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The rhizobacterium *Variovorax paradoxus* 5C-2, containing ACC deaminase, promotes growth and development of *Arabidopsis thaliana* via an ethylene-dependent pathway

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Abstract

Many plant-growth-promoting rhizobacteria (PGPR) associated with plant roots contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and can metabolize ACC, the immediate precursor of the plant hormone ethylene, thereby decreasing plant ethylene production and increasing plant growth. However, relatively few studies have explicitly linked ethylene emission and/or action to growth promotion in these plant–microbe interactions. This study examined effects of the PGPR *Variovorax paradoxus* 5C-2 containing ACC deaminase on the growth and development of *Arabidopsis thaliana* using wild-type (WT) plants and several ethylene-related mutants (*etr1-1*, *ein2-1*, and *eto1-1*). Soil inoculation with *V. paradoxus* 5C-2 promoted growth (leaf area and shoot biomass) of WT plants and the ethylene-overproducing mutant *eto1-1*, and also enhanced floral initiation of WT plants by 2.5 days. However, these effects were not seen in ethylene-insensitive mutants (*etr1-1* and *ein2-1*) even though bacterial colonization of the root system was similar. Furthermore, *V. paradoxus* 5C-2 decreased ACC concentrations of rosette leaves of WT plants by 59% and foliar ethylene emission of both WT plants and *eto1-1* mutants by 42 and 37%, respectively. Taken together, these results demonstrate that a fully functional ethylene signal transduction pathway is required for *V. paradoxus* 5C-2 to stimulate leaf growth and flowering of *A. thaliana*.

Key words: ACC deaminase, *Arabidopsis*, ethylene, growth, floral transition, rhizobacteria.

Introduction

The regulation of growth and functioning of plant root systems has attracted increased scientific attention in studies which aim to increase crop production but decrease environmental impacts of agriculture by decreasing water and nutrient inputs (Lynch, 2007; Ghanem *et al.*, 2011). Manipulating rhizosphere microbial populations potentially offers a low-cost and flexible method to increase plant growth by regulating the growth and functioning of the root system. Plant-growth-promoting rhizobacteria (PGPR) can stimulate plant growth directly by producing or metabolizing plant hormones or enhancing plant nutrient uptake (Arshad and Frankenberger, 1991; Vessey, 2003; Dodd *et al.*, 2010; Dodd and Ruiz-Lozano, 2012) or indirectly by mechanisms such as biocontrol of phytopathogens (Kloepper *et al.*, 1992). A single PGPR strain can provide multiple beneficial effects to plants (Lugtenberg and Kamilova, 2009; Jiang *et al.*, 2012). Many PGPRs contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (ACCd), which can hydrolyse ACC (the immediate precursor of the plant hormone ethylene) to ammonia and α-ketobutyrate. These bacteria take up ACC from plant root exudates and use it as a nitrogen (Jacobson *et al.*, 1994; Glick *et al.*, 1995) and carbon (Belimov *et al.*, 2005) source.

Growth promotion of different plant species was repeatedly observed following substrate inoculation with various strains containing ACCd, particularly in plants subjected to environmental stresses that stimulate ethylene production (Glick *et al.*, 1998, 2007; Belimov *et al.*, 2001).

Ethylene can inhibit plant growth in several ways (Pierik *et al.*, 2006). The triple response of dark-grown seedlings is a classic example illustrating that ethylene inhibits plant growth by reducing hypocotyl elongation and root growth (Guzman and Ecker, 1990). Application of ethylene or its precursor ACC or the ethylene-releasing chemical ethephon (which both can be converted to ethylene by plants) reduced leaf expansion and shoot growth (Lee and Reid, 1997; Pierik *et al.*, 2006). In addition to plant growth regulation, floral transition time in *Arabidopsis* was delayed in WT plants which were supplied with 10 μM ACC to the root-zone, and in *ctr1-1* mutants in which the ethylene signal transduction pathway is constantly stimulated (Achard *et al.*, 2007).

Ethylene is perceived by a family of receptors including ETR1, ETR2, ERS1, ERS2, and EIN4 in *Arabidopsis* (Chang *et al.*, 1993; Hua *et al.*, 1995, 1998; Hua and Meyerowitz, 1998). In the presence of ethylene, ETR1 inactivates the negative regulator CTR1 (a Ser/Thr kinase) which is activated by air (Kieber *et al.*, 1993). The positive regulator EIN2, which is downstream of CTR1 (Guzman and Ecker, 1990), can activate EIN3 and EIN3-like (EIL) transcription factors (Roman *et al.*, 1995), thus inducing the expression of ethylene-responsive genes (Solano *et al.*, 1998). Both *etr1-1* and *ein2-1* (the mutants used in this study) showed an ethylene-insensitive phenotype in the triple response assay (Guzman and Ecker, 1990; Chang *et al.*, 1993).

PGPR containing the enzyme ACCd decreased root ACC concentrations (Penrose *et al.*, 2001) and mitigated ethylene's inhibitory effects on shoot growth (Glick *et al.*, 1998; Belimov *et al.*, 2001, 2009a; Glick, 2005). Inoculation of plants with rhizobacteria containing ACCd attenuated an increase in xylem sap ACC levels induced by soil drying (Belimov *et al.*, 2009b) and decreased whole-seedling ethylene accumulation (Penrose *et al.*, 2001; Mayak *et al.*, 2004a, b; Madhaiyan *et al.*, 2006). Inhibitors of ethylene biosynthesis (aminoethoxyvinylglycine) or action (silver ions) had similar physiological effects to those of bacterial inoculation (Hall *et al.*, 1996; Belimov *et al.*, 2002, 2007), suggesting that bacterial growth stimulation was mediated by ethylene. Furthermore, transposon mutagenesis of microorganisms to downregulate ACCd activity reduced or eliminated their growth-promoting effect, in plant–microbe interactions such as canola–*Enterobacter cloacae* (Li *et al.*, 2000), tomato–*Pseudomonas brassicacearum* (Belimov *et al.*, 2007), pea–*Variovorax paradoxus* (Belimov *et al.*, 2009b) and canola–*Trichoderma asperellum* (Viterbo *et al.*, 2010). These findings suggested that ACCd plays a key role in promoting plant growth. However, relatively few studies have examined the effects of bacterial inoculation on ethylene production of adult plants, the ethylene-signalling pathway involved, or the possible regulation of flowering by this group of rhizobacteria. Previously, preliminary studies in potato demonstrated that *V. paradoxus* 5C-2 accelerated tuber sprouting in potato, thereby decreasing time to flowering (Belimov *et al.*, 2009a), although the possible mechanism causing this developmental regulation was not fully explored.

