Pak. J. Bot., 46(5): 1905-1910, 2014.

ISOLATION AND CHARACTERIZATION OF NOVEL BACTERIA CONTAINING ACC DEAMINASE FROM THE RHIZOSPHERE RESOURCE ON DRY-FARMING LANDS

PENG ZHENG[1], LIXIN ZHANG[1*], LI TIAN[1], LING ZHANG[1], FUCAI CHEN[1], BING ZHI LI[2] AND ZHIYAN CUI[1]

[1]College of Life Sciences, Northwest A&F University, Yangling 712100, P. R. China
[2]College of Horticulture, Northwest A&F University, Yangling 712100, P. R. China
[*]Correspondence author's e-mail: zhanglixin@nwsuaf.edu.cn;bingzhili@yahoo.com; Ph: +0086-29-87092262

Abstract

Soil-microbe-plant interactions are known to be intricate and they can greatly influence the crop vigor and yield. Plant growth promoting rhizobacteria (PGPR) containing ACC deaminase can markedly affect plant metabolic processes under stress conditions. In the present study, we isolated 300 bacterial strains from the rhizosphere of maize or apple grown in drought-hit soil including four different locations of the Loess Plateau, China. Of all isolated strains, four with ACC deaminase activity (ranging from 28.88 to 155.12 nmol alpha-ketobutyrate mg^{-1} h^{-1}) were further studied by determining their biological characters and sequencing the 16S rRNA gene. All four strains showed positive performance in terms of arabinose, citrate utilization, urease, indol, glucose and melibiose. In connection with the results of biochemical characters and phylogenetic analysis, these strains commonly belong to three different genera: *Klebsiella, Pseudomonas* and *Raoultella* and four different species: *Klebsiella oxytoca*, *Klebsiella variicola, Pseudomonas fluorescens* and *Raoultella planticola*. Although some researchers have reported their performance under stress conditions, we are the first to report *Klebsiella oxytoca*, *Klebsiella variicola* and *Raoultella planticola* containing ACC deaminase under drought stress. These findings are a reasonable explanation to their superb ability of causing stress-resistance in maize (*Zea mays*) or apple (*Malus domestica*) plants. The presence of diverse PGPR possessing potential ACC deaminase activity may be beneficial for enhancing crop production under different stress conditions.

Key words: Drought stress; 1-aminocyclopropane-1-carboxylate deaminase; Plant growth promoting rhizobacteria; *Zea mays* L.

Introduction

Scarcity of water is considered to be one of the most important ecological factors limiting plant survival and development, particularly in arid and semi-arid regions (Ashraf, 2010). Of a variety of drought-induced changes in plants, production of ethylene by plant tissues is also a common feature (Morgan & Drew, 1997). The enhanced ethylene levels in plants are directly associated with the accumulation of an ethylene precursor, 1-aminocyclopropane-1-carboxylate (ACC) (Glick *et al*., 1997). The increased synthesis of ethylene in response to drought stress may suppress growth, particularly seed germination and root growth in most plants (Saravanakumar *et al*., 2011). Thus, reducing the stress-induced ethylene level can alleviate some of the stress-induced adverse effects on plants (Glick, 2004).

Microorganisms isolated from the rhizosphere, which can effectively improve plant health as well as increase yield, are known as plant growth promoting rhizobacteria (PGPR) (Saleem *et al*., 2007). It has been found that certain PGPR containing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase can decrease ethylene production by cleaving ACC into ammonia and alpha-ketobutyrate. The rhizosphere bacteria possessing ACC deaminase can take up the plant ACC which the seeds or roots release. The ammonium released can be utilized as nitrogen (N) source by the bacteria (Ashraf, 2010). As a consequence, the decrease in ACC concentration in the plant in turn decreases the endogenous ethylene levels, which alleviates the ethylene-induced adverse effects on plants (Glick, 2004; Glick *et al*., 2007).

