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Molecules and Cells

Priming by Rhizobacterium Protects Tomato Plants from Biotrophic and Necrotrophic Pathogen Infections through Multiple Defense Mechanisms

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A selected strain of rhizobacterium, *Pseudomonas putida* strain LSW17S (LSW17S), protects tomato plants (*Lycopersicon esculentum* L. cv. Seokwang) from bacterial speck by biotrophic *Pseudomonas syringae* pv. *tomato* strain DC3000 (DC3000) and bacterial wilt by necrotrophic *Ralstonia solanacearum* KACC 10703 (Rs10703). To investigate defense mechanisms induced by LSW17S in tomato plants, transcription patterns of pathogenesis-related (*PR*) genes and H_2O_2 production were analyzed in plants treated with LSW17S and subsequent pathogen inoculation. LSW17S alone did not induce transcriptions of employed *PR* genes in leaves and roots. DC3000 challenge following LSW17S triggered rapid transcriptions of *PR* genes and H_2O_2 production in leaves and roots. Catalase infiltration with DC3000 attenuated defense-related responses and resistance against DC3000 infection. Despite depriving H_2O_2 production and *PR1b* transcription by the same treatment, resistance against Rs10703 infection was not deterred significantly. H_2O_2 is indispensable for defense signaling and/or mechanisms primed by LSW17S and inhibition of bacterial speck, however, it is not involved in resistance against bacterial wilt.

INTRODUCTION

Specific rhizobacteria is beneficial for plant growth by exerting its activity to diverse mechanisms such as nitrogen fixation, stimulating uptake of phosphorus and micronutrient, and enhancing photosynthesis efficiency (Kloepper et al., 1980; Zhang et al., 2008). The plant growth-promoting rhizobacteria (PGPR) often produce antibiotics and siderophores scavenging and depriving metal ions, therefore, its application suppress the growth of plant pathogens and deleterious microbe benign rhizosphere. In addition to direct antagonisms inhibiting pathogen's propagation, some PGPR has special capacity to lead a plant in a resistant state systemically. This indirect but active disease protection conferred by PGPR application has been termed as induced systemic resistance (ISR) (Kloepper et al., 2004; van Loon et al., 1998).

Plants have evolved defense mechanisms to cope with pathogen invasion. Prior to counteract against the pathogen challenge, pathogen recognition is prerequisite for the initiation of defense responses. After secretion of a bunch of microbial factors from the pathogen, pathogen-associated molecular patterns (PAMPs), into the intercellular space of the host, pattern recognition receptors (PRRs) anchored in the plasma membrane perceive non-self signals and then PAMP-triggered immunity (PTI) is activated. In the presence of resistance machinery matching specifically with effector(s) within the pathogen's secretome, interaction between them provokes sequential intracellular defense-related mechanisms and results in HR (Jones and Dangl, 2006). In the absence of the resistance machinery, effector-triggered susceptibility (ETS) attenuates the PTI and/or basal resistance and then virulent pathogens proliferate within infected cells and distal uninfected cells. Mass of investigations indicates the PGPR-mediated host protection from the virulent pathogen. Most plausible explanations for ISR are the rapid up regulation of the PTI or basal resistance sufficient for abrogation of the ETS. Both PAMPs and microbe (including PGPR)-associated molecular patterns (MAMPs) are perceived as slowly evolving secretomes, therefore, they should share a lot of cognate factors capable of inducing PTI or PTI-like resistance (Ahmad et al., 2010; Van Wees et al., 2008). A part of recent investigations have focused on elucidation of ISR factors from PGPR, plant proteins whose expression was altered by PGPR treatment, and pathogen PAMPs triggering PTI-induced basal resistance (Bittel and Robatzek, 2007; Kandasamy et al., 2009).

Treatment of PGPR prior to challenge with virulent pathogen often triggers rapid and effective defense-related responses and results in distinctive disease protection (Van der Ent et al., 2008). There are no distinctive differences between plants treated with mock or with PGPR. PGPR alone did not induce transcription of pathogenesis-related (*PR*) genes. In spite of ETS, followed challenge with compatible pathogens trigger fortified and accelerated defense mechanisms. This rapid and augmented disturbance of ETS brings up the activation of defense systems, priming, in immunized animals (Cho et al., 2002; Conrath et al., 2006; Ludewig et al., 1998; Serbina et al., 2008; Yang et al.,

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2009). Priming does not directly activate the plant's defense responses, but rather conditions the plant for a faster and stronger activation of stress-specific defense mechanisms against pathogens. Although accurate explanation for the relation between abrogation of ETS and priming is still enigmatic, several PGPRs are capable of reprogramming transcriptomes (Pozo et al., 2008; Verhagen et al., 2004; Wang et al., 2005). Much more diverse JA-responsive genes were primed and transcribed in the PGPR-treated and inoculated plants directly or indirectly (Pozo et al., 2008). This result implies the selective enrichment of JA defense-related genes by treatment with PGPR. Further, a docking site for the transcription factor MYC2 was significantly overrepresented in the plant treated with PGPR and challenged with JA-inducing pathogens.

