academicJournals

Vol. 7(50), pp. 5664-5668, 18 December, 2013
DOI: 10.5897/AJMR2013.1573
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Isolation and characterization of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-containing plant growth-promoting rhizobacteria from carnation soil and roots

Haiquan Huang[1,3]*#, Meijuan Huang[1]*#, Guosheng Fan[1]*, Xuefei Liu[1], Jihua Wang[2], Qing Duan[2] and Qixiang Zhang[3]*

[1]School of Landscape Architecture, Southwest Forestry University, Kunming 650224, China.
[2]Institute of Flower Research, Yunnan Provincial Academy of Agricultural Sciences, Kunming 650205 China.
[3]School of Landscape Architecture, Beijing Forestry University, Beijing 100083, China.

Accepted 8 February, 2013

Five strains of plant growth-promoting rhizobacteria (PGPR) with 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity were isolated from carnation soil and roots using ACC as the sole nitrogen source. Based on their growth morphological, microscopic cell properties and 16S rRNA sequence analysis, the results showed that three strains were identified as *Enterobacter* section and one as *Erwinia* among four strains from the soil, and one from carnation roots wAS identified as *Acinetobacter*; there are some differences in ACC deaminase activities among all isolated strains in this study. It is suggested that ACC deaminase-containing PGPR could be a cost-effective, environment-friendly and promising potential strategy to promote plant growth, alleviate biotic and abiotic stresses and ensure sustainable agriculture, especially for ethylene-sensitive flowers production.

Key words: 1-Aminocyclopropane-1-carboxylate (ACC), deaminase, plant growth-promoting rizobacteria, carnation, salinity stress.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are considered as advantageous bacteria in the rhizosphere, and helpful for sustainable agriculture by assisting plant growth and development directly or indirectly (Glick, 1995). Diverse mechanisms of different PGPR are reported on the basis of previous studies, including nitrogen fixation, phytohormone synthesis, mineral solubilization and phytopathogen prevention (biocontrol) (Glick and Bashan, 1997; Muhammad et al., 2007). PGPR exert some of these functions by means of specific enzymes, which agitate certain physiological and biochemical changes in plants. Among these enzymes, 1-aminocyclopropane-1-carboxylate (ACC) deaminase catalyses ACC, the immediate precursor of ethylene in higher plants, into ammonia and α-ketobutyrate (Glick et al., 1998). Ethylene is a well-known gaseous phytohormone among plant growth hormones, whose endogenous production has been correlated with various senescence processes (Abeles, 1973; Lieberman, 1979). Flowers are divided into two groups, ethylene-sensitive and ethylene-insensitive,

*Corresponding authors. E-mails: zqx@bjfu.edu.cn; 274955551@qq.com; meijuanhuang.swfu@gmail.com; haiquanhuang@gmail.com.

#These authors contributed equally to this work.

according to their productions of endogenous ethylene. A transient, rapid increase in ethylene concentration was indicated to be the cause of aging of ethylene-sensitive cut flowers (Halevy and Mayak, 1981; Woltering and van Doorn, 1988; Ludovica et al., 2011).

About half of arable lands in the world are saline (Jalili et al., 2009; Zahir et al., 2009). Yunnan province, located at the southwest part of China, is a semiarid region and the largest hub of carnation cultivation, in which most carnations are grown in the greenhouse. Because both the temperature and humidity in the greenhouse are always high, it results in high groundwater evaporation and plant transpiration and leads to salts solved in the ground water continuously moving toward the cultivation layer (De Clercq et al., 2009). Both biotic and abiotic stresses (such as salinity, heavy metals, drought and flooding) result in accelerating biosynthesis of ethylene (Kende, 1993; Johnson and Ecker, 1998) and affecting plant growth and crop production (Shao et al., 2007; Sajid et al., 2010). Carnation is economically one of the most important cut flowers cultivated for the flower market in the world. However, carnation has also been considered as a typical ethylene-sensitive flower, which suffer the saline and ethylene stresses in the greenhouse. Increasingly serious soil salinization in the cultivated layer leads to enormous losses of carnation yields and quality. Many researchers have clarified the positive effects of ACC deaminase-containing bacteria in the rhizosphere on degrading ACC to decrease or inhibit the ethylene production and alleviating different stresses on plant growth (Arshad et al., 2007; Cheng et al., 2007; Glick et al., 2007; Jalili et al., 2009; Wu et al., 2012). In this study, the bacterial strains with ACC deaminase activity from carnation cultivated soil and roots were screened and identified by using phenotypical, microscopic characters and 16S rDNA sequence analyses, which could be used as a bioinoculant to protect carnations against salinity stress environment, reduce or curb their ethylene output, promote their growth and prolong their supply time for the flower market.