Earlier published reports have shown that a variety of bacteria including species of *Azospirillum, Arthrobacter, Alcaligenes, Azotobacter, Burkholderia, Bacillus, Enterobacter, Pseudomonas, Klebsiella,* and *Serratia* could be regarded as PGPR (Kloepper *et al*., 1989; Jha & Kumar, 2007; Singh *et al*., 2011). Of these genera, *Pseudomonas* (Glick, 1995) and *Enterobacter* (Shah *et al*., 1998) are known to effectively produce ACC deaminase. Further, in many studies it has been shown that ACC deaminase-producing bacteria can effectively mitigate the stress-induced adverse effects on plant growth. For example, *Pseudomonas putida* and *Pseudomonas fluorescens* possessing ACC deaminase activity increased root length, plant height and grain yield of wheat (*Triticum aestivum*) seedlings (Nadeem *et al*., 2010). *Pseudomonas brassicacearum* and *Pseudomonas marginalis* with ACC deaminase activity effectively prevented Cd-induced growth inhibition in pea (*Pisum sativum*) plants (Safronova *et al*., 2006). Furthermore, while characterizing the ACC deaminase gene (*acdS*) from *Enterobacter cloacae* UW4 deficient mutants, it was found that the ability of the gene to elongate canola roots under aseptic conditions was reasonably impaired (Li *et al*., 2000).

It is believed that PGPR with ACC deaminase activity can promote stress resistance in plants. Thus, it is essential to screen more effective strains in terms of ACC deaminase production under natural field conditions so that we can explore their considerable potential of improving drought resistance in plants. In view of the aforementioned reasons, the present study was planned to isolate and characterize new rhizospheric bacteria strains producing ACC deaminase from drought soil particularly inhabiting maize (*Zea mays*) or apple (*Malus domestica*) plants.

Materials and Methods

Soil sampling: All soil samples were taken at a depth of 15 cm in August 2012. They were sieved through a plastic mesh (3 mm), and stored moist in sterile plastic bags in darkness at 4°C for 10 days before use. A total of 12 soil samples (three replications from each field) were randomly collected from dry-farming lands of three counties, XunYi, Fu (two differently sampling sites) and Qian in Loess Plateau.

Isolation of ACC- utilizing bacteria: One gram of soil was added to 50 ml sterile NA (Nutrient Agar) medium (per litre) with 3 g beef extract, 5 g peptone, 1 g glucose, 1 g NaCl in a 250 ml flask. The flask and its contents were incubated in a shaking water bath (200 rpm.) for 30 min at 30°C in the absence of light. The suspension so formed was allowed to stand. Then 1 ml aliquot from the supernatant was transferred to 50 ml of sterile DF salts (Dworkin & Foster, 1958) in a 250 ml flask, and then incubated at 200 rpm. in a shaking water bath at 30°C for 24 h. The DF salts per litre contained: 6.0 g Na_2HPO_4, 4.0 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 2.0 g glucose, 2.0 g citric acid and 2.0 ml gluconic acid with the following trace element salts: 10 mg H_3BO_3, 11.19 mg $MnSO_4 \cdot H_2O$, 1 mg $FeSO_4 \cdot 7H_2O$, 78.22 mg $CuSO_4 \cdot 5H_2O$, 124.6 mg $ZnSO_4 \cdot 7H_2O$, 10 mg MoO_3, pH 7.2 and 2.0 g $(NH_4)_2SO_4$ as a N source. In this experiment, the DF minimal medium was prepared as follows: (1) the trace elements (11.19 mg $MnSO_4 \cdot H_2O$, 10 mg H_3BO_3, 78.22 mg $CuSO_4 \cdot 5H_2O$, 124.6 mg $ZnSO_4 \cdot 7H_2O$, and 10 mg MoO_3) were dissolved in 100 ml sterile distilled water and then stored in a refrigerator at 4°C; (2) $FeSO_4 \cdot 7H_2O$ (100 mg) was dissolved in 10 ml sterilized distilled water and stored at 4°C; (3) all the other components such as, 4.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 2.0 g $(NH_4)_2SO_4$, 0.2 g $MnSO_4 \cdot H_2O$, 2.0 g glucose, 2.0 g citric acid, 2.0 ml gluconic acid, and 0.1 ml of each of the solutions of different trace elements and $FeSO_4 \cdot 7H_2O$ were dissolved in 1 L distilled water and subsequently autoclaved for 20 min at 120°C. The DF salt incubation procedure was under the same conditions. These incubations caused increase in bacterial population but reduced that of fungi.

