Cyclic Dipeptides (CDPs) are one of the simplest compounds produced by living organisms. Plant-growth promoting rhizobacteria (PGPRs) also produce CDPs that can induce disease resistance. Bacillus vallismortis strain BS07 producing various CDPs has been evaluated as a potential biocontrol agent against multiple plant pathogens in chili pepper. However, plant signal pathway triggered by CDPs has not been fully elucidated yet. Here we introduce four CDPs, cyclo(Gly-L-Pro) previously identified from Aspergillus sp., and cyclo(L-Ala-L-Ile), cyclo(L-Ala-L-Leu), and cyclo(L-Leu-L-Pro) identified from B. vallismortis BS07, which induce disease resistance in Arabidopsis against Pseudomonas syringae infection. The CDPs do not directly inhibit fungal and oomycete growth in vitro. These CDPs require PHYTOALEXIN DEFICIENT4, SALICYLIC ACID INDUCTION DEFICIENT2, and NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 important for salicylic acid-dependent defense to induce resistance. On the other hand, regulators involved in jasmonate-dependent event, such as ETHYLENE RECEPTOR1, JASMONATE RESPONSE1, and JASMONATE INSENSITIVE1, are necessary to the CDP-induced resistance. Furthermore, treatment of these CDPs primes Arabidopsis plants to rapidly express PATHOGENESIS-RELATED PROTEIN4 at early infection phase. Taken together, we propose that these CDPs from PGPR strains accelerate activation of jasmonate-related signaling pathway during infection.

Keywords: Bacillus vallismortis, cyclic dipeptide, induced-resistance, jasmonate-dependent defense, salicylic acid-dependent defense

Handling Associate Editor: Hong, Jeum Kyu

Land plants absorb nutrients and water from soils. Seeing at an evolutionary point of view, mutualism between ancient plants and diverse microorganisms, especially endomycorrhizae, led the most important leap so that plants successfully colonized in the land (Pirozynski and Malloch, 1975; Parniske, 2008). Plant-growth promoting rhizobacteria (PGPRs), that colonize root surface and rhizosphere, positively regulate plant growth, and also enhance disease resistance against plant’s natural enemies (Kloepper, 1994; Lugtenberg and Kamilova, 2009). The beneficial effects of PGPRs are mainly due to production of various metabolites that can directly and indirectly modulate plant physiology related to plant growth and immunity (Kloepper and Schroth, 1981; Lugtenberg and Kamila, 2009; Zamioudis and Pieterse, 2012).

Induced-resistance is often classified into two groups based on kinds of causal biological agents and plant signaling pathways triggered by the inducers. First, induced-resistance accelerated by PGPRs usually harnesses
jasmonate (JA)-mediated signaling machinery and the induced-resistance is called as induced systemic resistance (ISR) (Pieterse et al., 1998; Choudhary and Johri, 2009). On the other hand, foliar immunization with virulent and/or avirulent pathogens, which induce severe necrosis and hypersensitive response, respectively, also activates systemic acquired resistance (SAR) (Ross, 1961; Ryals et al., 1996). In case of SAR, plants need salicylic acid (SA) accumulation both in local and distal systemic tissues, and generate long distance mobile signals to establish successful immune response against secondary pathogen infection (Malamy et al., 1990; Gaffney et al., 1993; Dempsey and Klessing, 2012). Several genetic analyses reveal that mutant plants of Arabidopsis (A. thaliana L.) defective in JA- and SA-dependent signaling are insensitive to exogenous applications of PGPRs and SAR-inducing microbes (Pieterse et al., 1998; Jung et al., 2009; Zamiodus and Pieterse, 2012).

The exogenous stimulation of plant immune response by microorganisms can directly turn on SA- and/or JA-dependent defense responses regardless of subsequent pathogen infection (Hahn, 1996; Pieterse et al., 1998; Wiesel et al., 2014). Even if the treatment effectively confers disease resistance, a proper energy allocation is disturbed, which results in growth defect in plants (Walters et al., 2013). A different mode of action of beneficial microorganisms is to prime plants to rapidly and strongly activate defense-related events during pathogen infection (Conrath et al., 2002; Conrath, 2011). In this case, plants do not show any significant physiological changes without pathogen infection (Conrath et al., 2015). Thus the priming of defense response is noteworthy phenomenon for effective management of plant diseases (van Hulten et al., 2006).

