but only 11 isolates belonging to the genera *Bacillus*, *Staphylococcus* and *Kocuria* were capable of promoting seed germination and radicle growth. Together with the results of the correlation analysis revealed that the completion of seed germination may not be due to IAA production, phosphate solubilization, pellicle formation, and ACC deaminase, protease and lipase production by endophytic bacteria, but may be closely related with amylase and cellulase production. Further, endophytic bacterial isolates with plant growth-promoting traits may also provide beneficial effects to host plants at different growth stages. Thus, these results are of value for understanding the ecological roles of endophytic bacteria in host plant habitats and can serve as a foundation for further studies of their potential in plant regeneration.

***Keywords*:** *Ammodendron bifolium*, endophytic bacteria, plant growth-promoting traits, seed germination

Introduction

In arid desert regions, all organisms, including plants and bacteria, will inevitably be confronted with harsh environmental conditions, such as high temperature, low soil moisture and nutrient deficiency (Boor 2006; Su et al. 2011; Soussi et al. 2016; Liu et al. 2016). We were currently focusing on what strategies these organisms adopt to alleviate abiotic stresses and survive in this adverse environment. Currently, some researchers believe that microorganisms possess special adaptation mechanisms and are able to create stable associations with plants in desert environments (Bashan and de-Bashan 2010; Soussi et al. 2016). Thus, the identification of associated and endophytic microorganisms of plants is considered a promising approach in the establishment of plants and restoration of eroded desert lands (Bashan et al. 2012).

Endophytic bacteria are a class of microorganisms that colonize the interior tissues of host plants (Hallmann et al. 1997; Kuklinsky-sobral et al. 2004). They can exert many beneficial effects on plant growth by establishing symbiotic relationships with their host (Nair and Padmavathy 2014). Numerous studies have shown that endophytic bacteria promote host plant growth by fixing atmospheric nitrogen (Verma et al. 2001; Hurek et al. 2002; Iniguez et al. 2004), solubilizing inorganic phosphate (Taurian et al. 2010; Andrade et al. 2014), producing siderophores (Chen et al. 2010), and synthesizing phytohormones (Sessitsch et al. 2002; Cavalcante et al. 2007; Luo et al. 2012). Moreover, endophytic bacteria can protect host

plants from environmental stresses, including drought, salinity and chilling stresses (Creus et al. 1998; Saravanakumar et al. 2011; Ding et al. 2011; Rolli et al. 2014; Yaish et al. 2015), thereby helping their host adapt to the environment. Therefore, the evaluation of plant growth promoting abilities of new and beneficial endophytic bacteria is a significant area of research for the improvement of plant health and stress resistance.

Ammodendron bifolium (Pall.) Yakovl. is a rare psammophilic shrub that grows in the Takeermohuer Desert, located in the west of Ili, Xinjiang of China. Because of the narrow distribution of *A. bifolium* and the ecological deterioration of the habitat in which it grows, it has been classified as second-class protected endangered plants in China (Li et al. 2013*a*; Lü et al. 2014*b*). Most importantly, *A. bifolium* plays an important ecological role in this desert area, such as in drought resistance, as a windbreak, and in sand fixation (Li et al. 2013*b*; Lü et al. 2014*a*). However, the adaptive strategies and survival mechanisms of *A. bifolium* in the desert ecosystem remain unclear. The aim of this study was to analyse the plant growth-promoting traits of endophytic bacteria from *A. bifolium* and evaluate their abilities to promote host plant growth. The obtained data will contribute to the exploration of their potential use in *A. bifolium* growth and development and allow for the further study of *A. bifolium* adaptation mechanisms in the desert environment.

Materials and Methods

Bacterial strains and growth conditions

The endophytic bacterial isolates used in the present study were previously isolated from the root and leaf tissues of *A. bifolium* (growing in the Takeermohuer Desert, Xinjiang of China) and stored at – 80 °C in 20% (v/v) glycerol. Total genomic DNA for each isolate was extracted and the 16S rRNA genes were amplified from genomic DNA as described before (Byers et al. 1998). The purified PCR products were sequenced at Beijing Dingguo Changsheng Biotechnology Co. Ltd. (China). The determined sequences were analyzed by NCBI Blast program (E value = 0) and the phylogenetic tree was constructed based on partial 16S rRNA sequences from *A. bifolium* endophytic bacterial isolates and closely related sequences (reference strains) using neighbor-joining algorithms in MEGA5 software (Tamura et al. 2011). In order to simplify the tree structure, the representative isolate was selected based on higher similarity among some isolates. The nucleotide sequences obtained in this study have also been submitted to NCBI GeneBank and assigned the accession numbers KR045813 thru KR045857. The most closely related genus/species and their similarities with the isolates used in this study are listed in Table 1.

To generate bacterial suspensions, all isolates were incubated in nutrient broth medium (NB, 3.0 g beef extract, 10.0 g proteose peptone and 5.0 g NaCl in 1 l of distilled water, pH 7.2) for 18 h at 30 °C with shaking at 150 rpm. Next, the bacterial cells were pelleted by centrifugation (8000 × ***g***, 10 min), and then resuspended in a 0.85% saline solution to a final OD600 of 1.0 (approximately 10^8 CFU ml^{-1}) for subsequent assays.

Indole-acetic acid (IAA) quantification

IAA production was determined by the capacity of the isolates to produce IAA and similar molecules using 0.5 mg ml^{-1} of L-tryptophan in peptone water medium (PW; 10.0 g proteose peptone and 5.0 g NaCl in 1 l of distilled water, pH 7.2) (Glickmann and Dessaux 1995). Briefly, 1 ml of each bacterial cell suspension was transferred into 20 ml of PW medium as described above, and incubated for 24 h at 30 °C. Subsequently, 1 ml of the cell-free extracts was mixed with 2 ml of Salkowski reagent (Gordon and Weber 1951) at room temperature for 20 min, after which the absorbance was determined at 530 nm using pure IAA (Sigma-Aldrich, USA) as a standard.

Measurement of ACC deaminase activity

The ACC deaminase activity of isolates was evaluated according to a previously described method (Penrose and Glick 2003). Briefly, 0.75 ml of a bacterial cell suspension was transferred into 15 ml of NB medium and incubated at 30 °C for 24 h. The accumulated biomass was harvested by centrifugation at 4 °C (10000 × ***g***, 10 min) and washed with DF salts minimal medium described by Dworkin and Foster (1958). Next, the cells were suspended in 7.5 ml of DF salts minimal medium supplemented with 45 µl of 0.5 M ACC solution and incubated at 30 °C for 24 h with constant shaking at 150 rpm. The ACC deaminase activity in cell-free DF salts minimal medium was measured by monitoring the absorbance of α-ketobutyrate (α-KB) at 540 nm. The α-ketobutyrate (Sigma-Aldrich, USA)

was used as a standard, ranging between 0.1 and 1.0 µmol.

Determination of phosphate-solubilizing activity

Phosphate solubilization by the isolates was analysed in NBRIP (National Botanical Research Institute's phosphate growth medium) (Nautiyal 1999). In brief, for each isolate, 1 ml of bacterial cell suspension was inoculated into 20 ml of NBRIP medium with non-inoculated medium used as a control. After 7 days of incubation at 30 °C with constant shaking at 150 rpm, the solubilized phosphorus in the culture supernatant was determined by Murphy and Riley method (Watanabe and Olsen 1965) with the pH of the supernatant measured at the same time.

Detection of growth capacity of the isolates in nitrogen-free medium

The nitrogen fixation ability of the isolates was assessed according to the method of Andrade et al. (2014) with some modifications. Briefly, for each isolate, 5 µl of bacterial cell suspension was inoculated on nitrogen-free NFb semisolid medium (Döbereiner 1995). After seven days of incubation at 28 °C, plates were assessed for the formation of a halo of bacterial growth within the medium as an indicator of pellicle formation, which is a characteristic of free-living diazotrophs.

