Biochemical and Biophysical Research Communications 461 (2015) 361–365

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Promotion of plant growth by *Pseudomonas fluorescens* strain SS101 *via* novel volatile organic compounds

Yong-Soon Park [a, 1], Swarnalee Dutta [a, 1], Mina Ann [a], Jos M. Raaijmakers [b], Kyungseok Park [a, *]

[a] Agricultural Microbiology Division, National Academy of Agricultural Science (NAAS), Rural Development Administration, Wanju 565-851, South Korea
[b] Department of Microbial Ecology, Netherlands Institute of Ecology, 6708 PB, Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 2 April 2015
Available online 16 April 2015

Keywords:
Pseudomonas fluorescens SS101
VOCs
GC–MS
13-Tetradecadien-1-ol
Plant growth promotion

ABSTRACT

Volatile organic compounds (VOCs) from plant growth-promoting rhizobacteria (PGPR) play key roles in modulating plant growth and induced systemic resistance (ISR) to pathogens. Despite their significance, the physiological functions of the specific VOCs produced by *Pseudomonas fluorescens* SS101 (*Pf*.SS101) have not been precisely elucidated. The effects of *Pf*.SS101 and its VOCs on augmentation of plant growth promotion were investigated *in vitro* and *in planta*. A significant growth promotion was observed in plants exposed *Pf*.SS101 under both conditions, suggesting that its VOCs play a key role in promoting plant growth. Solid-phase micro-extraction (SPME) and a gas chromatography-mass spectrophotometer (GC–MS) system were used to characterize the VOCs emitted by *Pf*.SS101 and 11 different compounds were detected in samples inoculated this bacterium, including 13-Tetradecadien-1-ol, 2-butanone and 2-Methyl-n-1-tridecene. Application of these compounds resulted in enhanced plant growth. This study suggests that *Pf*.SS101 promotes the growth of plants *via* the release of VOCs including 13-Tetradecadien-1-ol, 2-butanone and 2-Methyl-n-1-tridecene, thus increasing understanding of the role of VOCs in plant-bacterial inter-communication.

1. Introduction

Volatile organic compounds (VOCs) produced by plant growth-promoting rhizobacteria (PGPR) play key roles in plant growth promotion and induced systemic resistance (ISR) to pathogens [1–3]. A number of bacterial species, from diverse genera including *Bacillus, Pseudomonas, Serratia, Arthrobacter* and *Stenotrophomonas*, produce VOCs influencing plant growth [4–6]. Acetoin and 2,3-butanediol from *Bacillus* are the best known of these compounds and are implicated in significant improvements to plant growth [1]. In addition to promoting growth, VOCs produced by bacilli may elicit ISR [2], indicating several potential roles for VOCs within a plant.

The *Pseudomonas fluorescens* strain SS101 (*Pf*.SS101), a nonpathogenic rhizobacterium, was isolated from the rhizosphere of wheat (*Triticum aestivum*) [7]. This strain can produce a cyclic lipopeptide surfactant, massetolide A, which is an important molecule in a variety of traits, including swarming motility, biofilm formation, manipulation of zoospores, defense against protozoan predators and ISR [7–11]. It has been shown more recently that induced resistance to *Pseudomonas syringae* pv *tomato* (*Pst*) mediated by *Pf*.SS101 depends upon salicylic acid (SA) signaling and *NONEXPRESSOR OF PR1* (*NPR1*) but not on the jasmonic acid/ethylene (JA/ET) signaling pathway in *Arabidopsis* [12]. Although previous researchers have focused on such important issues, the roles of VOCs derived from *Pf*.SS101 facilitating plant growth promotion remain poorly understood.

