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# ACC Deaminase from *Lysobacter gummosus* OH17 Can Promote Root Growth in *Oryza sativa* Nipponbare Plants

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Supporting Information

**ABSTRACT:** Although *Lysobacter* species are a remarkable source of natural compounds with antibacterial and antifungal activities, the ability of these bacteria to produce plant growth promoters remains practically unknown. In this work, the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) has been isolated from the secretions of *Lysobacter gummosus* OH17, indicating the presence of an ACC deaminase, which was shown to be encoded in the gene *peg_1256*. The recombinant enzyme could not only deaminate ACC to provide 2-oxobutanoic acid but also catalyzed the amination of the 2-oxobutanoic acid, demonstrating, for the first time, that ACC deaminases can produce ACC. After the treatment of rice *Oryza sativa* Nipponbare plants with OH17 ACC deaminase, the ethylene production levels were 44% higher in comparison with the control experiments, allowing significant improvements in root, 10%, and stem, 14%, growth.

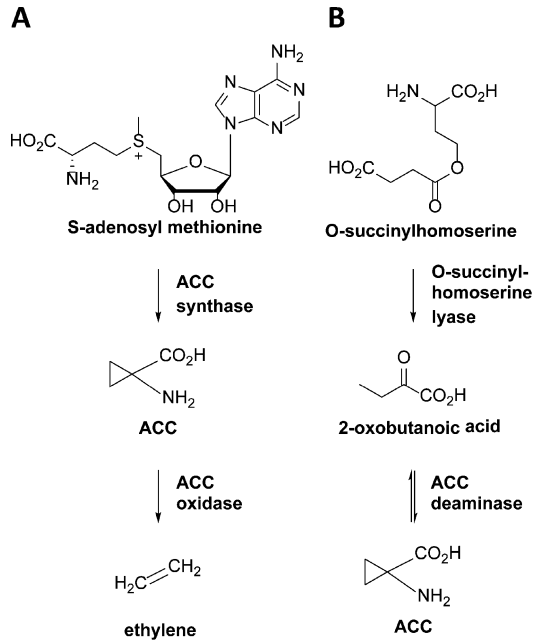
**KEYWORDS:** *ACC deaminase, Lysobacter, Oryza sativa Nipponbare, plant growth, rice growth promoter*

## INTRODUCTION

Ethephon is the most frequent plant growth regulator and has contributed in a significant manner to spectacular improvements in crop productivity.[1−4] For example, ethephon is commonly used to promote the growth of wheat, coffee, tobacco, cotton, vine, or rice to mature fruits or to initiate the reproductive development of pineapple. However, the toxicity of this agent inducing the synthesis of peroxides and then enhancing the ozone damage in plants, as well as the demonstrated cytotoxicity in animals, has stimulated a global concern and the regulation in several countries.[5−10] In this sense, the use of environmental-friendly alternatives, including 1-aminocyclopropane-1-carboxylate (ACC) deaminases, has been postulated as one of the most suitable ways to overcome the above-mentioned problems.[11,12] ACC is an intermediate included in the methionine cycle of plant roots which is obtained throughout ACC synthase-catalyzed hydrolysis and the oxidation of S-adenosyl-L-methionine, and it can be transformed to a plant growth hormone ethylene in the presence of ACC-oxidase (Scheme 1A).[13] ACC deaminases have been found in several soil bacteria.[14−17] ACC deaminases are capable of catalyzing the deamination of ACC to provide 2-oxobutanoic acid (Scheme 1B). It has been reported that bacteria which encode ACC deaminases are able to promote plant growth in plants when the bacteria interact with the plant root.[18−22]

*Lysobacter* is a soil bacterium which has been shown to be an interesting source of active secondary metabolites.[23] For example, antifungal metabolites "heat stable antifungal factor" (HSAF) and *p*-aminobenzoic acid were found in *Lysobacter enzymogenes* OH11 and *Lysobacter antibioticus* OH13, respectively, or a new family of cyclic lipodepsipeptides, WAP-8294A, with potent activity against Gram-positive bacteria was discovered in both *Lysobacter* sp. 8294 and *Lysobacter enzymogenes* OH11.[24−27] In spite of the relevant advances in

**Scheme 1. 1-Aminocyclopropane-1-carboxylic Acid (ACC) in Biosynthetic Routes[a]**




[a](A) Biosynthesis of ethylene in plants. (B) Biosynthetic route to ACC in *Lysobacter gummosus* OH17 starting from *O*-succinylhomoserine.

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the discovery of new biocontrol agents with antibacterial and antifungal activities for the treatment of plant diseases, the ability of these bacteria to produce plant growth promoters remained practically unknown. In this field, Fürnkranz and co-workers reported in 2012 an increase in germination rates as well as in the mean values of harvest yields of Styrian oil pumpkins after treatment of the pumpkin seeds with *Lysobacter gummosus* L101.[28] Although this important contribution suggested the presence of plant growth regulators in *Lysobacter* species apart from antibacterial and antifungal metabolites, no plant regulator was reported. For this reason, we decided to identify the active agent responsible for this function in *Lysobacter gummosus* to demonstrate that *Lysobacter* species can be an interesting tool for plant growth promotion. In this work, ACC has been isolated from *Lysobacter gummosus* OH17 secretions, indicating OH17 encodes an ACC deaminase. The gene encoding the 1-aminocyclopropane-1-carboxylate deaminase in OH17 has been identified. Finally, the effect of OH17 ACC deaminase in *Oryza sativa* Nipponbare metabolism and growth has been studied.

## MATERIALS AND METHODS

**General.** All reagents and chemicals were used as received from commercial suppliers without further purification or modification. NMR spectra were registered on a Bruker AV-400 instrument using the deuterated DMSO residual solvent signal as the internal standard. ESI mass spectrum was obtained on an AB (QTRAP 6500) mass spectrometer.

**Data Analysis.** The statistical analyses were performed using SPSS (Statistical Package, Version 20.0). The variables were subjected to student *t* test and were tested for significance at the $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$(****) level.

**Bacterial Strains.** *Lysobacter gummosus* OH17 was isolated from the rhizosphere of *Oryza sativa* Nipponbare rice. The *E. coli* DH5*α* strain was used as the host cell for general DNA propagation and *E. coli* BL21(DE3) for the overexpression of the ACC deaminase. *Lysobacter gummosus* OH17 (CGMCC No.8649) and *E. coli* were grown in lysogeny broth (LB) medium (10 g of triptone, 5 g of yeast extract, 10 g of sodium chloride, pH 7.0−7.2, in one liter of distilled water).