Qualitative detection of extracellular enzyme activity

Amylase, cellulase, protease, and lipase activities of endophytic bacterial isolates were assayed with starch agar plates (containing 0.2% starch, m/v), CMC agar plates (containing

0.2% carboxymethyl cellulase (CMC) sodium salt, m/v), milk agar plates (containing 1% skim milk, v/v) and peptone agar plates (containing 1% Tween 80, v/v), respectively (Akpan et al 1999; Kasana et al. 2008; Saran et al 2007; Sierra 1957). For each isolate, 2 µl of bacterial cell suspension was spot-inoculated onto each of the 4 different agar plates, which were then incubated at 30 °C for 2 days. The formation of transparent circles around the bacterial colonies indicated a positive reaction.

Seed germination test

A. bifolium seeds were surface-sterilized for 12 min with 75% ethanol and 15 min with a 5% sodium hypochlorite solution and then were rinsed 6 times in sterilized distilled water. The disinfection process was checked based on a previously described method (Kuklinsky-sobral et al. 2004). Subsequently, a small incision were made in the seed coats with a sterile razor blade. The surface-sterilized seeds were soaked in a 0.85% saline solution alone or in different bacterial suspensions for 4 h. Next, 5 replicates of 30 seeds each were placed in petri dishes containing filter paper wetted with sterilized water and incubated at 20 °C in the dark for 15 days. Germination was scored when the radicle had emerged, and the radicle length was also determined and averaged based on all germinated and non-germinated seeds 15 days after imbibition.

Data analysis

Each experiment was repeated at least three times. Data are presented as the mean values ± SE. Analysis of variance (ANOVA) followed by Tukey's test was carried out to identify significant effects between the control and endophytic bacterial treatments using SPSS 19.0 software (IBM company, USA). Differences were considered to be significant at the $P < 0.05$ level. Germination data were arcsine transformed to ensure homogeneity before statistical analysis (Yao et al. 2010), and the assumptions of the statistical tests indicated that data followed a normal distribution.

In addition, the correlation analysis between the growth characters of *A. bifolium* and the plant growth-promoting traits of endophytic bacteria was conducted according to Spearman correlation coefficient in SPSS 19.0 software. Spearman's coefficient does not require any assumptions about the distributions of the variables, but the data will be assigned the rank for categorical variables (Sedgwick 2014; Hauke and Kossowski 2011). Thus, positive results (+) were transformed to 1 and negative results (−) were turned into 0 in this study before correlation analysis.

Results

Analysis of the abilities of isolates to grow in NFb medium

Of the 45 isolates, a total of 15 strains (one third) were able to grow and form a pellicle in the NFb medium (Table 1). Among these isolates, 3 were of the genus *Bacillus*, 4 were of the genus *Pantoea*, 4 were of the genus *Erwinia*, 2 were of the genus *Klebsiella*, and 2 were of

the genus *Pseudomonas* (Table 1). In addition, it can be observed in Fig. 1 that these isolates belonged to *Firmicutes* and γ-*Proteobacteria*, while the isolates belonging to *Actinobacteria* were not able to grow and form a pellicle in the NFb medium.

Inorganic phosphate solubilization

All the isolates evaluated in this study showed distinct phosphate solubilizing abilities, especially between different genera. The isolates showing the significant ability to solubilize calcium phosphate accounted for 44.4% of all isolates, and most of these isolates belonged to γ-*Proteobacteria* phylum. While the majority of isolates (42.2% of all isolates) belonging to *Firmicutes* and *Actinobacteria* phyla did not show obvious phosphate-solubilizing activity (Supplemental Table S1). In term of the level of phosphate solubilization, *Pantoea* sp. BG25 exhibited the highest solubilization ability at the level of 699.16 μg ml^{-1} and low solubilization ability of 68.05 μg ml^{-1} was detected in *Klebsiella* sp. AG48 (Fig. 2). Microorganisms are able to solubilize insoluble phosphates and produce organic acids, leading to a reduction in the pH of the surrounding medium. Our results also showed that the increased phosphate solubilizing capacity by different isolates was accompanied by a decrease of the pH in the NBRIP medium (Fig. 2). Furthermore, there was a significant negative correlation between phosphate solubilizing ability and the pH decline according to Spearman correlation coefficient.

IAA production

Of all the isolates assayed in this study, 53.3% (24 isolates) were able to significantly produce IAA (Supplemental Table S1). All isolates belonging to the genera *Pantoea*, *Kocuria* and *Klebsiella* showed the highest abilities to produce IAA, which ranged from 17.09 to 58.66 μg ml^{-1}. Although *Pseudomonas*, *Sporosarcina*, *Bacillus* and *Erwinia* sp. isolates produced only low amount of IAA ranging from 1.21 to 9.88 μg ml^{-1}, their IAA productivity was significantly more than that of the control (Fig. 3). Among the 24 positive isolates, there were 14 isolates belonging to γ-*Proteobacteria*.

ACC deaminase activity

Of the 45 isolates analysed, 51.1% (23 isolates) showed significantly higher ACC deaminase activity as compared to the control (Supplemental Table S1). Among these isolates, all *Kocuria* sp. isolates were able to produce ACC deaminase activity over a range of 0.19-0.89 μmol mg^{-1} h^{-1}. While *Staphylococcus* sp. AY7, AY8 and AY13 showed the highest ACC deaminase activity at levels of α-KB greater than 1.08 μmol mg^{-1} h^{-1} (Supplemental Table S1 and Fig.4). In addition, some strains belonging to other genera also showed ACC deaminase activity over the range of 0.26-0.83 μmol mg^{-1} h^{-1}, except for *Acinetobacter*, *Sporosarcina*, *Cellulomonas* and *Oceanobacillus* sp. (Supplemental Table S1 and Fig.4).

Extracellular enzyme production

Enzymatic activities of all *A. bifolium* endophytic bacterial isolates presented in Table 1 showed the presence of hydrolytic enzymes, such as amylase, cellulase, protease and lipase.

All isolates of the genera *Bacillus*, *Kocuria*, *Arthrobacter*, *Cellulomonas* and *Paenibacillus* (40.0% of all isolates) showed positive results for amylase and cellulase tests. Only 5 *Bacillus* sp. and 1 *Paenibacillus* sp. (13.3% of all isolates) had the ability to produce protease. With respect to the lipase test, 24 isolates (53.3% of all isolates), including 7 *Bacillus* sp., 4 *Pantoea* sp., 3 *Staphylococcus* sp., 2 *Erwinia* sp., 3 *Acinetobacter* sp., 1 *Pseudomonas* sp., 2 *Sporosarcina* sp., 1 *Oceanobacillus* sp. and 1 *Paenibacillus* sp. were able to produce lipase. It can be seen from Fig. 1 that the amylase and cellulase-producing isolates belonged to *Firmicutes* and *Actinobacteria*, the protease-producing isolates belonged to *Firmicutes*, and the lipase-producing isolates belonged to *Firmicutes* and γ-*Proteobacteria*.

Plant growth promotion

It is well known that seed germination is the most important stage in plant growth and development. The germination test was used to assess the plant growth-promoting effects of the *A. bifolium* endophytic bacteria. As shown in Supplemental Table S1, the isolates showing the ability to significantly promote seed germination and radicle elongation (24.4% of all isolates) were distributed in the genera *Bacillus*, *Staphylococcus* and *Arthrobacter*, and they belonged to *Firmicutes* phylum except one *Arthrobacter* sp. By inoculation with these isolates, the seed germination percentage increased by 15 - 40% compared to the control (13.3%), while the radicle length was also increased by 1.5 or 4 times (Fig. 5).

Furthermore, we analyzed the correlations between the growth characters of *A. bifolium* and the plant growth-promoting traits of endophytic bacteria (Table 2). The results suggested that seed germination and radicle growth may be closely related with amylase and cellulase production by endophytic bacteria, but not with ACC deaminase, protease and lipase production. Conversely, IAA production and phosphate solubilization may have adverse effects on seed germination and radicle growth.