Bacillus is one of well-identified genera, acting as beneficial PGPRs (Kloepper and Ryu, 2006; Choudhary and Johri, 2009). In addition to direct formulation of Bacillus spp. as PGPR inoculants, tens of different antibiotics were identified in the genus (Compant et al., 2005). During the last 20 years, we isolated B. vallismortis EXTN-1, B. amyloliquefaciens and B. vallismortis BS07 strains from the rhizosphere soils of chili pepper (Capsicum annuum L.), that not only promote plant growth, but also control the plant disease (Park et al., 2001, 2006a, 2006b, 2007, 2013). Especially, treatment with the B. vallismortis BS07 on pepper seedlings confers disease resistance against infection with Phytophthora capsici and Colletotrichum acutatum, the most destructive pathogens to pepper plants (Park et al., 2013). Additionally, PATHOGENESIS-RELATED PROTEIN1 (PR1, a marker for SA-dependent defense response), but not PDF1.2 (a marker for JA-dependent defense response), is expressed in leaves of Arabidopsis 24 hours after treatment with the B. vallismortis BS07 strain. Interestingly, PR1 expression in B. vallismortis BS07-treated plants disappeared in leaves of Arabidopsis NahG plants expressing salicylate hydroxylase that converts SA to catechol (Park et al., 2013). These results suggest that induced-resistance by the B. vallismortis BS07 strain may require the accumulation of SA in plants.

Cyclic dipeptides (CDPs) are one of the simplest compounds that are derived from bacteria to human (Prasad, 1995; Bellezza et al., 2014). Bacillus genus also generates lots of CDPs conjugated with lipid molecules, such as iturins, fengycin, and surfactins, which trigger activation of plant immune response (Ongena and Jacques, 2008; Park et al., 2016). In addition, CDPs produced by Streptomyces sp. and Bacillus spp. have antibacterial and antifungal activities (Kumar et al., 2014; Wattana-Amorn et al., 2016). These previous reports strongly suggest that CDPs are good candidates for plant disease management.

Here we introduce novel biological activities of four different CDPs to induce disease resistance in Arabidopsis plants. Both a synthetic CDP, cyclo(Gly-L-Pro) and three different CDPs, cyclo(L-Ala-L-Ile), cyclo(L-Ala-L-Leu), and cyclo(L-Leu-L-Pro) isolated from B. vallismortis BS07, which do not have direct antimicrobial activity in vitro, require certain proteins important for SA- and/or JA-dependent defense responses to successfully inhibit bacteria growth in Arabidopsis. Additionally, exogenous treatment of these CDPs can prime plants to strongly express PR4 gene involved in JA-related defense in Arabidopsis.

Materials and Methods

Plants. Wild-type Arabidopsis thaliana L. ecotype Columbia-0 (Col-0) and mutant plants, whose parental ecotype is an ecotype Col-0, grew in an environmentally controlled growth chamber (21±1°C, 50–60% relative humidity, 12 h day and 12 h night). phytoalexin deficient4 (pad4), salicylic acid induction deficient2 (sid2), nonexpressor of pathogenesis-related proteins1 (npr1), ethylene receptor1 (etr1), jasmonate response1 (jra1) and jasmonate insensitive1 (jin1) mutants were used in this study (Bleecker et al., 1988; Staswick et al., 1992; Berger et al., 1996; Cao et al., 1997; Jirage et al., 1999; Nawrath and Métraux, 1999). Seeds of wild type and mutant plants were sterilized with 50% bleach solution, and kept at 4°C for 3 days until sowing. Plants were watered twice a day until experiments.

Extraction and synthesis of CDPs. Tryptic soy agar media (20 L) (15 g tryptone, 5 g soytone, and 5 g NaCl/L) incubated with B. vallismortis BS07 at 28°C for 7 days