To fill this gap in our current knowledge of the functions of VOCs produced by *Pf*.SS101, we investigated whether this bacterium affected plant growth promotion and also how its VOCs modulated plant growth and development. We performed an I-plate assay in which *Pf*.SS101 and plants were placed on each half of an I-plate, thus avoiding physical contact between them. Increased plant biomass, compared to the control, was observed when *Pf*.SS101 was inoculated onto one half of the I-plate. In agreement with this finding, tobacco plants exposed to *Pf*.SS101 were significantly taller and bigger than control plants under soil conditions. These results suggest that VOCs derived from *Pf*.SS101 are key components in

* Corresponding author. Fax: + 82 63 238 3834.
E-mail address: kspark3383@korea.kr (K. Park).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.bbrc.2015.04.039
0006-291X/

plant growth promotion. Therefore, we analyzed the biochemical profiles of VOCs produced by growing samples of *Pf*.SS101 using solid-phase micro-extraction (SPME) and a gas chromatography-mass spectrophotometer (GC–MS) system. This clearly identified 11 different chemical compounds presenting in samples inoculated with *Pf*.SS101. Moreover, tobacco plants exposed to various concentrations of 13-Tetradecadien-1-ol, 2-butanone and 2-Methyl-n-1-tridecene showed clear improvements in plant growth promotion. Collectively, our results suggest that *Pf*.SS101 enhances plant growth by producing VOCs, including 13-Tetradecadien-1-ol, 2-butanone and 2-Methyl-n-1-tridecene.

2. Materials and methods

2.1. Bacterial strain and culture media

The bacterial strain *P. fluorescens* SS101 (*Pf*.SS101) was used in all experiments. King's B broth (KB) plus agar was used as the substrate in experiments to determine interactions between *Pf*.SS101 and plants. Stock cultures of *Pf*.SS101 were maintained at −80 °C in tryptic soy broth (TSB, Difco, MI, USA) containing 20% glycerol.

2.2. Growth promotion by Pf.SS101 in vitro and in planta

For *in vitro* assays, surface-sterilized tobacco (*Nicotiana tabacum* cv. Xanthi-nc) seeds were placed in one half of an I-plate containing 0.5 × Murashige and Skoog (MS) medium (Duchefa Biochemie B.V, The Netherlands, 5 seeds per plate) plus agar. After 1 week of plant growth, a suspension of *Pf*.SS101 (20 μl, 10^7 cfu ml^{-1}) was dropped onto a sterile paper disc, which was then placed on the other half of the I-plate. The plates were completely sealed with parafilm and incubated at 25 °C for 3 weeks under a 12 h light/12 h dark photoperiod. The growth of plants incubated with *Pf*.SS101 was assessed using plant fresh weight.

For *in planta* assays, a plate inoculated with *Pf*.SS101 was placed beneath the soil at the bottom of a plastic container. A filter ensured that only VOCs from *Pf*.SS101 could transfer between the plate and the soil. Surface-sterilized tobacco seeds were sown onto the soil and the seedlings were grown at 25 °C for 4 weeks under a 12 h light/12 h dark photoperiod. Plant growth was assessed 4 weeks after sowing.

2.3. Identification of volatiles compounds produced by Pf.SS101

Pf.SS101 cultures were grown in KB broth in 20 ml glass headspace bottles (Supelco, Bellefonte, PA, USA) at 28 °C for 24 h before collection and analysis of volatiles using SPME and gas chromatography-mass spectrophotometry (GC–MS), as described previously [13] with minor modifications. Commercially available SPME fibers (Supelco, Bellefonte, PA, USA) were used to analyze volatiles from *Pf*.SS101. SPME fibers were desorbed at 60 °C for 2 min in the injection port of a Bruker Scion-SQ GC–MS (Bruker, Billerica, MA, USA) with Combi-Pal Autosampler and BR-5ms columns (Bruker, Billerica, MA, USA; length: 30 m, i.d.: 0.25 mm, 0.25 film thickness).

2.4. Verification of the selected synthetic VOCs on plant growth promotion

To confirm that the VOCs identified from *Pf*.SS101 promoted plant growth we tested three synthetic VOCs, 2-Butanone, 13-Tetradecadien-1-ol and 2-Methyl-n-1-tridecene, at three different concentrations. The procedures for preparation and incubation of tobacco seeds were as described above. The sealed plates were incubated at 25 °C for 4 weeks under a 12 h light/12 h dark photoperiod and the fresh weights of plants were measured after incubation.