Discussion

In the present study, a total of 45 endophytic isolates belong to γ-*Proteobacteria*, *Firmicutes* and *Actinobacteria* phyla with 13 different genera. Among them, the main bacterial genera were *Bacillus* and *Pantoea*, followed by *Kocuria*. However, the most common endophytic bacteria reported by previous researcher were *Pseudomonas*, *Pantoea*, *Bacillus*, *Rhizobium*, and *Enterobacter* sp. in red clover, carrots, and agricultural crops (Sturz et al. 1997; Surette et al. 2003; Kuklinsky-sobral et al. 2004; Palaniappan et al. 2010; Deng et al. 2011). Moreover, these most common bacteria mainly belong to α-*Proteobacteria*, γ-*Proteobacteria* and *Firmicutes* phyla. So the species composition of endophytic bacterial population from *A. bifolium* differs from the other plants and has its own characteristics.

Nitrogen loss and the limited bioavailability of phosphorus are the main reasons for restricting plant growth and development, especially in arid and semiarid areas (Evans and Belnap 1999; Rodríguez and Fraga 1999; Delgado et al. 2014). Numerous studies have shown

246 that endophytic bacteria are able to promote plant growth by producing a series of nutrients
247 and by facilitating plant nutrient uptake through atmospheric nitrogen fixation and phosphate
248 solubilization (Gothwal et al. 2008; Hariprasad and Niraniana 2009; Yu et al. 2011; Yaish et
249 al. 2015). In this study, some different species from the genus *Bacillus* were observed to have
250 the potential ability to fix nitrogen, but not to solubilize phosphate, in agreement with
251 previous studies (Sgroy et al. 2009; Andrade et al. 2014; Jasim et al. 2014). Furthermore, our
252 results also show that most of strains belonging to γ-*Proteobacteria* were able to both
253 solubilize phosphate and to grow in NFb medium as described by Kuklinsky-sobral et al.
254 (2004), but these isolates were unable to promote seed germination. Together with the results
255 of the correlation analysis imply that the completion of seed germination need not supply
256 nitrogen and phosphorus elements. However, nitrogen-fixing and phosphate-solubilizing
257 endophytic bacteria may exert many beneficial effects at certain stages of the plant life cycle
258 under natural conditions, which may be an important *A. bifolium* research topic in further
259 study.

260 Among the endophytic bacteria-induced growth promoting mechanisms resulting from
261 plant hormones, IAA is the most common and extensively studied phytohormone. Recent
262 studies have shown that more than 40% of all endophytic or rhizospheric bacteria are able to
263 produce IAA (Palaniappan et al. 2010; Li et al. 2012; Andrade et al. 2014; Hussein and Joo
264 2015), which is in agreement with our results (Fig. 2). The phytohormones produced by

endophytic bacteria are known to directly act on plant growth (Lodewyckx et al. 2002). However, endophytic bacteria containing ACC deaminase can indirectly promote plant growth through inhibition of ethylene production by their host plants (Ali et al. 2014). The accelerating effects of ethylene on seed germination have been well documented (Petruzzelli et al. 2000, Ishibashi et al. 2013, Corbineau et al. 2014, Yu et al. 2016), but comparatively little is known about the effects of IAA on seed germination (Liu et al. 2013). Our results do not show that seed germination was influenced through ACC deaminase or IAA production by endophytic bacterial isolates, and no positive correlation between seed germination and ACC deaminase or IAA production was observed. Consequently, the mechanisms by which ACC deaminase or IAA producing-endophytic bacteria from *A. bifolium* act upon plant growth will await further study.

Several researchers believe that endophytic bacteria may also be able to promote plant growth by extracellular hydrolytic enzyme production (Deivanai et al. 2014; Siddikee et al. 2015). Other experts insist that the production of hydrolytic enzymes by endophytes could assist in the plant invasion process, and thus improve plant growth (Verma et al. 2001; Adriano-Anaya et al. 2006; Mostajeran et al. 2007). In angiosperms, the mobilization of polymers, such as proteins and carbohydrates, is crucial for seed germination and successful seedling establishment (Pritchard et al. 2002; Eckstein et al. 2016), and the synthesis of hydrolytic enzymes facilitates the implementation of this process. Our results suggest that *A.*

bifolium seed germination and radicle growth maybe have resulted from extracellular amylase or cellulase production by endophytic bacteria, but not every isolate capable of promoting seed germination have the ability to produce the two enzymes. Thus, Whether or not hydrolytic enzymes mediate *A. bifolium* endophytic bacteria-stimulated seed germination remains unclear.

The results based on seed germination test show that 24.4% of all isolates were found to be plant growth promoting, 60.1% remained plant growth neutral, and only 15.5% inhibited plant growth (Supplemental Table S1). The 3 different effects on plant growth were also found in other plant species under laboratory conditions (Surette et al. 2003). Some researchers found that the effect of a single isolate inoculation on plant growth was quite different from mixed inoculation of two strains (Sturz et al. 1997). Therefore, growth promotion or inhibition by endophytic bacteria seems to depend upon the interaction among internal microorganism population (Sturz and Christie 1995). Through inoculation with growth-promoting bacteria, the seed germination percentage increased by 15 - 40%, and radicle length increased by 1.5 - 4 times (in this study). In the case of the tomato plants, germination and radicle length were found to be 6 - 17% and 0.2 - 0.7 times by paper towel method (Hariprasad and Niranjana 2009). For cacti grown in semi-arid region, its seedlings growth was not significantly increased after endophytic bacterial colonization in artificially prepared soil (Lima et al. 2015). Although *A. bifolium* endophytic bacteria had obvious

growth-promoting effects on seed germination and radicle growth, the effects of these strains on seedling growth and other growth stage still need further investigation.

Conclusions

All isolates analysed in this study had at least 1 plant growth promoting trait, but not every isolate had the ability to promote *A. bifolium* seed germination and radicle elongation. Moreover, the strains capable of promoting seed germination and radicle growth did not present common growth promoting traits. The correlation analysis shows that germimation and radicle growth of *A. bifolium* may be closely related with amylase and cellulase production by endophytic bacteria. In fact, the specific growth promoting mechanisms exerted by endophytic bacteria still remain to be elucidated. Furthermore, endophytic bacterial isolates with plant growth-promoting traits may also provide beneficial effects to host plants at different growth stages. Thus, the results obtained in this study will be helpful for evaluating the ecological roles of endophytic bacteria under natural conditions and can serve as a foundation for further study of their potential in vegetation restoration.

Acknowledgement

The work described here was supported by a grant from the National Natural Science Foundation of China (No. 31260060).

Conflict of Interest Statement

The authors declare that there is no conflict of interest.

References

Adriano-Anaya, M.L., Salvador-Figueroa, M., Ocampo, J.A., and García-Romera, I. 2006. Hydrolytic enzyme activities in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) roots inoculated with *Gluconacetobacter diazotrophicus* and *Glomus intraradices*. Soil Biol. Biochem. **38**: 879–886. doi:10.1016/j.soilbio.2005.08.004.

Akpan, I., Bankole, M.O., and Adesemowo, A.M. 1999. A rapid plate culture method for screening of amylase producing micro-organisms. Biotechnol. Tech. **13**: 411–413. doi:10.1023/A:1008965808641.

Ali, S., Charles, T.C., and Glick, B.R. 2014. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol. Biochem. **80**: 160–167. doi:10.1016/j.plaphy.2014.04.003.

Andrade, L.F., De Souza, G.L.O.D., Nietsche, S., Xavier, A.A., Costa, M.R., Cardoso, A.M.S., et al. 2014. Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth. J. Microbiol. **52**(1): 27–34. doi:10.1007/s12275-014-3019-2.

Bashan, Y., Salazar, B.G., Moreno, M., Lopez, B.R., and Linderman, R.G. 2012. Restoration of eroded soil in the Sonoran Desert with native leguminous trees using plant growth-promoting microorganisms and limited amounts of compost and water. J. Environ. Manage. **102**: 26–36. doi:10.1016/j.jenvman.2011.12.032.

Bashan, Y., and De-bashan, L.E. 2010. Microbial populations of arid lands and their potential for restoration of deserts. *In* Soil biology and agriculture in the tropics. Edited by P. Dion. Springer-Verlag Berlin Heidelberg. pp. 109–137. doi:10.1007/978-3-642-05076-3_6.

Boor, K.J. 2006. Bacterial stress responses: what doesn't kill them can make them stronger. PLoS Biol. **4** (1): 18–20. doi:10.1371/journal.pbio.0040023.