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were harvested with methanol (20 L×2) twice and the extracts were concentrated under vacuum. The concentration was subsequently dissolved in 50% aqueous methanol (500 mL) followed by extractions with n-butanol (4 L×3). The n-butanol phase was evaporated to dryness, affording 43.5 g of a brown gum, which was chromatographed on a C18 flash column (90 id×70 mm, C18, 40×63 μm, Merck) using a 10% stepwise gradient elution of increasing methanol concentration in H2O (1 L each) to afford eleven fractions (A to K: 28.0 g, 3.0 g, 2.0 g, 3.0 g, 2.0 g, 1.5 g, 4.5 g, 2.0 g, 1.5 g, and 1.0 g, respectively). Fractions C (2.0 g) was fractionated on a normal phase MPLC (50 id×220 mm, Silica Redi Sep Rf: Teledyne Isco) eluted with ethyl acetate/methanol/H2O (80:20:0 to 30:63:7) to give eighteen sub-fractions (C1 to C18) from fraction C. Sub-fraction C2 (130 mg) was purified by preparative C18 HPLC (Cosmosil, 5C18, 20 id×250 mm, 25-30% aqueous methanol) to [6.0 mg, tR 25.0 min, cyclo(L-Ala-L-Leu)], [12.0 mg, tR 28.0 min, cyclo(L-Ala-L-Leu)], and [2.5 mg, tR 39.0 min, cyclo(L-Leu-L-Pro)]. The cyclic dipeptides were synthesized according to the previously reported method (Thajudin et al., 2010). Briefly, N-Boc protected amino acid (1 mmol) was dissolved in dimethyl formamide. N,N-disopropylethylamine (1.1 mmol) and 2-(1H-benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexa-fluorophosphate (1.1 mmol) were added at room temperature, followed by addition of amino acid methyl ester HCl salt (1 mmol). The mixture was stirred for 3 hours. The reaction mixture was diluted with ethyl acetate, washed with water, 1 M NH4Cl solution, saturated sodium bicarbonate solution, and saline. The resulting linear dipeptide was dissolved in water and autoclaved at 130°C for 4 hours and water was evaporated under vacuum. The remaining residue was purified by silica gel column chromatography (hexane/ethyl acetate/MeOH or CH2Cl2/MeOH).

**Chemical treatment.** Different concentrations of cyclo(Gly-L-Pro), cyclo(L-Ala-L-Ile), cyclo(L-Ala-L-Leu), and cyclo(L-Leu-L-Pro) were dissolved in distilled water before treatment. Both 1 and 10 ppm (part per million) of each CDP solution were infiltrated in leaves of Arabidopsis with a needless syringe and 100 ppm of them was applied onto plants with a sprayer 1 day prior to pathogen infection. The CDP-treated plants were cultured in a typical growth conditions as described above.

**Bacterial inoculation.** A virulent Pseudomonas syringae pv. maculicola ES4326 was employed in this study. The strain was cultured in King’s B liquid media (10 g proteose peptone, 1.5 g K2HPO4, 15 g glycerol and 5 mM MgSO4/L) supplemented with 50 μg/ml streptomyacin at 28°C. The freshly cultured strain was diluted to OD600=0.0001 (to test disease resistance) or OD600=0.01 (to test gene expression) in 10 mM MgSO4. We infiltrated the diluted bacteria into leaves of wild type and mutant plants with a needleless syringe. After infection, the infected plants were covered with a transparent plastic dome to maintain high humidity, and incubated in a growth chamber during 3 days (21±1°C, 12 h day and 12 h night). Number of bacteria was counted on day 3 after infection.

**Induction of priming response and quantitative real-time PCR.** One ppm of these CDP solutions were infiltrated into leaves of wild-type Arabidopsis. One day after treatment, *P. syringae* pv. maculicola ES4326 (OD600=0.01) was infiltrated into the pretreated leaves with each CDP. During infection, plants were kept in a growth chamber. The infiltrated leaves were collected at the indicated time after infection. Total RNAs were isolated with the TriZol® reagent according to the manufacturer’s instruction (Thermo Fisher Scientific, MA, USA). To minimize DNA contamination, TURBO DNase (Thermo Fisher Scientific) was treated in the total RNAs at 37°C for 30 min. About 1 μg of total RNA was used to synthesize first-strand cDNAs with Superscript II reverse transcriptase (Thermo Fisher Scientific). qRT-PCR was performed with SYBR Green PCR mixture (Takara Bio, Japan) using the cycling program as following: 95°C for 5 min. followed by 40 cycles at 95°C for 15s, 60°C for 30s, and 72°C for 30s (Bio-Rad, CA, USA) (Jung et al., 2009; Lee et al., 2016). *Actin2* (At3g18780) was used as the internal control. Oligo-nucleotide sequences used in this study will be provided under a request.