2.5. Statistical analysis

The data were subjected to analysis of variance using SAS JMP software (SAS Institute, Cary, NC, USA). Significant differences in the treatment means of each sample were determined using the Least Significance Difference (LSD) Test at $P = 0.05$. All experiments were performed at least twice. The data from each experiment were analyzed separately.

3. Results

3.1. Plant growth promotion by Pf.SS101

To examine whether *Pf*.SS101 affected plant growth, surface-sterilized tobacco seeds were sown on 0.5 × MS medium plus agar in a square plate and incubated for 1 week. At this point, small plates inoculated with *Pf*.SS101 were placed within the same square plate and the plants were grown for an additional 3 weeks at 25 °C. Plants exposed to *Pf*.SS101 showed an increase in shoot and root biomass relative to the controls (Supplementary Fig. S1). This suggests that *Pf*.SS101 positively modulates plant growth and that VOCs derived from *Pf*.SS101 play a role in plant growth promotion.

3.2. Elicitation of plant growth promotion by Pf.SS101-mediated VOCs in vitro and in planta

To investigate the effect of VOCs produced by *Pf*.SS101 on plant growth, we performed I-plate assays as these avoid any physical contact between tobacco plants and Pf.SS101. One-week-old tobacco seedlings growing in one half of an I-plate were co-incubated with a suspension of *Pf*.SS101 in the other half of the plate for 3 weeks at 25 °C. Biomass production was stimulated in plants exposed to *Pf*.SS101 in this manner (Fig. 1A). Following

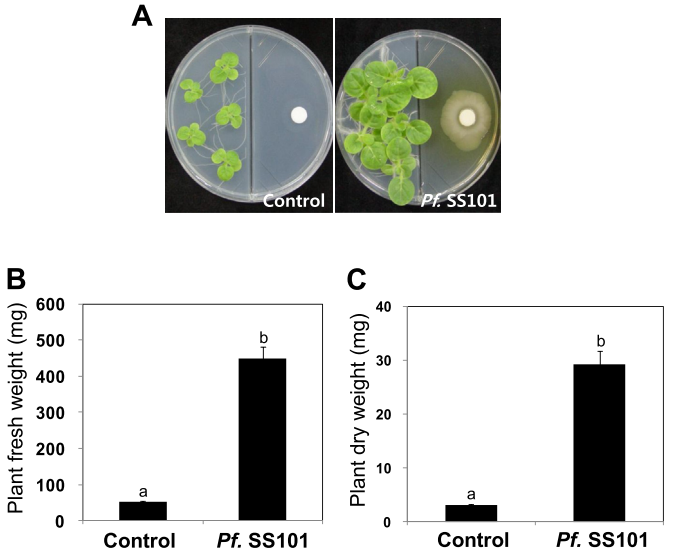


Fig. 1. Effect of VOCs from *Pf*.SS101 on plants grown in the I-plate assay. (A) One-week-old seedlings were exposed to VOCs from *Pf*.SS101 and incubated for 3 weeks at 25 °C. The representative photographs were taken four weeks after sowing. Plant fresh (B) and dry (C) weights were recorded. Error bars indicate the standard error of the mean (n = 6). The letters above the columns indicate significant differences between conditions ($P = 0.05$). Experiments were replicated at least three times producing similar results.

exposure to *Pf*.SS101, the fresh and dry weights of plants increased significantly by 8.8- and 9.5-fold, respectively, relative to controls (Fig. 1B and C).