V. paradoxus 5C-2 also produced indoles *in vitro* (Belimov *et al.*, 2005; Jiang *et al.*, 2012). Canola (*Brassica campestris*) root inoculation with the rhizobacterium *Methylobacterium fujisawaense* that contains ACCd not only decreased ethylene accumulation, but also increased indole-3-acetic acid concentrations in young seedlings (Madhaiyan *et al.*, 2006). However, it is not clear whether there is a correlation between increased indole-3-acetic acid concentrations and growth promotion effects from *M. fujisawaense*. Although *V. paradoxus* 5C-2 increased xylem abscisic acid (ABA) concentrations of pea plants exposed to drying soil (Belimov *et al.*, 2009b), it decreased root ABA concentration (and xylem ABA flows) of well-watered and well-fertilized pea plants (Jiang *et al.*, 2012). A minimum endogenous ABA concentration seems necessary to maintain optimal shoot and root development (Sharp *et al.*, 2000; Spollen *et al.*, 2000), even under well-watered conditions. Thus growth promotion by *V. paradoxus* 5C-2 may involve other phytohormonal mechanisms.

Several phytohormone-insensitive mutants of *Arabidopsis thaliana* were used to investigate effects of PGPR such as *Bacillus megaterium* (Lopez-Bucio *et al.*, 2007) and *Phyllobacterium brassicacearum* (Galland *et al.*, 2012) on root architecture. Results suggested that the effects of *B. megaterium* were ethylene and auxin independent, while ethylene was involved in mediating effects of *P.brassicacearum*. To determine whether the ethylene-signalling pathway regulated effects of *V. paradoxus* 5C-2 on growth and development, several ethylene-related *A. thaliana* mutants were used in this study. Furthermore, to determine whether rhizobacterial inoculation of the growing substrate affected long-distance ethylene signalling, ACC concentrations and ethylene accumulation of rosette leaves were measured.

Materials and methods

Seed lines

The *A. thaliana* lines used in this study were Columbia (Col) wild-type (WT), the ethylene-insensitive mutants *etr1-1* (Bleecker *et al.*, 1988) and *ein2-1* and the ethylene over-producing mutant *eto1-1* (Guzman and Ecker, 1990). Col-O, *etr1-1*, and *ein2-1* were kindly given by Mike Roberts (Lancaster Environment Centre, Lancaster University, UK). *eto1-1* was obtained from the European *Arabidopsis* Stock Centre (NASC, University of Nottingham). All mutant lines were derived from parental *A. thaliana* Columbia. In an attempt to avoid any surface contamination from microbes, all seeds were surface sterilized before sowing in the substrate by rinsing with 70% ethanol, followed by 95% ethanol for 1 min. Surface-sterilized seeds were kept at 4 °C for 2 days and then sown on top of the growth medium (All Purpose Growth medium, Sinclair Hort Products, Lincoln, UK, mixed with sand and vermiculite at a ratio of 3:1:0.5, v/v/v) into individual 130-ml pots (63 mm high and 65 mm diameter). The compost was sieved and then sterilized by autoclaving (121 °C for 15 minutes) before mixing with sand and vermiculite, which were also autoclaved individually under the same conditions. Pots were placed in propagator trays (56 × 37.5 cm) and covered with a propagator lid (comprising six ventilation holes 1 × 5 cm) to minimize the ingress of airborne microbes. Plants were well watered throughout and grown on a bench in a walk-in controlled environment room

with an average temperature of 23 °C ± 2 °C, and at 230 ± 20 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) with a 16/8 h light/dark cycle.

Bacterial cultures and inoculation

V. paradoxus 5C-2 was obtained from the All-Russia Research Institute for Agricultural Microbiology (St Petersburg). Bacteria were cultured on Bacto *Pseudomonas* F (BPF) medium and used for liquid suspension preparation as previously described (Belimov *et al.*, 2001). Liquid suspension was applied to the plant growth medium by thorough mixing prior to filling the pots. The final bacterial concentration in the growth medium was 10^6 cells g^{-1} substrate. *Arabidopsis* seeds were planted (four seeds per pot) on the surface of the soil. Ten days after planting (DAP), germinated seedlings were thinned to one per pot.

Estimation of bacteria on roots

Colonization of roots by *V. paradoxus* 5C-2 was determined as previously described (Belimov *et al.*, 2009b). Briefly, plant roots were removed from the growth substrates by gently shaking to remove any adhering substrate particles. Both primary and lateral roots were used for analysis immediately after sampling. Root samples were homogenized in sterile tap water with a sterile mortar and pestle, the homogenates serially diluted in 10-fold steps, and 50 μl aliquots plated in duplicate on BPF medium supplemented with (l^{-1}) 20 mg rifampicin and 30 mg kanamycin (to which *V. paradoxus* 5C-2 shows resistance) and 40 mg nystatin (to prevent fungal growth). Colony-forming units were counted by comparing with the morphology of the original strain grown on BPF agar after incubation of plates for 4 days at 28 °C. Roots from uninoculated plants were the control treatment. Plates from these roots had few (if any) bacterial colonies, none of which morphologically resembled the inoculated strain (Supplementary Fig. S1, available at *JXB* online).