A 0.5 M solution of ACC was filtered using a 0.2 µm membrane and the filtrate so collected was frozen at -20°C. Before inoculation, 300 µl of the ACC solution were added to 50 ml sterile DF salts minimal medium lacking $(NH_4)_2SO_4$ (ADF medium). All the procedures mentioned above were finished under the aseptic condition and soaked all the glass apparatus in sterilized distilled water for 12 h so as to remove any type of N source. 1 ml of DF solution was transferred to ADF. The flasks were then placed in a shaking water bath at 200 rpm. and grown at 25-30°C for 24 h. In this study, this step was conducted for 10 times in a row to ensure that the screened organisms only used ACC as N source. After 10 times incubations in the ADF medium, 12 samples were spread onto plates with 15 ml solid ADF medium containing 1.8% agar. The isolated stains were selected and stored in a refrigerator at -80°C (Penrose & Glick, 2003).

ACC Deaminase activity assay: ACC deaminase activity was assayed following the method described by Penrose and Glick, which measures the concentration of alpha-ketobutyrate resulting from the action of ACC-deaminase on ACC. The concentration of alpha-ketobutyrate (nmol) was determined using a standard curve prepared using 0.1 to 1.0 nmol alpha-ketobutyrate solution at 540 nm. Experiments were undertaken in duplicate and all samples were assayed three times (Penrose & Glick, 2003).

Growth curve: To determine the growth curve, the isolated strains were grown in 250 ml flasks filled with sterile 50 ml NA (Nutrient Agar) medium, including 3.0 g beef extract, 1.0 g glucose, 5.0 g peptone, and 1.0 g NaCl. The growth curve was measured using optical density at 600 nm (OD_{600}) determined using a SHIMADZU UV-3600 spectrophotometer. Bacteria were incubated in a shaking water bath at 200 rpm in the absence of light at 30°C. The optical density was recorded at 600 nm after each 30 min. Each strain was determined for three times (Zhou, 2006).

Morphological and biochemical characterization of strains with ACC deaminase activity: The KOH test was conducted to ascertain Gram attributes. One to two drops of 3% KOH were placed on a glass slide. Isolated bacterial strains were carefully pulled out from the surface of the solid medium using an inoculation loop. The material was stirred in the KOH solution. After 5-10 s stirring, the inoculation loop was raised from the drop. If the KOH solution had become viscous, a thread of slime followed the loop 0.5-2 cm or more. This was a positive reaction and was regarded as Gram-negative bacteria. If there was a watery suspension which did not follow the loop, the strain was referred to as Gram-positive bacteria (Gregersen, 1978).

The morphological properties of the isolated strains listed in Table 1 were determined using standard methods according to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Physiological characterization of strains F1, F2, YX2 and YL2 were determined using BoJian Gram-negative Bacteria Identification System (QingDao Hopebiol Technology Co. Ltd.) according to the manufacturer's instructions.

Identification based on 16S rRNA gene sequencing and phylogenetic analysis: For molecular characterization, 12 screened strains were subjected to 16S rRNA gene sequence analysis. Genomic DNA was isolated from the culture by using Genomic DNA isolation kit. The 16S rRNA gene was amplified using universal forward 27F (5'-AGAGTTTGATCMTGGCTCAGC-3') and reverse 1492R (5'-GGTTACCTTGTTACGACTT-3') primers. An Applied Biosystems model ABI 3730XL automated DNA sequencing system (Applied BioSystems, USA) was employed to resolve the sequencing products. The PCR cycle used for amplification was as follows: 5 min at 95 °C, followed by 35 cycles of 30 s at 95°C, 30 s at 57°C, 1.5 min at 72°C and a final extension of 3 min at 72°C. The amplified 16S rRNA gene was purified with a PCR purification kit and outsourced for sequencing. The obtained 16S rRNA gene sequences were aligned and the affiliations deduced, using BLAST analysis. The partial 16S rRNA gene sequences were deposited in the GenBank data base.