MATERIALS AND METHODS

Isolation of bacteria

The soil and healthy root samples of carnation cultivated in greenhouse used for bacterial isolation were collected from several sites in Kunming, Yunnan Province, P. R. China. The bacteria were isolated using the dilution plate technique with Dworkin and Foster (DF) minimal salt medium containing $(NH_4)_2SO_4$ as the sole nitrogen source (Penrose and Glick, 2003), and ACC Dworkin and Foster minimal salt medium (ADF) with 3.0 mM ACC instead of $(NH_4)_2SO_4$ as the sole nitrogen source. The soil samples were treated by using the method of Glick et al. (2007) and roots using the method of Qin et al. (2008) with some modifications. Colonies that developed on the plates were subcultured repeatedly to acquire pure single colony, which was preserved on agar slants for further characterization and identification.

Growth curve

Five single colonies of the strains on the ADF medium plates were inoculated into the DF and ADF culture media at 28°C with vigorous shaking speed of 200 rpm, respectively. The photometric density of liquid culture at 600 nm was measured at specific intervals of time by repeating it thrice. The growth curve of different strains in different media was then drawn.

Measurement of ACC deaminase enzyme activity

Based on the initial experimental results, it was found that all the five strains could utilize ACC to grow well in ADF culture medium. The ACC deaminase activity of the strains was assayed according to the method of Penrose and Glick (2003). The calibration curve was plotted according to α-ketobutyric acid and protein concentration of cellular suspension after using toluene to treat cells. In order to draw the calibration curve of protein, bovine serum albumin (BSA) was used (Barbosa et al., 2009).

Bacterial characterization and 16S rDNA identification

The morphological and microscopic cell properties of the isolated strains were determined by using standard methods according to Bergey's Manual of Determinative Bacteriology (Holt, 1994). Besides, five bacterial strains were genetically confirmed by 16S rRNA gene sequence (Sangon Biotech (Shanghai) Co., Ltd. SongJiang Industrial Park) on the basis of comparative analysis on the GenBank database using the NCBI Blast program (Drancourt et al., 2001).

RESULTS AND DISCUSSION

It was found that carnation soil and roots samples contained several groups of bacteria when they were cultured on DF media. In order to search for some bacteria with ACC-degrading ability, carnation soil and roots were plated in DF medium without $(NH_4)_2SO_4$ supplemented with ACC as the sole nitrogen source for the isolation of ACC-degrading bacteria, meanwhile, *Bacillus subtilis* without ACC deaminase-containing activity was used as the control during all the screening procedures (Figure 1). The five colonies of ACC deaminase-containing bacteria, including four from soil (designated CS1, CS2, CS3 and CS4) and one from roots (designated CR1), were successfully screened by ADF media, respectively.

All the isolated bacteria had a marked ACC deaminase activity after incubation at 28°C, which could use ACC as a sole nitrogen substrate and catalyse it into ammonia and α-ketobutyrate. ACC deaminase activities of five different strains isolated in this study were measured and shown (Figure 2). We found that there existed much difference among the strains in the activities of ACC degradation, especially CS2 strain which had the highest level of ACC deaminase activity among the five isolated strains (Figure 2). Microscopic examination revealed that all the strains isolated in this study were Gram-negative, the cells appeared rods, and different strains had their own growth morphological characters (data not shown).