To demonstrate that VOCs were the major regulators of this enhancement of plant growth and development, we created the *in planta* system shown in Fig. 2A. In brief, surface-sterilized tobacco seeds were sown onto autoclaved soil and grown for a week. At this time, a plate containing either *Pf*.SS101 or a control was placed beneath a filter at the bottom of each soil-filled plastic container, ensuring only VOCs produced by *Pf*.SS101 could come into contact with the tobacco plants under soil conditions. The tobacco seedlings were incubated for a further 3 weeks. Growth of tobacco plants in soil was significantly stimulated following exposure to *Pf*.SS101 in comparison to that of control plants (Fig. 2B), with fresh weight of exposed plants being increased by 1.5-fold relative to that of controls (Fig. 2C). Thus, our data indicate that plant growth is promoted by exposure to *Pf*.SS101 both *in vitro* and *in planta*, suggesting that VOCs emitted by *Pf*.SS101 regulate plant growth and development.

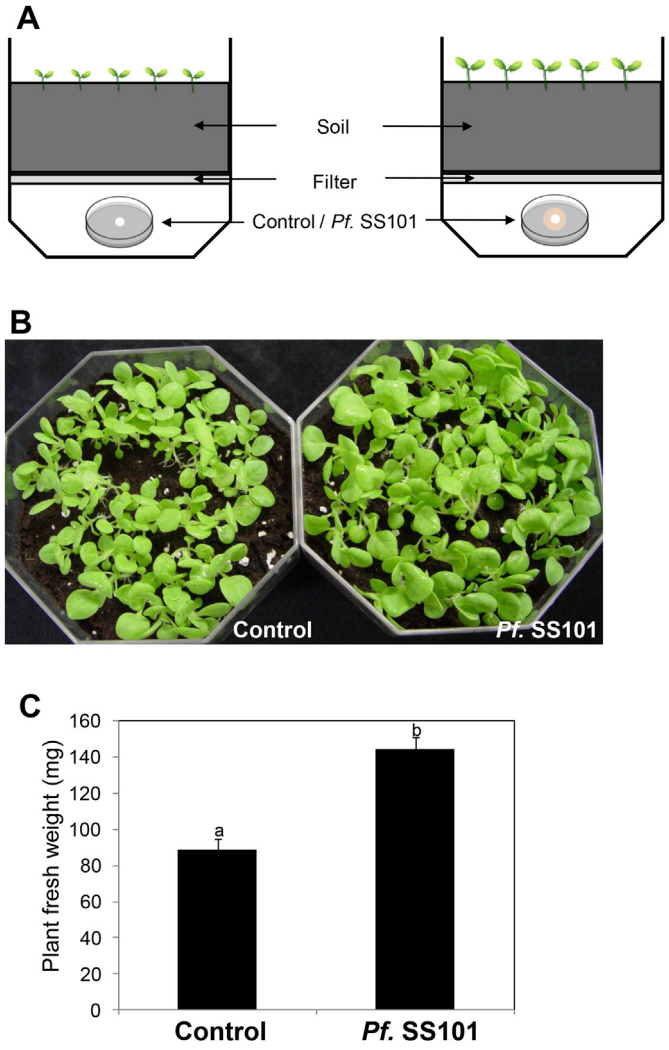


Fig. 2. Effect of VOCs from *Pf*.SS101 on plants grown in soil. (A) Schematic representation of the experimental setup. (B) Surface-sterilized seeds were sown on soil and exposed to VOCs emitted by *Pf*.SS101. The representative photograph was taken three weeks after sowing. (C) Fresh weights of plants of plants exposed to *Pf*.SS101 were higher 3 weeks after sowing. Error bars indicate the standard error of the mean (n = 5). The letters above the columns indicate a significant difference between conditions ($P = 0.05$). Experiments were replicated at least three times producing similar results.