Table 1. Physiological and biochemical characterization of four isolated strains

Characters	Bacterial Isolates			
	F1	F2	YX2	YL2
Accession number	KJ465988	KJ465989	KJ465990	KJ465991
Morphological				
Gram reaction	-	-	-	-
Strain shape (Fig. 3)	Rod	Rod	Rod	Rod
Strain color (Fig. 3)	Ivory	Ivory	Yellowish	Yellowish
Biochemical				
ONPG	+	+	-	+
Arginine decarboxylase	-	-	+	-
Lysine decarboxylase	+	+	-	+
Ornithine decarboxylase	-	-	-	-
Citrate utilization	+	+	+	+
H_2S	-	-	-	-
Urease	+	+	+	+
Lactose	-	+	-	+
Indol	+	+	+	+
Voges- Proskaaaer	+	+	+	-
Gelatinase	-	-	-	-
Glucose	+	+	+	+
Mannitol	+	+	-	+
Inositol	+	+	-	+
Sorbitol	+	+	-	+
Rhamnose	+	+	-	+
Sucrose	+	+	-	+
Melibiose	+	+	+	+
Amygdalin	+	+	-	-
Arabinose	+	+	+	+
Oxidase	-	-	+	-

+: Positive growth; −: Negative growth (after 18-24h of incubation at 30°C)

Phylogenetic analyses were conducted using MEGA version 5.22 after multiple alignments of the data by CLUSTAL X. Distances were obtained using options following the Kimura two-parameter model, and clustering was performed using the neighbor-joining method. The statistical confidence of the nodes was estimated by bootstrapping using 1000 replications.

Statistical analysis: The data were analyzed in a Microsoft Excel 2010 spread sheet for calculating means and standard deviations.

Nucleotide sequence accession numbers: The National Center for Biotechnology Information GenBank accession numbers for the sequences of drought-tolerant strains are from KJ465988 to KJ465991.

Results

ACC deaminase activity: The ACC-deaminase present in isolated microorganisms hydrolyzes ACC into alpha-ketobutyrate which can restrict ethylene production for stimulating plant growth. The highest ACC-deaminase activity obtained from strain YX2 (155.12 nmol alpha-ketobutyrate $mg^{-1}h^{-1}$) followed by F2 (86.88 nmol alpha-ketobutyrate $mg^{-1}h^{-1}$), F1 (74.19 nmol alpha-ketobutyrate $mg^{-1}h^{-1}$) and YL-2 (28.88 nmol alpha-ketobutyrate $mg^{-1}h^{-1}$) (Fig. 1).

Bacterial growth curve: The growth of isolated bacteria was also monitored in a liquid NA medium supplemented with four screened bacterial cells, F1 (KJ465988), F2 (KJ465989), YX2 (KJ465990) and

YL2 (KJ465991). In accordance with the normal bacterial growth, all these strains went through lag phase, log phase and stationary phase. YL2 was the most active strain among them, which suggested that it could be more adaptive in rhizosphere (Fig. 2).

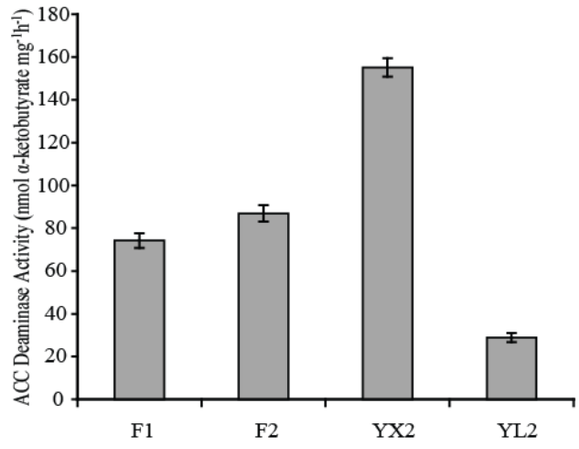

Fig. 1. ACC deaminase activity of four isolated bacteria.

