Plant Soil (2016) 401:259–272
DOI 10.1007/s11104-015-2743-7

REGULAR ARTICLE

Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects

Dongmei Zhou · Xing-Feng Huang · Jacqueline M. Chaparro · Dayakar V. Badri · Daniel K. Manter · Jorge M. Vivanco · Jianhua Guo

Received: 24 August 2015 / Accepted: 12 November 2015 / Published online: 26 November 2015
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Abstract
Background and aims Plant growth-promoting rhizobacteria (PGPR) have garnered interest in agriculture due to their ability to influence the growth and production of host plants. ATP-binding cassette (ABC) transporters play important roles in plant-microbe interactions by modulating plant root exudation. The present study aimed to provide a more precise understanding of the mechanism and specificity of the interaction between PGPR and host plants.
Methods In the present study, the effects of interactions between a PGPR strain, *Bacillus cereus* AR156, and *Arabidopsis thaliana* wild type (Col-0) or its ABC transporter mutants on plant growth have been studied.
Results B. cereus AR156 promoted the shoot growth of Col-0 and *Atabcg30* but repressed the growth of *Atabcc5*. Bacterial volatiles and secretion promoted the shoot growth of Col-0 and *Atabcg*30 but had no effect on *Atabcc5*. We also found that root exudates of Col-0 induced the expression of *B. cereus* AR156 genes related to siderophore and chitinase production; while root exudates of *Atabcc5* inhibited the expression level of those genes. Further analysis of root exudates revealed that amino acids, organic acids, and sugars were

Responsible Editor: Jesus Mercado-Blanco.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-015-2743-7) contains supplementary material, which is available to authorized users.

D. Zhou · J. Guo (✉)
Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China
e-mail: jhguo@njau.edu.cn

D. Zhou · J. Guo
Engineering Center of Bioresource Pesticide in Jiangsu Province, Nanjing Agricultural University, Ministry of Education, Nanjing 210095, China

D. Zhou · J. Guo
National and Local Joint Green Pesticide Formulation and Application Technology Engineering Research Center, Nanjing Agricultural University, Ministry of Education, Nanjing 210095, China

D. Zhou · J. Guo
Key Laboratory of Integrated Management of Crop Diseases and Pests, Nanjing Agricultural University, Ministry of Education, Nanjing 210095, China

D. Zhou · X.-F. Huang · J. M. Chaparro · D. V. Badri · J. M. Vivanco
Center for Rhizosphere Biology, Colorado State University, Fort Collins, CO 80523, USA

X.-F. Huang
Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO 80523, USA

D. K. Manter
USDA-ARS Soil–Plant–Nutrient Research Unit, Fort Collins, CO 80526, USA

significantly less abundant in *Atabcc5* when compared to Col-0.
Conclusions Our findings highlight that both host plant and PGPR play active roles in the outcome of the plant-microbe interaction.

Keywords Plant growth-promoting rhizobacteria · Root exudates · ABC transporters · *Bacillus cereus* · Bacterial secretions

Introduction

Plant growth-promoting rhizobacteria (PGPR) were initially characterized due to their ability to stimulate the growth of plants in several ways via solubilizing nutrients such as phosphorus and iron, fixing atmospheric nitrogen, and producing phytohormones when grown in association with plant roots (Gray and Smith 2005; Idris et al. 2007; Kloepper and Schroth 1978). In addition to plant growth promotion, PGPRs also exhibit biological control traits including secretion of antibiotics, competition for nutrients, production of lytic enzymes, and induction of systemic resistance in plants (Chet 1990; Chet and Inbar 1994; Loon and Bakker 2006; Weller 1988). Furthermore, an increasing number of studies have shown that PGPRs play important roles in conferring plant tolerance to abiotic stresses such as drought and salinity (Malhotra and Srivastava 2009; Mayak et al. 2004; Wang et al. 2012). *Bacillus* strains from species such as *B. subtilis*, *B. cereus*, *B. licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, *B. polymyxa*, and *B. megaterium* have been reported to successfully colonize the roots and rhizosphere of several plants such as tomato, banana, canola, wheat, apple, red pepper, and *Arabidopsis* resulting in promotion of plant growth and yield, disease resistance, drought tolerance, and heavy metal remediation (Chen et al. 2013; Joo and Chang 2005; Karlidag et al. 2007; Khalid et al. 2004; Mayak et al. 2004; Sgroy et al. 2009). A large body of knowledge has shown the advantages of PGPRs; however, the shortcomings of PGPRs including poor survival rate (Acea et al. 1988) and inconsistent efficacy (Labuschagne et al. 2011) should not be overlooked. Few reports have attempted to comprehend the reasons for the variable effects of PGPRs, showing that these organisms could be highly specific to plant species, cultivars, and genotypes (Figueiredo et al. 2011; Gupta et al. 2000; Lucy et al. 2004; Siddiqui and Shaukat 2003). In addition, the presence of competing microbes and soil-factors such as temperature, water content, oxygen, and pH influence the effect of PGPR on host plants (Dutta and Podile 2010; Frans et al. 2007; Hrynkiewicz et al. 2010).

It is well documented that plant root exudates play key roles in the mediation of plant-microbe interactions in the rhizosphere (Badri et al. 2008, 2009; Chaparro et al. 2013). The composition of plant root exudates is determined by plant species, cultivar, developmental stage, and numerous environmental factors including soil type, temperature, humidity, pH, and nutrient availability (Bais et al. 2006; Brimecombe et al. 2001; Nicholas 2007; Rovira 1969). Root exudates are also responsible for biological control by eliciting microbial biofilm formation on the root surfaces which is now widely recognized as a form of biological control of plant pathogens by weakening pathogens' competition for nutrients and space (Bais et al. 2004; Chen et al. 2012; Davey and O'Toole 2000). A previous study reported that L-malic acid found in the root exudates of tomato strongly stimulated biofilm formation *ex planta* of *B. subtilis* (Chen et al. 2012); thus promoting its biocontrol of the bacterial disease caused by *Ralstonia solanacearum* (Chen et al. 2013).