Physiological and biochemical measurements

Flowering time of *Arabidopsis* was determined by recording the total number of rosette leaves (excluding cotyledons) when the floral stem was 1 cm long as described (Achard *et al.*, 2007); and recording days at the same time. To determine rosette fresh weight, leaf number, and leaf area, WT plants were harvested at three points during the growth period: 17, 22, and 33 DAP. To ensure similarities of developmental stages between WT and mutants at harvesting, the mutants were harvested at 33 DAP for *etr1-1*, 35 DAP for *ein2-1*, and 28 DAP for *eto1-1*.

To determine rates of ethylene production, rosette leaves (0.5 g fresh weight) from WT plants and the *eto1-1* mutant were taken when the floral stem emerged and placed in 7.8-ml glass vials to incubate for 1 h before 1 ml of headspace was withdrawn and injected into a gas chromatograph for the quantification of ethylene as described previously (Dodd *et al.*, 2009). Leaf samples for ACC measurement were collected when the floral stem emerged, frozen in liquid nitrogen and stored at –20 °C. ACC content was determined by GC-MS as previously described (Dodd *et al.*, 2009).

Statistics

Two-way analysis of variance (ANOVA) was performed to determine effects of rhizobacteria, genotype, and their interactions, using SPSS version 19 (SPSS, Chicago, USA). One-way ANOVA with Tukey's HSD ($P < 0.05$) was used to discriminate means. Pairwise comparisons used Student's t-test and standard error (SE) in SigmaPlot for Windows version 7.0 (Jandel Scientific, Erkrath, Germany).

Results

Inoculation of *V. paradoxus* 5C-2 significantly ($P < 0.01$) increased fresh biomass of WT plants by 34–47% throughout development (Fig. 1). Leaf areas (cm^2) of different genotypes from uninoculated controls were 41 ± 1 for WT, 6.9 ± 0.2 for *eto1-1*, 64 ± 2 for *etr1-1*, and 62 ± 2 for *ein2-1*; while fresh weights (g) for control plants were 1.4 ± 0.04 for WT, 0.2 ± 0.01 for *eto1-1*, 2.0 ± 0.1 for *etr1-1*, and 2.6 ± 0.1 for *ein2-1*. Effects of genotype and rhizobacterial inoculation on shoot biomass (% of control) were analysed using two-way ANOVA. Both
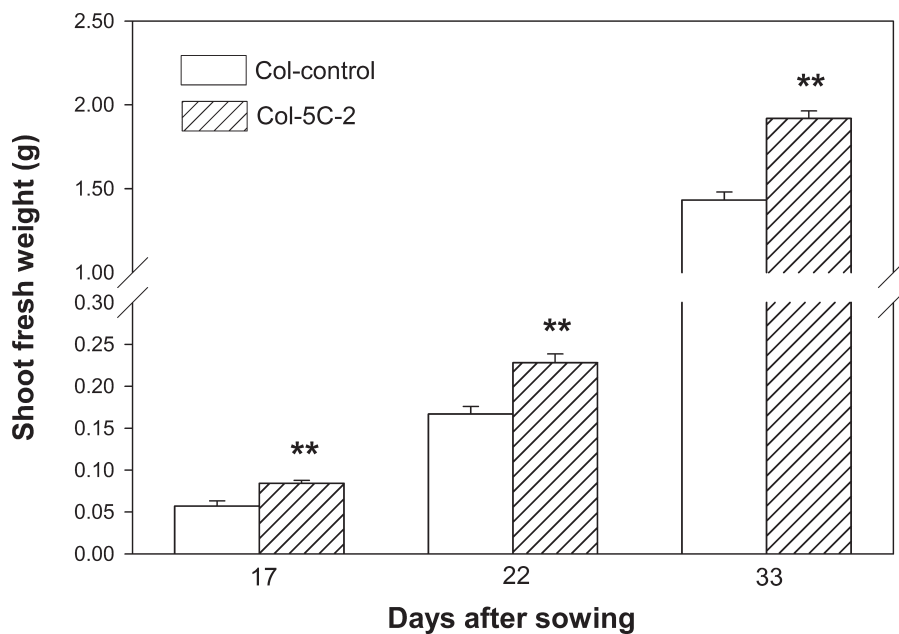

Fig. 1. Shoot fresh weight (g) of WT plants harvested at 17, 22, and 33 days after planting in response to *V. paradoxus* 5C-2 inoculation. Bars indicate mean ± SE (*n* = 25–30). Asterisks indicate significant differences (*P* < 0.01).

factors were highly significant (*P* < 0.001), as was the interaction (*P* < 0.001; Fig. 2), indicating that bacterial inoculation significantly (*P* < 0.01) stimulated growth of the WT and *eto1-1* plants but not the *etr1-1* and *ein2-1* mutants (Fig. 2). Similarly, bacterial inoculation and genotype significantly (*P*<0.001) affected leaf area and again, there was a significant genotype × inoculation interaction (*P* < 0.001; Fig. 3). *V. paradoxus* 5C-2 inoculation significantly (*P* < 0.01) increased whole-plant leaf area of the WT and *eto1-1* plants, but there was no promotion effect on the *etr1-1* and *ein2-1* mutants (Fig. 3).

Both genotype and rhizobacterial inoculation and also their interaction had significant effects on both days to flowering and rosette leaf number at flowering. WT plants inoculated with *V. paradoxus* 5C-2 flowered significantly earlier than control plants (*P* < 0.01; Fig. 4). However, inoculation of the ethylene-insensitive mutants (*etr1-1*, and *ein2-1*) caused no statistically significant (*P* > 0.1; Fig. 4) decreases in days to flowering. Inoculation with *V. paradoxus* 5C-2 significantly (*P* < 0.01) decreased the number of WT rosette leaves at flowering (Fig. 5) but this did not occur in the ethylene-insensitive mutants *etr1-1* and *ein2-1* (*P* > 0.1; Fig. 5). Thus developmental effects of *V. paradoxus* 5C-2 were dependent on plant genotype.