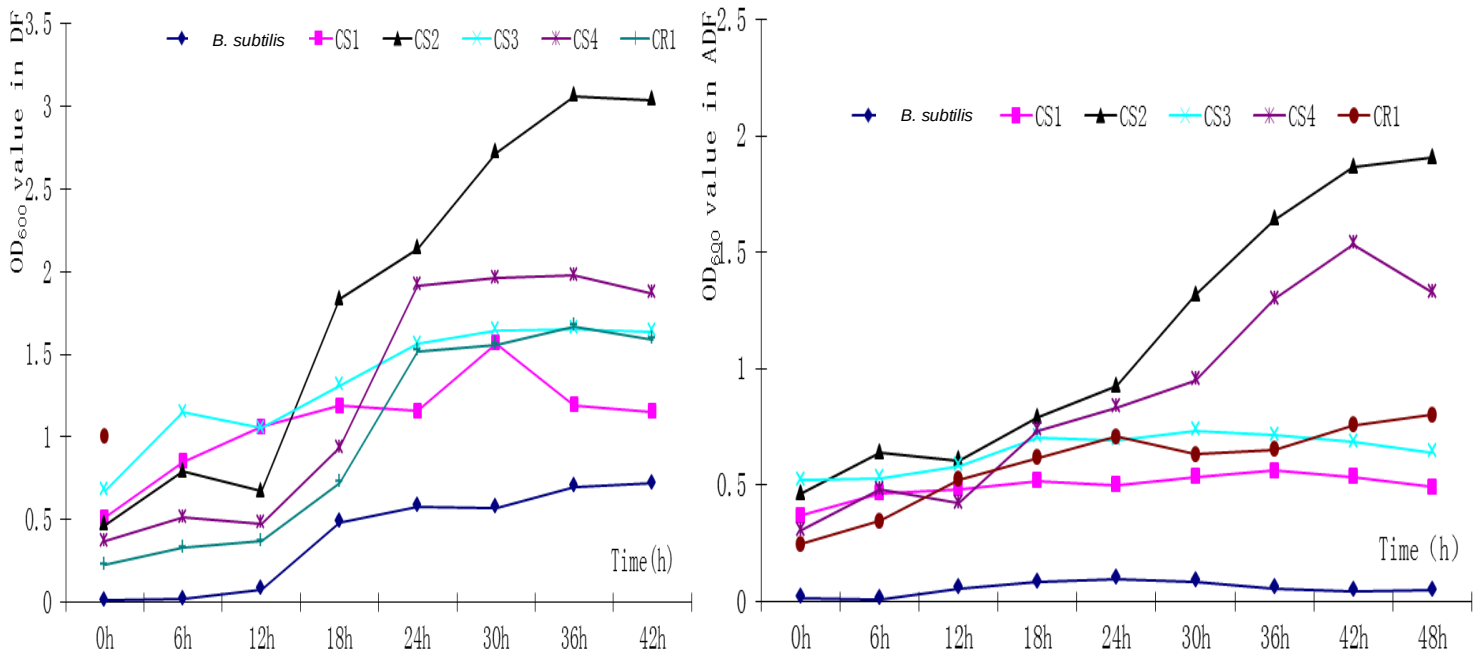

Figure 1. Growth of different isolated strains in DF and ADF culture media.

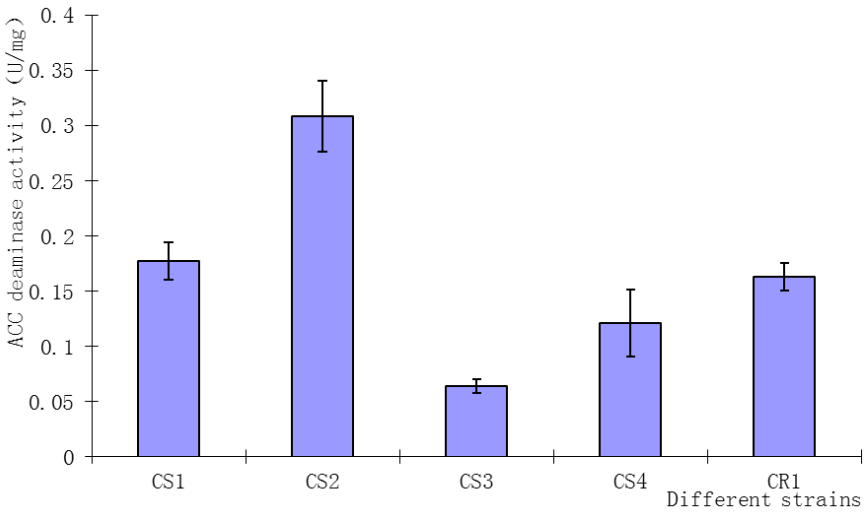

Figure 2. ACC deaminase activities of different strains isolated in this study. Error bar represents ± S.D (standard deviation) from the average of triplicates.