**Results and Discussion**

**Exogenous treatment with CDPs effectively inhibited bacterial growth in Arabidopsis.** In order to examine whether or not CDPs could inhibit microbial growth in Arabidopsis, first, we synthesized cyclo(Gly-L-Pro) (compound A), that is one of well-documented CDPs from *Aspergillus* spp, according to the literature (Thajudin et al., 2010; Ortiz-Castro et al., 2011; Li et al., 2012). Additionally, we also recently isolated 17 different CDPs from *B. vallismortis* BS07 strain. By means of initial screening to identify bioactive compounds, we selected three different CDPs, cyclo(L-Ala-L-Ile) (compound B), cyclo(L-Ala-L-Leu) (compound C), and cyclo(L-Leu-L-Pro) (compound D) conferring disease resistance on several crop plants (data not shown). The molecular structures of these CDPs was described in Fig. 1A.

In order to characterize function of these CDP in plants, we used *Arabidopsis* plants. Wild-type *Arabidopsis* Col-
CDPs Require Host Proteins to Induce Disease Resistance

0 plants were cultivated in potting mixture (Dongbu Farm Hannong, Korea), in an environmentally controlled growth chamber. Different concentrations of each CDP (1, 10, and 100 ppm) were prepared in distilled water. Each solution was treated on plants 1 day prior to inoculation, as mentioned above. A virulent \textit{P. syringae} pv. \textit{maculicola} ES4326 strain was infiltrated in the pretreated leaves with the each compound (OD$_{600}$=0.0001). Treatment of 1 ppm of the compounds inhibited \textit{Pseudomonas} growth in plants, as compared with mock-treatment (Fig. 1B, left). Direct infiltration of 10 ppm of the compounds, however, was not effective, except compound C (Fig. 1B, left), indicating that concentration of the CDPs may be important for controlling bacterial disease. Additionally, number of bacteria was also reduced in plants applied with 100 ppm of each CDP (Fig. 1B, right). Next, to test whether or not these CDPs had a direct antimicrobial activity to phytopathogenic fungi, and oomycetes, we performed a paper disc-agar diffusion assay of the compound. \textit{Alternaria alternata}, \textit{C. acutatum}, \textit{Fusarium oxysporum}, \textit{Rhizoctonia solani}, and \textit{P. capsici} were co-incubated with paper discs containing different concentration of the compound. Mycelial growth of these microorganisms was not inhibited by these compounds \textit{in vitro} (data not shown), which indicate that these CDPs do not have direct antifungal and antioomycete activity. Thus, we hypothesize that biological activity of these CDPs is due to the activation of plant’s built-in defense response, rather than direct inhibition of microbial growth in plants.

**Induced-resistance triggered by CDPs required several key regulators important for SA- and/or JA-dependent defenses.** As described above, it is known that disease resistance induced by PGPRs usually depends on JA-related defense response (Pieterse et al., 1998; Zamioudis and Pieterse, 2012). On the other hand, systemic resistance initiated by primary local infection with avirulent or virulent pathogens relies on SA-related events both in local and distal tissues and generation of long distance mobile signals in local tissues (Dempsey and Klessing, 2012). However, it is not yet clear which defense-related signaling pathways are activated by these
CDPs. To address this question, first we prepared 100 ppm of each CDP and applied them on various *Arabidopsis* mutants lacking a SA-dependent event as following; pad4 (SA regulation) (Jirage et al., 1999), sid2 (SA biosynthesis) (Nawrath and Métraux, 1999), and npr1 (SA responsiveness) (Cao et al., 1997). Twenty six-day old plants were used for the experiments. One day after treatment, a virulent *P. syringae* pv. *maculicola* ES4326 strain (OD<sub>600</sub>=0.0001) was infiltrated into leaves of plants, and number of bacteria was counted in the infected leaves 3 day after inoculation. Bacterial growth was only reduced in sid2 mutant, but not pad4 and npr1 mutants, as compared with that in mock-treated plants, after treatment of compound A, which indicates that compound A requires PAD4 and NPR1 for inducing disease resistance (Fig. 2A). In case of compound B, PAD4, SID2, and NPR1 are inessential to induce resistance response against *Pseudomonas* infection, because these mutant plants were still sensitive to application of compound B (Fig. 2A). Exogenous treatment of compound C effectively inhibited bacterial growth in leaves of pad4 mutant, but not sid2 and npr1 (Fig. 2A). The result proposes that the compound C requires SA biosynthesis and responsiveness for establishing disease resistance in *Arabidopsis*. On the other hand, mutation of SID2 and NPR1 did not affect compound D-induced resistance in plants, whereas PAD4 was necessary for the resistance (Fig. 2A). Based on these results, we conclude that each CDP tested in this study can differentially influence SA-dependent defense response in *Arabidopsis*.