**Large Scale Fermentation and Isolation of ACC.** *Lysobacter gummosus* OH17 was cultivated for 24 h in a 250 mL flask containing 50 mL of LB medium at 28 °C. The culture was then transferred into 200 mL of production medium in a 1 L flask (total of 50 flasks). The flasks were shaken at 180 rpm and 28 °C for 24 h ($OD_{600} \approx 2.0$). The culture was centrifuged for 20 min at 20 000 rpm at 4 °C, and the supernatant was collected. Then, the solvent was evaporated under reduced pressure. The residue was washed with ethyl acetate (200 mL, 3 times) and with ethyl acetate/methanol 1:1 (200 mL, 3 times). The resulting oil (6.2 g) was dissolved in a mixture acetone/water 1:1 (300 mL) and treated with fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) (1.5 g, 5.6 mmol) in the presence of sodium hydrogen carbonate (1 g, 11.9 mmol). The reaction was stirred at room temperature for 16 h. Fmoc-labeled ACC was purified by silica gel chromatography using a mixture of EtOAc/MeOH 9:1 as the eluent (Rf = 0.7). The purification was monitored by HPLC. HPLC conditions: reverse phase HPLC (Shimadzu MS-2020, Tokyo, Japan) at 310 nm using a C-18 column (250 × 4.6 mm, Phenomenex). The mobile phase was 20−30% $CH_3CN$ in $H_2O$ from 0 to 20 min, 90% $CH_3CN$ in $H_2O$ from 20 to 30 min, and 20% $CH_3CN$ in $H_2O$ from 30 to 35 min ($H_2O$ containing 0.04% trifluoroacetic acid). The peak corresponding to the Fmoc-labeled ACC appeared at 6.2 min. Fractions which contained the desired pure compound were evaporated to dryness under reduced pressure. The purified compound (7.2 mg) was characterized by NMR and mass spectrometry. Mentioned labeling conditions allowed the detection of ACC during the silica gel purification but not the quantification of ACC.

$^1$H NMR (400 MHz, DMSO-$d_6$, $\delta$): 7.94 (s, 1H), 7.89 (d, 2H, $J$ = 7.6 Hz), 7.70 (d, 2H, $J$ = 7.2 Hz), 7.41 (t, 2H, $J$ = 7.2 Hz), 7.33 (t, 2H, $J$ = 7.6 Hz), 4.31−4.24 (m, 2H), 4.23−4.18 (m, 1H), 1.30 (dd, 2H, $J$ = 7.2, 4.4 Hz), 0.99 (dd, 2H, $J$ = 7.2, 4 Hz). $^{13}$C NMR (100 MHz, DMSO-$d_6$, $\delta$): 170.43, 156.35, 143.84, 140.72, 127.63, 127.07, 125.27, 120.11, 72.50, 65.47, 63.09, 16.60. HRMS (ESI): $[M + Na]^+$ Calcd. for $C_{19}H_{17}NNaO_4$, 346.1055; found, 346.1053.

**Identification of the Biosynthetic Pathway and Overexpression Experiments.** The protein sequences of 1-aminocyclopropane-1-carboxylate deaminase (EC 3.5.99.7) and *O*-succinylhomoserine lyase (EC 2.5.1.48 or EC 4.2.99.9) were identified by local BLAST (BioEdit Version 7.0.9.0) using the protein sequences of *Pseudomonas entomophila* strain PS−PJH (FJ882923.1) and *Cicer arietinum* cultivar ICC4958 (CM003628.2), respectively, as templates. The sequences of *peg_1256* (1-aminocyclopropane-1-carboxylate deaminase, Genbank number MG182711) or *peg_5321* (*O*-succinylhomoserine lyase, Genbank number MG182712) were used to design overexpression-specific primers. The *peg_1256* DNA fragment was amplified by PCR using the following primers: 5′-CCCAAGCTTCATGTCCGCCGATCCGATCGC-3′ and 5′-TGCTCTAGATCAGAAGTGCAAGCCTTCGC-3′. The *peg_5321* DNA fragment was amplified by PCR using the following primers: 5′-CCCAAGCTTCCCGCCAATCTACGCCACCTCG-3′ and 5′-TGCTCTAGACTATGCACCCAAGCGAGGCGCCGATCG-3′. The genes were amplified by a polymerase chain reaction (PCR) using Tks Gflex DNA Polymerase (Takara) and genomic DNA as templates according to the manufacturer's instructions. Briefly, the PCR amplification was performed using 30 PCR cycles consisting of denaturation at 98 °C for 10 s, annealing at 55 °C for 15 s, and elongation at 68 °C for 1 min. The PCR fragments were double digested by *Xba*I and *Hind*III, ligated to the overexpression vector pBBR1MCS-5 (a $P_{lac}$ broad-host-range vector), and transferred to DH5*α*. Then, the desired recombinant vectors were confirmed by using universal primers M13F/M13R.[29] Sequenced recombinant vectors were transformed into wild-type OH17 by electroporation for constructing the overexpression strain.

**Disruption of *peg_1256*.** The disrupted mutant of *peg_1256* was constructed after amplifying the internal fragment of *peg_1256* by PCR using the following primers: 5′- CGCGGATCCTTCGAAAACGACCTGGCCCAT-3′ and 5′- TGCTCTAGAACAGCACATAGGTGCGGTTGCC-3′; which was later digested using *Xba*I and *BamH*I and ligated to the suicide vector pJQ200SK. The suicide vector pJQ200SK contained a vegetative replication region of plasmid R6K, but lacked the *pir* gene, avoiding its replication in OH17. The suicide vector also contained the origin of a conjugal transfer region, so it could be mobilized by the transfer system. Thus, the suicide vector containing the internal fragment of *peg_1256* could be transferred into wild-type OH17 by electroporation and inserted into the internal region of *peg_1256* by single crossover recombination. The transconjugant showed gentamycin resistance (25 mg/L).

**Quantification of ACC in OH17 Secretions.** OH17 containing the overexpression vectors of *peg_1256* and *peg_5321* or the disruption vector of *peg_1256* was cultivated in 10 mL LB medium at 28 °C 180 rpm in the presence of 25 mg/L gentamycin. After 48 h, the culture showed $OD_{600} \approx 2$ and was centrifuged (6 min, 6000 rpm). The upper phase was collected. One hundred microliters of this solution was mixed with 40 $\mu$L of sodium bicarbonate (1 M) and 100 $\mu$L of Marfey's reagent solution (0.1 M) in acetone. The reaction solution was stirred at 180 rpm at 37 °C for 2 h. Fifty microliters of the reaction was directly injected in the HPLC. HPLC conditions: reverse phase HPLC (Shimadzu MS-2020, Tokyo, Japan) at 350 nm using a C-18 column (250 × 4.6 mm, Phenomenex) at 35 °C. The mobile phase was 0−60% $CH_3CN$ in $H_2O$ from 0 to 60 min, 90% $CH_3CN$ in $H_2O$ from 60 to 70 min, and 0% $CH_3CN$ in $H_2O$ from 70 to 75 min. Labeled ACC appeared at 40.2 min retention time. The concentration was calculated according to the peak area. Commercial ACC was used as the standard for the linear calibration between 0.5 and 10 mg/L. OH17 containing the commercial overexpression vector pBBR1MCS-5 without any additional gene was used as the control. To confirm that the peak at 40.2 min in OH17 corresponded to ACC (Marfey's


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reagent appeared at 44.1 min), the peak was purified by HPLC and studied by mass spectrometry (AB (QTRAP 6500) mass spectrometer) detecting a $m/z$ value of 352.0896, which was in agreement with the expected molecular weight $[M - H]^-$ = 352.0893.