ATP-binding cassette (ABC) transporters encompass a large protein family and play key roles in the transportation of compounds in plant cells both extracellularly over the plasma membrane and intracellularly into the vacuoles (Kang et al. 2010; Rea 2007; Yazaki 2005; Yazaki et al. 2009). ABC transporters are important for plant root exudation and play key roles during interaction with rhizosphere organisms (Badri et al. 2008, 2009; Loyola-Vargas et al. 2007; Sugiyama et al. 2007). For instance, the ABC transporter mutant *Atabcg30* secreted more phenolic compounds than sugars in their root exudates compared to the wild type which modified the rhizosphere microbial community (Badri et al. 2009). ABC transporters also play a role in plant-fungus symbiont interactions by acting as a cellular strigolactone exporter in the case of PDR1 in *Petunia hybrida* which serves to regulate the development of arbuscular mycorrhizae and axillary branches (Kretzschmar et al. 2012). Atabcg30 belongs to the pleiotropic drug resistance protein (PDR) subfamily which is involved in exporting antifungal compounds, in disease resistance, and in heavy metal tolerance (Fourcroy et al. 2014; Stein et al. 2006). Atabcc5 belongs to the multidrug resistance-associated protein

(MRP) and acts as an auxin conjugate transporter as increased auxin levels were found in Atabcc5 plants (Jasinski et al. 2003). It had been documented that Atabcc2 (AtMRP2) contributed to detoxification, vacuolar organic anion transport and chlorophyll degradation (Frelet-Barrand et al. 2008). Moreover, it had been reported that *Arabidopsis* Atabcg36 (AtPDR8) and Atabcg37 (AtPDR9) had an involvement in regulation of auxin homeostasis and plant development by directly transporting the auxin precursor indole-3-butyric acid (Strader and Bartel 2009; Růžička et al. 2010).

B. cereus AR156 has been previously described as a PGPR which significantly increased biomass and induced systemic resistance in *Arabidopsis thaliana* against *Pseudomonas syringae* pv. *lycopersici* (DC3000) by concurrently activating salicylate- and jasmonate/ethylene-dependent signaling pathways (Niu et al. 2011). Moreover, another study showed that *B. cereus* AR156 enhanced drought tolerance of cucumber (Wang et al. 2012). In previous works, *B. cereus* AR156 had the ability to produce siderophore and chitinases in vitro. Siderophores are produced by many PGPRs and have the potential of stimulating plant growth by binding a variety of iron-containing molecules (Ahmed and Holmström 2014; Crowley 2006; Kloepper et al. 1980; Miethke and Marahiel 2007). Chitinases produced in a variety of *Bacillus* spp. are involved in plant growth promotion (Lee et al. 2005; Sharp 2013) and in biocontrol of plant pathogens and pests (Herrera-Estrella and Chet 1999). In a previous study, *B. cereus* AR156 functioned as a biocontrol agent against *Pseudomonas syringe* and *Meloidogyne incognita* (Niu et al. 2011; Wei et al. 2010). Yet PGPR's ability to positively influence plant growth and productivity is inconsistent, thus their application in the field does not always produce desired outcomes (Lambert and Joos 1989; Martínez-Viveros et al. 2010). Here, we aimed at getting a better understanding of the specificity of the interaction between PGPR and host plants in order to determine possible reasons that, in some instances, PGPR inoculations are ineffective. We hypothesized that root exudates could play an important role in PGPR - host plant interactions. To achieve this goal, we set up a system by using *B. cereus* AR156 and ABC transporter mutants of *Arabidopsis*. ABC transporter mutants of *Arabidopsis* were selected because they have been shown to be important in plant root exudation and root exudates play a significant role in initiating and maintaining plant-PGPR interactions. In this study, we show how the PGPR affected the plant's growth due to bacterial secretions and emissions, and how root exudation modulated biochemical determinants in the PGPR that could affect the performance of the host plant.

Materials and methods

Plant materials, bacterial strains, and growth conditions

Seeds of *Arabidopsis thaliana* wild type Col-0 and ABC-transporter mutants (*Atabcg30*, *Atabcg36*, *Atabcg37*, *Atabcc2*, and *Atabcc5*) (Badri et al. 2008, 2009) were surface-sterilized in 2.5 % NaClO for 1 min, washed five times with sterile water and then placed at 4 °C for 4 days to break dormancy. Seeds were planted on Murashige and Skoog medium (MS) (Murashige and Skoog 1962) agar plates containing 1 % sucrose and placed vertically in a growth chamber at 25 °C under a 16/8 h photoperiod.

The PGPRs in the present study were *B. cereus* AR156 and its kanamycin-resistant mutant *B. cereus* AR156-Ka. The kanamycin-resistant mutant was used in root colonization studies under soil conditions when kanamycin was used to exclude other bacteria from being accounted (Niu et al. 2011). *B. cereus* AR156 was grown on Luria-Bertani (LB) (Bertani 1951) agar plates and *B. cereus* AR156-Ka was grown on LB agar plates supplemented with 200 mg/L of kanamycin at 30 °C for 24 h. A single colony of each strain was transferred to liquid LB medium and incubated at 30 °C, 200 rpm for 24 h. Bacterial cells were centrifuged (8000 × g, 10 min) and re-suspended in sterile Hoagland's solution.

Bacterial suspension experiment

Seven days old *Arabidopsis* seedlings were transferred into pots (6 cm × 3.6 cm × 6 cm) containing a mixture of sterile sand and vermiculite (1:1, volume) and were placed in a growth chamber (25 °C, 16/8 h photoperiod, and relative humidity of 80–85 %). Three mL of *B. cereus* AR156 suspension (2×10^8 CFU/mL) in Hoagland's solution was inoculated to 14 days-old plants. The same amount of Hoagland's solution was used as control. There were eight plants in each tray and three trays for each treatment (24 plants in total). Plant shoot weight was recorded at 35 days-old.