Byers, H.K., Stackebrandt, E., Hayward, C., and Blackall, L.L. 1998. Molecular investigation of a microbial mat associated with the great artesian basin. FEMS Microbiol. Ecol. **25**: 391–403. doi:10.1111/j.1574-6941.1998.tb00491.x.

Cavalcante, J.J.V., Vargas, C., Nogueira, E.M., Vinagre, F., Schwarcz, K., Baldani, J.I., et al. 2007. Members of the ethylene signalling pathway are regulated in sugarcane during the association with nitrogen-fixing endophytic bacteria. J. Exp. Bot. **58**(3): 673–686. doi:10.1093/jxb/erl242.

Chen, L., Luo, S.L., Xiao, X., Guo, H.J., Chen, J.L., Wan, Y., et al. 2010. Application of plant growth-promoting endophytes (PGPE) isolated from *Solanum nigrum* L. for phytoextraction of Cd-polluted soils. Appl. Soil Ecol. **46**: 383–389. doi:10.1016/j.apsoil.2010.10.003.

Corbineau, F., Xia, Q., Bailly, C., and El-Maarouf-Bouteau, H. 2014. Ethylene, a key factor in the regulation of seed dormancy. Front. Plant Sci. **5**: 1–13. doi:10.3389/fpls.2014.00539.

Creus, C.M., Sueldo, R.J., and Barassi, C.A. 1998. Water relations in *Azospirillum*-inoculated wheat seedlings under osmotic stress. Can. J. Bot. **76:** 238–244. doi:10.1139/cjb-76-2-238.

Deivanai, S., Bindusara, A.S., Prabhakaran, G., and Bhore, S.J. 2014. Culturable bacterial endophytes isolated from Mangrove tree (*Rhizophora apiculata* Blume) enhance seedling growth in Rice. J. Nat. Sc. Biol. Med. **5**(2): 437–444. doi:10.4103/0976-9668.136233.

Delgado, M., Mendez, J., Rodríguez-Herrera, R., Aguilar, C.N., Cruz-Hernández, M., and Balagurusamy, N. 2014. Characterization of phosphate-solubilizing bacteria isolated from the arid soils of a semi-desert region of north-east Mexico. Biol. Agric. Hortic. **30**(3): 211–217. doi:10.1080/01448765.2014.909742.

Deng, Z.S., Zhao, L.F., Kong, Z.Y., Yang, W.Q., Lindström, K., Wang, E.T., et al. 2011. Diversity of endophytic bacteria within nodules of the *Sphaerophysa salsula* in different regions of Loess Plateau in China. FEMS Microbiol. Ecol. **76**: 463–475. doi:10.1111/j.1574-6941.2011.01063.x.

Ding, S., Huang, C.L., Sheng, H.M., Song, C.L., Li, Y.B., and An, L.Z. 2011. Effect of inoculation with the endophyte *Clavibacter* sp. strain Enf12 on chilling tolerance in *Chorispora bungeana*. Physiol. plantarum **141**: 141–151. doi:10.1111/j.1399-3054.2010.01428.x.

Döbereiner, J. 1995. Isolation and identification of aerobic nitrogen-fixing bacteria from soil and plants. *In* Methods in Applied Soil Microbiology and Biochemistry. Edited by K. Alef and P. Nannipieri. Academic Press, London, San Diego, New York, Boston, Sydney, Tokyo, Toronto. pp 134–141.

379 Dworkin, M., and Foster, J.W. 1958. Experiments with some microorganisms which utilize
380 ethane and hydrogen. J. Bacteriol. **75**: 592–603. PMID: 13538930
381 Eckstein, A., Jagiello-Flasińska, D., Lewandowska, A., Hermanowicz, P., Appenroth, K-J.,
382 and Gabryś, H. 2016. Mobilization of storage materials during light-induced germination of
383 tomato (*Solanum lycopersicum*) seeds. Plant Physiol. Biochem. **105**: 271–281.
384 doi:10.1016/j.plaphy.2016.05.008.
385 Evans, R.D., and Belnap, J. 1999. Long-term consequences of disturbance on nitrogen
386 dynamics in an arid ecosystem. Ecology **80**(1): 150–160. doi:10.2307/176986.
387 Glickmann, E., and Dessaux, Y. 1995. A critical examination of the specificity of the
388 Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl.
389 Environ. Microbiol. **61**(2): 793–796. PMID: 16534942
390 Gordon, S.A., and Weber, R.P. 1951. Colorimetric estimation of indoleacetic acid. Plant
391 Physiol. **26**(1): 192–195. PMID: 16654351
392 Gothwal, R.K., Nigam, V.K., Mohan, M.K., Sasmal, D., and Ghosh, P. 2008. Screening of
393 nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants.
394 Appl. Ecol. Env. Res. **6**(2): 101–109.
395 Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F., and Kloepper, J.W. 1997. Bacterial
396 endophytes in agricultural crops. Can. J. Microbiol. **43**: 895–914. doi:10.1139/m97-131.

Hariprasad, P., and Niranjana, S.R. 2009. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant and Soil **316**: 13–24. doi:10.1007/s11104-008-9754-6.

Hauke, J., and Kossowski, T. 2011. Comparison of Values of Pearson's and Spearman's Correlation Coefficients on the Same Sets of Data. Quaestiones Geographicae **30**: 87-93. doi:10.2478/v10117-011-0021-1.

Hurek, T., Handley, L.L., Reinhold-Hurek, B., and Piché, Y. 2002. *Azoarcus* grass endophytes contribute fixed nitrogen to the plant in an unculturable state. Mol. Plant-Microbe Interact. **15**: 233–242. doi:10.1094/MPMI.2002.15.3.233.

Hussein, K.A., and Joo, J.H. 2015. Isolation and characterization of rhizomicrobial isolates for phosphate solubilization and indole acetic acid production. J. Korean Soc. Appl. Biol. Chem. **58**: 847–855. doi:10.1007/s13765-015-0114-y.

Iniguez, A.L., Dong, Y., and Triplett, E.W. 2004. Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. Mol. Plant-Microbe Interact. **17**: 1078–1085. doi:10.1094/MPMI.2004.17.10.1078.

Ishibashi, Y., Koda, Y., Zheng, S.H., Yuasa, T., and Iwaya-Inoue, M. 2013. Regulation of soybean seed germination through ethylene production in response to reactive oxygen species. Ann. Bot. **111**: 95–102. doi:10.1093/aob/mcs240.

Jasim, B., Joseph, A.A., John, C.J., Mathew, J., and Radhakrishnan, E.K. 2014. Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of *Zingiber officinale*. 3 Biotech. **4**: 197–204. doi:10.1007/s13205-013-0143-3.

Kasana, R.C., Salwan, R., Dhar, H., Dutt, S., and Gulati, A. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr. Microbiol. **57**: 503–507. doi:10.1007/s00284-008-9276-8.

Kuklinsky-Sobral, J., Araújo, W.L., Mendes, R., Geraldi, I.O., Pizzirani-Kleiner, A.A., and Azevedo, J.L. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ. Microbiol. **6**: 1244–1251. doi:10.1111/j.1462-2920.2004.00658.x.

Li, L., Sinkko, H., Montonen, L., Wei, G.H., Lindström, K., and Räsänen, L.A. 2012. Biogeography of symbiotic and other endophytic bacteria isolated from medicinal *Glycyrrhiza* species in China. FEMS Microbiol. Ecol. **79**: 46–68. doi:10.1111/j.1574-6941.2011.01198.x.

Li, J., Huang, L.P., Lü, H.Y., and Wang, X.A. 2013*a*. Distribution patterns of main populations of *Ammodendron argenteum* Communities in the Takeermohuer Desert. Arid Zone Res. **30**: 634–639.

Li, J., Wang, X.A., Lü, H.Y., and Wang, S.X. 2013*b*. Interspecific relationships of major species in *Ammodendron argenteum* communities of Takeermohuer Desert. Guihaia **33**: 482–

487.