**Detection and Quantification of 2-Oxobutanoic Acid in OH17.** To detect 2-oxobutanoic acid by HPLC, 2-oxobutanoic acid was labeled according to the conditions reported by Laborda and co-workers for the labeling of sialic acids.[30] Thirty milliliters of the overexpression experiments of *peg_1256* and *peg_5321* or disruption experiment of *peg_1256* in LB medium ($OD_{600} \approx 2$) in the presence of 25 mg/L gentamycin was submitted to cell lysis for 20 min at 0 °C using an ultrasonic machine (Ultrasonic Homogenizer, Scientz-IID). The resulting suspension was centrifuged (6000 rpm, 5 min), and the upper phase was evaporated under reduced pressure. The obtained oil was dissolved in 5 mL water. One hundred and twenty microliters of this solution was mixed with 20 μL of the aqueous solution of 1,2-diaminobenzene (0.1 M) and $NaHSO_3$ (0.2 M). The reaction mixture was kept at 80 °C for 1 h. Fifty microliters of the reaction was directly injected in the HPLC. HPLC conditions: reverse phase HPLC (Shimadzu MS-2020, Tokyo, Japan) at 450 nm using a C-18 column (250 × 4.6 mm, Phenomenex) at 35 °C. The mobile phase was 0−60% $CH_3CN$ in $H_2O$ from 0 to 60 min, 90% $CH_3CN$ in $H_2O$ from 60 to 70 min, and 0% $CH_3CN$ in $H_2O$ from 70 to 75 min. Labeled 2-oxobutanoic appeared at a 31.7 min retention time. The concentration was calculated according to the peak area. Commercial 2-oxobutanoic acid was used as the standard for the linear calibration between 0.05 and 1 mg/L. OH17 containing the commercial overexpression vector was used as the control. To confirm that the peak at 31.7 min in OH17 corresponded to 1,2-diaminobenzene-labeled 2-oxobutanoic acid, the peak was purified by HPLC and studied by mass spectrometry (AB (QTRAP 6500) mass spectrometer) detecting a $m/z$ value of 175.0872, which was in agreement with the expected molecular weight $[M + H]^+$ = 175.0871.

**Cloning, Expression in *E. coli*, and Purification of ACC Deaminase from OH17.** The total *peg_1256* DNA fragment was amplified by PCR using the following primers: 5′-GGGAATTCCA-TATGTCCGCCGATCCGATCGC-3′ and 5′- GACAAGCTTTCA-GAAGTGCAAGCCTTCGCGCCCGGCGAT-3′. Amplification was performed by PCR using Tks Gflex DNA Polymerase (Takara) and genomic DNA as templates according to the manufacturer's instructions. Briefly, the PCR amplification was performed using 30 PCR cycles consisting of denaturation at 98 °C for 10 s, annealing at 55 °C for 15 s, and elongation at 68 °C for 40 s. The PCR fragments were double digested by *Nde*I and *Hind*III, ligated to the over-expression vector pCold II (Takara), and transferred to *E. coli* DH5α to construct the overexpression plasmid. Then, the desired recombinant vectors were confirmed using universal primers pCOLD-F/pCOLD-R. Sequenced recombinant vectors were transformed into *E. coli* BL21(DE3) competent cells (Takara) by electroporation for constructing the overexpression strain. *E. coli* BL21(DE3) cells containing pCOLDII-*peg_1256* were cultured in 2 L of LB medium with 100 μg/mL of ampicillin at 180 rpm and 37 °C to $OD_{600} \approx 0.5$. Then, 0.5 mM IPTG were added to the culture, which was incubated at 180 rpm and 15 °C for 24 h. Then, the cells were collected by centrifugation at 4 °C and suspended in 10 mL Tris-NaCl buffer (300 mM NaCl, 50 mM Tris-Cl, pH 7.9). Cell lysis was carried out through sonication using an Ultrasonic Disruptor SCIENTZ-IID (Scientz, China) for 10 min. The supernatant was analyzed by SDS-PAGE (8% gel) to confirm the overexpression of the enzyme. The target recombinant ACC deaminase was purified using a His60 Ni Superflow Resin column (Takara). The concentration of dialyzed recombinant ACC deaminase was detected using a BCA Protein Assay Kit (Takara).

**In Vitro Amination and Deamination Reactions Using Recombinant ACC Deaminase.** Amination and deamination reactions were performed in 1 mL eppendorf tubes shaken at 180 rpm and 37 °C for 24 h. For the deamination reaction, 5 μL of a 0.1 M solution of ACC was added into an aqueous solution (195 μL) containing 323 μg of recombinant ACC deaminase. No buffer was used in the deamination reaction. For the amination reaction, 10 μL of a 0.1 M solution of 2-oxobutanoic acid and 150 μL of a 0.1 M solution of ammonia (pH = 8.0) were added into an aqueous solution (40 μL) containing 323 μg of recombinant ACC deaminase. No buffer was used in the amination reaction. To control the reaction, 1 μL of the reaction solution was poured in a TLC plate after 0, 1, 2, 4, 12, and 24 h of reaction time. A mixture of EtOAc/MeOH/AcOH 2:2:1 was used as the eluent. ACC (Rf = 0.5) was observed after staining with a ninhydrin-containing solution (stain solution was prepared through the addition of 250 mg of ninhydrin in a 200 mL EtOH/AcOH 3:1 solution).

**Detection of Ethylene in *Oryza sativa* ssp. *japonica* cv. Nipponbare Plants.** To carry out the studies, a sterilized minimal medium consisting of 200 mg of glucose, 100 mg of ammonium chloride, 100 mg of magnesium chloride, and 100 mg of calcium chloride per liter of distilled water was used. Four parallel experiments were performed. Thirty rice seeds were used for each experiment. Rice seeds were submerged in 4 bottles containing 100 mL of water. After 16 h at 37 °C, the water was discarded and *Lysobacter gummosus* OH17 in a minimal medium (100 mL), minimal medium (100 mL), and minimal medium with 1.2 mg/L ACC (100 mL) or mutant OH17 after disruption of gene *peg_1256* in minimal medium (100 mL) were poured in each bottle. The 3 last experiments were carried out as control for further comparisons. To prepare the culture of wild-type and disrupted OH17 in minimal medium, 50 μL of a LB culture containing the corresponding strain ($OD_{600} \approx 2$) was poured into 100 mL of minimal medium ($1 \times 10^6$ cells/mL). A minimal medium including 25 mg/L gentamycin was used for the disruption bacteria. The four suspensions were incubated at 28 °C and 80 rpm during 12 h. Then, the seeds of each bottle were transferred into 8.5 cm diameter Petri dishes with cotton. Three milliliters of fresh water were poured in each dish to maintain a high level of humidity. The dishes were incubated at 37 °C for 48 h. Then, the germinated seeds were transferred to 4 different pots containing 800 mL of water. The pots containing the germinated rice seeds were incubated for 7 days at 30 °C (for 12 h per day) and 28 °C (for 12 h per day) under artificial light (12 h of artificial light per day). The root was submerged in water during the cultivation but not the stem, hydroponic culture according to conditions reported by Liang and co-workers.[31,32] After 7 days, 7−8 plants (with average size in comparison with the rest of the plants in the pot) were stored in a closed 20 mL vial containing 1 mL of water for 1 day. Two milliliters of the gas inside the vial were injected in a gas chromatography instrument (Agilent Technologies 7890A GC System, Agilent HP-PLOT-Q capillary column). Chromatograms were recorded using a WEL-PLO Q column, FID detector, and 1 mL/min gas flow rate. Column temperature: 32 °C from 0 to 1 min, 32−70 °C from 1 to 2 min, 70 °C from 2 to 3 min, and 70−160 °C from 3 to 12 min. The injector and detector temperature were 200 and 250 °C, respectively. The peak corresponding to ethylene appeared at 5.8 min. Ethylene concentration in the plant was calculated according to the peak area. Commercial ethylene was used for the calibration between 10 and 0.05 ppb. The experiments were repeated 5 times.