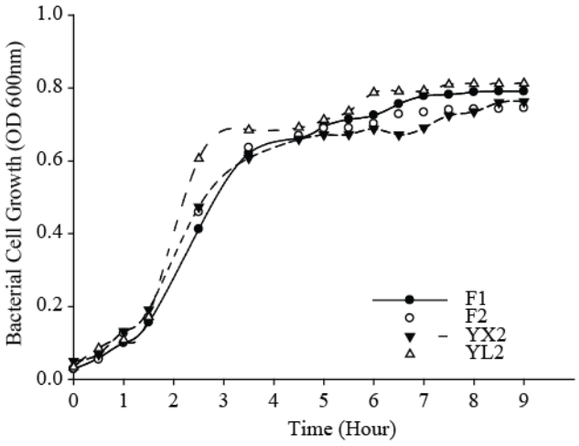

Fig. 2. Growth curves of F1, F2, YX2 and YL2 in DF medium. The cultivations were performed with 1% of the inculcated amount at 30°C and 200 rpm for 10 h in a rotary shaker. Each value is the mean of triplicates.

Physiological and biochemical characterization: Following the determination of ACC deaminase activity, all strains were tested for a number of characters related to their ability to facilitate plant growth and proliferate in the soil environment. Morphological and biochemical characteristics of four bacterial strains are given in Table 1. All the isolates grew at the temperature 30°C in the absence of light. Following morphological characterization, the isolates were compared with those of the standard species using Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Based on the biological performances, the software calculated confidence of all isolated strains. The confidence levels were above 80% which were regarded as reliable results (Fig. 3).

Identification and phylogenetic analysis of bacterial isolates: All the strains containing ACC deaminase were further identified by 16S rRNA gene sequencing analysis in combination with their biological traits to confirm taxonomical positions. According to the results of BLAST from the NCBI database, the representative four bacterial strains belonged to four different genera of class *Gammaproteobacteria*. The strain F1 showed a 99% similarity with the 16S rRNA gene sequences of the species *Klebsiella oxytoca* strain SHD-1; F2 exhibited a 99% similarity to *Klebsiella variicola* strain XF16; YX2 exhibited 99% homology to *Pseudomonas fluorescens* and YL2 was 99% homologous to *Raoultella planticola* strain ALK314 (Table 2). The related sequences were retrieved from the NCBI and used for the phylogenetic analysis with MEGA 5 using neighbor-joining method with 1000 bootstrap replications and the results are shown in Fig. 4.

Discussion

Microbial diversity is considered as one of the most useful resources for sustainable agriculture (Singh *et al*., 2011). PGPR possessing ACC deaminase activity help plants to overcome a stress (biotic or abiotic) by reducing the level of stress-induced ethylene. In the present study, we have isolated four PGPR containing ACC deaminase from 12 rhizospheric soil samples of maize or apple planted in the Loess Plateau. We have demonstrated that there is a diversity of PGPR being able to produce ACC deaminase and promote plant growth. In connection with the results of biochemical characters and phylogenetic analysis, these strains commonly belong to three different genera: *Klebsiella, Pseudomonas and Raoultella* and four different speceis: *Klebsiella oxytoca*, *Klebsiella variicola, Pseudomonas fluorescens* and *Raoultella planticola*. In the last few decades, several genera have been reported that they can produce ACC deaminase under stress conditions (Mayak *et al*., 2004; Toklikishvili *et al*., 2010). However, to our knowledge, we are the first to report that *Klebsiella oxytoca*, *Klebsiella variicola* and *Raoultella planticola* under drought stress contained that vital enzyme ACC-deaminase.

Table 2. Identification of the bacterial isolates by 16S rRNA similarity search.

Isolate	Accession number	Closest species in NCBI	Percentage of identity
F1	KJ465988	*Klebsiella Oxytoca* strain SHD-1 (GU361112)	99%
F2	KJ465989	*Klebsiella Variicola* strain XF16 (KC853308)	99%
YX2	KJ465990	*Pseudomonas Fluorescens* strain RWX31 (JN020938)	99%
YL2	KJ465991	*Raoultella Planticola* strain ALK314 (JQ01417)	99%