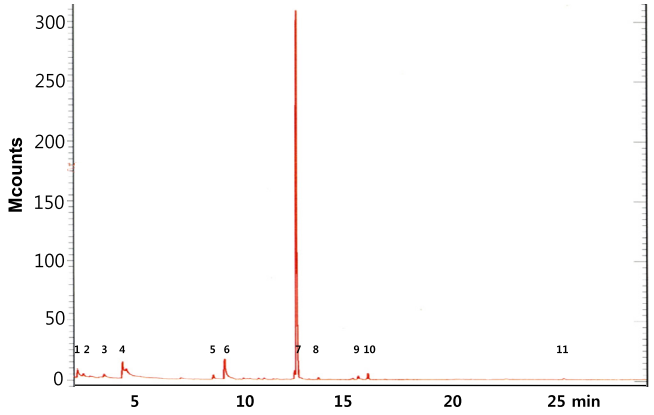
3.3. Identification of VOCs emitted by Pf.SS101

Since particular VOCs might play key roles in promoting plant growth, we characterized the VOCs emitted by *Pf*.SS101. To obtain a chemical profile of the VOCs produced by *Pf*.SS101, volatile emissions were collected from *Pf*.SS101 cultures grown in KB broth in 20 ml vials at 28 °C for 24 h and analyzed using SPME coupled with GC–MS. The spectrum of VOCs emitted by *Pf*.SS101 contained many peaks but 11 peaks were clearly and consistently identified in experimental samples relative to the controls (Fig. 3; data not shown). All these compounds had a retention time between 2 and 26 min and their molecular weights ranged from 72 to 240 (Fig. 3). Five of the identified compounds were alcohols: 2-Methyl-1-propanol, 3-methyl-1-butanol, 13-Tetradecadien-1-ol, 1-Cyclohexyl-1-pentanol and Trans-9-Hexadecen-1-ol. In addition, two hydrocarbons (1-Nonene and 2-Methyl-n-1-tridecene), a ketone (2-Butanone), an ester (Methyl thiolacetate) and an ether (Decyl-Oxirane) were present in the samples collected from *Pf*.SS101 (Fig. 3). The alcohol 13-Tetradecadien-1-ol displayed the highest peak of the 11 compounds detected in the VOC spectrum emitted by *Pf*.SS101 (Fig. 3).

3.4. Assessment of plant growth promotion by specific VOCs released by Pf.SS101

Of the 11 compounds identified as prominent VOCs released by *Pf*.SS101, we examined the effects of three, 13-Tetradecadien-1-ol, 2-Methyl-n-1-tridecene and 2-Butanone, on the promotion of plant growth. Surface-sterilized tobacco seeds were sown and grown for 1 week in half of an I-plate, as described above, and then one of the compounds, dissolved in methanol to a final concentration of 5, 50 and 500 ng, was applied to the other half of the plate (Fig. 4A). The plates were incubated for 4 weeks at 25 °C to allow plant growth.

Visual inspection revealed that plant growth was enhanced by all three compounds compared to the control treatment (Fig. 4A). The fresh weight of tobacco seedlings was increased approximately 3-fold by the presence of 13-Tetradecadien-1-ol (50 ng) and 2-fold by 2-Methyl-n-1-tridecene (5 ng), compared to growth under control conditions (Fig. 4B). In addition, a significant increase in fresh weight was observed in response to all tested concentrations



1: 2-Butanone; 2: 2-Methyl-1-propanol; 3: Methyl thiolacetate; 4: 3-methyl-1-butanol; 5: 1-Nonene; 6: 2,5-dimethylpyrazine; 7: 13-Tetradecadien-1-ol; 8: 2-Methyl-n-1-tridecene; 9: 1-Cyclohexyl-1-pentanol; 10: Decyl-Oxirane; 11: Trans-9-Hexadecen-1-ol

Fig. 3. Identification of VOCs emitted by *Pf*.SS101. *Pf*.SS101 cultures were grown in KB broth in headspace bottles at 28 °C for 24 h to enable collection of emitted VOCs. Analysis of VOCs derived from *Pf*.SS101 was performed using an SPME-GC-MS system. The numbers of the peaks indicate the individual chemical compounds identified during analysis.