**Effect of *Lysobacter gummosus* OH17 in *Oryza sativa* ssp. *japonica* cv. Nipponbare Plants Growth.** To calculate *Oryza sativa* growth, the treatments and cultivation procedures described in the Detection of Ethylene in Oryza sativa ssp. japonica cv. Nipponbare Plants Section were followed. After 7 days of cultivation of the germinated seeds, stem and root length were measured, and the root hair of each plant was studied using an Olympus MDX6-T microscope. Experiments were repeated 5 times. Standard deviation calculated using Microsoft Excel 2010 was used to quantify the dispersion between the average values of each experiment.

## RESULTS AND DISCUSSION

**Purification and Identification of ACC in OH17 Secretions.** The improvement of the harvest yields of Styrian oil pumpkin in the presence of *Lysobacter gummosus* reported by Fürnkranz and co-workers, as well as the genome of this bacteria, suggested that OH17 encoded an ACC deaminase.[28] ACC is a nonproteinogenic α-amino acid which consists in a


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J. Agric. Food Chem. 2018, 66, 3675−3682

cyclopropane ring, wherein the carboxylic acid and the amine are linked to the same carbon. Thus, it is a nonaromatic polar structure difficult to purify. For this reason, we decided to label ACC with fluorenylmethyloxycarbonyl (Fmoc), a big polar aromatic protecting group selective for amines. The labeled compound could be easily purified by silica gel chromatography. In order to guarantee the correct isolation of Fmoc-labeled ACC, silica gel purification was monitored not only by thin-layer chromatography but also by HPLC. The peak corresponding to ACC appeared at 5.8 min in the HPLC chromatogram (Figure 1A). The retention time of the isolated compound was in accordance with commercial ACC, suggesting the existence of ACC in OH17 cultures. To confirm the desired structure, monodimensional experiments, $^{1}$H NMR and $^{13}$C NMR, were collected in deuterated dimethyl sulfoxide (Figures S1 and S2). $^{1}$H NMR spectrum of Fmoc-labeled ACC showed 2 doublets of doublets at 1.30 and 0.99 ppm with coupling constants of 7.6 and 4.4 Hz, corresponding to the hydrogens of the cyclopropane ring. The protons in the *cis* position with respect the carboxylic acid appeared more deshielded, at 1.30 ppm, in comparison with the protons in the *trans* position, 0.99 ppm. Since there is a plane of symmetry in the bond which links both protonated carbons of the cyclopropane ring, the 2 protons in *cis* of the carboxylic acid appear as a single signal as do the 2 protons in *trans* of the carboxylic acid. The rest of the signals of the $^{1}$H NMR spectrum were assigned to the Fmoc moiety. The $^{13}$C NMR spectrum showed a signal at 174.30 ppm, matching with the carboxylic acid. The signal corresponding to the tetrasubstituted carbon of the cyclopropane ring appeared at 65.47 ppm. As expected, only one signal at 16.60 ppm could be detected for the other 2 carbons of the cyclopropane ring due to the plane of symmetry. Mass spectrometry revealed a 346.1053 $m/z$ peak, which is in well accordance with the calculated mass of $[M + Na]^+$ = 346.1055 (Figure 1B).

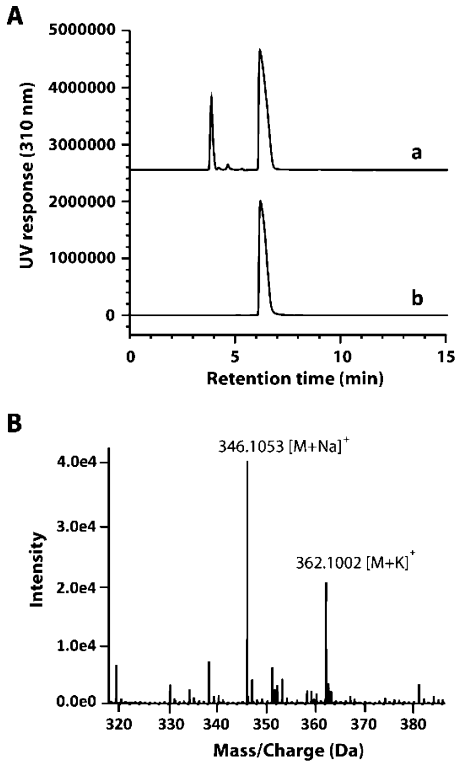


**Figure 1.** Purification and identification of ACC in *Lysobacter gummosus* OH17 secretions. (A) HPLC-based study of ACC after labeling with fluorenylmethyloxycarbonyl chloride (Fmoc-Cl). (a) Chromatogram corresponding to the secreted metabolites from OH17. (b) Chromatogram corresponding to Fmoc-labeled ACC used as a standard. (B) Mass spectrometry analysis of Fmoc-labeled ACC isolated from OH17 secretions.

**Identification of ACC Biosynthetic Pathway.** As mentioned in the Introduction Section, there are important differences between the biosynthetic pathways of ACC in plants and in bacteria. The rational biosynthetic pathway in bacteria consists in the transformation of succinylhomoserine into 2-oxobutanoic acid, and then 1-aminocyclopropane-1-carboxylate deaminase-catalyzed amination of 2-oxobutanoic acid provides ACC.[33] *O*-succinylhomoserine links the biosynthesis of ACC to bacterial methionine and cysteine metabolism. Since ACC was found in OH17 secretions, we could assume that 1-aminocyclopropane-1-carboxylate deaminase and *O*-succinylhomoserine lyase would be encoded in the OH17 genome. Bioinformatic analysis was used to search for the corresponding genes on the *Lysobacter gummosus* OH17 genome. Homology analysis revealed that *peg_5321*, encoding 391 amino acids, was homologous to *O*-succinylhomoserine lyase proteins from *Cicer arietinum* (56%), *Mycobacterium ulcerans* (69%), and *Helicobacter pylori* (70%). We also found that *peg_1256*, encoding 408 amino acids, shared a high identity in comparison with 1-aminocyclopropane-1-carboxylate deaminase proteins from *Azospirillum lipoferum* (39%), *Bradyrhizobium diazoefficiens* (38%), and *Pseudomonas entomophila* (39%) (Figures S3 and S4).