However, the contribution of ACC deaminase activity could be induced by ADF media. Growth of all the isolated strains was measured with the increase of turbidity at 600 nm. Figure 1 shows typical growth of the strains in the presence of $(NH_4)_2SO_4$. Growth was visualized with a perceivable lag phase and growth climax was obtained after 36 h. Relatively, weak growth occurred on ADF medium, unless it contained $(NH_4)_2SO_4$. The experiments showed that there existed some differences in the growth of different strains in ADF medium, which could be due to their different ACC deaminase activities.

The bacterial strains were consecutively confirmed by sequencing of 16S rRNA and genomic DNA analysis (Figure 3), which revealed that CS2 had 98.0% identity to the sequence of *Erwinia amylovora* (NR_041970.1), *Erwinia tasmaniensis* (NR_042422.1) and *Erwinia rhapontici* (NR_041976.1); CR1 showed 97.0% identity to the sequence of *Acinetobacter*; CS1, CS3 and CS4 possessed 99% identity to the sequence of *Enterobacter*. Thus, sequence analysis showed that different bacteria strains with ACC deaminase activity from diverse genus and family were able to degrade ACC.

Carnation, a typical ethylene-sensitive flower, produces ethylene by its autocatalytic pathway and accelerates the

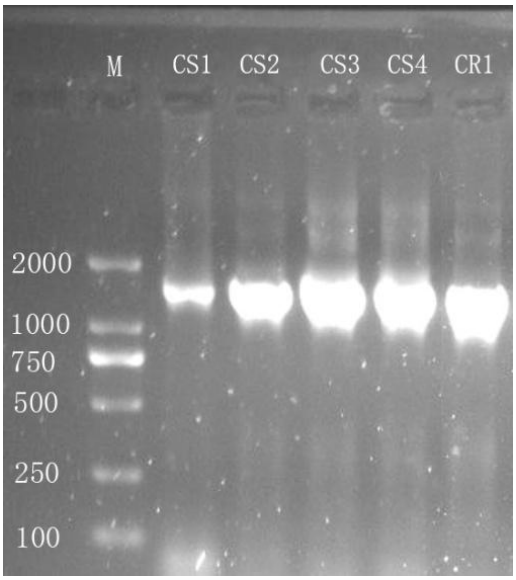

Figure 3. Agarose gel electrophoresis of PCR-amplified 16S rRNA of different isolated strains.

flower senescence (Rahemi and Jamali, 2011). Ethylene biosynthesis is influenced by many factors including inside and outside the tissues of flowers or plants. Some rhizospheric plant growth-promoting bacteria containing ACC deaminase have previously been proved to be able to slow the aging process of fresh cut carnation flower petals (Nayani et al., 1998). It has been demonstrated that ACC deaminase-containing bacterial endophytes can effectively delay the flower senescence (Ali et al., 2012). In this study, it was demonstrated that all the isolated strains had ACC deaminase activity, which were helpful to alleviate biotic and abiotic ethylene production and accumulation, and improve crop yield and quality, especially some ethylene-sensitive flowers and fruits. Furthermore, some strains may be applied as bio-inoculant for remediation of flower, fruit and crop cultivation, and their post-harvest technologies. It offers an environment-friendly and efficient method to regulate the plant ethylene for the production of some flowers and crops. More studies need to be undertaken to elucidate the mechanisms of different ACC deaminase-containing strains to promote different flower growth, strengthen their stress resistances, improve their production and quality, and extend their supply time of market and shelf life. The application of ACC deaminase-containing PGPR together with other innovations could prove to be cost-effective and environment-friendly strategy to ensure sustainable agriculture.

ACKNOWLEDGEMENTS

This work was supported by grants-in-aid from Natural Science Foundation of Yunnan Province, P. R. China (2011FB067), Provincial Key Discipline of Landscape Plants and Ornamental Horticulture, Yunnan Province, P. R. China and Provincial Key Lab of Colleges and Universities in Landscape Plants and Ornamental Horticulture, Yunnan Province, P. R. China, and Large Apparatuses Sharing Platform of Southwest Forestry University.