PGPR frequently offers disease resistance effective against a broad spectrum of virulent pathogens, however, it does not always overcome to other classes of pathogens (Choi et al., 2007; Jetiyanon and Kloepper, 2002; Jetiyanon et al., 2003; Murphy et al., 2000; Ryu et al., 2004). These results indicate that ISR and/or ISR-priming have a certain resistance spectrum. Most of ISR conferred by PGPR is dependent on JA, therefore, these resistance was disappeared in the Arabidopsis ecotypes or defense mutants insensitive to JA (Barriuso et al., 2008; Kloepper et al., 2004; Korolev et al., 2008; Matthes et al., 2010). Similarly, JA-dependent ISR or priming is ineffective for the plant protection if the resistance is dependent on different messenger(s) other than JA or peculiar defense mechanisms.

Virulent pathogens induce rapid cellular defense responses such as AOS accumulation, callose and phenolic compound deposition, and augmented transcriptions of defense-related responses in plants primed by PGPR (Benhamou et al., 2002; Faize et al., 2004; Jeun et al., 2004; Pare et al., 2005; Park et al., 2002; van Loon et al., 2008; Verhagen et al., 2010; Whipps, 2001; Yedidia et al., 2003). Besides arresting pathogen proliferation *in planta*, AOS are involved in cell wall fortification (Olivain et al., 2003) and protein cross-linking, and acts as signaling molecules mediating systemic resistance.

In the previous research, we have described effect of *Pseudomonas putida* strain LSW17S as a biocontrol agent conferring ISR to Arabidopsis and tomato (Ahn et al., 2007; Lee et al., 2005). Treatment of strain LSW17S protected Arabidopsis from *P. syringae* pv. *tomato* infection through priming of defense-related mechanisms (Ahn et al., 2007) which is dependent on jasmonic acid and *NPR1*. These results suggest that this bacterial strain should be a potent candidate of an agricultural agent, however, no information is now available for the ISR spectrum caused by LSW17S.

The aims of this study were to investigate the biocontrol potential of LSW17S in the inhibition of bacterial speck and bacterial wilt caused by biotrophic *P. syringae* pv. *tomato* strain DC3000 and necrotrophic *Ralstonia solanacearum* strain Rs10703 and to analyze their defense mechanisms in the cellular and molecular levels.

MATERIALS AND METHODS

Plants and pathogen challenge

Tomato plants (cv. Seokwang) were grown in a green house at 25-30°C and 60% to 70% relative humidity under natural light. We grew tomato plants using commercial, circulating hydroponic system (Jayeunmi, Korea) containing half strength of Yamazaki nutrient solution (NO_3-N, 7; NH_4-N, 0.67; PO_4-P, 2; K, 4; Ca, 3; Mg, 2 me per liter) with full strength of micronutrient (Fe, 2.0 mg; B, 0.2 mg; Mn, 0.2 mg; Zn, 0.02 mg; Cu, 0.01 mg; Mo, 0.005 mg; Cl, 1.0 mg per liter). Five to six-week-old plants were used for bacterial and chemical treatments. The DC3000 strain of *Pseudomonas syringae* pv. *tomato* (DC3000) was grown on King's medium B containing 50 μg ml^{-1} kanamycin for 2 days at 28°C. *Ralstonia solanacearum* Rs10703 (race 1, biovar 3, rifampicin-resistant), virulent on tomato plants and resistant to 100 μg ml^{-1} rifampicin, was obtained from Korean Agricultural Culture Collection, Rural Development Administration, Korea and was grown on casamino acid-peptone-glucose (CPG) agar (Kelman, 1954) supplemented with 100 μg ml^{-1} rifampicin for 2 days at 28°C. Bacterial stock was preserved as cell suspension amended with 20% glycerol at -70°C until use.

To inoculate tomato plants, bacterial cells were retrieved with 0.85% NaCl solution and the concentration was adjusted to 1×10^8 (DC3000) or 1×10^7 (Rs10703) colony-forming units (cfu) ml^{-1}. Twenty tomato plants were inoculated 5 days later treatment with LSW17S by spraying bacterial suspension of DC3000 (supplemented with 250 μg ml^{-1} Tween 20) until all the leaves were covered with fine droplets or pouring Rs10703 suspension into hydroponic system containing 20 liter Yamazaki solution. The DC3000-inoculated plants were kept in a dew chamber for 16 h at 25°C, 100% relative humidity and then transferred to a greenhouse. After Rs10703 inoculation, plants were return to the greenhouse. Disease assessment and estimation of pathogen growth *in planta* were carried out as described (Lin and Martin, 2005; Saile et al., 1997). Five days after inoculation, DC 3000 and speck numbers were estimated for five representative areas (2×2 cm^2) which showed discrete lesions. Fully expanded leaflets (usually the third or fourth leaves from the top of the plant) from five plants were used. Ten days after inoculation, *in planta* Rs10703 growth were recorded for 1 g root tissue on the tetrazolium medium supplemented with 50 μg ml^{-1} tetrazolium (Sigma, USA) and 100 μg ml^{-1} rifampicin as described previously (Kelman, 1954). Disease severity was measured by the rate at which plants wilted. The numbers of leaves wilted and cfu of Rs10703 were recorded for each of 10 plants.