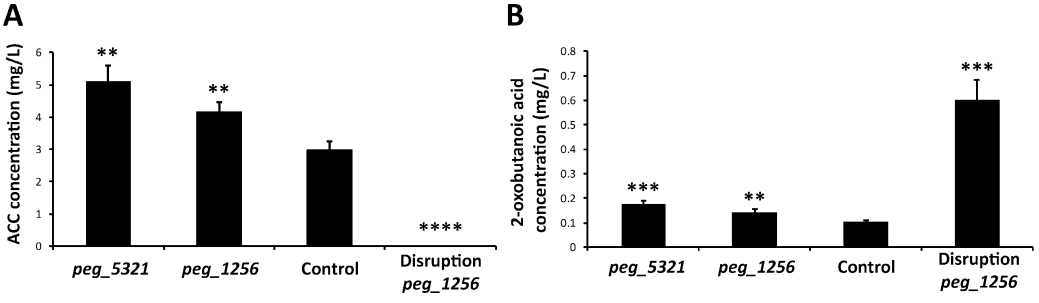


**Figure 2.** Analysis of the concentration of (A) ACC (mg/L) and (B) 2-oxobutanoic acid (mg/L) in the overexpression experiments of genes *peg_1256* (1-aminocyclopropane-1-carboxylate deaminase) and *peg_5321* (*O*-succinylhomoserine lyase) in OH17, in OH17 containing the commercial expression vector without the incorporation of any gene (as control experiment), and mutant OH17 after disruption of *peg_1256* (1-aminocyclopropane-1-carboxylate deaminase).



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J. Agric. Food Chem. 2018, 66, 3675−3682

Overexpression of genes *peg_1256* and *peg_5321* in *Lysobacter gummosus* OH17 showed higher concentration levels of ACC in comparison with the control bacteria, indicating that both genes are involved in the biosynthesis of ACC (Figure 2A). ACC (2.97 mg/L) could be detected using OH17 after the transformation of the commercial overexpression vector without any additional genes, whereas the overexpression of *peg_1256* and *peg_5321* provided 4.17 and 5.12 mg/L ACC, respectively. After the disruption of gene *peg_1256*, the signal corresponding to ACC disappeared demonstrating that an ACC deaminase is encoded in *peg_1256* (Figure S5). In order to confirm the role of the encoded enzymes, the concentration of the intermediate 2-oxobutanoic acid in the overexpression experiments was calculated (Figures 2B and S6). The concentration of 2-oxobutanoic acid increased in the overexpression of *peg_5321*, 0.18 mg/L, in comparison with the concentration detected in the control experiment, 0.10 mg/L. This result can be explained considering that *peg_5321* encodes an *O*-succinylhomoserine lyase which transforms *O*-succinylhomoserine into 2-oxobutanoic acid, and thus the concentration of 2-oxobutanoic acid increases. On the other hand, the concentration of 2-oxobutanoic acid in the overexpression of *peg_1256*, 0.14 mg/L, was higher in comparison with the control experiment, which confirmed that *Lysobacter gummosus* OH17 ACC deaminase is able to catalyze the reversible conversion between 2-oxobutanoic acid and ACC, producing an equilibrium between both structures. As we can see in Figure 2, the concentrations of ACC in OH17 were higher in comparison with the detected concentrations of 2-oxobutanoic acid, suggesting that the equilibrium must be mainly situated in the ACC site. In agreement with these results, the concentration of 2-oxobutanoic acid significantly increased after disruption of *peg_1256*, 0.60 mg/L. Obtained results situate *Lysobacter gummosus* OH17 among the soil bacteria, which encodes 1-aminocyclopropane-1-carboxylate deaminase and, for this reason, an interesting tool for plant growth promotion. Homologous genes as *peg_1256* could be found in other *Lysobacter* species, including *Lysobacter antibioticus*, *Lysobacter capsici* AZ780, *Lysobacter daejeonensis* GH1−9, *Lysobacter enzymogenes*, *Lysobacter* sp. Root690, or *Lysobacter spongiicola* DSM2.

Although the ability of ACC deaminase to catalyze the deamination of ACC to provide 2-oxobutanoic acid has been deeply explored,[18,34] the capacity of this enzyme to catalyze the amination of 2-oxobutanoic acid to ACC has never been reported before. In order to prove the new role of ACC deaminase, the ACC deaminase from OH17 was cloned, expressed in *E. coli*, and purified. The recombinant enzyme was shown to be able to catalyze the deamination reaction of ACC (Figure S7A) but not the deamination of other amino acids, such as L-Ser and L-Cys.[16] ACC deaminase-catalyzed synthesis of ACC via 2-oxobutanoic acid amination was observed in the presence of ammonia, confirming that the ACC deaminase is capable of producing ACC in the presence of high concentrations of ammonia (Figure S7B). Thus, the equilibrium between ACC and 2-oxobutanoic acid is controlled by the concentration of ammonia in the medium, which is consistent with the higher concentration of ACC in comparison with 2-oxobutanoic acid observed in the bacteria cultures using a medium rich in nitrogenated compounds.

**Influence of OH17 in the Metabolism of *Oryza sativa* Nipponbare Plants.** The participation of ACC in the biosynthesis of ethylene in rice plants has been reported before.[35−37] In this field, Zarembinski and Theologis described that both anaerobiosis and plant growth hormones, mainly auxins, were able to induce 2 genes encoding ACC synthases in *Oryza sativa* plants, promoting the plant growth.[36] Later, Zhou and co-workers described the rapid induction of a novel ACC synthase in deep water *Oryza sativa* seedlings, indicating a direct connection between the anaerobiosis and the production of auxins.[37] It was suggested that the oxygen deprivation promoted the production of auxins and, thus, the production of ethylene in the rice plants.[37]

To study the influence of *Lysobacter gummosus* OH17 ACC deaminase in the metabolism of rice plants, the concentration of ethylene in treated plants after 7 days of cultivation was measured by gas chromatography (Figures 3 and S8). The

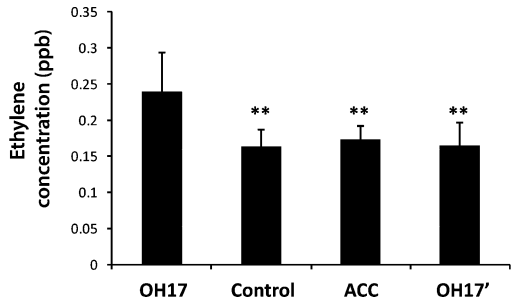


**Figure 3.** Concentration of ethylene (ppb) in *Oryza sativa* Nipponbare plants after treatment with *Lysobacter gummosus* OH17 (OH17). Control experiments were performed using minimal medium (control), 1.2 mg/L ACC in minimal medium (ACC), and *peg_1256* disrupted mutant in minimal medium (OH17′). Plants were cultivated for 7 days.