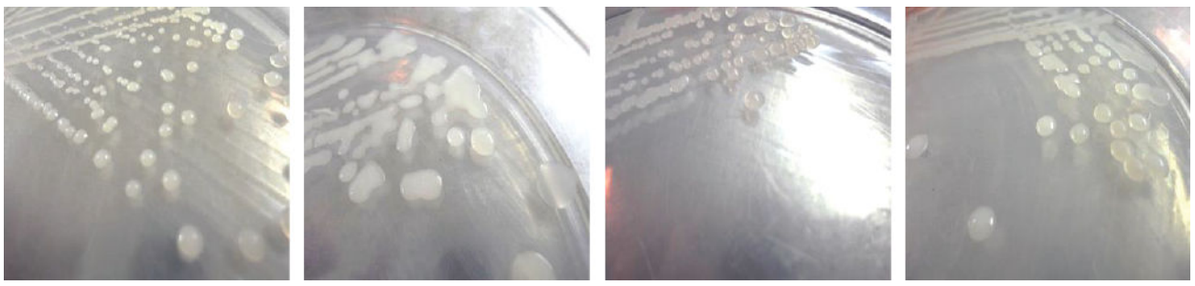

Fig. 3. Shape and color of four isolated strains in the ADF medium. From left to right were F1, F2, YX2 and YL2, respectively.

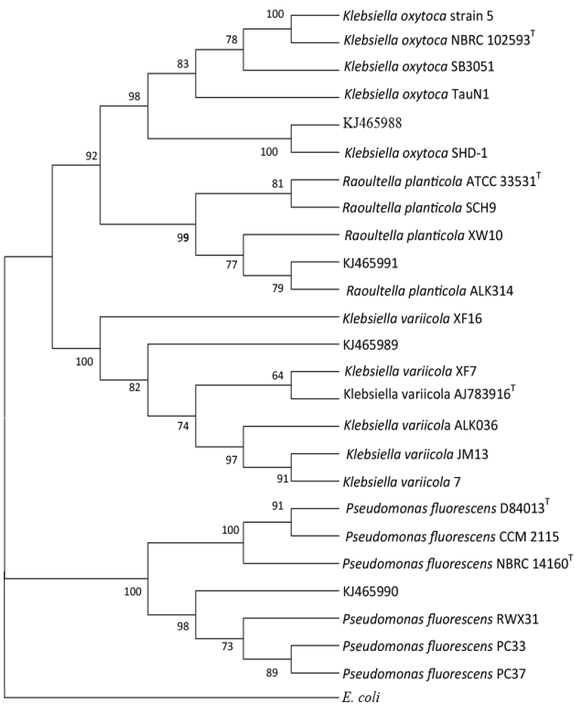

Fig. 4. Phylogenetic analysis of four isolated bacterial strains and other typical authentic species based on 16S rRNA sequences. The MEGA5 program using the neighbor-joining cluster algorithm was employed to construct the tree. The numbers at the branches are bootstrap values (confidence limits). Escherichia coli was used as an out-group to root the tree.

It is now well evident that PGPR play a marked role in promoting growth and development of most plants both under non-stress and stress conditions (Khalid *et al*., 2004; Glick *et al*., 2007; Nadeem *et al*., 2010). Researchers have demonstrated the great potential of *Klebsiella* and *Pseudomonas* in sustainable agriculture (Tilak *et al*., 2005). In the last few decades, the interaction between microbes and plant roots stimulates great interest among scientists. Researchers often found that *Klebsiella* can have a consistent symbiosis with the host (Podschun & Ullmann, 1998). The *Klebsiella variicola* and *Klebsiella oxytoca* species including clinical and plant-associated isolates were identified in 2004 (Rosenblueth *et al*., 2004). *Klebsiella* spp. is also known to have endophytic association as diazotrophs with plants like wheat, maize, rice (*Oryza sativa* L.), etc. (Fisher *et al*., 1992; Adachi *et al*., 2002; Jha & Kumar, 2007). As to the genus of *Pseudomonas*, more achievements have been accessed than *Klebsiella* as to the performance under stress condition. Cho *et al*., (2008) reported that *Pseudomonas chlororaphis* strain O6 could mediate stomatal closure and drought resistance by producing 2R, 3R-butanediol. Then, Fu *et al*., (2010) proposed an interesting mechanism for the alleviation of salt stress. After the eggplant (*Solanum melongena*) was inoculated with *Pseudomonas* sp. DW1 under salt stress, the response to saline condition may involve decreasing mineral uptake and increasing activities of antioxidant enzyme meanwhile. Mung bean (*Vigna radiata*) plants treated with *Pseudomonas fluorescens* had greater resistance to drought stress than untreated ones (Saravanakumar *et al*., 2011). Although there have been large numbers of studies conducted with *Klebsiella, Pseudomonas* and *Raoultella* under stress conditions, a few of them mentioned the importance of ACC deaminase in reducing stress-induced ethylene production.