Treatment of microbe and chemicals

Pseudomonas putida strain LSW17S and *Escherichia coli* strain DH5α kept at -70°C as 15% glycerol stock, were grown on tryptic soy agar (TSA; Difco Lab., USA) supplemented with 100 μg ml^{-1} streptomicin at 28°C for 48 h and on Luria-Bertani agar (LBA) at 37°C for 20 h. Strains LSW17S and DH5α were applied by drenching (final 10^7 CFU ml^{-1} Yamazaki solution concentration) 5 days after treatment. Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH; Sigma, USA) was applied by drenching 1.2 mM solutions 5 days prior to pathogen challenge. Five replicates were performed for each treatment (10 plants per treatment). To investigate the effect of H_2O_2 on priming by LSW17S, DC3000 (5×10^4 cfu ml^{-1}) or Rs10703 (1×10^6 cfu ml^{-1}) suspension and catalase (5000 U ml^{-1}) were also infiltrated into the leaf parenchyma tissue using 1 ml needleless plastic syringe. The inoculated plants were kept in a dew chamber for 16 h at 25°C, 100% relative humidity and then transferred to a growth chamber with a 16 hour-photoperiod at 25°C, 65% relative humidity. Disease severity was assessed by determining the cfu within $\pm$ 1 g (fresh weight) of tomato leaves from five plants through plating appropriate dilutions on King's medium B containing 100 μg ml^{-1} streptomicin.

Detection of LSW17S within rhizosphere and nutrient solutions

Roots ($\pm$ 1 g fresh weight) were recovered 5 days after LSW17S treatment, surface-sterilized with 5% sodium hypochlorite, and spun in 9 ml of sterilized 0.85% NaCl solution at 400 $\times$ *g*. Nutri-

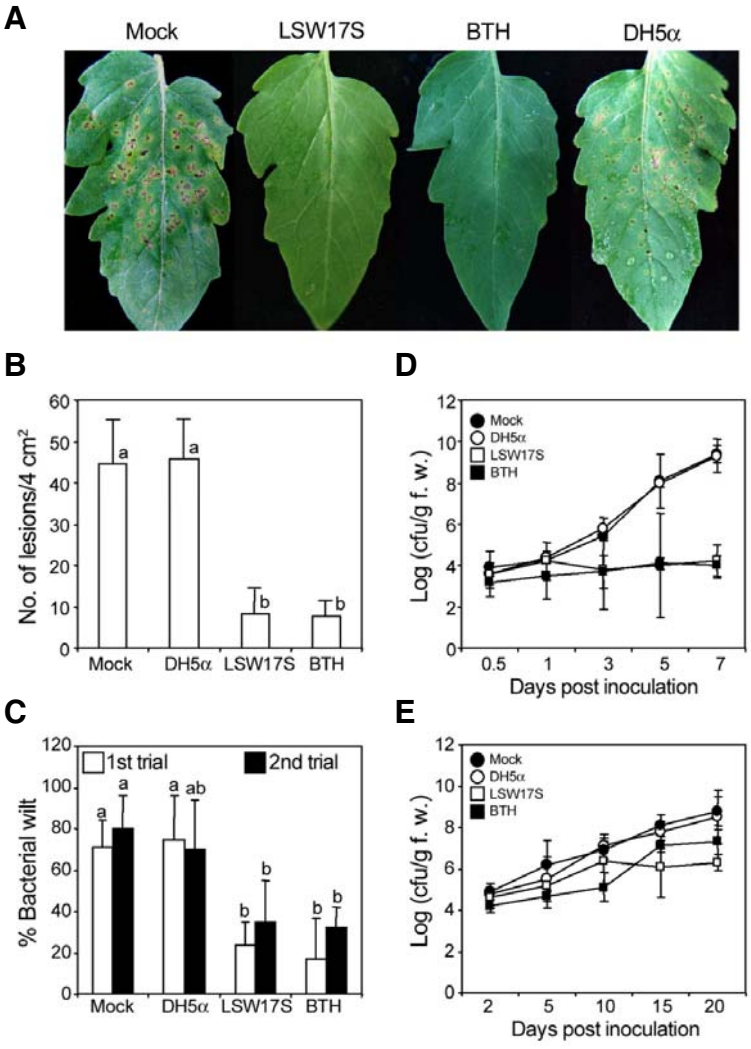

Fig. 1. Responses of *Lycopersicon esculentum* L. cv. Seokwang to *Pseudomonas syringae* pv. *tomato* strain DC3000 (DC3000) and *Ralstonia solanacearum* Rs10703 (Rs10703) infection after *Pseudomonas putida* strain LSW17S treatment. (A) Necrotic lesions normally caused on tomato leaves by DC3000 are suppressed by LSW17S treatment. Tomato plants treated with *Escherichia coli* strain DH5α or 1.2 mM benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) were also inoculated 5 days later with DC3000 as controls. A representative leaflet from each treatment was photographed 5 days after inoculation. (B) Quantification of systemic resistance against DC3000 infection. Tomato plants were treated with LSW17S, 1.2 mM BTH, or DH5α (+) in 0.85% NaCl or 0.85% NaCl only (Mock). Five days later, leaves of the plants were sprayed with DC 3000 suspension. Data are means and standard errors of lesion number within 2 × 2 cm^2 leaf area from 5 leaves from 5 plants. (C) Quantification of systemic resistance against Rs10703 infection in tomato. Plants were treated with LSW17S, 1.2 mM BTH, or DH5α (+) in 0.85% NaCl or 0.85% NaCl only (Mock). Five days later, plants inoculated with Rs10703 suspension by drenching. Data are means [percentage of symptomatic plants relative to control plants (100%)] with standard errors from 20 plants. (D) Time course of bacterial growth in the leaves treated with mock, DH5α, LSW17S, or BTH and inoculated with DC3000. Data points are means (colony-forming units per gram fresh weight) with standard errors from 25 randomly selected leaves. (E) Time course of bacterial growth in the roots treated with mock, DH5α, LSW17S, or BTH and inoculated with Rs10703. Data points are means (colony-forming units per gram fresh weight) with standard errors from 10 randomly selected roots. In (B), (C), (D), and (E), different letters indicate statistically significant differences among treatments (Duncan's multiple range tests; $P < 0.05$). All experiments were repeated more than three times and similar results were obtained.