*Oryza sativa* Nipponbare variety, which is one of the most essential crops in the world providing main food supplies to China, Korea, and Japan populations, was used in the experiments. First, seeds were treated with OH17 in minimal medium, and minimal medium, 1.2 mg/L ACC in minimal medium, and mutant OH17 after disruption of *peg_1256* in minimal medium were used as control experiments. ACC (1.2 mg/L) was employed since the concentration of ACC after the bacterial treatment was 1.2 mg/L. It must be noticed that no ACC was observed in the medium using the disrupted OH17 bacteria. $OD_{600} \approx 0.55$ was detected at the end of the treatment with OH17, whereas the $OD_{600}$ value in the treatment was lower than 0.05 in all of the repetitions, indicating that the disrupted enzyme had difficulties to grow in the treatment medium. This result can be explained considering that the disrupted enzyme has no access to the ACC of the seeds, and thus the carbon source is limited to the low concentration of glucose present in the minimal medium. Observed ethylene concentration in the surroundings of the stored rice plant treated with OH17, 0.25 ppb, were significantly higher with respect to the control experiments, between 0.14 and 0.16 ppb. These results indicated that ACC deaminase of OH17 is capable of interacting with the rice seeds reducing internal ACC levels, and thus OH17 produced stress in the rice plants, which led to ethylene production levels 44% higher after 7 days of cultivation.[18] Obtained results are in agreement with the reported effect of *R. leguminosarum* bv. *trifolii* SN10 ACC deaminase, which was demonstrated to be able to colonize the rice root of different rice species.[38] Since no significant alterations in the levels of ethylene could be detected in the rice plants treated with ACC in comparison with the plants


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J. Agric. Food Chem. 2018, 66, 3675−3682

treated with minimal medium, we could confirm that ACC deaminase is the agent responsible for the growth promotion and not ACC. When disrupted OH17 was used, the concentration of ethylene remained unchanged in comparison with the other control experiments, indicating that only the ACC deaminase produced the effects in the rice metabolism.

**Examination of *Oryza sativa* Nipponbare Growth after Treatment with OH17.** *Oryza* sativa, also called Asian rice, is one of the most produced and consumed food crops worldwide, and it provides approximately 19% of the daily supply of calories (545 kcal) for the world population.[39] For this reason, new methodologies able to promote *Oryza sativa* growth cause significant interest in order to ensure and improve *Oryza sativa* crops. In this sense, Hurek and co-workers reported that *Azoarcus* sp. BH72 can colonize and invade roots of *Kallar* grass and rice *Oryza sativa* seedlings.[40] Later, Lee and co-workers described *Oryza sativa* growth promotion by production of phytohormone of $N_2$-fixing methylotrophic isolates.[41] Madhaiyan et al. reported the growth promotion of *Oryza sativa* rice after treatment with *Methylobacterium* spp.[42,43]

In this work, *Lysobacter gummosus* OH17 ACC deaminase has been used for the promotion of *Oryza sativa* Nipponbare growth by treatment of the rice seeds with OH17 cultures in minimal medium (Figure S9).[44] The differences between the mean values were checked using SPSS. The average root and stem lengths of the plants treated with OH17 were 14.80 ± 0.18 and 9.80 ± 0.99 cm, respectively, whereas the root and stem lengths of the plants treated with disrupted OH17 were 13.38 ± 0.52 cm and 8.31 ± 1.03 cm, respectively. The root length of the plants treated with minimal medium and ACC were 8.42 ± 0.99 and 8.48 ± 0.80 cm, respectively, and the stem lengths were 13.34 ± 0.45 and 11.69 ± 0.62 cm, respectively. Thus, the root and stem lengths of plants treated with OH17 were 10% and 15% longer, respectively, compared with the length of the plants treated with disrupted OH17, 10% and 14% longer, respectively, in comparison with the length of the plants treated with minimal medium, 21% and 13% longer, respectively, in comparison with the length of the plants treated with ACC (Figure 4). OH17 could not produce the obvious growth promotion effects in *Oryza sativa* varieties 9311 and IR24. Although Bhattacharjee and co-workers and Bal and co-workers reported the root growth promotion in other rice species caused by ACC deaminases from *R. leguminosarum* bv. *trifolii* SN10 and soil bacteria, respectively,[38,45] OH17 ACC deaminase is the first ACC deaminase able to promote *Oryza sativa* Nipponbare growth. After 7 days of cultivation, roots were examined using a microscope indicating that the root hair of the plants treated with OH17 was more developed in comparison with the root hair observed in the control plants (Figure 5). Detected root development can be explained considering the observed high levels of ethylene after treatment with OH17, which have been proven to stimulate lateral root formation in other plant species.[18,46−49] Root hair has been demonstrated to be essential for water and nutrients absorption, which explicates the higher stem and root length observed in the plants treated with OH17.[50]

In summary, we have demonstrated that *Lysobacter gummosus* OH17 encodes an ACC deaminase in the gene *peg_1256*. *Lysobacter gummosus* OH17 ACC deaminase has been shown to be able to increase the levels of hormone ethylene in rice *Oryza sativa* Nipponbare plants, promoting root and stem growth. This ACC deaminase is the first growth plant regulator found in *Lysobacter* species. Further studies in soil plant systems using

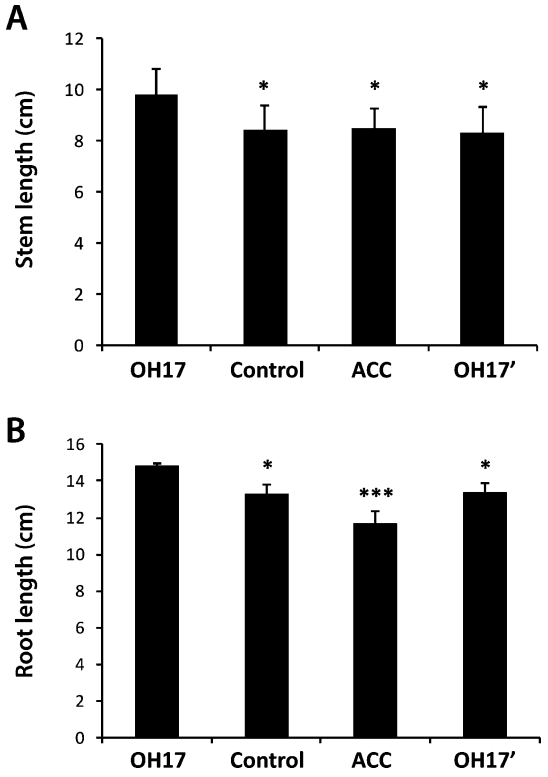


**Figure 4.** Study of the effect of *Lysobacter gummosus* OH17 in the growth of rice *Oryza sativa* Nipponbare plants. (A) Stem height (cm) and (B) root length (cm) of rice plants after priming with *Lysobacter gummosus* OH17 cultures in minimal medium (OH17), minimal medium (control), 1.2 mg/L ACC in minimal medium (ACC), and *peg_1256* disrupted mutant in minimal medium (OH17′). Plants were cultivated for 7 days.

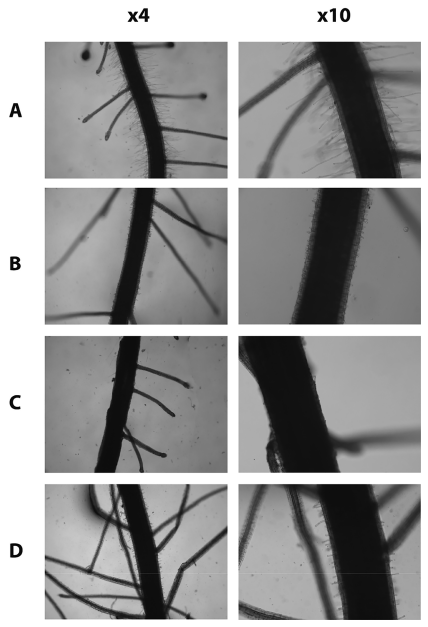


**Figure 5.** Images showing the root hair development in *Oryza sativa* plants after treatment with (A) *Lysobacter gummosus* OH17 cultures in minimal medium, (B) minimal medium, (C) 1.2 mg/L ACC in minimal medium, and (D) *peg_1256* disrupted mutant in minimal medium. Plants were cultivated for 7 days.