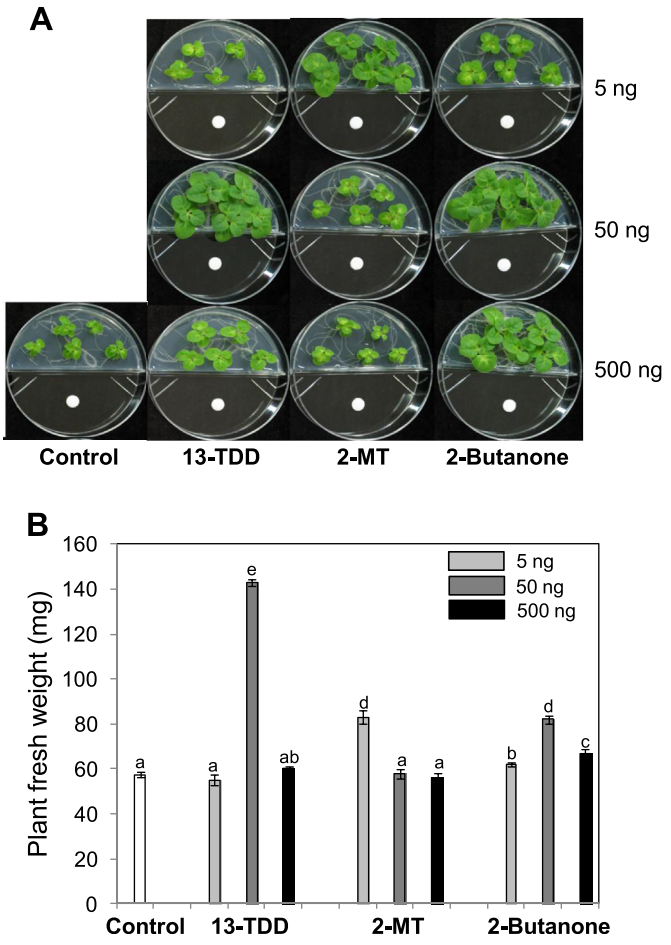


Fig. 4. Assessment of the effects of individual VOCs emitted by *Pf*.SS101. (A) The synthetic VOCs 13-Tetradecadien-1-ol (13-TDD), 2-Methyl-n-1-tridecene (2-MT) and 2-Butanone were dissolved in methanol and applied to sterile paper discs at three different concentrations (5, 50 and 500 ng). A disc was placed onto half of an I-plate 1 week after tobacco seeds had been sown on the other half of the plate. The pictures were taken 4 weeks after sowing. (B) Plant fresh weights were monitored and recorded after four weeks. Error bars indicate the standard error of the mean (n = 5). The letters above the columns indicate a significant difference between conditions ($P = 0.05$). Experiments were replicated at least three times producing similar results.

of 2-Butanone, with the maximum effect seen in plants exposed to 50 ng 2-Butanone (Fig. 4B). Our results provide strong evidence that VOCs produced by *Pf*.SS101 mediate the promotion of plant growth.

4. Discussion

Previous studies of *Pf*.SS101 reveal that it is required for ISR and other aspects [7–12]. However, despite these studies, the full roles of *Pf*.SS101 and/or VOCs derived from *Pf*.SS101 in promoting plant growth and development have been rarely reported. Our study produced several novel results: (1) *Pf*.SS101 stimulated plant growth promotion *in vitro* and *in planta*; (2) VOCs derived from *Pf*.SS101 were key regulators of plant growth promotion; and (3) exposure to three specific VOCs produced by *Pf*.SS101, 13-Tetradecadien-1-ol, 2-Methyl-n-1-tridecene and 2-Butanone, increased plant biomass. These results provide new insight into the inter-communication between tobacco plants and *Pf*.SS101, increasing our knowledge of the roles of VOCs in *Pf*.SS101-mediated plant growth.

Since the development of an *in vitro* I-plate system in 2003 [1], it has evaluated the effect of specific PGPR on plant growth. That initial study assessed the effects on *Arabidopsis* growth promotion by *Bacillus subtilis* GB03 and *B. amyloliquefaciens* IN937a and showed a significant improvement in the growth of plants exposed to these bacteria, compared to controls [1]. One of the intriguing findings of our study was that *Pf*.SS101 positively affected plant growth (Fig. 1).