Obviously, the increased level of ethylene in response to drought stress results in inhibition of root elongation and even death. Thus, decreasing the level of ACC, a precursor of ethylene, is an effective method to encounter ethylene synthesis (Glick *et al*., 2007). Researchers have found that PGPR with ACC deaminase activity can overcome the negative influences on drought-induced ethylene production in plants. A bacterium is able to grow on ACC and acts as a PGPR as long as the ACC deaminase activity reaches to approximately 20 nmol alpha-ketobutyrate mg^{-1} h^{-1} (Penrose & Glick, 2003). In our study, the ACC deaminase activities of all screened strains were above 20 nmol alpha-ketobutyrate mg^{-1} h^{-1} (Fig. 1). It is worth noting that the enzyme activity of the strain YX2 rose to 155 nmol alpha-ketobutyrate mg^{-1} h^{-1} far more than the known standard. Regarding that interesting result, Penrose & Glick (2003) have proposed a reasonable explanation by experiments. Namely, microorganisms with different levels of ACC deaminase activity do not have a consequent relationship with PGPR traits (Table 1). Same finding has been reported by Bal *et al*., (2013). Although the ACC deaminase activities of isolated strains were various, they exhibited similar performance in respect to some promoting traits, such as root length, root dry weight, shoot length. Thus, inoculation with rhizobacteria possessing ACC deaminase could play a vital role in alleviating the drought-induced injurious effects on the growth and development of plants.

Conclusion

Various plant growth-promoting traits such as arabinose, citrate utilization, urease, indol, glucose and melibiose have been found in the strains F1 (KJ465988), F2 (KJ465989), YX2 (KJ465990), and YL2 (KJ465991). They also exhibited some important characters, such as arginine decarboxylase, lysine decarboxylase, ornithine decarboxylase and citrate utilization (Table 1). The ACC deaminase activities ensure that the strains can survive using ACC as the only N source. Although *Klebsiella, Pseudomonas* and *Raoultella* have been studied under stress conditions, this is the first time that we found *Klebsiella oxytoca, Klebsiella variicola, Pseudomonas fluorescens* and *Raoultella planticola* producing ACC deaminase on dry-farming lands.

Acknowledgments

This research was supported by the Special Fund for Agro-Scientific Research in the Public Interest (201303104) and West Light Foundation of CAS (2060299-14), Environment Protection Program (2012-47) and Sci-tech Coordinating Innovative Engineering Projects of Shaanxi Province (2011KTZB02-02-05). Peng Zheng and Lixin Zhang give the greatest contributions to this work.

References

Adachi, K., M. Nakatani and H. Mochida. 2002. Isolation of an endophytic diazotroph, Klebsiella oxytoca, from sweet potato stems in Japan. *Soil. Sci. Plant Nutr*., 48: 889-895.

Ashraf, M. 2010. Inducing drought tolerance in plants: recent advances. *Biotechnol. Adv.*, 28: 169-183.

Bal, H.B., S. Das, T.K. Dangar and T.K. Adhya. 2013. ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. *J. Basic. Microbiol*., 53: 972-984.

Cho, S.M., B.R. Kang, S.H. Han, A.J. Anderson, J.Y. Park, Y.H. Lee, B.H. Cho, K.Y. Yang, C.M. Ryu and Y.C. Kim. 2008. 2R, 3R-butanediol, a bacterial volatile produced by Pseudomonas chlororaphis O_6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. *Mol. Plant Microbe Interaction*., 21: 1067-1075.

Dworkin, M. and J. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *J. Bacteriol*., 75: 592.

Fisher, P., O. Petrini and H.L. Scott. 1992. The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). *New Phytol*., 122: 299-305.