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J. Agric. Food Chem. 2018, 66, 3675−3682

OH17 or other *Lysobacter* species would be interesting in order to demonstrate the applicability of *Lysobacter* in agriculture as a growth promotion agent.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b00063.

$^{1}$H and $^{13}$C NMR spectra of purified Fmoc-labeled ACC, alignment of amino acids sequences from bacterial *O*-succinylhomoserine lyases and ACC deaminases (including the ones from *Lysobacter gummosus* OH17), HPLC-based analysis of ACC and 2-oxobutanoic acid in OH17 secretions, TLC of the in vitroamination and deamination reactions using recombinant ACC deaminase from OH17, SDS-PAGE of the purification of ACC deaminase from OH17, images showing the hydroponic rice cultures, measurement of ethylene in *Oryza sativa* plants, and the effect of gentamycin on *Oryza sativa* growth (PDF)

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### Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS USED

ACC, 1-aminocyclopropane-1-carboxylic acid; ACC deaminase, 1-aminocyclopropane-1-carboxylate deaminase; Fmoc-Cl, fluorenylmethyloxycarbonyl chloride; Fmoc, fluorenylmethyloxycarbonyl; HPLC, high-performance liquid chromatography; HSAF, heat stable antifungal factor; OH17, *Lysobacter gummosus* OH17; NMR, nuclear magnetic resonance.

## ■ REFERENCES


(1) Davis, T. D.; Curry, E. A.; Steffens, G. L. Chemical-regulation of vegetative growth. *Crit. Rev. Plant Sci.* **1991**, *10*, 151−188.

(2) Szyjewicz, E.; Rosner, N.; Kliewer, W. M. Ethephon ((2-chloroethyl)phosphonic acid, etherel, CEPA) in vinicultura - A review. *Am. J. Enol. Viticult.* **1984**, *35*, 117−123.

(3) Dennis, F. G. The history of fruit thinning. *Plant Growth Regul.* **2000**, *31*, 1−16.

(4) Bartholomew, D. P. Inflorescence development of pineapple (*Ananas comosus* [L.] Merr.) induced to flower with ethephon. *Bot. Gaz.* **1977**, *138*, 312−320.

(5) Yazar, S.; Baydan, E. The subchronic toxic effects of plant growth promoters in mice. *Ankara Uni. Vet. Fak.* **2008**, *55*, 17−21.

(6) Yurdakok, B.; Baydan, E.; Okur, H.; Gurcan, I. S. Cytotoxic effects of etephon and maleic hydrazide in Vero, Hep2, HepG2 cells. *Drug Chem. Toxicol.* **2014**, *37*, 459−465.

(7) Wang, K. S.; Lu, C. Y.; Chang, S. H. Evaluation of acute toxicity and teratogenic effects of plant growth regulators by *Daphnia magna* embryo assay. *J. Hazard. Mater.* **2011**, *190*, 520−528.

(8) Stover, E. W.; Greene, D. W. Environmental effects on the performance of foliar applied plant growth regulators: A review focusing on tree fruits. *Horttechnology* **2005**, *15*, 214−221.

(9) Mehlhorn, H.; O'Shea, J. M.; Wellburn, A. R. Atmospheric ozone interacts with stress ethylene formation by plants to cause visible plant injury. *J. Exp. Bot.* **1991**, *42*, 17−24.

(10) Altuntas, H.; Demirci, S. N. S.; Duman, E.; Ergin, E. Toxicological and physiological effects of ethephon on the model organism, *Galleria mellonella* L. 1758 (Lepidoptera: Pyralidae). *Turkish Journal of Entomology* **2016**, *40*, 413−423.

(11) Van Der Straeten, L.; Van Der Straeten, D. Accumulation and transport of 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: current status, considerations for future research and agronomic applications. *Front. Plant Sci.* **2017**, *8*, 38.

(12) Singh, R. P.; Shelke, G. M.; Kumar, A.; Jha, P. N., Biochemistry and genetics of ACC deaminase: a weapon to "stress ethylene" produced in plants. *Front. Microbiol.* **2015**, *6*, Article number: 937.10.3389/fmicb.2015.01255

(13) Van de Poel, B.; Van Der Straeten, D. 1-Aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene! *Front. Plant Sci.* **2014**, *5*, 640.

(14) Ma, W. B.; Guinel, F. C.; Glick, B. R. *Rhizobium leguminosarum* biovar viciae 1-aminocyclopropane-1-carboxylate deaminase promotes nodulation of pea plants. *Appl. Environ. Microb.* **2003**, *69*, 4396−4402.

(15) Jacobson, C. B.; Pasternak, J. J.; Glick, B. R. Partial-purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from plant-growth promoting rhizobacterium *Pseudomonas putida* GR12−2. *Can. J. Microbiol.* **1994**, *40*, 1019−1025.

(16) Nascimento, F. X.; Rossi, M. J.; Soares, C. R. F. S.; McConkey, B. J.; Glick, B. R. New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. *PLoS One* **2014**, *9*, e99168.

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

(18) Glick, B. R. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol. Res.* **2014**, *169*, 30−39.

(19) Kaushal, M.; Wani, S. P. Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. *Ann. Microbiol.* **2016**, *66*, 35−42.

(20) Singh, R. P.; Shelke, G. M.; Kumar, A.; Jha, P. N., Biochemistry and genetics of ACC deaminase: a weapon to "stress ethylene" produced in plants. *Front. Microbiol.* **2015**, *6*, Article number: 937.10.3389/fmicb.2015.00937

(21) Vejan, P.; Abdullah, R.; Khadiran, T.; Ismail, S.; Boyce, A. N. Role of plant growth promoting rhizobacteria in agricultural sustainability - A review. *Molecules* **2016**, *21*, 573.

(22) Zaidi, A.; Ahmad, E.; Khan, M. S.; Saif, S.; Rizvi, A. Role of plant growth promoting rhizobacteria in sustainable production of vegetables: Current perspective. *Sci. Hortic.* **2015**, *193*, 231−239.

(23) Xie, Y. X.; Wright, S.; Shen, Y. M.; Du, L. C. Bioactive natural products from. *Nat. Prod. Rep.* **2012**, *29*, 1277−1287.

(24) Lou, L. L.; Qian, G. L.; Xie, Y. X.; Hang, J. L.; Chen, H. T.; Zaleta-Rivera, K.; Li, Y. Y.; Shen, Y. M.; Dussault, P. H.; Liu, F. Q.; Du, L. C. Biosynthesis of HSAF, a tetramic acid-containing macrolactam from *Lysobacter enzymogenes*. *J. Am. Chem. Soc.* **2011**, *133*, 643−645.

(25) Zhao, Y. Y.; Qian, G. L.; Ye, Y. H.; Wright, S.; Chen, H. T.; Shen, Y. M.; Liu, F. Q.; Du, L. C. Heterocyclic aromatic *N*-oxidation in the biosynthesis of phenazine antibiotics from *Lysobacter antibioticus*. *Org. Lett.* **2016**, *18*, 2495−2498.