![Fig. 2. Genetic requirement of the CDPs-induced resistance response in Arabidopsis.](image-url)

Mutant plants, whose parental plant is *Arabidopsis* Columbia-0, grew in an environmentally controlled growth chamber. One hundred ppm of each compound, as well as sterilized distilled water as a mock treatment (M), were applied on mutant plants 1 day prior to the infection with *Pseudomonas syringae* pv. *maculicola* ES4326 strain (OD<sub>600</sub>=0.0001). Bacterial growth in the leaves of pad4, sid2, and npr1 (A), etr1, jar1, and jin1 (B) pre-treated with these different CDPs was counted 3 days after inoculation. Note that we cannot directly compare the numbers of bacteria in mock-, compound A-, and compound B-treated plants (left side of dotted lines) with those in mock-, compound C-, and compound D-treated plants (right side of dotted lines), because we have used different flats for these experiments. The columns present the average with standard error. The asterisks indicate statistically significant differences between mock-treated plants and each CDP-treated plants (n=8, Student t-test, *P* < 0.01). The experiment was repeated twice with the same results.
Exogenous treatment of *B. vallismortis* strain BS07 induces *PR1* expression, but not *PDF1.2*, in *Arabidopsis* (Park et al., 2013). In general, however, JA-dependent signaling is important for induced-resistance triggered by PGPRs. Since these CDPs were produced by *Aspergillus* sp. (compound A), and *B. vallismortis* (compound B, C, and D), we expected that key components of JA-dependent events might also be required for the resistance response induced by these CDPs. To test this, we applied 100 ppm of these CDPs on *etr1* (ethylene perception) (Bleecker et al., 1988), *jar1* (JA-Ile production) (Staswick et al., 1992), and *jin1* (JA responsiveness) (Berger et al., 1996) mutants 1 day prior to *P. syringae pv. maculicola* ES4326 inoculation (OD$_{600}$=0.0001). Treatment of both compound A and B failed to suppress bacterial growth in *etr1* and *jin1* mutants, whereas bacterial growth was decreased in leaves of *jar1* mutants, as compared with those in mock-treated plants (Fig. 2B). These indicate that both compound A and B require ETR1 or ETR1-regulated proteins and JIN1 to trigger disease resistance against *Pseudomonas* infection. In case of compound C and D, numbers of bacteria in *etr1*, *jar1*, and *jin1* mutants were comparable with those in mock-treated plants, which proposes that ethylene and JA-related events are crucial for compound C- and/or D-induced disease resistance in *Arabidopsis*.

**PR4 transcription strongly occurred in the CDP-treated plants at early infection phase.** There are two different mechanisms of action of exogenous stimuli to execute induced-resistance in plants. Benzothiadiazole (BTH), a well-known elicitor for plant disease resistance, directly activates SA-related defense response after treatment (Lawton et al., 1996). Priming of defense response is another mechanism of action for induced-resistance (Conrath et al., 2002). Exogenous treatment of chemical primers does not cause any detectable dramatic shift of defense response before pathogen infection. However, immune response is strongly activated in primed plants more than in non-primed plants (Jung et al., 2009; Conrath et al., 2015). Indeed, expression levels of a few defense-related genes in *Arabidopsis* treated with compound A, B, C, and D were similar to those in mock-treated plants in the absence of pathogen infection (data not shown). The observation allowed us to examine whether or not these chemical inducer acted as primers for establishing disease resistance.