To study the dosage effect of *B. cereus* AR156 on *Arabidopsis* plant growth, five concentrations of 3 mL bacterial suspensions of *B. cereus* AR156 (2×10^4 CFU/mL, 2×10^6 CFU/mL, 2×10^7 CFU/mL, 2×10^8 CFU/mL, and 2×10^9 CFU/mL) were inoculated to 14 days-old *A. thaliana* seedlings. There were eight plants in each tray and three trays for each treatment (24 plants in total). Plant shoot fresh weight and shoot dry weight of *Arabidopsis* plants was recorded when plants were 35 days-old.

Colonization of *B. cereus* AR156 in *Arabidopsis* rhizosphere

The rhizosphere samples of *Arabidopsis* plants treated with *B. cereus* AR156-Ka were collected (three plants of each mutant per replication) at 21 day after inoculation (Huang et al. 2015; Niu et al. 2011). One gram of thoroughly mixed rhizosphere samples from three plants was re-suspended in 9 mL of sterile 0.85 % NaCl and was shaken at 200 rpm for 30 min. Serial dilutions of the rhizosphere sample were placed on LB agar plates supplemented with 200 mg/L kanamycin. The number of CFU per gram of rhizosphere soil was recorded after incubation at 30 °C for 24 h. The quantification of colonization of *B. cereus* AR156 in the rhizosphere of different *Arabidopsis* mutants was repeated three times.

Infiltration assay

Leaves of 18 days-old *Arabidopsis* seedlings were infiltrated with bacterial suspension of *B. cereus* AR156 (2×10^8 CFU/mL) which were suspended in sterile distilled water. Sterile distilled water was used as control. The treated seedlings were covered in plastic wrap to maintain humidity. Disease symptoms were measured at 24 h after inoculation. There were 6 plants in each treatment and 3 leaves of each plant were infiltrated with *B. cereus* AR156 or sterile water.

Effect of bacterial volatiles on the shoot growth of *A. thaliana*

The effect of volatiles secreted by *B. cereus* AR156 on the shoot growth of Col-0 and ABC transporter mutants was studied as described by Ryu et al. (2003) and Huang et al. (2015). *Escherichia coli* (DH5α) and LB liquid medium were used as negative controls. There were three plates for each treatment and three plants on each plate. Plates were sealed with Parafilm and incubated in a growth chamber (25 °C, continuous light). Plant shoot fresh weight was recorded after 14 days.

Effect of bacterial culture filtrate on the shoot growth of *A. thaliana*

The effect of bacterial culture filtrate on Col-0 and ABC transporter mutants' growth was studied. *B. cereus* AR156 was cultured in 500 mL M9 liquid medium at 30 °C to reach $OD_{600}=2.0$, then adjusted to 2×10^8 CFU/mL and centrifuged at 12,000 rpm for 10 min to collect the supernatant. The supernatant was filtered through 0.2 μm membrane (Cat No. 722–2520, Thermo Scientific) to remove the bacterial cells. Three mL culture filtrate of *B. cereus* AR156 was applied to 14 days-old seedlings. The same amount of M9 liquid medium was used as control. The plants were placed in a growth chamber at 25 °C under a 16/8 h photoperiod. Each treatment contained 24 plants. Plant shoot fresh weight was measured at 35 days-old.

Root exudate collection

Root exudates were collected according to the methods of Badri et al. (2008; 2009). Seven-d-old *Arabidopsis* seedlings were transferred to six-well culture plates (Cat No. 08-772-1B, Fischer Co.) with 5 mL liquid MS medium containing 1 % sucrose in each well. The six-well plates were placed on a shaker (25±2 °C, 90 rpm, and 16/8 h photoperiod). When plants reached 18 days-old, they were washed three times with sterile water and transferred into sterile six-well plates containing fresh 5 mL MS liquid medium (without sucrose). The exudates were collected after 3 days following plants' transfer (plants were 21 day-old). Every treatment had 4 replicates and each replicate contained 12 individual plants. To remove root-border-like cells and root sheathing, root exudates were filtered through 0.45 μm nylon filters (Millipore).

Effect of root exudates on the growth of *B. cereus* AR156

The effect of root exudates on the growth of *B. cereus* AR156 was determined by counting the bacterial colonies on LB agar plates. *B. cereus* AR156 was grown in LB liquid medium at 30 °C to reach $OD_{600}=2.0$, then adjusted to a final density of 1×10^4 CFU/mL. One

hundred μL cell suspensions of *B. cereus* AR156 were mixed with 100 μL root exudates (Col-0, *Atabcg30*, and *Atabcc5*) or MS and placed on LB plates and incubated at 30 °C for 18 h. Root exudates were collected as described above. The colony forming units (CFUs) were counted. Data shown were from at least three replicates. The experiment was repeated twice.

Effect of root exudates on gene expression of *B. cereus AR156*

Root exudates from *Atabcc5* and Col-0 were collected as described above. One mL root exudates or MS liquid medium (control) was added into a flask containing 100 mL LB liquid medium (root exudate at a final concentration of 1 % v/v) (Chen et al. 2012). *B. cereus* AR156 was added to the flask to a final concentration of 1.0×10^4 CFU/mL. Flasks were put in a shaker at 30 °C and 200 rpm. Bacterial cells were collected from three replications at 7 h and 12 h after inoculation. The selected genes were *BACI_c19650* (siderophore biosynthesis protein) and *chiA* (chitinase). Previous studies have shown that *B. cereus* AR156 produces siderophore and chitinase, which play important roles in promoting plant growth (unpublished data from the Guo's lab). In addition, genes related to the production of siderophore and chitinase have been found by sequencing the whole genome of *B. cereus* AR156 (unpublished data). Total RNA of bacterial cells was extracted with the SV Total RNA Isolation System (Promega, Corporation) and cDNA was made with SuperScript® III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. Semi-quantitative RT-PCR was performed by using gene-specific primers (Table S1). The experiment was repeated twice.