At the end of experiments, *V. paradoxus* 5C-2 was detected on roots of inoculated WT plants and ethylene-insensitive mutants (Table 1), but there was no significant genotypic effect. Bacterial root colonization was not determined in the *eto1-1* mutant, as it was difficult to extract root tissues from the small root system of this mutant.

V. paradoxus 5C-2 inoculation significantly (*P* < 0.05) decreased ACC concentrations of rosette leaves of mature WT plants (Table 2). Furthermore, bacterial inoculation significantly decreased (*P* < 0.01) ethylene emission from rosette leaves of mature WT and *eto1-1* mutants (Fig. 6). Ethylene emission of both genotypes responded similarly to inoculation, as indicated by the lack of a significant genotype × inoculation interaction (*P* > 0.05; Fig. 6).

Discussion

Since *V. paradoxus* 5C-2 containing ACCd had multiple impacts on plant growth by stimulating leaf expansion, biomass accumulation, and flowering (Figs. 1–5), further evidence of the importance of ethylene in its interaction with *A. thaliana* was sought here by comparing growth responses of WT plants and ethylene-insensitive mutants (*etr1-1* and *ein2-1*) to soil inoculation with this PGPR. Previously, both *etr1-1* (Ruzicka *et al.*, 2007) and *ein2-1* (Stepanova *et al.*, 2007) showed a response of root growth to exogenous auxin similar to WT plants. Although *V. paradoxus* 5C-2 can produce auxins *in vitro* (Belimov *et al.*, 2005; Jiang *et al.*, 2012), differences in growth and flowering between ethylene-insensitive mutants and WT plants (Figs. 2–5) suggests that ethylene, rather than auxin, was responsible for the growth promotion observed.

An alternative possible explanation for the lack of growth promotion in the ethylene-insensitive mutants may be that there was insufficient bacterial colonization of the root
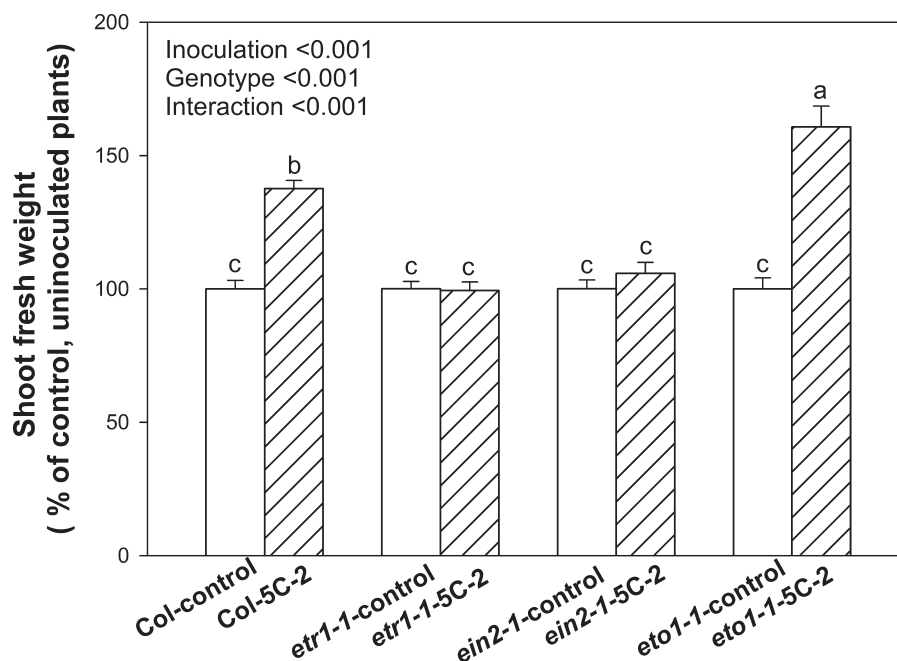

Fig. 2. Shoot fresh weight (% of control plants of each genotype) of WT, *eto1-1*, *etr1-1*, and *ein2-1* plants in response to *V. paradoxus* 5C-2 inoculation. WT plants were harvested at 29 DAP, while *eto1-1*, *etr1-1* and *ein2-1* were harvested at the corresponding development stage. Values are mean ± SE. Data for control, *etr1-1* and *ein2-1* were from two or three independent experiments and data for *eto1-1* from one representative experiment; 25–30 replicates were used for each experiment. Different letters above bars indicate significant differences (*P* < 0.05; Tukey test). *P*-values are shown for two-way ANOVA for bacterial inoculation, genotype, and their interaction.
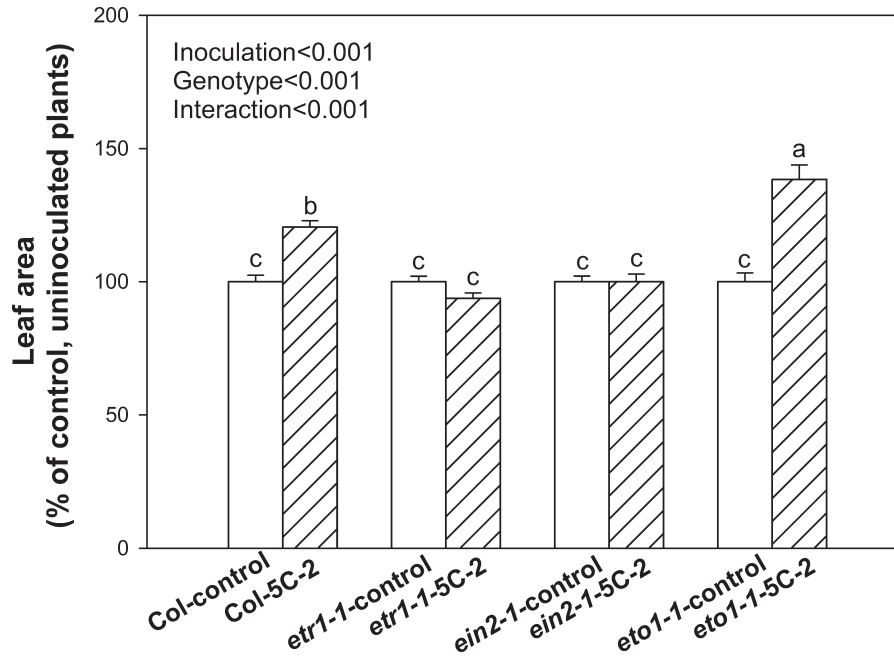
Fig. 3. Leaf area (% of control plants of each genotype) of WT, *eto1-1*, *etr1-1*, and *ein2-1* plants in response to *V. paradoxus* 5C-2 inoculation. WT plants were harvested at 29 DAP, while *eto1-1, etr1-1* and *ein2-1* were harvested at the corresponding development stage. Values are mean ± SE. Data of control, *etr1-1* and *ein2-1* were from two or three independent experiments and data for *eto1-1* from one representative experiment; 25–30 replicates were used for each experiment. Different letters above bars indicate significant differences (*P* < 0.05; Tukey test). *P*-values are shown for two-way ANOVA for bacterial treatment inoculation, genotype, and their interaction.