Lima, J.V.L., Weber, O.B., Correia, D., Soares M.A., and Senabio, J.A. 2015. Endophytic bacteria in cacti native to a Brazilian semi-arid region. Plant Soil **389**: 25-33. doi:10.1007/s11104-014-2344-x.

Liu, X.D., Zhang, H., Zhao, Y., Feng, Z.Y., Li, Q., Yang, H.Q., et al. 2013. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated *ABI3* activation in *Arabidopsis*. PNAS **110**(38): 15485–15490. doi:10.1073/pnas.1304651110.

Liu, M.L., Zhu, R.Q., Zhang, Z.S., Liu, L.C., Hui, R., Bao, J.T., et al. 2016. Water use traits and survival mechanisms of psammophytes in arid ecosystems. Arid Land Res. Manage. **30**(2): 166–180. doi:10.1080/15324982.2015.1090498.

Lodewyckx, C., Vangronsveld, J., Porteus, F., Moore, E.R.B., Taghavi, S., Mezgeazy, M., et al. 2002. Endophytic bacteria and their potential applications. Crit. Rev. Plant Sci. **21**(6): 583–606. doi:10.1080/0735-260291044377.

Lü, H.Y., Wang, X.A., Li, J., and Dang, Q.F. 2014*a*. Structure and dynamics of China rare plant *Ammodendron bifolium* (Pall.) Yakovl natural population. Acta Bot. Boreal. **34**: 0177–0183.

Lü, H.Y., Cao, M.H., and Li, J. 2014*b*. Study on the breeding system of the rare plant *Ammodendron argenteum*. Guihaia **34**(6): 763–767.

Luo, S.L., Xu, T.Y., Chen, L., Chen, J.L., Rao, C., Xiao, X., et al. 2012. Endophyte-assisted

promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. SLS18. App. Microbiol. Biotechnol. **93**: 1745–1753. doi:10.1007/s00253-011-3483-0.

Mostajeran, A., Amooaghaie R., and Emtiazi, G. 2007. The participation of the cell wall hydrolytic enzymes in the initial colonization of *Azospirillum brasilense* on wheat roots. Plant Soil **291**: 239–248. doi:10.1007/s11104-006-9189-x.

Nair, D.N., and Padmavathy, S. 2014. Impact of endophytic microorganisms on plants, environment and humans. The Scientific World J. **2014**: 250693. doi:10.1155/2014/250693.

Nautiyal, C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol. Lett. **170**: 265–270. doi:10.1111/j.1574-6968.1999.tb13383.x.

Palaniappan, P., Chauhan, P.S., Saravanan, V.S., Anandham, R., and Sa, T. 2010. Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. Biol. Fertil. Soils **46**: 807–816. doi:10.1007/s00374-010-0485-5.

Penrose, D.M., and Glick, B.R. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plantarum **118**: 10–15. doi:10.1034/j.1399-3054.2003.00086.x.

Petruzzelli, L., Coraggio, I., and Leubner-Metzger, G. 2000. Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of 1-aminocyclo-

propane-1-carboxylic acid oxidase. Planta **211**: 144–149. doi:10.1007/s004250000274.

Pritchard, S.L., Charlton, W.L., Baker, A., and Graham, I.A. 2002. Germination and storage reserve mobilization are regulated independently in *Arabidopsis*. Plant J. **31**(5): 639–647. doi:10.1046/j.1365-313X.2002.01376.x.

Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M.L., et al. 2014. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. Environ. Microbiol. **17**(2): 316–331. doi:10.1111/1462-2920.12439.

Rodríguez, H., and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. **17**: 319–339. doi:10.1016/S0734-9750(99)00014-2.

Saran, S., Isar, J., and Saxena, R.K. 2007. A modified method for the detection of microbial proteases on agar plates using tannic acid. J. Biochem. Biophys. Methods **70**: 697–699. doi:10.1016/j.jbbm.2007.03.005.

Saravanakumar, D., Kavino, M., Raguchander, T., Subbian, P., and Samiyappan, R. 2011. Plant growth promoting bacteria enhance water stress resistance in green gram plants. Acta Physiol. Plant **33**: 203–209. doi:10.1007/s11738-010-0539-1.

Sedgwick, P. 2014. Spearman's rank correlation coefficient. BMJ **349**: g7327. doi:10.1136/bmj.g7327.

Sessitsch, A., Howieson, J.G., Perret, X., Antoun, H., and Martíane-Romero, E. 2002. Advances in *Rhizobium* research. Crit. Rev. Plant Sci. **21**(4): 323–378. doi:10.1080/0735-260291044278.

Sgroy, V., Cassán, F., Masciarelli ,O., Papa, M.F.D., Lagares, A., and 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. doi:10.1007/s00253-009-2116-3.

Siddikee, M.A., Sundaram, S., Chandrasekaran, M., Kim, K., Selvakumar, G., and Sa, T. 2015. Halotolerant bacteria with ACC deaminase activity alleviate salt stress effect in canola seed germination. J. Korean Soc. Appl. Biol. Chem. **58**: 237-241. doi:10.1007/s13765-015-0025-y.

Sierra, G. 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie Van Leeuwenhoek **23**: 15–22. doi:10.1007/BF02545855.

Soussi, A., Ferjani, R., Marasco, R., Guesmi, A., Cherif, H., Rolli, E., et al. 2016. Plant-associated microbiomes in arid lands: diversity, ecology and biotechnological potential. Plant Soil **405**: 357–370. doi:10.1007/s11104-015-2650-y.

Sturz, A.V., and Christie, B.R. 1995. Endophytic bacterial systems governing red clover growth and development. Ann. Appl. Biol. **126**: 285–290. doi:10.1111/j.1744-

509 7348.1995.tb05366.x.

510 Sturz, A.V., Christie, B.R., Matheson, B.G., and Nowak, J. 1997. Biodiversity of endophytic

511 bacteria which colonize red clover nodules, roots, stems and foliage and their influence on

512 host growth. Biol. Fertil. Soils **25**: 13–19. doi:10.1007/s003740050273.

513 Su, Y.G., Zhao, X., Li, A.X., Li, X.R., and Huang, G. 2011. Nitrogen fixation in biological

514 soil crusts from the Tengger desert, northern China. Eur. J. Soil Biol. **47**: 182–187.

515 doi:10.1016/j.ejsobi.2011.04.001.

516 Surette, M.A., Sturz, A.V., Lada, R.R., and Nowak, J. 2003. Bacterial endophytes in

517 processing carrots (*Daucus carota* L. var. sativus): their localization, population density,

518 biodiversity and their effects on plant growth. Plant Soil **253**: 381–390.

519 doi:10.1023/A:1024835208421.

520 Tamura, K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S. 2011. MEGA5:

521 Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary

522 Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. **28**: 2731–2739.

523 doi:10.1093/molbev/msr121.

524 Taurian, T., Anzuay, M.S., Angelini, J.G., Tonelli, M.L., Ludueña, L., Pena, D., et al. 2010.

525 Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting

526 activities. Plant Soil **329**: 421–431. doi:10.1007/s11104-009-0168-x.

527 Verma, S.C., Ladha, J.K., and Tripathi, A.K. 2001. Evaluation of plant growth promoting and
528 colonization ability of endophytic diazotrophs from deep water rice. J. Biotechnol. **91**: 127–
529 141. doi:10.1016/S0168-1656(01)00333-9.
530 Watanabe, F.S., and Olsen, S.R. 1965. Test of an ascorbic acid method for determining
531 phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. J. **29**: 677–678.
532 doi:10.2136/sssaj1965.03615995002900060025x.
533 Yaish, M.W., Antony, I., and Glick, B.R. 2015. Isolation and characterization of endophytic
534 plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their
535 potential role in salinity tolerance. Antonie van Leeuwenhoek **107**: 1519–1532.
536 doi:10.1007/s10482-015-0445-z.
537 Yao, S., Chen, S., Zhao J., Xu D., Lan H., and Zhang, F. 2010. Effect of three salts on
538 germination and seedling survival of dimorphic seeds of *Chenopodium album*. Botany **88**:
539 821–828. doi:10.1139/B10-052.
540 Yu, X., Liu, X., Zhu, T.H., Liu, G.H., and Mao, C. 2011. Isolation and characterization of
541 phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus
542 mobilization. Biol. Fertil. Soils **47**: 437–446. doi:10.1007/s00374-011-0548-2.
543 Yu, Y., Wang, J., Shi, H., Gu, J., Dong, J., Deng, X.W., et al. 2016. Salt stress and ethylene
544 antagonistically regulate nucleocytoplasmic partitioning of COP1 to control seed germination.
545 Plant Physiol. **170**: 2340–2350. doi:10.1104/pp.15.01724.