ent solutions were also recovered at the same time. Stem (± 1 g fresh weight) was harvested at the same time were ground with 9 ml of 0.85% NaCl containing 0.5 g of glass beads. Dilutions of each were plated on TSA supplemented with 100 μg ml^{-1} streptomycin to estimate streptomycin-resistant LSW17S. After 36 h incubation at 28°C, the number of streptomicin-resistant cfu per gram of root and leaf fresh weight was estimated.

Detection and quantification of H_2O_2

To investigate whether LSW17S and/or pathogen challenge affected the priming and thereby plant responses such as oxidative burst and callose deposition, LSW17S- or mock-treated leaves were left untreated or challenged with virulent DC3000 by spraying or infiltration 5 days after LSW17S treatment. Histochemical detection of H_2O_2 was performed as described previously (Wohlgemuth et al., 2002) with minor modification. To visualize accumulation of H_2O_2, leaves were stained with 0.1% (w/v) diaminobenzidine (DAB) and then cleared with 96% (v/v) ethanol and preserved in 50% (v/v) ethanol. DAB staining was indicated as red-brown under the light microscope. H_2O_2 levels were determined using Autolumat LB953 luminometer (EG & G Derthold, Germany) as described previously (Baker et al., 2002). Each reaction mixture contained 0.72 U peroxidase and 77.6 mM luminol.

RNA isolation and expression analyses

Total RNA was extracted from tomato by lithium chloride precipitation (Davis and Ausubel, 1989). For hybridization analysis, 10 μg of total RNA were separated electrophoretically in denaturing formaldehyde-agarose gels (8% formaldehyde, 0.5 × MOPS, 1.5% agarose) and blotted onto Hybond-N+ membrane (Amersham Pharmacia Biotech, UK) by capillary transfer. Equal sample loading was confirmed by EtBr staining of rRNA. RNA gel blots were hybridized, washed, and exposed to X-ray film. Complementary DNAs of *PR1b*, *PR4*, and *PR5* were obtained from Dr. Doil Choi, Seoul National University, Seoul, Korea. DNA probes were labeled with [^{32}P]-dCTP by random primer labeling (Boehringer Mannheim, Germany).

RESULTS

Pseudomonas putida strain LSW17S protects tomato from bacterial infection

The bioprotection capacity of *Pseudomonas putida* strain LSW17S was assessed in *Lycopersicon esculentum* cv. Seokwang, which is susceptible to *Pseudomonas syringae* pv. *tomato* strain DC3000 (DC3000) and *Ralstonia solanacearum* strain Rs10703. In addition, benzothiadiazole (BTH) treatment was included as a resistant control. Previous reports showed that BTH induced systemic resistance against bacterial speck and bacterial wilt in a susceptible tomato plant (Fidantsef et al., 1999; Pradhanang et al., 2005). Plants pretreated with systemic resistance-activating LSW17S, systemic resistance non-activating *Escherichia coli* DH5α, or BTH were then challenged with DC3000 are shown in Fig. 1A. Leaflets from tomato plants pretreated with mock or *E. coli* DH5α and inoculated with DC3000 showed no observable symptoms within 36 h post inoculation (hpi). Typical bacterial speck lesions were apparent by 72 to 96 hpi. More than 40% of leaf surface was covered with lesions and halo by 7 days post inoculation (dpi). Similar with above, mock or DH5α pretreatment did not influence bacterial wilt development. These Rs10703-inoculated plants exhibited withering of upper leaves by 96 hpi. Root surface turned into light brown at the same time. Whole plant wilting and withering were evident within 7 dpi, and yellowish ooze drop was frequently observed on the cross section of stem. Interestingly, LSW17S treatment attenuated typical development of both diseases. Minute brown spots were observed within 24 hpi with DC3000 and remained unchanged without further symptom development (Figs. 1A, 1B, and 1C). The same treatment also inhibited incidence of bacterial wilt by 76%. Similar significant disease protection was achieved by BTH treatment. To further confirm these effects, cfu number of DC3000 or Rs10703 within infected leaf or root tissues were estimated (Figs. 1D and 1E). These results are coincided with the above results of LSW17S on both diseases. All experiments were repeated three times with five replicates independently and almost the same tendency was obtained.