Although the I-plate *in vitro* system is useful in determining intra- and inter-kingdom communications, its main drawback is the absent of biological relevance. To overcome this weakness, alternative methods are required for further analysis. We therefore used an alternative *in planta* system to support the I-plate methodology, determining that *Pf*.SS101 also stimulates plant growth under soil conditions (Fig. 2). Collectively, our results suggest that plant growth can be promoted by *Pf*.SS101-mediated VOCs in the absence of any contact between Pf.SS101 and plants.

That specific PGPR promote plant growth and ISR in the absence of any physical contact with plants, suggesting that VOCs are normally involved in these processes [12]. However, few bacterial VOCs with positive or negative impacts on plant growth have been studied [6]. For this reason, it is noteworthy that we have identified specific VOCs derived from *Pf*.SS101 (Fig. 3) and confirmed that they improve plant growth (Fig. 4). These results shed new light on how inter-communications between plants and *Pf*.SS101 *via* VOCs.

Several published studies indicate that VOCs released by common PGPR can be classified into numerous chemical groups, including alcohols, hydrocarbons, ketones, acids, ethers, esters, S-containing compounds and inorganic compounds [13–15]. As with these earlier reports, we detected alcohols, hydrocarbons, ketones, ethers and esters using the SPME-GC-MS system (Fig. 3). 2-butanone, 2-methyl-1-propanol, 3-methyl-1-butanol and Tridecane have been reported previously as compounds emitted by PGPR [13,15]. Quantification of the levels of VOCs produced by *Pf*.SS101 and of their effects on facilitating plant growth indicated that 13-Tetradecadien-1-ol was the most important compound. In contrast to other well-known VOCs, 13-Tetradecadien-1-ol has been reported in a relatively narrow context as a component of many lepidopterous sex pheromones [16]. These pheromones can be divided into two independent subgroups by means of their chemical structure [17]. The first subgroup contains pheromones composed of C_{10} to C_{18} acetates, alcohols, aldehydes and their derivatives and the second contains pheromones composed of C_{17} to C_{23} molecules; pheromones belonging to the second subgroup have been identified in specific species of Lepidoptera [18]. Therefore, although we cannot yet elucidate the underlying mechanisms triggered by 13-Tetradecadien-1-ol, our results indicate that this insect-associated VOC plays a critical role in interactions between *Pf*.SS101 and plants.

This study demonstrates the relationship between *Pf*.SS101 and plants *in vitro* and *in planta*. We provide new evidence that VOCs released by *Pf*.SS101 positively modulate plant growth promotion. Biochemical analysis of *Pf*.SS101 samples identified 11 independent VOCs. Characterization of these shows a clear role for 13-Tetradecadien-1-ol in plant growth and thus it is a novel candidate for a *Pf*.SS101 specific VOC. Our results provide new insight into plant growth promotion by identifying 13-Tetradecadien-1-ol as a VOC produced by *Pf*.SS101, thus broadening the range of functions of VOCs in plant-bacterial interactions.

Conflict of interests

We declare that we have no conflict of interests.

Acknowledgments

This study was supported by the International Cooperation Projects, RDA (PJ010807) and Research Program for Agricultural

Science & Technology Development and NAAS, RDA, South Korea (PJ009991).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.039.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.039