system. However, all studied genotypes showed similar levels of bacterial colonization of roots (Table 1). Comparatively little is known about putative plant regulation of rhizobacterial populations, and it is not clear whether root ACC exudation could stimulate colonization by bacteria containing ACCd.

Circumstantial evidence showed that rhizobacterial populations of *Hordeum spontaneum* plants were enriched in ACCd-containing organisms in sun-exposed plants (which may have been more stressed) compared to plants grown on the opposite (shaded) sides of the valley (Timmusk *et al.*, 2011). Although
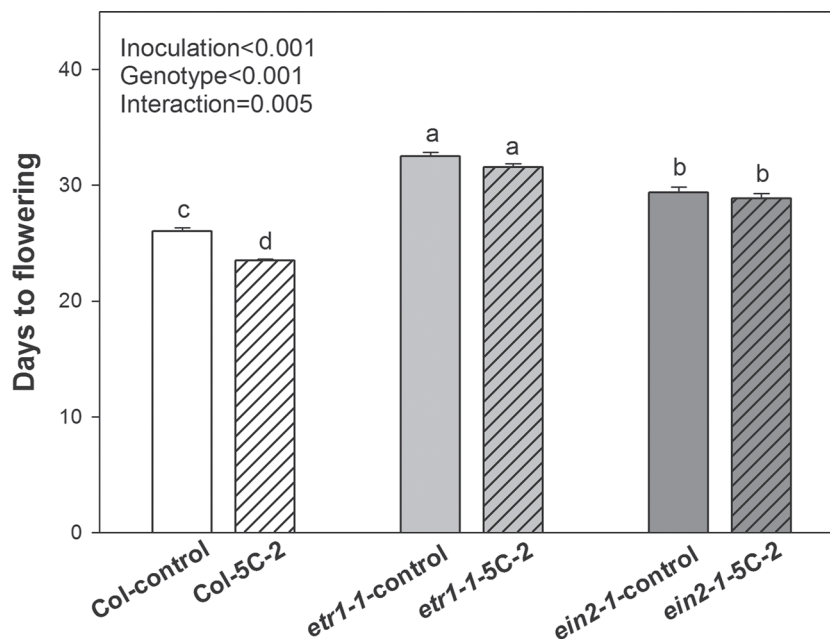

Fig. 4. Days to flowering of *V. paradoxus* 5C-2-treated WT, *etr1-1*, and *ein2-1* plants when the floral stem extended to 1 cm. Values are mean ± SE (*n* = 30). Different letters above bars indicate significant differences (*P* < 0.05; Tukey test). *P*-values are shown for two-way ANOVA for bacterial treatment inoculation, genotype, and their interaction.
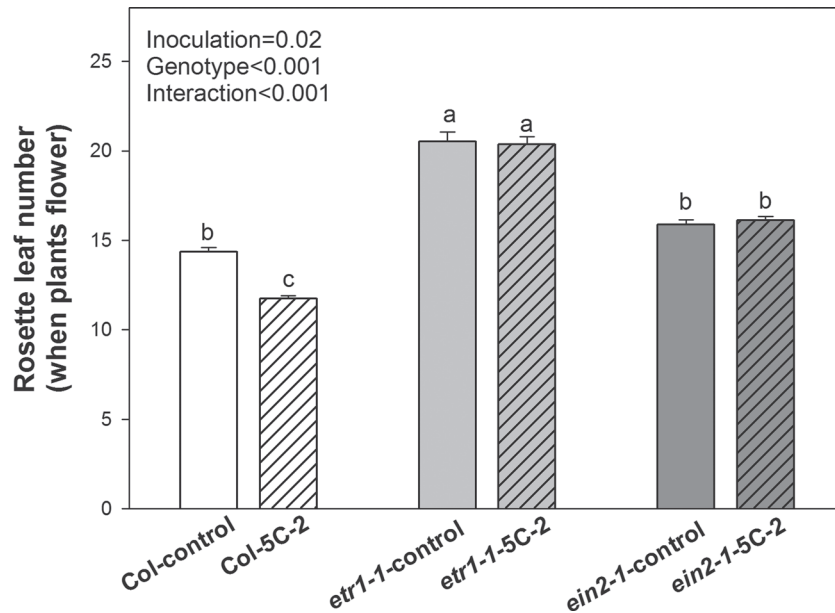
Fig. 5. Rosette leaf numbers of *V. paradoxus* 5C-2-treated WT, *etr1-1*, and *ein2-1* plants when the floral stem was extended to 1 cm. Values are mean ± SE (*n* = 30). Different letters above bars indicate significant differences (*P* < 0.05; Tukey test). *P*-values are shown for two-way ANOVA for bacterial treatment inoculation, genotype, and their interaction.

the impaired ability of the *etr1-1* and *ein2-1* plants to perceive ethylene was no impediment to colonization by rhizobacteria containing ACCd (Table 1), it is unknown whether root ACC efflux is independent of the ethylene-signalling pathway or rhizobacterial colonization depends on ACC efflux.