Gas chromatography–mass spectrometry (GC-MS)

Root exudates for GC-MS analysis were collected as described above with some modifications. The exudates were collected after 3 days following plants' transfer (plants were 21 days-old), freeze dried and derivatized using the standard methoximation and trimethylsilylation procedure (Broeckling et al. 2005). For each replicate (n=5) 60 mL of exudates from 12 individually grown *Arabidopsis* plants were collected. Ribitol was used as an internal standard for each sample. GC-MS was carried out at the Samuel Roberts Nobel Foundation. An Agilent 6890 GC coupled with a 5973 MS at a split ratio of 1:1 was used to inject 1 μL of each sample. Separation was achieved using a 60 m DB-5MS (J & W Scientific) at a flow rate of 1 mL/min. Oven was held at 80 °C for two min, ramped at 5 °C/min to a final temperature of 315 °C and held for 12 min.

Data transformation and statistical analyses

One-way analysis of variance (ANOVA) was carried out and followed with Tukey's HSD (Honestly Significant Difference) test to compare the difference in fresh shoot weight of by Col-0 and mutants by inoculating B. cereus AR156 at different concentrations. The *t*-test was conducted to compare the difference in bacterial growth and colonization of the treatments and controls. Peak detection and deconvolution for GC-MS data was achieved through the Automated Mass Spectral Deconvolution and Identification System (AMDIS) (Halket et al. 1999). The quantitative peak area values were extracted using metabolomics ion-based extraction algorithm (MET-IDEA) (Broeckling et al. 2005). All compounds obtained via GC-MS analysis (Table S2) were normalized to the ribitol internal standard. For multivariate statistical analysis redundant peaks were removed and peak areas were pareto scaled. Statistical significant differences of identified root exudate compounds between wildtype and *Atabcc5* were determined by Bonferroni corrected *t*-test.

Results

Effect of *B. cereus* AR156 on plant growth of *Arabidopsis* wild type and ABC transporter mutants

Shoot fresh weight was measured and recorded at 21 days after inoculation of 2×10^8 CFU/mL *B. cereus* AR156 (Fig. S1). As shown in Fig. S1A, *B. cereus* AR156 showed distinct effects on the shoot weight of wild type and ABC transporter mutants. Fresh shoot weight of Col-0 and *Atabcg* mutants (*Atabcg30*, *Atabcg36*, and *Atabcg37*) significantly increased after the inoculation of *B. cereus* AR156 exhibiting a higher fresh shoot weight ranging from 0.07 to 0.20 g (16–72 % increase) compared to their respective uninoculated controls (*t*-test, $p<0.05$). *B. cereus* AR156 had no effect on the shoot weight of *Atabcc*2. In contrast, *Atabcc5* had 0.19 g lower (56 % decrease) fresh shoot weight than its control (*t*-test, $p<0.05$) (Fig. S1).

To test the effect of the dosage of *B. cereus* AR156 on the plat growth, *Atabcg30* and *Atabcc5* were selected for further experimentation because the shoot weight of *Atabcg30* was significantly promoted while the shoot weight of *Atabcc5* was significantly repressed compared to their respective controls. As the concentration of *B. cereus* AR156 increased Col-0 and the mutants showed distinct growth effects (Fig. 1a). The shoot fresh weight of Col-0 significantly increased at 2×10^4 CFU/mL, 2×10^6 CFU/mL, and 2×10^7 CFU/mL (Tukey's HSD test, $p<0.05$) compared to un-inoculated plants. The shoot fresh weight of Col-0 decreased significantly (Tukey's HSD test, $p<0.05$) at concentration 2×10^9 CFU/mL in comparison with plants treated at 2×10^4 CFU/mL, 2×10^6 CFU/mL, and 2×10^7 CFU/mL. *B. cereus* AR156 increased the shoot fresh weight of *Atabcg30* mutant plants at all concentrations. Moreover, there were significant increases of shoot fresh weight at 2×10^4 CFU/mL and 2×10^6 CFU/mL compared to the control plants (Tukey's HSD test, $p<0.05$) (Fig. 1a). In contrast, in *Atabcc5* the shoot fresh weight decreased significantly in comparison with control plants as the concentrations of *B. cereus* AR156 increased to 2×10^8 CFU/mL and 2×10^9 CFU/mL (Tukey's HSD test, $p<0.05$) (Fig. 1a); all other concentrations of *B. cereus* AR156 did not have an effect on shoot dry weight compared to the control.

The shoot dry weight of all plants was not completely consistent with the shoot fresh weight (Fig. 1b). No differences between treatments were seen in Col-0. In *Atabcg30*, *B. cereus* AR156 significantly increased the dry biomass at concentrations 2×10^6 CFU/mL, 2×10^8 CFU/mL, and 2×10^9 CFU/mL (Tukey's HSD test, $p<0.05$) (Fig. 1b). The shoot dry weight of *Atabcc5* significantly decreased by *B. cereus* AR156 at concentrations of 2×10^8 CFU/mL and 2×10^9 CFU/mL (Tukey's HSD test, $p<0.05$) (Fig. 1b).