References

[1] C.M. Ryu, M.A. Farag, C.H. Hu, M.S. Reddy, H.X. Wei, P.W. Pare, J.W. Kloepper, Bacterial volatiles promote growth in Arabidopsis, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 4927–4932.
[2] C.M. Ryu, M.A. Farag, C.H. Hu, M.S. Reddy, H.X. Wei, J.W. Kloepper, P.W. Pare, Bacterial volatiles induce systemic resistance in *Arabidopsis*, Plant Physiol. 134 (2004) 1017–1026.
[3] M.A. Farag, H. Zhang, C.M. Ryu, Dynamic chemical communication between plants and bacteria through airborne signals: induced resistance by bacterial volatiles, J. Chem. Ecol. 39 (2013) 1007–1018.
[4] M. Kai, B. Piechulla, Plant growth promotion due to rhizobacterial volatiles – an effect of CO_2? FEBS Lett. 583 (2009) 3473–3477.
[5] M. Kai, B. Piechulla, Impact of volatiles of the rhizobacteria *Serratia odorifera* on the moss *Physcomitrella patens*, Plant Signal. Behav. 5 (2010) 444–446.
[6] A. Bailly, L. Weisskopf, The modulating effect of bacterial volatiles on plant growth, Plant Signal. Behav. 7 (2012) 1–7.
[7] J.T. De Souza, M. De Boer, P. De Waard, T.A. Van Beek, J.M. Raaijmakers, Biochemical, genetic, and zoosporical properties of cyclic lipopeptide surfactants produced by *Psedomonas fluorescens*, Appl. Environ. Microbiol. 69 (2003) 7161–7172.
[8] I. de Bruijn, M.J. de Kock, P. de Waard, T.A. van Beek, J.M. Raaijmakers, Massetolide A biosynthesis in *Psedomonas fluorescens*, J. Bacteriol. 190 (2008) 2777–2789.
[9] M. Mazzola, I. de Bruijn, M.F. Cohen, J.M. Raaijmakers, Protozoan induced regulation of cyclic lipopeptide biosynthesis is an effective predation defense mechanism for *Psedomonas fluorescens*, Appl. Environ. Microbiol. 75 (2009) 6804–6811.
[10] J.E. van de Mortel, H. Tran, F. Govers, J.M. Raaijmakers, Cellular responses of the late blight pathogen *Phytophthora infestans* to cyclic lipopeptide surfactants and their dependence on G proteins, Appl. Environ. Microbiol. 75 (2009) 4950–4957.
[11] J.M. Raaijmakers, I. De Bruijn, O. Nybroe, M. Ongena, Natural functions of lipopeptides from *Bacillus* and *Psedomonas*: more than surfactants and antibiotics, FEMS Microbiol. Rev. 34 (2010) 1037–1062.
[12] J.E. van de Mortel, R.C. de Vos, E. Dekkers, A. Pineda, L. Guillod, K. Bouwmeester, J.J. van Loon, M. Dicke, J.M. Raaijmakers, Metabolic and transcriptomic changes induced in *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101, Plant Physiol. 160 (2012) 2173–2188.
[13] M.A. Farag, C.M. Ryu, L.W. Sumner, P.W. Pare, GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants, Phytochemistry 67 (2006) 2262–2268.
[14] B. Lee, M.A. Farag, H.B. Park, J.W. Kloepper, S.H. Lee, C.M. Ryu, Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*, Plos One 7 (2012) e48744.
[15] B. Audrain, M.A. Farag, C.M. Ryu, J.M. Ghigo, FEMS Microbiol. Rev. (2015) in press.
[16] T. Anto, Y. Hasegawa, M. Uchiyama, Separation of Lepidopterous sex pheromones by reversed-phase thin layer chromatography and high performance liquid chromatography, Agric. Biol. Chem. 50 (1986) 2935–2937.
[17] R. Yamakawa, Y. Takubo, K. Ohbayashi, H. Naka, T. Ando, Female sex pheromone of Cystidia couaggaria couaggaria (Lepidoptera: geometridae): identification and field attraction, Biosci. Biotechnol. Biochem. 76 (2012) 1303–1307.
[18] T. Ma, Y. Li, Z. Sun, X. Wen, (Z,E)-9,12-Tetradecadien-1-ol: a major sex pheromone component of *Euzophera pyriella* (Lepidoptera:Pyralididae) in Xinjiang, China, Fla. Entomol. 97 (2014) 496–503.