Persistence of LSW17S effects

In the first, number of LSW17S was estimated in the root and leaf of tomato and nutrient solution (Fig. 2A) 5, 10, 15, and 20 days after bacterization. No LSW17S was detected in the stem. The number of LSW17S in nutrient solution and within root tissue (endophytic) was decreased rapidly by 10 days post treatment (dpt) and maintained thereafter. In the next, we investigated the duration of disease resistance by LSW17S (Fig. 2B). LSW17S treatment interdicted developments of bacterial speck by 74, 72, and 56% when pathogen was challenged 5, 10, and 20 days post bacterization. This rhizobacterium also inhibited incidence of bacterial wilt by 54, 71, and 64%, respectively. The level of disease control by LSW17S was comparable or not significantly different with that achieved by BTH.

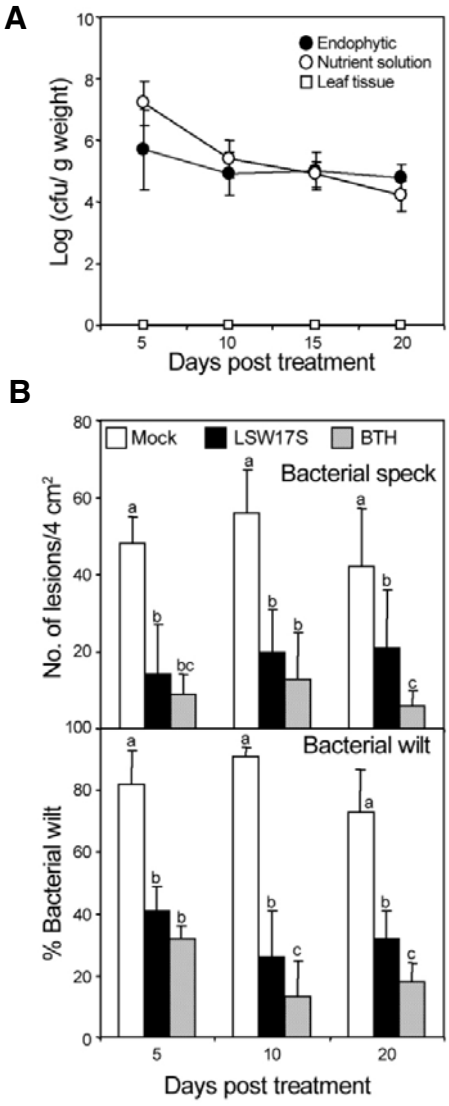

Fig. 2. Treatment of LSW17S suppresses bacterial infection of tomato (cv. Seokwang) for long periods through the induction of resistance responses. (A) Number of LSW17S within roots (endophytic) and stems from 20 plants or nutrient solutions from 5 hydroponic culture systems. Sampling was performed 5, 10, 15, and 20 days post treatment. Each data point represents the mean of bacterial colony forming units (cfu) with standard errors. (B) Bacterial disease developments in mock-, DH5α-, BTH-, and LSW17S-treated tomato leaves and roots. Treated tomato was inoculated with *P. syringae* pv. *tomato* strain DC3000 (bacterial speck) or *R.solanacearum* strain Rs10703 (bacterial wilt) at 5, 10, and 20 days after LSW17S treatment. Different letters indicate statistically significant differences among treatments (Duncan's multiple range tests; $P < 0.05$).

Production of H_2O_2

The effects of LSW17S and DC3000 or LSW17S and Rs10703 on H_2O_2 accumulation were determined (Fig. 3). Pathogen was challenged 5 days post LSW17S treatment and leaf and root samples were harvested 0 and 6 hpi. This response was one of the reliable evidence whether plants are primed or not (Ahn et al., 2007). H_2O_2 accumulation was barely detectable in LSW17S-, mock-, or DH5α-treated tomato leaves and roots. DC3000 challenge also did not trigger H_2O_2 production in mock-, or DH5α-treated plant leaflets (Fig. 3A). In contrast, the amendment with bacteria and subsequent DC3000 challenge induced robust H_2O_2 accumulation. Rs10703 challenge provoked H_2O_2 accumulation in LSW17S- or mock-treated tomato roots without significant difference. In addition, BTH treatment induced H_2O_2 accumulation *per se* and these cellular responses were highly triggered by DC3000 challenge rather than Rs10703 inoculation. Microscopic observation and quantification of H_2O_2 are also similar with these observations (Figs. 3B and 3C).