Fu, Q., C. Liu, N. Ding, Y. Lin and B. Guo. 2010. Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas* sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. *Agr. Water Manage.*, 97: 1994-2000.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol*., 41: 109-117.

Glick, B.R. 2004. Bacterial ACC deaminase and the alleviation of plant stress. *Advan. Appl. Microbiol*., 56: 291-312.

Glick, B.R., C. Liu, S. Ghosh and E.B. Dumbroff. 1997. Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Soil Biol. Biochem*., 29: 1233-1239.

Glick, B.R., Z. Cheng, J. Czarny and J. Duan. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. In: *New perspectives and approaches in plant growth-promoting rhizobacteria research*. (Eds.): P.A.H. Bakker, J. Raaijmakers, P. Lemanceau and G. Bloemberg. Springer, pp. 329-339.

Gregersen, T. 1978. Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur. J. Appl. Microbiol. Biotechnol.*, 5: 123-127.

Holt, J.G., N.R. Krieg, P.H. Sneath, J.T. Staley and S.T. Williams. 1994. *Bergey's manual of determinative bacteriology*. Williams and Wilkins, Baltimore.

Jha, P. and A. Kumar. 2007. Endophytic colonization of Typha australis by a plant growth-promoting bacterium *Klebsiella oxytoca* strain GR-3. *J. Appl. Microbiol.*, 103: 1311-1320.

Khalid A., M. Arshad and Zahir Z.A.. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol*., 96(3): 473-480.

Kloepper, J.W., R. Lifshitz and R.M. Zablotowicz. 1989. Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol.*, 7: 39-44.

Li, J., D.H. Ovakim, T.C. Charles and B.R. Glick. 2000. An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. *Current Microbiol.*, 41: 101-105.

Mayak S., T. Tirosh and Glick B.R.. 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Sci*., 166(2): 525-530.

Morgan, P.W. and M.C. Drew. 1997. Ethylene and plant responses to stress. *Physiol. Plant*, 100: 620-630.

Nadeem, S.M., Z.A. Zahir, M. Naveed, H.N. Asghar and M. Arshad. 2010. Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. *Soil Sci. Soc. Amer. J.*, 74: 533-542.

Penrose, D.M. and B.R. Glick. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant*, 118: 10-15.

Podschun, R. and U. Ullmann. 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev.*, 11: 589-603.

Rosenblueth, M., L. Martínez, J. Silva and E. Martínez-Romero. 2004. *Klebsiella variicola*, a novel species with clinical and plant-associated isolates. *Syst. Appl. Microbiol.*, 27: 27-35.

Safronova, V.I., V.V. Stepanok, G.L. Engqvist, Y.V. Alekseyev and A.A. Belimov. 2006. Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol. Fert. Soils*, 42: 267-272.

Saleem, M., M. Arshad, S. Hussain and A.S. Bhatti. 2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J. Ind. Microbiol. Biot.*, 34: 635-648.

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

Shah, S., J. Li, B.A. Moffatt and B.R. Glick. 1998. Isolation and characterization of ACC deaminase genes from two different plant growth-promoting rhizobacteria. *Can. J. Microbiol.*, 44: 833-843.

Singh, J.S., V.C. Pandey and D. Singh. 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agr. Ecosyst. Environ.*, 140: 339-353.

Tilak, K., N. Ranganayaki, K. Pal, R. De, A. Saxena, C.S. Nautiyal, S. Mittal, A. Tripathi and B. Johri. 2005. Diversity of plant growth and soil health supporting bacteria. *Curr. Sci.*, 89: 136-150.

Toklikishvili, N., N. Dandurishvili, A. Vainstein, M.Tediashvili, N. Giorgobiani, S. Lurie, E. Szegedi, B. Glick and L. Chernin. 2010. Inhibitory effect of ACC deaminase-producing bacteria on crown gall formation in tomato plants infected by *Agrobacterium tumefaciens* or A. *vitis*. *Plant pathol.*, 59: 1023-1030.

Zhou D.Q. 2006. *Microbiology Experimental Microbiology*. (3rd Ed) Higher Education Press.

(Received for publication 10 October 2013)