Colonization of *B. cereus* AR156 in the rhizosphere of Col-0, *Atabcg30*, and *Atabcc5*

The colonization of *B. cereus* AR156 in the rhizospheres of Col-0, *Atabcg30*, and *Atabcc5* was monitored at 21 days post-treatment (Fig. 1c). It was observed that *B. cereus* AR156 colonized the rhizosphere of *Arabidopsis* wild type and the two mutants at all five inoculation concentrations. Mutant *Atabcc5* had significantly (Tukey's HSD test, $p<0.05$) more *B. cereus* AR156 cells in the rhizosphere when compared to mutant *Atabcg30* and Col-0 at the inoculation concentrations of 2×10^4 CFU/mL, 2×10^6 CFU/mL, and 2×10^7 CFU/mL. Furthermore, both mutants *Atabcg30* and *Atabcc5* exhibited more *B. cereus* AR156 colonization in the rhizosphere compared to Col-0 at the inoculation concentrations of 2×10^8 CFU/mL and 2×10^9 CFU/mL (Tukey's HSD test, $p<0.05$) (Fig. 1c).

B. cereus AR156 is not pathogenic to *Arabidopsis* plants

As an explanation for the inhibition of the growth of *Atabcc5*, we hypothesized a possible pathogenic effect of *B. cereus* AR156 towards *Atabcc5*. To test this possibility, we infiltrated the leaves of 18 days-old seedlings with *B. cereus* AR156 at 2×10^8 CFU/mL. After 24 h of treatment, there were no disease symptoms on the leaves of both *Atabcc5* and Col-0 (Fig. S2). We kept the plants under the same conditions for another three days but no disease symptoms appeared (data not shown).

Effect of bacterial volatile compounds on shoot weight of Col-0, *Atabcg30*, and *Atabcc5*

The involvement of *B. cereus* AR156 volatile compounds on plant growth promotion was tested by using partitioned media plates. *B. cereus* AR156 showed no effect on *Atabcc5* shoot weight compared to the controls (LB and *E. coli*) (Fig. 2a). However, the volatiles of *B. cereus* AR156 had a positive effect on *Atabcg30* growth as fresh weight significantly increased. The plant fresh weight increased 0.02 g (54 % increase) than that of control plants (Fig. 2a; *t*-test, $p<0.05$).

Effect of bacterial culture filtrate on shoot weight of Col-0, *Atabcg30*, and *Atabcc5*

The effect of bacterial culture filtrate was tested on Col-0, *Atabcg30*, and *Atabcc5* plants (Fig. 2b). The bacterial culture filtrate of *B. cereus* AR156 had a significant positive effect on the shoot weight of *Atabcg30* from 0.18 to 0.26 g (43.3 % increase) while that of Col-0 increased from 0.17 to 0.26 g (48.2 % increase) (Fig. 2b; *t*-test, $p<0.05$). However, the bacterial culture filtrate did not show significant effect on the growth of *Atabcc5* (Fig. 2b).

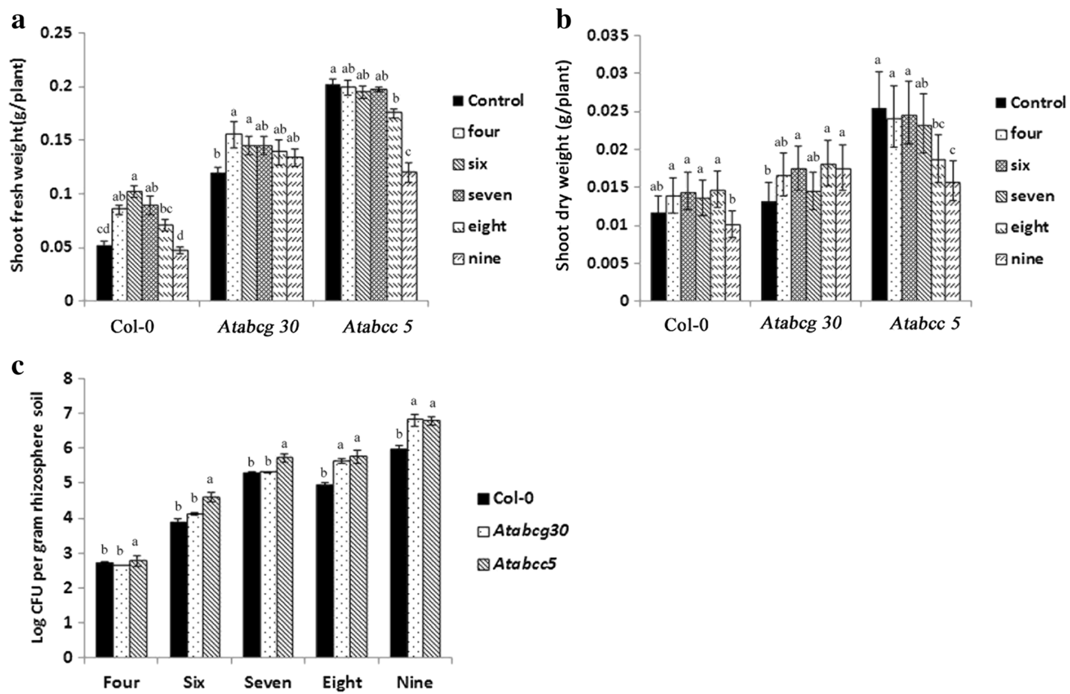

Fig. 1 Effects of different concentrations of AR156 on shoot fresh weight biomass (**a**) and shoot dry weight (**b**) of 35 days-old *Atabcg30*, *Atabcc5* and Col-0, and colonization of AR156 in the rhizosphere of those plants (**c**). Two week old plants were inoculated with cell suspensions of five concentrations of AR156: 2×10^6 CFU/mL (Six), 2×10^7 CFU/mL (Seven), 2×10^8 CFU/mL (Eight), and 2×10^9 CFU/mL (Nine) re-suspended in Hoagland's solution; Hoagland's solution alone was used as a control. Letters indicate statistically significant differences between the treatments of a given plant type with different concentrations of AR156 (Tukey's honest significance test; $p<0.05$)

Effect of *Arabidopsis* Col-0 and mutants' root exudates on the growth of *B. cereus* AR156

Root exudates of Col-0, *Atabcg30*, and *Atabcc5* plants were collected from 21 days-old seedlings and applied to *B. cereus* AR156 to determine the effect of root exudates from Col-0 and different *Arabidopsis* mutants on the growth of *B. cereus* AR156 by counting CFUs on LB agar plates. As shown in Fig. 3, root exudates of Col-0, *Atabcg30*, and *Atabcc5* significantly (*t*-test, $p<0.05$) promoted the growth of *B. cereus* AR156 when compared to the control (MS media alone) (Fig. 3).