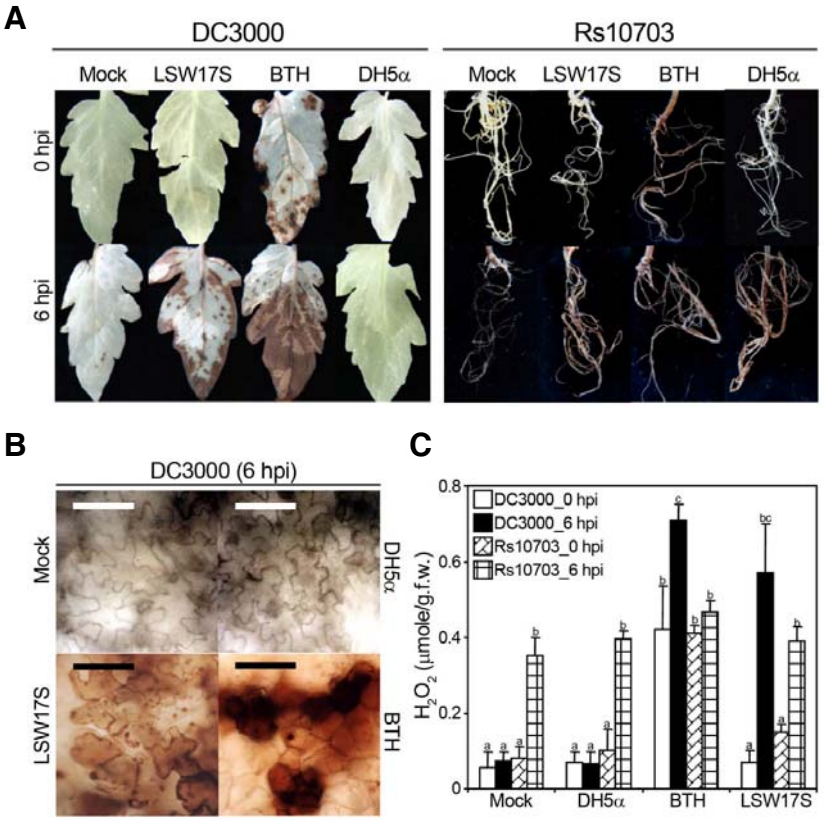

Fig. 3. Effect of priming by LSW17S on H_2O_2 accumulation in tomato leaves and roots. Five to six-week-old tomato plants grown in hydroponic systems were drenched with 10^8 cfu ml^{-1} LSW17S (+) in 0.85% NaCl (mock) or with the mock alone (-). Five days later, leaves or roots were inoculated with virulent *P. syringae* pv. *tomato* strain DC3000 (10^8 cfu ml^{-1}) or *R. solanacearum* strain Rs10703 (10^7 cfu ml^{-1}). Zero and 6 h after pathogen challenge, more than 25 leaves or 10 roots were harvested from 10 randomly selected tomatoes. (A) Accumulation of H_2O_2 in leaves and roots from tomato pretreated with mock, LSW17S, benzothiadiazole (BTH), and DH5α and challenged with DC3000 or Rs10703. Samples were harvested 0 and 6 h after inoculation. (B) Microscopic observation of H_2O_2 accumulation in leaves from tomato pretreated with mock, LSW17S, BTH, and DH5α and challenged with DC3000. Leaves were harvested 0 and 6 h after inoculation. Bars = 50 μm. (C) Quantification of H_2O_2 accumulation in leaves and roots of tomatoes pretreated with mock, LSW17S, BTH, and DH5α and challenged with DC3000 or Rs10703. Leaves and roots were harvested 0 and 6 h after inoculation. Different letters indicate statistically significant differences among treatments (Duncan's multiple range tests; $P < 0.05$). All experiments were repeated more than three times and similar results were obtained.

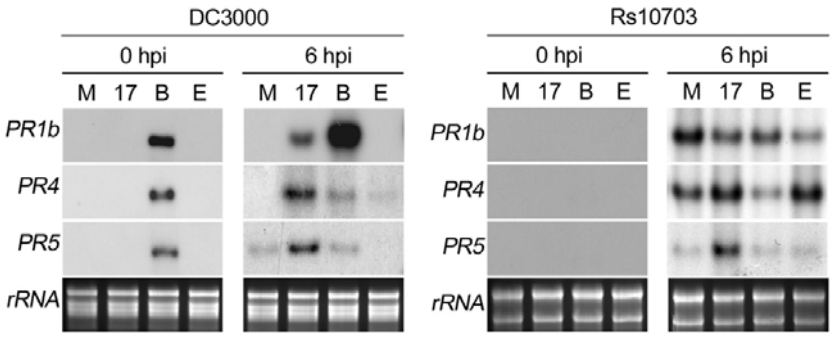

Fig. 4. Effect of LSW17S and pathogen challenge on the transcriptions of *PR1b*, *PR4*, and *PR5*. Tomato plants (n = 25) were treated with mock (M; 0.85% NaCl), LSW17S in 0.85% NaCl (17), benzothiadiazole (B; BTH), or DH5α (E) and challenged 5 days later with virulent *P. syringae* pv. *tomato* strain DC3000 or *R. solanacearum* strain Rs10703. hpi designates hours post inoculation. Equal sample loading was confirmed by ethidium bromide straining of the rRNA in the gel.

Effects of LSW17S and pathogen challenge on defense-related gene transcription

To investigate the mechanisms of disease protection conferred by LSW17S, transcript accumulations of three pathogenesis-related (*PR*) genes were analyzed (Fig. 4). Pathogen was challenged 5 days post LSW17S treatment and leaf and root samples were harvested 0 and 6 hpi. LSW17S alone as well as mock- or DH5α treatment did not induce *PR1b*, *PR4*, and *PR5* mRNA accumulations in tomato leaflets and roots. Although BTH induced transcriptions of all three tested genes in leaves, this treatment did not trigger expressions of them in roots. DC3000 inoculation had no effects on *PR1b* and *PR4* genes' transcriptions, but induced fortified transcriptions of the two genes in leaves of tomato pretreated with LSW17S. In contrast, Rs10703 induced expressions of *PR1b* and *PR4* genes *per se*. *PR5* transcription was observed in leaves and roots of tomato pretreated with LSW17S and inoculated with DC3000 or Rs10703.