(26) Wang, Y.; Qian, G. L.; Liu, F. Q.; Li, Y. Z.; Shen, Y. M.; Du, L. C. Facile method for site-specific gene integration in *Lysobacter enzymogenes* for yield improvement of the anti-MRSA antibiotics

WAP-8294A and the antifungal antibiotic HSAF. *ACS Synth. Biol.* **2013**, *2*, 670−678.
(27) Laborda, P.; Zhao, Y.; Ling, J.; Hou, R.; Liu, F. Production of antifungal *p*-aminobenzoic acid in *Lysobacter antibioticus* OH13. *J. Agric. Food Chem.* **2018**, *66*, 630−636.
(28) Furnkranz, M.; Adam, E.; Muller, H.; Grube, M.; Huss, H.; Winkler, J.; Berg, G. Promotion of growth, health and stress tolerance of Styrian oil pumpkins by bacterial endophytes. *Eur. J. Plant Pathol.* **2012**, *134*, 509−519.
(29) Kovach, M. E.; Elzer, P. H.; Hill, D. S.; Robertson, G. T.; Farris, M. A.; Roop, R. M.; Peterson, K. M. 4 new derivatives of the broad-host-range cloning vector PBBR1MCS, carrying different antibiotic-resistance cassetes. *Gene* **1995**, *166*, 175−176.
(30) Laborda, P.; Wang, S.-Y.; Lu, A.-M.; He, M.; Duan, X.-C.; Qian, Y.-J.; Jung, Y.-S.; Liu, L.; Voglmeir, J. Diastereoselective one-step synthesis of 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) analogues as templates for the development of influenza drugs. *Adv. Synth. Catal.* **2017**, *359*, 3120−3125.
(31) Liang, C.; Zheng, G.; Li, W.; Wang, Y.; Hu, B.; Wang, H.; Wu, H.; Qian, Y.; Zhu, X. G.; Tan, D. X.; Chen, S.-Y.; Chu, C. Melatonin delays leaf senescence and enhances salt stress tolerance in rice. *J. Pineal Res.* **2015**, *59*, 91−101.
(32) Liang, C.; Li, A.; Yu, H.; Li, W.; Liang, C.; Guo, S.; Zhang, R.; Chu, C. Melatonin regulates root architecture by modulating auxin response in rice. *Front. Plant Sci.* **2017**, *8*, 134.
(33) Hardoim, P. R.; van Overbeek, L. S.; van Elsas, J. D. Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.* **2008**, *16*, 463−471.
(34) Fedorov, D. N.; Ekimova, G. A.; Doronina, N. V.; Trotsenko, Y. A. 1-Aminocyclopropane-1-carboxylate (ACC) deaminases from *Methylobacterium radiotolerans* and *Methylobacterium nodulans* with higher specificity for ACC. *FEMS Microbiol. Lett.* **2013**, *343*, 70−76.
(35) VanDerStraeten, D.; Anuntalabhochai, S.; VanCaeneghem, W.; Zhou, Z. Y.; Gielen, J.; VanMontagu, M. Expression of three members of the ACC synthase gene family in deepwater rice by submergence, wounding and hormonal treatments. *Plant Sci.* **1997**, *124*, 79−87.
(36) Zarembinski, T. I.; Theologis, A. Anaerobiosis and plant-growth hormones induce 2 genes encoding 1-aminocyclopropane-1-carboxylae synthase in rice (*Oryza sativa* L.). *Mol. Biol. Cell* **1993**, *4*, 363−373.
(37) Zhou, Z. Y.; Vriezen, W.; Van Caeneghem, W.; Van Montagu, M.; Van Der Straeten, D. Rapid induction of a novel ACC synthase gene in deepwater rice seedlings upon complete submergence. *Euphytica* **2001**, *121*, 137−143.
(38) Bhattacharjee, R. B.; Jourand, P.; Chaintreuil, C.; Dreyfus, B.; Singh, A.; Mukhopadhyay, S. N. Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. *trifolii* SN10 promote rice growth, and in the process undergo colonization and chemotaxis. *Biol. Fertil. Soils* **2012**, *48*, 173−182.
(39) Kim, B.; Woo, S.; Kim, M. J.; Kwon, S. W.; Lee, J.; Sung, S. H.; Koh, H. J. Identification and quantification of flavonoids in yellow grain mutant of rice (*Oryza sativa* L.). *Food Chem.* **2018**, *241*, 154−162.
(40) Hurek, T.; Reinholdhurek, B.; Vanmontagu, M.; Kellenberger, E. Root colonization and systemic spreading of *Azoarcus* sp. strain-BH72 in grasses. *J. Bacteriol.* **1994**, *176*, 1913−1923.
(41) Lee, H. S.; Madhaiyan, M.; Kim, C. W.; Choi, S. J.; Chung, K. Y.; Sa, T. M. Physiological enhancement of early growth of rice seedlings (*Oryza sativa* L.) by production of phytohormone of N-2-fixing methylotrophic isolates. *Biol. Fertil. Soils* **2006**, *42*, 402−408.
(42) Madhaiyan, M.; Poonguzhali, S.; Senthilkumar, M.; Seshadri, S.; Chung, H. Y.; Yang, J. C.; Sundaram, S.; Sa, T. M. Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by *Methylobacterium* spp. *Bot. Bull. Acad. Sinica* **2004**, *45*, 315−324.
(43) Senthilkumar, M.; Madhaiyan, M.; Sundaram, S. P.; Kannaiyan, S. Intercellular colonization and growth promoting effects of *Methylobacterium* sp with plant-growth regulators on rice (*Oryza sativa* L. Cv CO-43). *Microbiol. Res.* **2009**, *164*, 92−104.
(44) Contesto, C.; Desbrosses, G.; Lefoulon, C.; Béna, G.; Borel, F.; Galland, M.; Gamet, L.; Varoquaux, F.; Touraine, B. Effects of rhizobacterial ACC deaminase activity on *Arabidopsis* indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria. *Plant Sci.* **2008**, *175*, 178−189.
(45) Bal, H. B.; Das, S.; Dangar, T. K.; Adhya, T. K. ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. *J. Basic Microbiol.* **2013**, *53*, 972−984.
(46) Negi, S.; Ivanchenko, M. G.; Muday, G. K. Ethylene regulates lateral root formation and auxin transport in. *Plant J.* **2008**, *55*, 175−187.
(47) Tucker, M. L.; Xue, P.; Yang, R. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration and ACC synthase expression in soybean roots, root tips, and soybean cyst nematode (*Heterodera glycines*)-infected roots. *J. Exp. Bot.* **2010**, *61*, 463−472.
(48) Shaharoona, B.; Arshad, M.; Zahir, Z. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Lett. Appl. Microbiol.* **2006**, *42*, 155−159.
(49) Li, J.; Ovakim, D. H.; Charles, T. C.; Glick, B. R. An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. *Curr. Microbiol.* **2000**, *41*, 101−105.
(50) Nye, P. The effect of the nutrient intensity and buffering power of a soil, and the absorbing power, size and root hairs of a root, on nutrient absorption by diffusion. *Plant Soil* **1966**, *25*, 81−105.


DOI: 10.1021/acs.jafc.8b00063
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