As shown previously in potato (Belimov *et al*. 2009a), promotion of floral initiation was noted in WT *Arabidopsis* plants inoculated with *V. paradoxus* 5C-2 (Figs. 4, 5). Together with promotion of shoot growth (Figs. 1–3) following inoculation of *V. paradoxus* 5C-2, these data are consistent with an inhibitory effect of ethylene on flowering (Achard *et al.*, 2007) and growth (Lee and Reid, 1997). Although a systemic effect of *V. paradoxus* 5C-2 inoculation was suggested by the decreased xylem ACC concentrations of peas grown in drying soil (Belimov *et al.*, 2009b), this conclusion is based on the assumption that root ACC export to the xylem quantitatively correlates with shoot ethylene evolution (Else and Jackson, 1998). However, ACC synthase, the rate-limiting enzyme in ethylene production (Wang *et al.*, 2002), is encoded by multigene families which can be expressed in roots and shoots (Liang *et al.*, 1992; Johnson and Ecker, 1998), indicating that plants can regulate their ACC pool (and hence ethylene production) in many ways.

Even though *eto1-1* overproduces ethylene as a result of increased stability of the ACC synthesis protein 5 (Chae *et al.*, 2003), here the increased shoot biomass and leaf area (Figs. 2, 3) but decreased ethylene accumulation (Fig. 6) of *eto1-1* following root inoculation with *V. paradoxus* 5C-2 suggest that long-distance ACC signalling regulated by the bacteria can still be influential. Similar decreases in foliar ACC levels and ethylene evolution of mature WT plants (Table 2, Fig. 6), together with the results from *eto1-1*, further indicate that bacterial inoculation not only causes local effects on roots (Glick *et al.*, 1997; Madhaiyan *et al.*, 2006) but also systemic effects via long-distance ACC signalling.

Interestingly, ACCd enzyme activity is not only reported in bacteria or fungi, but also detected in *Arabidopsis*, tomato, and poplar (McDonnell *et al.*, 2009; Plett *et al.*, 2009). In particular, two putative ACCd genes were identified in the genome of *Arabidopsis*, and downregulating one of these two genes increased ethylene production of 3-day-old *Arabidopsis* seedlings (McDonnell *et al.*, 2009). However, data presented here suggest that root inoculation of rhizobacteria containing ACCd can stimulate leaf growth of *Arabidopsis*, even during early growth (Fig. 1), despite any impact of *Arabidopsis* ACCd genes. These genes of *Arabidopsis* ACCd may be tightly regulated at transcriptional and translational levels

Table 1. Number of bacteria isolated from roots of inoculated plants. Data are mean ± SE (*n* = 4). There were no significant differences (*P* > 0.05). DAP, days after planting.

	Genotypes		
	Col (29 DAP)	*etr1-1* (33 DAP)	*ein2-1* (35 DAP)
Number of bacteria (10⁶ CFU (g FW)⁻¹)	1.85±0.70	2.44±0.07	2.46±0.19

Table 2. ACC concentration of rosette leaves from control and inoculated WT plants. Plant leaves were harvested when the floral stem emerged. Data are mean ± SE (*n* = 7–8).

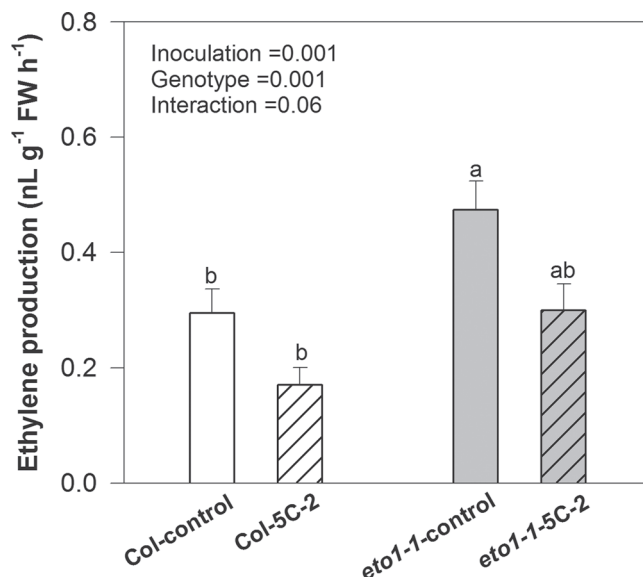|	Treatment		
	Control	Bacterial inoculation	*P*-value
ACC (pmol (g FW)⁻¹)	817±174	336±49	0.014

Fig. 6. Ethylene emission from mature leaves of control and uninoculated plants (WT and *eto1-1*). Plant leaves were harvested when the floral stem emerged from rosette leaves. Values are mean ± SE (Col *n* = 12, *eto1-1 n* = 6). Different letters above bars indicate significant differences ($P < 0.05$; Tukey test). *P*-values are shown for two-way ANOVA for bacterial treatment inoculation, genotype, and their interaction.

to regulate its ethylene production, but rhizobacterial ACCd seems relatively independent of plant regulation and thus could affect plant ethylene evolution further.