Effects of H_2O_2 accumulation on priming

Effects of catalase on the priming by LSW17S, leaf parenchyma tissues of mock- or LSW17S-treated tomato plants were infiltrated with DC3000 or Rs10703 to investigate the role of H_2O_2 on priming acclimated by rhizobacterium (Fig. 5). Catalase infiltration resulted in loss of H_2O_2 accumulation induced by LSW17S (Fig. 5A). Resistance against DC3000 infection by LSW17S was nullified by exogenous catalase scavenging H_2O_2, however, resistance against Rs10703 was not abrogated by

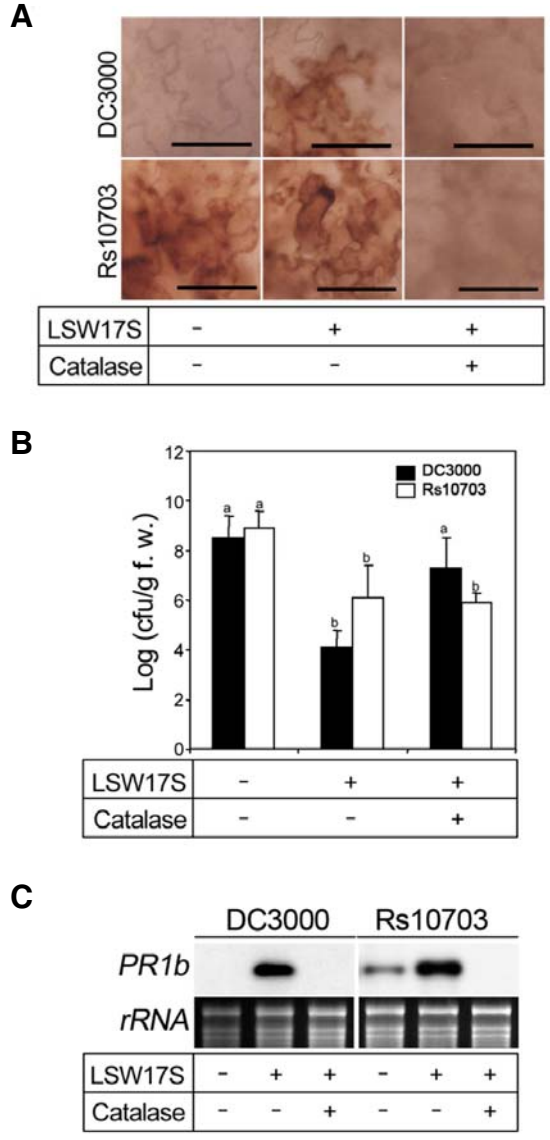

Fig. 5. Effects of exogenous application of catalase on H_2O_2 accumulation, disease resistance conditioned by LSW17S, and *PR1b* transcription. Virulent *P. syringae* pv. *tomato* DC3000 (5×10^4 cfu ml^{-1};+) or *R. solanacearum* Rs10703 (1×10^6 cfu ml^{-1};+) and 5000 U ml^{-1} catalase (+) were infiltrated with needless syringae into tomato leaf 5 days after mock (-) or LSW17S (+) drenching. (A) Effects of exogenous application of catalase on H_2O_2 accumulation. Six hours after infiltration of pathogen and catalase, 20 leaves were recovered from 10 plants and stained with diaminobenzidine and representative samples were photographed. Bars = 50 μm. (B) Titers of DC3000 and Rs10703 in leaves of tomato pretreated with LSW17S and infiltrated with pathogen (+) and catalase (+). After infiltration, 20 leaves from 5 plants were recovered at 5 days post inoculation and numbers of cfu were estimated. All experiments were conducted three times independently and similar results were obtained. Each bar represents the mean ± SE. Different letters indicate statistically significant differences between treatments (Duncan's multiple range tests; $P < 0.05$).

the same treatment (Fig. 5B). In addition, augmented transcriptions of *PR1b* transcriptions by priming and DC3000 challenge or Rs10703 infection were inhibited by catalase (Fig. 5C).

DISCUSSION

We previously found that disease development in *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato* strain DC3000 (DC3000) is significantly inhibited by soil amendment with a PGPR strain of LSW17S belong to *P. putida* (Ahn et al., 2007). Although bacterization itself did not cause any observable changes, subsequent pathogen challenge and cellular/molecular analyses revealed that LSW17S changes susceptible Arabidopsis in a concealed resistance state. This result is one of the typical examples of priming. To investigate whether priming by LSW17S is also functional in tomato plants and confers disease protection against biotrophic DC3000 and necrotrophic *Ralstonia solanacearum* strain Rs10703 infections in tomato (*Lycopersicon esculentum* L. cv. Seokwang), we analyzed disease protection and the representative cellular and molecular alterations by LSW17S.