**Fig. 3.** Priming of defense response in the leaves of the CDP-treated plants after pathogen infection. (A) A timetable for the experiment presented in (B). Sterilized distilled water (▪), compound A (■), B (□), C (▲), D (▲) and 1 μM of benzothiadiazole (BTH) (▲) were applied in leaves of wild-type *Arabidopsis* plants 1 day prior to inoculation. Inoculated leaves were taken for total RNA extraction at the indicated time points after infection with *Pseudomonas syringae pv. maculicola* ES4326 (OD$_{600}$=0.01). (B) mRNA expression of *PR1* and *PR4* genes in the leaves of wild-type *Arabidopsis* after infection. An *Arabidopsis Actin2* gene (At3g18780) was used as an internal reference gene. Expression levels of *PR1* and *PR4* were calculated by the comparative CT method ($2^{-\Delta\Delta CT}$) (Livak and Schmittgen, 2001). The asterisks indicate statistically differences between mock-treated plants and each CDP- or BTH-treated ones (*P < 0.0001, Student t-test). The experiment was repeated twice with the similar expression patterns.
resistance response in Arabidopsis. To test the possibility, 100 ppm of each CDP was applied on wild-type Arabidopsis. At 24 h after treatment, P. syringae pv. maculicola ES4326 (OD<sub>600</sub> = 0.01) were infiltrated into leaves of CDP-treated plants, and the infected leaves were taken 12 h and 18 h after inoculation (Fig. 3A). To test whether or not the treatment primed plants to rapidly express defense-related genes, we examine mRNA expression of PRI (At2g14610) and PR4 (At3g04720, Hevein-like protein, a marker for JA-dependent defense response) after subsequent pathogen infection (Fig. 3B) (De Vos et al., 2005; Lorenzo and Solano, 2005). Expression of PRI in compound A, B, and D-treated plants were comparable with that in mock-treated plants after Pseudomonas infection, whereas PRI expression level in compound C-treated plants was higher than mock-treated plants 18 hours after infection. This strongly suggests that compound C may have an ability to prime SA-dependent defense responses in Arabidopsis after pathogen infection (Fig. 3B, left panel). Interestingly, exogenous treatment of these CDPs triggered strong mRNA expression of PR4 in Arabidopsis leaves 12 h after subsequent pathogen infection (Fig. 3B, right panel). However the expression did not strongly continue until 18 h after infection in the CDP-treated plants, as compared with that in mock-treated plants. These results strongly suggest that CDPs tested in this study mainly trigger expression of JA-responsive genes during pathogen infection in Arabidopsis.

In conclusion, CDPs from B. vallismortis BS07 strain had different mode of action to induce disease resistance in Arabidopsis. Especially, the facts that most of compounds require JA-responsiveness and enhanced PR4 expression at early infection phase demonstrate that the CDPs tested in this study can prime JA-dependent defense response during pathogen infection. How do plants recognize these CDPs from soil-born bacteria? We are confident that the perception mechanism will be elucidated using genetic analysis in the near future.

Acknowledgments

This research was supported by Agenda research program from the Rural Development Administration (RDA) grant (PJ 009991032016) to Ho Won Jung.

References

Bellezza, I., Peirce, M. J. and Minelli, A. 2014. Cyclic dipeptides: from bugs to brain. Trends Mol. Med. 20:551-558.
Berger, S., Bell, E. and Mullet, J. E. 1996. Two methyl jasmonate-insensitive mutants show altered expression of AtVsp in response to methyl jasmonate and wounding. Plant Physiol. 111:525-531.
Bleecker, A., Estelle, M., Somerville, C., and Kende, H. 1988. Insensitivity to ethylene conferred by a dominant mutation in Arabidopsis thaliana. Science 241:1086-1089.
Cao, H., Bowling, S.A., Gordon, A.S., and Dong, X. 1997. Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6:1583-1592.
Choudhary, D. K. and Johri, B. N. 2009. Interactions of Bacillus spp. and plants-with special reference to induced systemic resistance (ISR). Microbiol. Res. 164:493-513.
Choudhary, D. K., Prakash, A. and Johri, B. N. 2007. Induced systemic resistance (ISR) in plants: mechanism of action. Indian J. Microbiol. 47:289-297.
Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E. A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 71:4951-4959.
Conrath, U. 2011. Molecular aspects of defence priming. Trends Plant Sci. 16:524-531.
Conrath, U., Beckers, G. J., Langenbach, C. J. and Jaskiwicz, M. R. 2015. Priming for enhanced defense. Annu. Rev. Phytopathol. 53:97-119.
Conrath, U., Piererse, C. M. and Mauch-Mani, B. 2002. Priming in plant-pathogen interactions. Trends Plant Sci. 7:210-216.
De Vos, M., van Oosten, V. R., van Peocke, R. M., van Pelt, J. A., Pozo, M. J., Mueller, M. J., Buchala, A. J., Métraux, J. P., van Loon, L. C., Dicke, M. and Pieterse, C. M. 2005. Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Mol. Plant-Microbe Interact. 18:923-937.
Dempsey, D. A. and Klessig, D. F. 2012. SOS - too many signals for systemic acquired resistance? Trends Plant Sci. 17:538-545.
Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. and Rylas, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261:754-756.
Hahn, M. G. 1996. Microbial elicitors and their receptors in plants. Annu. Rev. Phytopathol. 34:387-412.
Jirage, D., Tootle, T. L., Reuber, T. L., Frost, L. N., Feyes, B. J., Parker, J. E., Ausubel, F. M. and Glazebrook, J. 1999. Arabidopsis thaliana P AD4 encodes a lipase-like gene that is important for salicylic acid signaling. Proc. Natl. Acad. Sci. U.S.A. 96:13583-13588.
Jung, H. W., Tschaplinski, T. J., Wang, L., Glazebrook, J. and Greenberg, J. T. 2009. Priming in systemic plant immunity. Science 324:89-91.
Kloepper, J. W. 1994. Plant growth-promoting rhizobacteria (other systems). In: Azospirillum/Plant Associations, ed. by Y. Okon, pp. 111-118. CRC Press, Boca Raton, FL, USA.
Kloepper, J. W. and Ryu, C. M. 2006. Bacterial endophytes as elicitors of induced systemic resistance. In: Microbial Root Endophytes, eds. by B. Schulz, C. Boyle and T. Siebern, pp. 33-51. Springer-Verlag, Heidelberg, Germany.
Kloepper, J.W., and Schroth, M.N., 1981. Relationship of in
vitro antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology* 71:1020-1024.