Taken together, the work presented here elucidated that root inoculation of rhizobacteria containing ACCd promoted plant growth and floral initiation via an ethylene-dependent signalling pathway by regulating shoot ethylene production. Ethylene is generally regarded as a 'stress hormone' as it can be stimulated by various stresses such as drought (Morgan and Drew, 1997; Sobeih *et al.*, 2004). Chemical inhibitors of ethylene synthesis or action have been used in agriculture to alleviate ethylene-induced growth inhibition, but suffer from practical disadvantages such as expense (aminoethoxyvinylgly-cine), toxicity (silver ions), or localized action (1-methylcyclo-propene is only effective in disrupting shoot ethylene biology as it cannot penetrate the soil). Compared to these inhibitors, rhizobacteria containing ACCd offer relatively non-toxic, environmentally friendly, and low-cost benefits to mitigate effects of ethylene on both roots and shoots. Rhizobacteria containing ACCd may be used in agriculture to increase crop water use efficiency, especially by maintaining leaf expansion of plants in drying soil (Belimov *et al.*, 2009b), allowing greater coverage of the soil surface to lessen soil evaporation and greater light capture to maximize whole-plant assimilation. Furthermore, stimulating crop development (as suggested by the promotion of flowering observed here) may accelerate the cropping cycle, of advantage both in avoiding late-season water deficits or other stresses, but also under well-watered conditions.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Fig. S1. Plates from inoculated and control roots at different dilution levels.

References

Achard P, Baghour M, Chapple A, Hedden P, Van der Straeten D, Genschik P, Moritz T, Harberd NP. 2007. The plant stress hormone ethylene controls floral transition via DELLA-dependent regulation of floral meristem-identity genes. *Proceedings of the National Academy of Sciences, USA* **104,** 6484–6489.

Arshad M, Frankenberger WT. 1991. Microbial production of plant hormones. *Plant and Soil* **133,** 1–8.

Belimov A, Dodd IC, Hontzeas N, Theobald JC, Safronova VI, Davies WJ. 2009b. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. *New Phytologist* **181,** 413–423.

Belimov A, Dodd IC, Safronova VI, Davies WJ. 2009a. ACC deaminase-containing rhizobacteria improve vegetative development and yield of potato plants grown under water-limited conditions. *Aspects of Applied Biology* **98,** 163–169.

Belimov A, Dodd IC, Safronova VI, Hontzeas N, Davies WJ. 2007. *Pseudomonas brassicacearum* strain Am3 containing 1-aminocyclopropane-1-carboxylate deaminase can show both pathogenic and growth-promoting properties in its interaction with tomato. *Journal of Experimental Botany* **58,** 1485–1495.

Belimov A, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR. 2005. Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.). *Soil Biology and Biochemistry* **37,** 241–250.

Belimov A, Safronova VI, Mimura T. 2002. Response of spring rape (*Brassica napus* var. *oleifera* L.) to inoculation with plant growth promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate deaminase depends on nutrient status of the plant. *Canadian Journal of Microbiology* **48,** 189–199.

Belimov A, Safronova VI, Sergeyeva TA, *et al.* 2001. Characterization of plant growth promoting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. *Canadian Journal of Microbiology* **47,** 642–652.

Bleecker AB, Estelle MA, Somerville C, Kende H. 1988. Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* **241,** 1086–1089.

Chae HS, Faure F, Kieber JJ. 2003. The *eto1*, *eto2*, and *eto3* mutations and cytokinin treatment increase ethylene biosynthesis in *Arabidopsis* by increasing the stability of ACS protein. *The Plant Cell* **15,** 545–559.

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM. 1993. *Arabidopsis* ethylene-response gene *Etr1*– similarity of product to 2-component regulators. *Science* **262,** 539–544.

Dodd IC, Ruiz-Lozano JM. 2012. Microbial enhancement of crop resource use efficiency. *Current Opinion in Biotechnology* **23,** 236–242.

Dodd IC, Theobald JC, Richer SK, Davies WJ. 2009. Partial phenotypic reversion of ABA-deficient flacca tomato (*Solanum lycopersicum*) scions by a wild-type rootstock: normalizing shoot ethylene relations promotes leaf area but does not diminish whole plant transpiration rate. *Journal of Experimental Botany* **60,** 4029–4039.

Dodd IC, Zinovkina NY, Safronova VI, Belimov A. 2010. Rhizobacterial mediation of plant hormone status. *Annals of Applied Biology* **157,** 361–379.

Else MA, Jackson MB. 1998. Transport of 1-aminocyclopropane-1-carboxylic acid (ACC) in the transpiration stream of tomato (*Lycopersicon esculentum*) in relation to foliar ethylene production and petiole epinasty. *Australian Journal of Plant Physiology* **25,** 453–458.

Galland M, Gamet L, Varoquaux F, Touraine B, Touraine B, Desbrosses G. 2012. The ethylene pathway contributes to root hair elongation induced by the beneficial bacteria *Phyllobacterium brassicacearum* STM196. *Plant Science* **190,** 74–81.

Ghanem ME, Hichri I, Smigocki AC, Albacete A, Fauconnier ML, Diatloff E, Martinez-Andujar C, Lutts S, Dodd IC, Perez-Alfocea F. 2011. Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance. *Plant Cell Reports* **30,** 807–823.

Glick BR. 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiology Letters* **251,** 1–7.

Glick BR, Cheng Z, Czarny J, Duan J. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *European Journal of Plant Pathology* **119,** 329–339.

Glick BR, Karaturovic DM, Newell PC. 1995. A novel procedure for rapid isolation of plant-growth promoting pseudomonads. *Canadian Journal of Microbiology* **41,** 533–536.

Glick BR, Liu CP, Ghosh S, Dumbroff EB. 1997. Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Soil Biology and Biochemistry* **29,** 1233–1239.

Glick BR, Penrose DM, Li JP. 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of Theoretical Biology* **190,** 63–68.

Guzman P, Ecker JR. 1990. Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. *The Plant Cell* **2,** 513–523.

Hall JA, Peirson D, Ghosh S, Glick BR. 1996. Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Israel Journal of Plant Sciences* **44,** 37–42.