Effect of root exudates on gene expression of *B. cereus* AR156

Here we assessed the effect of root exudates of Col-0 and *Atabcc5* on the expression of genes of *B. cereus* AR156 related to the production of *BACI_c19650* (siderophore biosynthesis protein) and *chiA* (chitinase). As shown in Fig. 4, the transcript level of *BACI_c19650* was induced by the addition of Col-0 root exudates at 7 h post treatment. Furthermore, at 12 h post treatment, the expression level of both genes were induced by the addition of Col-0 root exudates and the control (MS) while no expression was observed in the treatment of root exudates of *Atabcc5*.

Analysis of root exudates of Col-0 and *Atabcc5* by GC-MS

Principal component analysis (PCA) revealed that the compounds detected in the root exudates of Col-0 and *Atabcc5* by GC-MS were significantly different from each other (Fig. 5). GC-MS analyses identified 537 unique features within *Atabcc5* root exudates and of these 89 features were annotated (Table S2). Furthermore it was observed that *Atabcc5* secreted significantly ($p<0.05$) less organic acids (malic acid, oxalic acid, fumaric acid, trihydroxybutyric acid, p-hydroxy benzoic acid, succinic acid, propionic acid, and citric acid) when compared to Col-0. Similarly, *Atabcc5* had significantly ($p<0.05$) lower levels of the sugars ribose

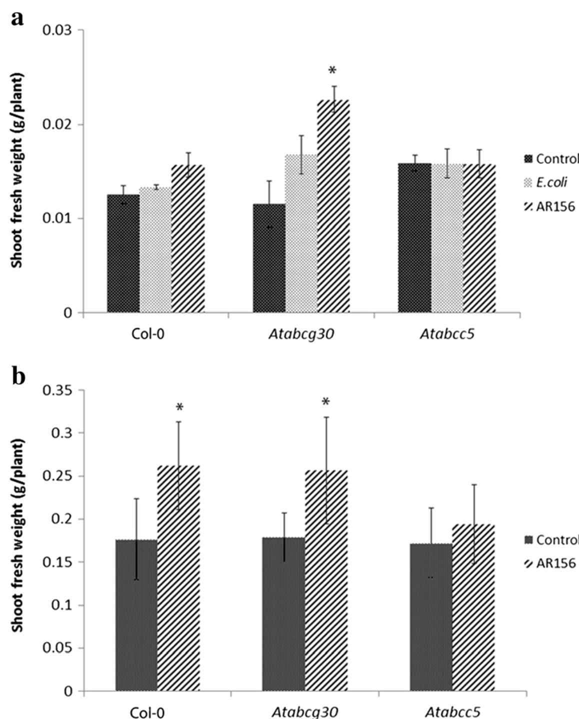

Fig. 2 Effects of AR156 volatile compounds (**a**) and culture filtrate (**b**) on *Arabidopsis* shoot fresh weight. **a** Shoot fresh weight of plants was measured after 14 days of inoculation of AR156 on the other side of the plate. The controls used were LB medium and *E. coli*. **b** Plant shoot fresh weight was measured when the seedlings were 35 days- old. The asterisks above the bars indicate significance relative to the control at $p<0.05$level (*t*-test)

and D-galactose as well as many amino acids (phenylalanine, valine, serine, tyrosine, proline, threonine, isoleucine, methionine, leucine, alanine, lysine, asparagine, and glycine) in the root exudates.

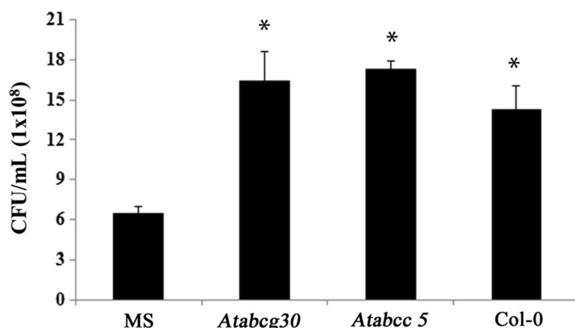

Fig. 3 Effect of root exudates on the growth of AR156. The asterisks above bars indicate significance relative to the control (MS) at the $p<0.05$level (*t*-test)

Discussion

The positive effect of PGPR on host plants is correlated with the colonization of these microbes in the rhizosphere (Knauth et al. 2005; Orr et al. 2011), and by the ability of these bacteria to express functions such as phosphate solubilization (Ramaekers et al. 2010; Richardson and Simpson 2011), and plant protection (Frapolli et al. 2010; Ryan et al. 2004). However, there is limited information on the mechanisms that govern the specificity of the interaction between PGPR and plants. In the present study, we aimed at understanding this specificity.