546 Fig. 1 Phylogenetic relationships of the endophytic bacterial isolates from *A. bifolium* based
547 on their partial 16S rRNA sequences and closely related sequences, based on neighbor-joining
548 algorithm (1000 bootstrap replicates performed). The accession numbers and the proportion
549 of the similar isolates in total isolates were presented in parentheses.

550 Fig. 2 Calcium phosphate solubilization by *A. bifolium* endophytic bacterial isolates in
551 NBRIP medium. (a) Different bacterial cell suspensions were inoculated into 20 ml of NBRIP
552 medium. The non-inoculated medium was used as a control. After seven days of incubation at
553 30 °C under constant shaking at 150 rpm, the solubilized phosphorus in the culture
554 supernatant was determined according to the Murphy and Riley method. (b) The pH of the
555 supernatant as described above was also measured. Data are displayed as the means ± SE (n =
556 3), with different letters indicating a significant different at $P < 0.05$.

557 Fig. 3 IAA production by *A. bifolium* endophytic bacterial isolates in peptone water (PW)
558 medium with L-tryptophan. Different bacterial cell suspensions were transferred into 20 ml of
559 PW medium and incubated for 24 h at 30 °C. The non-inoculated medium was used as a
560 control. The IAA concentration in the cell-free medium was measured based on the
561 colorimetric method described by Gordon and Weber (1951). Data are displayed as the means
562 ± SE (n = 3), with the different letters indicating a significant different at $P < 0.05$.

563 Fig. 4 ACC deaminase activity in DF salts minimal medium containing ACC by *A. bifolium*
564 endophytic bacterial isolates. ACC deaminase activity in cell-free DF salts minimal medium

565 was measured for all endophytic bacterial isolates according to the protocol described by
566 Penrose and Glick (2003). The non-inoculated medium was used as a control. Data are
567 displayed as the means ± SE (n = 3), with different letters indicating a significant different at
568 $P < 0.05$.

569 Fig. 5 Effects of inoculation with endophytic bacteria on *A. bifolium* seed germination (a) and
570 radicle length (b). The surface-sterilized seeds were soaked in a 0.85% saline solution
571 (Control) or different bacterial suspensions for 4 h, then placed in petri dishes containing filter
572 paper wetted with sterilized water and were incubated at 20 °C in the dark for 15 days after
573 which the seed germination rate and radicle length were determined. Data are displayed as the
574 means ± SE (n = 5), and significant differences between the control and the endophytic
575 bacterial treatments are indicated by different letters ($P < 0.05$).

576

577

578

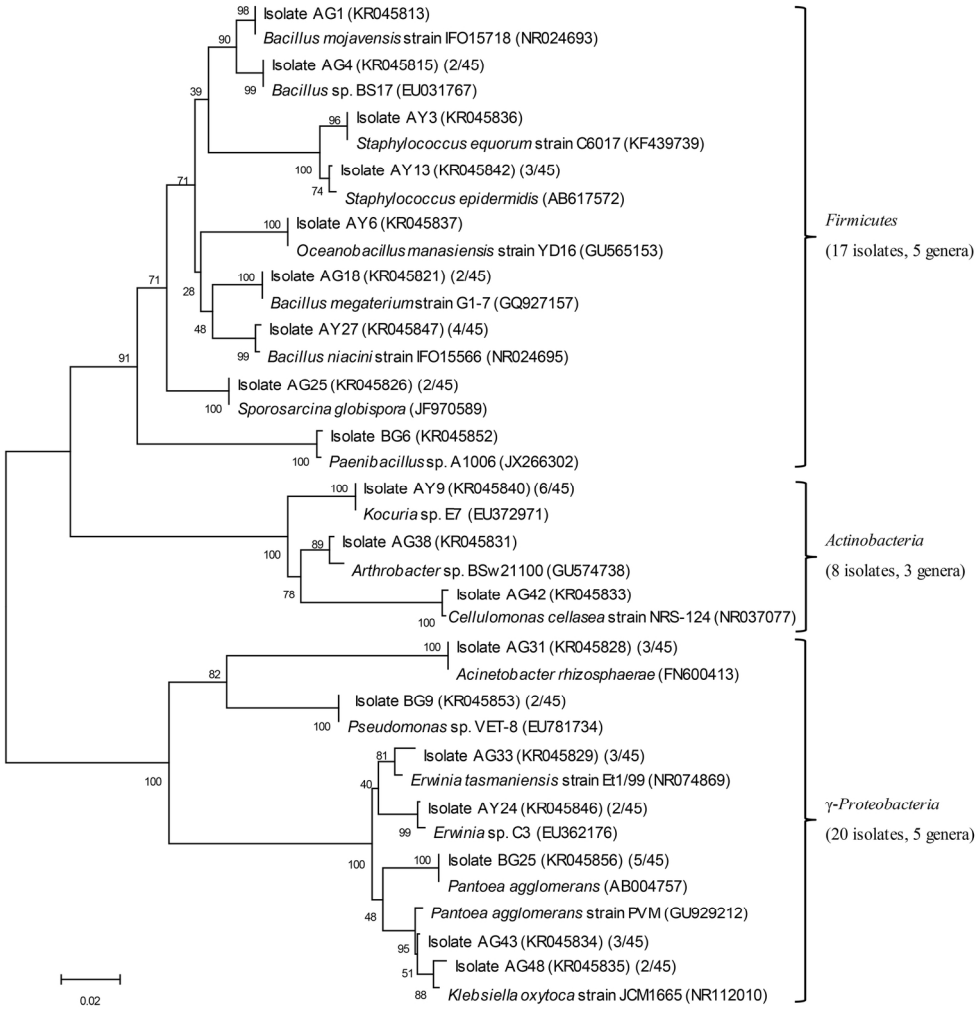

Fig. 1 Phylogenetic relationships of the endophytic bacterial isolates from *A. bifolium* based on their partial 16S rRNA sequences and closely related sequences, based on neighbor-joining algorithm (1000 bootstrap replicates performed). The accession numbers and the proportion of the similar isolates in total isolates were presented in parentheses.

168x177mm (300 x 300 DPI)