REFERENCES

Abeles F (1973). Ethylene in plant biology. Academic Press, London.
Ali S, Charles TC, Glick BR (2012). Delay of flower senescence by bacterial endophytes expressing 1-aminocyclopropane-1-carboxylate deaminase. J. Appl. Microbiol.113:1139-1144
Arshad M, Saleem M, Hussain S (2007). Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol. 25:356–362
Cheng Z, Park E, Glick BR (2007). 1-Aminocyclopropane-1-carboxylate deaminase from Pseudomonas putida UW4 facilitates the growth of canola in the presence of salt. Can. J. Microbiol. 53:912–918
De Clercq WP, Van Meirvenne M, Fey MV (2009). Prediction of the soil-depth salinity-trend in a vineyard after sustained irrigation with saline water. Agric. Water Manage. 96:395–404
Drancourt M, Bollet C, Carta A, Rousselier P (2001). Phylogenetic analyses of Klebsiella species delineate Klebsiella and Raoultella gen. nov., with description of Raoultella ornithinolytica comb. nov., Raoultella terrigena comb. nov. and Raoultella planticola comb. nov. Int. J. Syst. Evol. Microbiol. 51:925–932
Glick BR (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41:109–117
Glick BR, Bashan Y (1997). Genetic manipulation of plant growth promoting bacteria to enhance biocontrol of fungal phytopathogens. Biotechnol. Adv. 15:353–378
Glick BR, Cheng ZY, Czarny J, Duan J (2007). Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur. J. Plant Pathol. 119:329–339
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)
Halevy A, Mayak S (1981) Senescence and postharvest physiology of cut flowers. Part 2. Hort. Rev. 3:59–143
Holt JG (1994). Bergey's manual of determinative bacteriology, 3rd ed. Williams and Wilkins, Baltimore
Jalili F, Khavazi K, Pazira E, Nejati A, Rahmani HA, Sadaghiani HR, Miransari M (2009). Isolation and characterization of ACC deaminase - producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. J. Plant Physiol. 166: 667–674
Johnson PR, Ecker JR (1998). The ethylene gas signal transduction pathway: a molecular perspective. Annu. Rev. Genet. 32: 227–254.
Kende H (1993). Ethylene biosynthesis. Annu. Rev. Plant Biol. 44: 283–307.
Lieberman M (1979). Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol. 30:533–591
Ludovica S, Katia M, Marco D, Carlo R, Francesco T, Valentina S (2011). β-Cyclodextrin-based nanosponges as carriers for 1-MCP in extending the postharvest longevity of carnation cut flowers: an evaluation of different degrees of cross-linking. Plant Growth Regul. 65:505–511
Muhammad S, Muhammad A, Sarfraz H, Ahmad SB (2007). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J. Ind. Microbiol. Biotechnol. 34:635-648.
Nayani S, Mayak S, Glick BR (1998). Effect of plant growth-promoting rhizobacteria on senescence of flower petals. Indian J. Exp. Biol. 36:836–839.
Penrose DM, Glick BR (2003). Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plantarum 118:10–15
Qin S, Wang HB, Chen HH, Zhang YQ, Jiang CL, Xu LH, Li WJ(2008). Glycomyces endophyticus sp. nov., an endophytic actinomycete isolated from the root of Carex baccans Nees. Int. J. Syst. Evol. Microbiol. 58:2525–2528

Rahemi M, Jamali B (2011). Carnation flower senescence as influenced by nickel, cobalt and silicon. J. Biol. Environ. Sci. 5:147–152.
Sajid MN, Zahir AZ, Muhammad N, Muhammad A (2010). Microbial ACC-Deaminase: Prospects and Applications for Inducing Salt Tolerance in Plants. Crit. Rev. Plant Sci. 29: 360-393
Shao HB, Chu LY, Wu G, Zhang JH, Lu ZH, Hu YC (2007). Changes of some anti-oxidative physiological indices under soilwater deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. Colloids Surf. B 54: 143–149.
Woltering EJ, van Doorn WG (1988) Role of ethylene in senescence of petals—morphological and taxonomical relationships. J. Exp. Bot. 39:1605–1616
Wu ZS, Yue HT, Lu JJ, Li C (2012). Characterization of rhizobacterial strain Rs-2 with ACC deaminase activity and its performance in promoting cotton growth under salinity stress. World J. Microbiol. Biotechnol. 28:2383–2393
Zahir ZA, Ghani U, Naveed M, Nadeem SM, Asghar HN (2009). Comparative effectiveness of Pseudomonas and Serratia sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. Arch. Microbiol. 191: 415–424