Effect of *B. cereus* AR156 on plant is plant type-specific

We observed that *B. cereus* AR156 promoted the shoot weight of Col-0 and *Atabcg30*, which was not surprising since the same result has been shown in other plants such as cucumber, tomato, and pepper (Niu et al. 2011; Zhou et al. 2014). It has been reported that *B. cereus* stimulates plant growth through the release of phytohormones (Dawwam et al. 2013), volatile compounds such as dimethyl disulfide (Huang et al. 2012), and peptidoglycan (Peterson et al. 2006).. Different possibilities were considered and tested to get insights on the mechanisms used by *B. cereus* AR156 to reduce the shoot weight of *Atabcc5*. First, we tested whether the negative effect of the bacterium was concentration-dependent. The effect of *B. cereus* AR156 on the shoot weight of Col-0 and *Atabcc5* was consistent with a previous study showing that high concentration of a mixture of *B. subtilis* and *B. cereus* decreased the shoot weight and yield of pepper (Zhou et al. 2014). We found that *B. cereus* AR156 suppressed the shoot weight of Col-0 and *Atabcc5* at concentrations 10^9 CFU/ mL and 10^7 CFU/ mL or greater, respectively. In comparison, *B. cereus* AR156 increased the shoot weight of *Atabcg30* at concentrations. This indicates that the effect of *B. cereus* AR156 on Col-0, *Atabcg30*, and *Atabcc5* is plant type-specific.

It had been reported that rhizosphere microbes are able to enhance plant hydration and nutritional status (Aliasgharzad et al. 2006; Azcón et al. 2013; Paul and Lade 2014). Our results showed that *B. cereus* AR156 had a positive effect on the fresh weight of plants compared to the effect on the shoot dry weight, we suggest that this is likely due to the ability of *B. cereus* AR156 to improve water uptake by the plants; however,

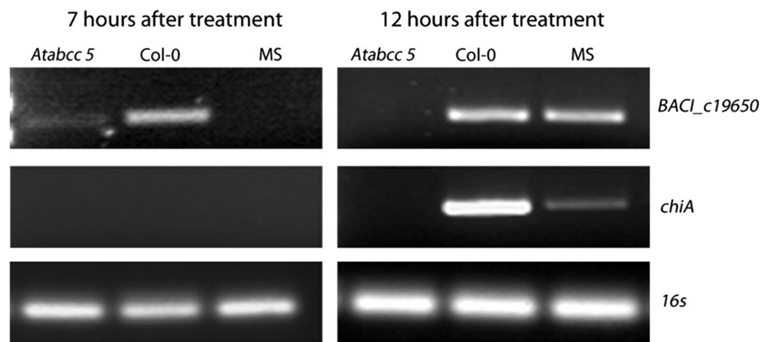

Fig. 4 Effect of root exudates on AR156 gene expression using RT-PCR. Gene expression level after 7 h (*left*) and 12 h (*right*) upon root exudates' treatment. The experiment had three replicates with similar results and the image represent one experiment

additional studies examining the impact of *B. cereus* AR156 on *Arabidopsis* root biomass and plant-water relations are needed.

Some *B. cereus* strains are food-borne pathogens (Stenfors Arnesen et al. 2008) but some of them are approved to be PGPRs to plants and were used for biocontrol of some plant diseases (Almaghrabi et al. 2013; Niu et al. 2011). Our infiltration assay confirmed that *B. cereus* AR156 was not pathogenic to *Atabcc5* or Col-0.

It is well known that ABC-transporters play important roles in the movement of different compounds both in and out of the cell (Kang et al. 2010; Yazaki 2005). Atabcc5 is an ion channel regulator in guard cells controlling stomata movement and the mutant *Atabcc5* is insensitive to abscisic acid (ABA) resulting in partial stomatal closure (Gaedeke et al. 2001). This information indicates that *Atabcc5* is incapable of detecting some compounds. The volatiles and secretions of *B. cereus* AR156 increased the shoot growth of Col-0 and *Atabcg30* but not the shoot growth of *Atabcc5*. These combined facts suggest that *Atabcc5* might lack the ability to sense and respond to the secretions of *B. cereus* AR156.

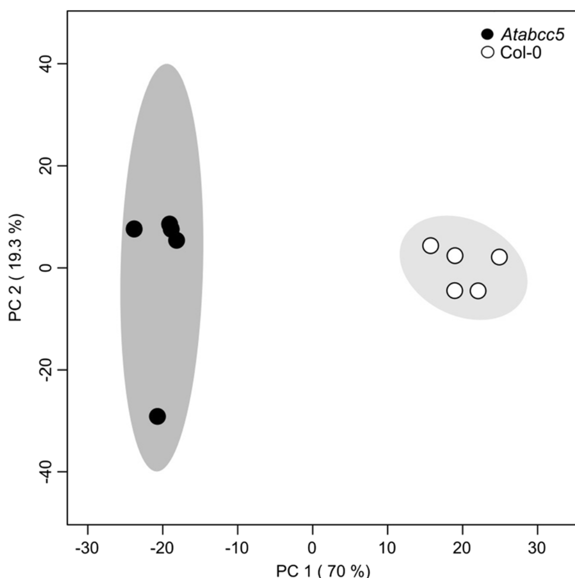

Fig. 5 Principal component analysis (PCA) of the root exudate profiles of Col-0 and *Atabcc5* obtained by GC-MS analysis. Ellipses are 95 % confidence intervals for each treatment

Effect of root exudates on PGPR

Root exudates contain many kinds of compounds including sugars, organic acids, amino acids, phenolic compounds, and some secondary metabolites (Bais et al. 2006), which are used as substrates for bacteria (Campbell and Greaves 1990). ABC transporters are strongly tied to root exudation (Badri et al. 2008, 2009). Recently, it was reported that root exudates from tobacco induced changes in exopolysaccharides and lipid-packing in the cell surface of *B. cereus*, and that these changes had a positive effect on bacterial colonization (Dutta et al. 2013). In the current study, we found that root exudates of Col-0, *Atabcg30*, and *Atabcc5* significantly promoted the growth of *B. cereus* AR156 when compared to MS media alone. The differences in root exudate composition may explain the observation that there was more bacterial colonization on mutants *Atabcg30* and *Atabcc5* compared to Col-0. For example, it has been reported that *Atabcg*30 showed increased phenolic compounds and decreased sugars in its root exudates which caused a change in the rhizosphere microbial community increasing the abundance of certain PGPRs (Badri et al. 2009). Moreover, phenolic compounds greatly influence the soil microbial community (Badri et al. 2013). Additionally, bacterial auxin produced in the rhizosphere is able to loosen plant cell walls resulting in increased plant root exudation (Glick

2012). This increase in root exudation could result in the enhanced bacterial colonization that we observed with *Atabcg30* and *Atabcc5*. Interestingly, bacterial colonization was the same in the treatments with higher *B. cereus* AR156 concentrations (2×10^8 CFU/mL and 2×10^9 CFU/mL) suggesting that the rhizosphere has an upper limit of *B. cereus* AR156 colonization that it can support.