Kumar, S. N., Nambsian, B. and Mohandas, C. 2014. Purification and identification of two antifungal cyclic dipeptides from *Bacillus cereus* subsp. *thuringiensis* associated with a rhizobid entomopathogenic nematode especially against *Fusarium oxysporum*. *J. Enzyme Inhib. Med. Chem.* 29:190-197.

Lawton, K. A., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann, H., Staub, T. and Ryals, J. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *Plant J.* 10:71-82.

Lee, M. W., Seo, R., Lee, Y. J., Bae, J. H., Park, J. K., Yoon, J. H., Lee, J. W. and Jung, H. W. 2016. *ALTERED MERISTEM PROGRAM 1* has conflicting effects on the tolerance to heat shock and symptom development after *Pseudomonas syringae* infection. *Biochem. Biophys. Res. Commun.* 480:296-301.

Li, X. J., Zhang, Q., Zhang, A. L. and Gao, J. M. 2012. Metabolites from *Aspergillus fumigatus*, an endophytic fungus associated with *Melia azedarach*, and their antifungal, antifeedant, and toxic activities. *J. Agric. Food Chem.* 60:3424-3431.

Livak, K. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^ΔΔCt method. *Methods* 25:402-408.

Lorenzo, O. and Solano, R. 2005. Molecular players regulating the jasmonate signalling network. *Curr. Opin. Plant Biol.* 8:532-540.

Lugtenberg, B. and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63:541-556.

Malamy, J., Carr, J. P., Klessig, D. F. and Raskin, I. 1990. Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science* 250:1002-1004.

Nawrath, C. and Métraux, J. P. 1999. Salicylic acid induction-deficient mutants of *Arabidopsis* express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen inoculation. *Plant Cell* 11:1393-1404.

Ongenaa, M. and Jacques, P. 2008. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol.* 16:115-125.

Ortiz-Castro, R., Díaz-Pérez, C., Martínez-Trujillo, M., del Río, R. E., Campos-García, J. and Lópezbucio, J. 2011. Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. *Proc. Natl. Acad. Sci. U.S.A.* 108:7253-7258.

Park, J.-W., Balaraju, K., Kim, J.-W., Lee, S.-W. and Park, K. 2013. Systemic resistance and growth promotion of chili pepper induced by an antibiotic producing *Bacillus vallismortis* strain BS07. *Biol. Control* 65:246-257.

Park, K. S., Ahn, I. P. and Kim, C. H. 2001. Systemic resistance and expression of the pathogenesis-related genes mediated by the plant growth-promoting rhizobacterium *Bacillus amyloliquefaciens* EXTN-1 against anthrancose disease in cucumber. *Mycobiology* 29:48-53.