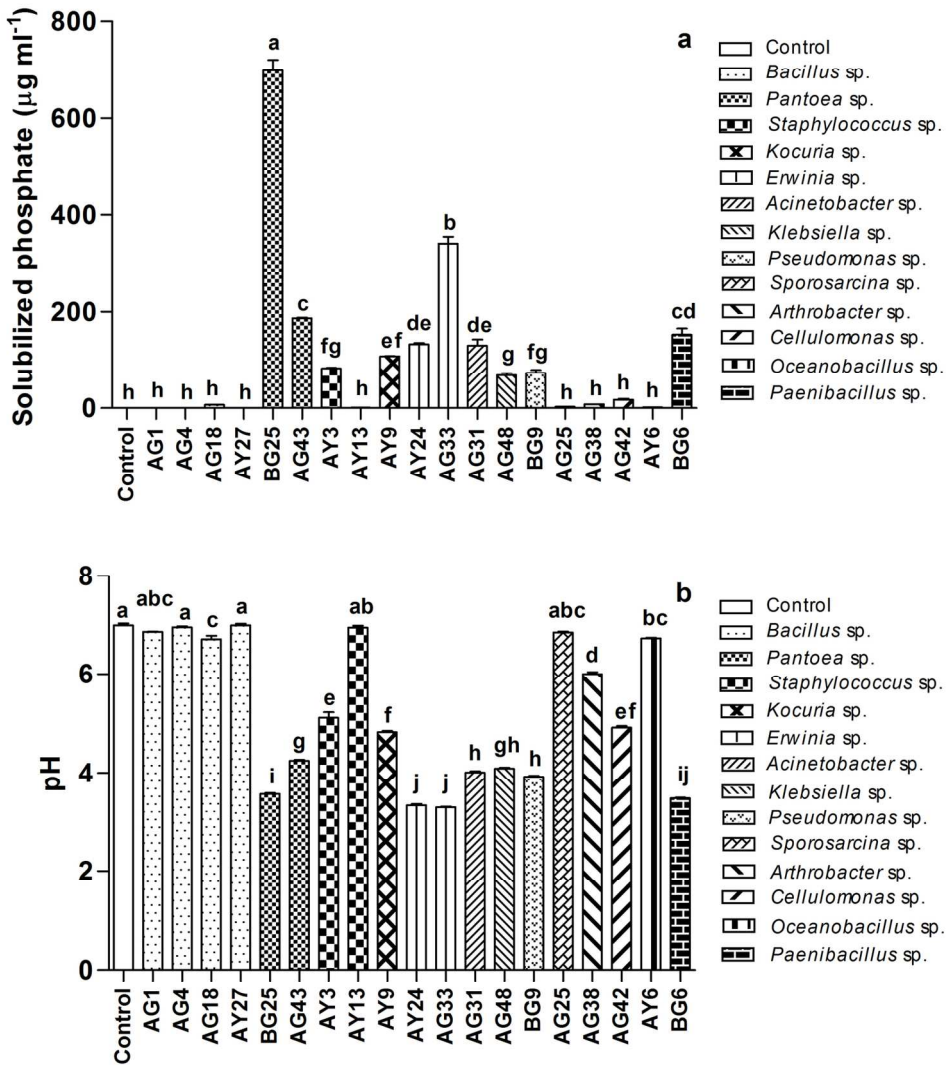

Fig. 2 Calcium phosphate solubilization by *A. bifolium* endophytic bacterial isolates in NBRIP medium. (a) Different bacterial cell suspensions were inoculated into 20 ml of NBRIP medium. The non-inoculated medium was used as a control. After seven days of incubation at 30 °C under constant shaking at 150 rpm, the solubilized phosphorus in the culture supernatant was determined according to the Murphy and Riley method. (b) The pH of the supernatant as described above was also measured. Data are displayed as the means ± SE (n = 3), with different letters indicating a significant different at $P < 0.05$.

144x161mm (300 x 300 DPI)

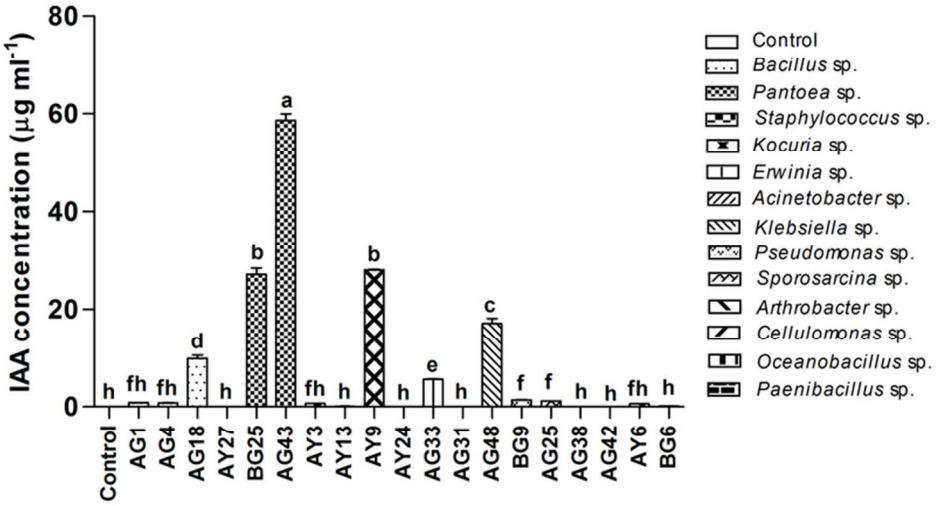

Fig. 3 IAA production by *A. bifolium* endophytic bacterial isolates in peptone water (PW) medium with L-tryptophan. Different bacterial cell suspensions were transferred into 20 ml of PW medium and incubated for 24 h at 30 °C. The non-inoculated medium was used as a control. The IAA concentration in the cell-free medium was measured based on the colorimetric method described by Gordon and Weber (1951). Data are displayed as the means ± SE (n = 3), with the different letters indicating a significant different at $P < 0.05$.

72x40mm (300 x 300 DPI)

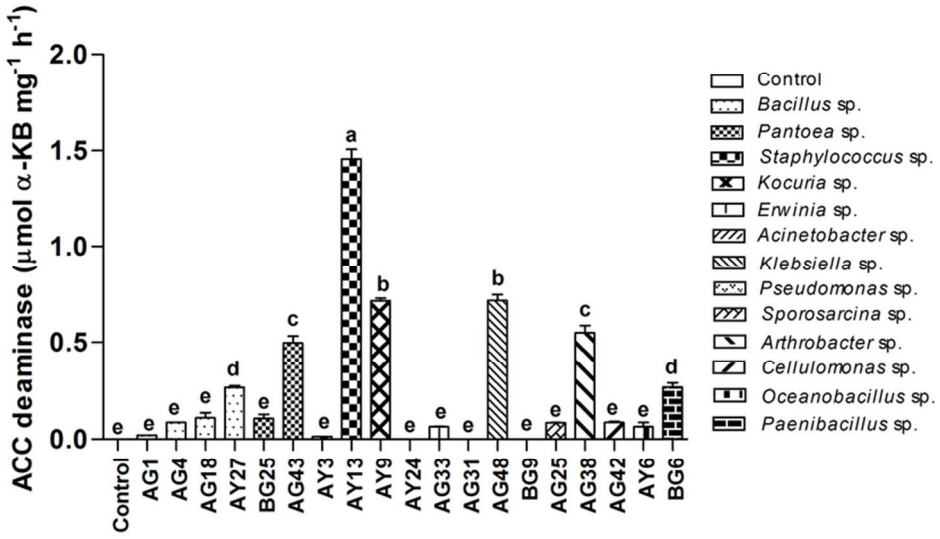

Fig. 4 ACC deaminase activity in DF salts minimal medium containing ACC by *A. bifolium* endophytic bacterial isolates. ACC deaminase activity in cell-free DF salts minimal medium was measured for all endophytic bacterial isolates according to the protocol described by Penrose and Glick (2003). The non-inoculated medium was used as a control. Data are displayed as the means ± SE (n = 3), with different letters indicating a significant different at $P < 0.05$.

75x43mm (300 x 300 DPI)