The expression of certain gene encoding functions in PGPR such as *Pseudomonas*, *Azospirillum*, and *Bacillus* that benefit plants has been shown to be regulated by root exudation (Chen et al. 2012; Notz et al. 2001; Prigent-Combaret et al. 2008; Přikryl and Vančura 1980; Somers et al. 2005; Zakharova et al. 2000). Components of root exudates such as sugars (Notz et al. 2001), defense or development involved compounds for plants such as 2, 4-diacetylphloroglucinol (DAPG) and pyoluteorin (PLT) (de Werra et al. 2011), amino acids (Li and Glick 2001; Malhotra and Srivastava 2006; Rothballer et al. 2005), vitamins, and organic acids (Keshav Prasad Shukla et al. 2011) could either up or down regulate the expression level of genes encoding antifungal compounds and indole acetic acids by the beneficial bacteria. In our studies, we determined the influence of root exudates on the expression of siderophore biosynthesis protein gene *BACI_c19650* and chitinase gene *chiA* of *B. cereus* AR156. These genes were selected because *B. cereus* AR156 has the ability to produce siderophore and chitinase. Here, we found that the siderophore biosynthesis protein gene *BACI_c19650* was induced by the root exudates of Col-0, but less induced by root exudates of *Atabcc5*. Thus, indicating this as one of the mechanisms used by *B. cereus* AR156 to facilitate the growth of Col-0. Organic acids can enhance the amount of siderophore produced by bacteria (Sayyed et al. 2010). Another study showed that amino acids are co-exuded with siderophore in plants (Fan et al. 1997). Our metabolomics analysis (GC-MS) of the root exudates of *Atabcc5* revealed that significantly less organic acids (malic acid, oxalic acid, fumaric acid, trihydroxybutyric acid, p-hydroxy benzoic acid, succinic acid, propionic acid, and citric acid) and amino acids were exuded when compared to Col-0. Accordingly, we speculate that decreased secretion of organic acids and amino acids by *Atabcc5* inhibits the production of siderophores by *B. cereus* AR156. Subsequently, this lack of siderophore functioning could have resulted in shoot growth repression of *Atabcc5*. In the present study, we found the chitinase gene *chiA* was up-regulated by root exudates of Col-0 compared to those of *Atabcc5* 12 h after treatment. This result implies that one mechanism of plant growth promotion by *B. cereus* AR156 could be the production of chitinase which can promote plant growth and protect plants against fungal pathogens (Kim et al. 2005; Lee et al. 2005; Sharp 2013). In summary, our results indicate that root exudates of different mutants of *Arabidopsis* have the potential to differentially regulate the expression of genes related to plant growth promotion and biological control in *B. cereus* AR156.

The interaction between root exudates and PGPR leads to distinct plant growth promotion

Transcription level analyses of *Atabcg30* roots revealed that the expression of genes involved in biosynthesis and transport of secondary metabolites were induced compared to Col-0 (Badri et al. 2009). Our results showed that the shoot growth of *Atabcg30* was enhanced by the secretions of *B. cereus* AR156 compared to Col-0 and *Atabcc*5, indicating that the secretions of *B. cereus* AR156 that induce plant growth could be potentially transported in *Atabcg30*. Additionally, our metabolomics analysis revealed that *Atabcc5* exudes significantly lower amounts of certain compounds (organic acid, sugars, and amino acids) into the rhizosphere compared to Col-0, which may be indicative of a diminished ability to transport compounds into and out of the rhizosphere. The shoot growth of *Atabcc5*, on the other hand, could not be promoted by secretions of *B. cereus* AR156 which suggests that Atabcc5 might be important in sensing and transporting these bacterial secretions. Another possible reason for the growth repression on *Atabcc5* could be due to the auxin and auxin-like substrates produced by *B. cereus* AR156 in the rhizosphere that in addition with the increased levels of auxin produced by the mutant might have produced a toxic effect that negatively impacted the mutants' growth (Pilet and Saugy 1987). For example, one study reported that a *Bacillus* strain OSU-142 promoted growth and yield of apricot, yet had negative effects on the growth of raspberry potentially due to the high amounts of auxin or secondary metabolites produced by OSU-142 (Orhan et al. 2006). We also speculate that Atabcc5 plays a role in the transport of substances produced by PGPR that could be involved in plant growth. Further experimentation is needed to warrant the validity of these speculations.

In summary, the distinct plant growth promotion effects by PGPR are related to both the host and PGPR interactions which mutually regulated secretions of bioactive compounds. The volatile and secretions from PGPR have the ability to promote plant growth, but this effect is affected by mutations; likewise, plant exudates can modulate bacterial growth and colonization as well as beneficial gene expression of PGPR.

Acknowledgments We thank members of Professors Vivanco's and Guo's groups for technical assistance and helpful discussions. We especially thank Prof. Congfeng Song (Nanjing Agricultural University, China) and Dr. Azeddine Driouich (Universite' de Rouen, France) for helpful suggestions. This work was supported by National Natural Science Foundation of China (No.31471812 and No.31171809) to JG, China Scholarship Council (No. 201206850028) to DZ, and by Colorado State University Agricultural Experiment Station.

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