Hua J, Chang C, Sun Q, Meyerowitz EM. 1995. Ethylene insensitivity conferred by *Arabidopsis ERS* gene. *Science* **269,** 1712–1714.

Hua J, Meyerowitz EM. 1998. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell* **94,** 261–271.

Hua J, Sakai H, Nourizadeh S, Chen QHG, Bleecker AB, Ecker JR, Meyerowitz EM. 1998. *EIN4* and *ERS2* are members of the putative ethylene receptor gene family in *Arabidopsis*. *The Plant Cell* **10,** 1321–1332.

Jacobson CB, Pasternak JJ, Glick BR. 1994. Partial-purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant-growth promoting rhizobacterium *Pseudomonas putida* Gr12-2. *Canadian Journal of Microbiology* **40,** 1019–1025.

Jiang F, Chen L, Belimov AA, Shaposhnikov AI, Gong F, Meng X, Hartung W, Jeschke DW, Davies WJ, Dodd IC. 2012. Multiple impacts of the plant growth-promoting rhizobacterium *Variovorax paradoxus* 5C-2 on nutrient and ABA relations of *Pisum sativum*. *Journal of Experimental Botany* **63,** 6421–6430.

Johnson PR, Ecker JR. 1998. The ethylene gas signal transduction pathway: a molecular perspective. *Annual Review of Genetics* **32,** 227–254.

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR. 1993. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. *Cell* **72,** 427–441.

Kloepper JW, Tuzun S, Kuc JA. 1992. Proposed definitions related to induced disease resistance. *Biocontrol Science and Technology* **2,** 349–351.

Lee SH, Reid DM. 1997. The role of endogenous ethylene in the expansion of *Helianthus annuus* leaves. *Canadian Journal of Botany-Revue Canadienne De Botanique* **75,** 501–508.

Li JP, Ovakim DH, Charles TC, Glick BR. 2000. An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. *Current Microbiology* **41,** 101–105.

Liang XW, Abel S, Keller JA, Shen NF, Theologis A. 1992. The 1-aminocyclopropane-1-carboxylate synthase gene family of *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **89,** 11046–11050.

Lopez-Bucio J, Campos-Cuevas JC, Hernandez-Calderon E, Velasquez-Becerra C, Farias-Rodriguez R, Macias-Rodriguez LI, Valencia-Cantero E. 2007. *Bacillus megatenum* rhizobacteria promote growth and alter root-system architecture through an auxin-and ethylene-independent signalling mechanism in *Arabidopsis thaliana*. *Molecular Plant–Microbe Interactions* **20,** 207–217.

Lugtenberg B, Kamilova F. 2009. Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology* **63,** 541–556.

Lynch JP. 2007. Roots of the second green revolution. *Australian Journal of Botany* **55,** 493–512.

Madhaiyan M, Poonguzhali S, Ryu J, Sa T. 2006. Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing *Methylobacterium fujisawaense*. *Planta* **224,** 268–278.

Mayak S, Tirosh T, Glick BR. 2004a. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry* **42,** 565–572.

Mayak S, Tirosh T, Glick BR. 2004b. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. *Plant Science* **166,** 525–530.

McDonnell L, Plett JM, Andersson-Gunneras S, Kozela C, Dugardeyn J, Van Der Straeten D, Glick BR, Sundberg B, Regan S. 2009. Ethylene levels are regulated by a plant encoded 1-aminocyclopropane-1-carboxylic acid deaminase. *Physiologia Plantarum* **136,** 94–109.

Morgan PW, Drew MC. 1997. Ethylene and plant responses to stress. *Physiologia Plantarum* **100,** 620–630.

Penrose DM, Moffatt BA, Glick BR. 2001. Determination of 1-aminocycopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. *Canadian Journal of Microbiology* **47,** 77–80.

Pierik R, Tholen D, Poorter H, Visser EJW, Voesenek L. 2006. The Janus face of ethylene: growth inhibition and stimulation. *Trends in Plant Science* **11,** 176–183.

Plett JM, McDonnell L, Regan S. 2009. Plant-encoded 1-aminocyclopropane-1-carboxylic acid deaminase activity implicated in different aspects of plant development. *Plant Signalling and Behavior* **4,** 1186–1189.

Roman G, Lubarsky B, Kieber JJ, Rothenberg M, Ecker JR. 1995. Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. *Genetics* **139,** 1393–1409.

Ruzicka K, Ljung K, Vanneste S, Podhorska R, Beeckman T, Friml J, Benkova E. 2007. Ethylene regulates root growth through effects on auxin biosynthesis and transport-dependent auxin distribution. *The Plant Cell* **19,** 2197–2212.

Sharp RE, LeNoble ME, Else MA, Thorne ET, Gherardi F. 2000. Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. *Journal of Experimental Botany* **51,** 1575–1584.

Sobeih WY, Dodd IC, Bacon MA, Grierson D, Davies WJ. 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial root-zone drying. *Journal of Experimental Botany* **55,** 2353–2363.

Solano R, Stepanova A, Chao QM, Ecker JR. 1998. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes and Development* **12,** 3703–3714.

Spollen WG, LeNoble ME, Samuels TD, Bernstein N, Sharp RE. 2000. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiology* **122,** 967–976.

Stepanova AN, Yun J, Likhacheva AV, Alonso JM. 2007. Multilevel interactions between ethylene and auxin in *Arabidopsis* roots. *The Plant Cell* **19,** 2169–2185.

Timmusk S, Paalme V, Pavlicek T, Bergquist J, Vangala A, Danilas T, Nevo E. 2011. Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates. *Plos One* **6,** e17968.

Vessey JK. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* **255,** 571–586.

Viterbo A, Landau U, Kim S, Chernin L, Chet I. 2010. Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. *FEMS Microbiology Letters* **305,** 42–48.

Wang KLC, Li H, Ecker JR. 2002. Ethylene biosynthesis and signalling networks. *The Plant Cell* **14,** S131–S151.