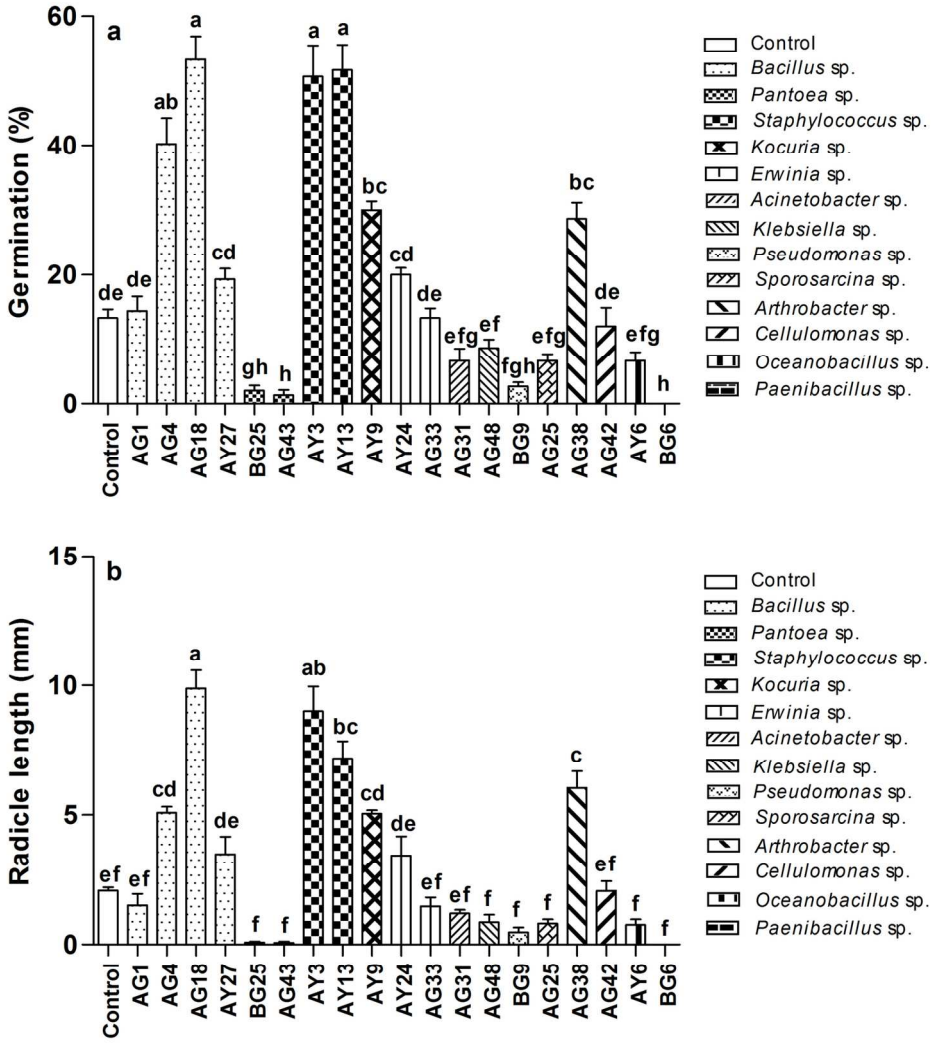

Fig. 5 Effects of inoculation with endophytic bacteria on *A. bifolium* seed germination (a) and radicle length (b). The surface-sterilized seeds were soaked in a 0.85% saline solution (Control) or different bacterial suspensions for 4 h, then placed in petri dishes containing filter paper wetted with sterilized water and were incubated at 20 °C in the dark for 15 days after which the seed germination rate and radicle length were determined. Data are displayed as the means ± SE (n = 5), and significant differences between the control and the endophytic bacterial treatments are indicated by different letters ($P < 0.05$).

144x161mm (300 x 300 DPI)

Table 1 Pellicle formation and extracellular enzyme production by *A. bifolium* endophytic bacterial isolates

Isolate	Most closely related genus/species (Accession no.)	Similarity %	Pellicle formation	Amylase	Cellulase	Protease	Lipase
AG1	*Bacillus mojavensis* strain IFO15718 (NR024693)	99	+	+	+	+	+
AG3	*Bacillus* sp. BS17 (EU031767)	100	+	+	+	+	+
AG4	*Bacillus* sp. BS17 (EU031767)	99	+	+	+	+	+
AG13	*Bacillus megaterium* isolate PDD-31b-1(HQ256822)	99	–	+	+	+	–
AG18	*Bacillus megaterium* strain G1-7 (GQ927157)	100	–	+	+	+	–
AY18	*Bacillus niacini* strain IFO15566 (NR024695)	99	–	+	+	–	+
AY27	*Bacillus niacini* strain IFO15566 (NR024695)	99	–	+	+	–	+
AY28	*Bacillus* sp. A50 (KC522837)	99	–	+	+	–	+
AY29	*Bacillus* sp. A50 (KC522837)	99	–	+	+	–	+
AG14	*Pantoea agglomerans* strain LMG 2565 (AF373196)	99	+	–	–	–	+
AG22	*Pantoea agglomerans* (AB004757)	99	–	–	–	–	–
AG23	*Pantoea agglomerans* (AB004757)	99	+	–	–	–	+
AG24	*Pantoea agglomerans* strain PVM (GU929212)	99	–	–	–	–	–
BG25	*Pantoea agglomerans* (AB004757)	99	+	–	–	–	+
BG31	*Pantoea agglomerans* strain PVM (GU929212)	99	–	–	–	–	–
AG34	*Pantoea agglomerans* (AB004757)	99	+	–	–	–	+
AG43	*Pantoea agglomerans* strain PVM (GU929212)	99	–	–	–	–	–
AY3	*Staphylococcus equorum* strain C6017 (KF439739)	100	–	–	–	–	–
AY7	*Staphylococcus epidermidis* (AB680360)	100	–	–	–	–	+
AY8	*Staphylococcus epidermidis* (AB617572)	99	–	–	–	–	+
AY13	*Staphylococcus epidermidis* (AB617572)	99	–	–	–	–	+
AY9	*Kocuria* sp. E7 (EU372971)	99	–	+	+	–	–
AY11	*Kocuria* sp. E7 (EU372971)	99	–	+	+	–	–
AY20	*Kocuria* sp. E7 (EU372971)	99	–	+	+	–	–
AG21	*Kocuria rosea* strain 2P03AA (EU977667)	99	–	+	+	–	–
AY35	*Kocuria* sp. E7 (EU372971)	99	–	+	+	–	–
AY38	*Kocuria carniphila* (AM237350)	99	–	+	+	–	–
AG9	*Erwinia tasmaniensis* strain Et1/99 (NR074869)	99	+	–	–	–	–
AG10	*Erwinia tasmaniensis* strain Et1/99 (NR074869)	99	+	–	–	–	–
AY24	*Erwinia* sp. C3 (EU362176)	99	–	–	–	–	+
AG33	*Erwinia tasmaniensis* strain Et1/99 (NR074869)	99	+	–	–	–	–
AG40	*Erwinia* sp. C3 (EU362176)	99	+	–	–	–	+
AG6	*Acinetobacter rhizosphaerae* (FN600413)	100	–	–	–	–	+
AY23	*Acinetobacter lwoffii* strain AM-72 (KF817684)	99	–	–	–	–	+
AG31	*Acinetobacter rhizosphaerae* (FN600413)	99	–	–	–	–	+
BG12	*Klebsiella oxytoca* strain JCM1665 (NR112010)	99	+	–	–	–	–
AG48	*Klebsiella oxytoca* strain JCM1665 (NR112010)	99	+	–	–	–	–

BG9	*Pseudomonas* sp. VET-8 (EU781734)	99	+	–	–	–	–
BG23	*Pseudomonas* sp. TFD39 (EU827489)	99	+	–	–	–	+
AG25	*Sporosarcina globispora* (JF970589)	100	–	–	–	–	+
AG26	*Sporosarcina globispora* (JF970589)	99	–	–	–	–	+
AG38	*Arthrobacter* sp. BSw21100 (GU574738)	99	–	+	+	–	–
AG42	*Cellulomonas cellasea* strain NRS-124 (NR037077)	99	–	+	+	–	–
AY6	*Oceanobacillus manasiensis* strain YD16 (GU565153)	99	–	–	–	–	+
BG6	*Paenibacillus* sp. A1006 (JX266302)	99	–	+	+	+	+

Note: "+" indicates a positive result, and "–" indicates a negative result.

Table 2 The correlation coefficients between the growth characters of *A. bifolium* and the plant growth-promoting traits of endophytic bacteria

Traits	**Germination**	**Radicle length**	**IAA production**	**ACC deaminase**	**PO_4^{3-} solubilization**	**Pellicle formation**	**Amylase**	**Protease**	**Cellulase**
Germination	1.000								
Radicle length	0.946**								
IAA production	−0.253*	−0.353**							
ACC deaminase	0.080	0.035	0.318*						
PO_4^{3-} solubilization	−0.473**	−0.472**	0.483**	0.046					
Pellicle formation	−0.151	−0.254*	0.373**	−0.143	0.070				
Amylase	0.335**	0.357**	−0.230	0.262*	−0.364**	−0.062			
Protease	0.158	0.107	0.054	−0.031	−0.269*	0.230	0.618**		
Cellulase	0.335**	0.357**	−0.230	0.262*	−0.364**	−0.062	1.000**	0.618**	
Lipase	−0.069	−0.118	−0.301*	−0.128	−0.236	0.030	0.037	0.266*	0.037

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).