LSW17S protects tomato plants from infections by DC3000 and Rs10703. Repeated challenge tests corroborated this disease-protecting capacity. Neither LSW17S nor its culture filtrate arrested *in vitro* growth of both tomato-infecting bacteria (data not shown). Disease resistance in the multiple hosts without direct antagonism against pathogen is a typical characteristic of ISR induced by PGPR (Jetiyanon et al., 2003; Kloepper et al., 2004; Krause et al., 2003; Raupach and Kloepper, 1998; Zhang et al., 2004) and implies that defense mechanisms against DC3000 infection in Arabidopsis and that in tomato might be identical. The reasonable interpretations of this ISR acclimated by LSW17S are; secretion of ISR determinant(s) from the PGPR and their recognition by the conserved or cognate PRR(s) shared by *Arabidopsis thaliana* and tomato plants. Further, this resistance indicates the abrogation of ETS. Similar interpretation of ISR by the determinant(s) within secretome from PGPR was described elsewhere (van Loon et al., 2008; Van Wees et al., 2008). In addition, defined and rapid cell death induction within short time after DC3000 inoculation suggests the up regulation of basal resistance equivalent to an effector-triggered immunity (ETI) level. LSW17S induces local or systemic resistance against *R. solanacearum*, a typical member of necrotrophic pathogens in tomato (Llorente et al., 2008; Takabatake et al., 2007). Although LSW17S confers resistance against Rs10703, it is uncertain that the above resistance and/or defense mechanisms protecting DC3000 are also effective for the inhibition of bacterial wilt. Especially, Rs10703 is a pathogen belonged to necrotrophs. Therefore, simultaneous protection of bacterial speck and wilt by LSW17S and these pathogens' biotrophic and necrotrophic nature imply that an ISR from LSW17S might exert its activity through diverse defense mechanisms.

LSW17S does not induce observable changes *perse* in Arabidopsis, however, followed DC3000 challenge virulent on wild type Col-0 triggered rapid defense responses (Ahn et al., 2007). H_2O_2 plays key roles in this resistance against DC3000. H_2O_2 production and accumulation have been perceived as an earliest event in the PRR-mediated basal resistance and this AOS could serve as a signaling molecule necessary for systemic translocation of this resistance against DC3000 (Houot et al., 2001; Hu et al., 2003; Neuenschwander et al., 1995; Ren et al., 2002; Siegrist et al., 2000). We have analyzed the accumulation of H_2O_2 by the LSW17S to unveil the factors responsible for priming, however, LSW17S *per se* did not induce H_2O_2 in leaflet as well as root (Figs. 3A and 3B). In addition, depriving H_2O_2 through catalase infiltration resulted in the nullification of

PR1b transcription (Fig. 5). These results suggest that H_2O_2 should act as a signaling molecule against DC3000. Although its production is an earlier event prior to rapid transcription of defense-related transcriptional changes, however, an interaction(s) among MAMP from LSW17S and PRR(s) does not trigger H_2O_2. Similar functions of MAMPs from PGPR have been hypothesized (Van der Ent et al., 2009; van Loon et al., 2008). Although the exact MAMP/PRR interaction remains uncertain, it might contribute to formation and maintaining primed status and resulted induction of H_2O_2 by a recognition of PAMP from DC3000 should trigger rapid up regulation of real basal resistance capable of overcoming ETS or avoidance of ETS expression. According to this interpretation, H_2O_2 production is one of the earliest responses induced by DC3000 infection in the primed plants. In contrast, Rs10703 triggered H_2O_2 accumulation in tomato plants in the absence of LSW17S pretreatment. In addition, this pathogen also provoked rapid transcription of defense-related genes employed (Fig. 4). Therefore, earlier induction of AOS and transcription of defense-related genes were not related with the ISR or priming effective for inhibition of bacterial wilt. PAMPs from necrotrophs frequently kill host cells to obtain and use their leakages for the pathogen's colonization. H_2O_2 has important functions in the diseased plants; an earlier signaling molecule translocating resistance systemically, fortification of apoptotic tissues, and making infected cell suicide. All of them are not proper for the inhibition of necrotrophic diseases. Although accurate defense mechanisms are still elusive, LSW17S should trigger resistance suitable for inhibition of bacterial wilt through (an) alternative defense mechanisms.

BTH is known as a representative agent for systemic acquired resistance (SAR) and also works as a priming agent in plants (Conrath et al., 2006). High concentration of this agent triggered defense-related responses, and even more, low dose was sufficient for maintaining priming. In our experiment, 1.2 mM BTH alone is capable of inducing H_2O_2 production and *PR* gene transcription in leaves and roots (Figs. 3 and 4). Therefore, BTH should induce a series of plant defense status in a dose-dependent manner.

In sum, our investigations revealed that defense signaling primed by LSW17S and dependent on H_2O_2 effectively perturbs infection by biotrophic *P. syringae* pv. *tomato*. However, the same mechanism(s) seems to be not involved in the resistance against necrotrophic *R. solanacearum* infection. These results clearly show that more than one disease defense mechanisms might be primed by LSW17S.

LSW17S colonises tomato root tissues endophytically and persists for more than 20 days post treatment within root tissues and nutrient solutions. Endophytic colonization, local and systemic translocation of broad spectrum resistance, and persistence for longer period are noticeable benefits of disease management using LSW17S.

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