Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae*

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Summary

• The mechanisms by which plant growth-promoting rhizobacteria (PGPR) mediate induced systemic resistance are currently being intensively investigated from the viewpoint of signal transduction pathways within plants.

• Here, we determined whether our well-characterized PGPR strains, which have demonstrated induced resistance on various plants, also elicit induced resistance in *Arabidopsis thaliana*. Nine different PGPR strains were evaluated for their capacity to cause induced resistance on *Arabidopsis* against two pathovars of *Pseudomonas syringae*. Six strains significantly reduced severity of *P. syringae* pv. tomato, whereas seven strains reduced severity of *P. syringae* pv. maculicola.

• From the initial screenings, four strains (90-166, SE34, 89B61 and T4) were selected because of their consistent induced resistance capacity. Elicitation of induced resistance with these strains depended on how disease severity was measured. Three strains (90-166, 89B61 and T4) induced resistance in NahG plants (SA-deficient), indicating a salicylic acid-independent pathway, which agrees with the previously reported pathway for induced resistance by PGPR. However, differences from the reported pathway were noted with strain 89B61, which did not require jasmonic acid or ethylene signaling pathways for induced resistance, and with strain T4, which induced resistance in *npr1* plants.

• These results indicate that strains 89B61 and T4 induce resistance via a new pathway or possibly a variation of the previously reported pathway. This information will broaden our understanding of ways in which microorganisms can signal physiological changes in plants.

Key words: plant growth-promoting rhizobacteria, *Arabidopsis thaliana*, *Pseudomonas syringae*, induced resistance.

Introduction

Plants have evolved numerous mechanisms to defend themselves against microbial pathogens. Some of these defense mechanisms are constitutive, such as the physical barriers of the cell wall, while others are induced (Agrios, 1997). Induced disease resistance occurs when a plant exhibits an increased level of resistance to infection by a pathogen after prior treatment with an inducing agent. Some selected strains of plant growth-promoting rhizobacteria (PGPR) have been found to activate plant defense via induced systemic resistance (ISR) (Kloepper *et al.*, 1992; van Loon *et al.*, 1998). The process of active resistance in ISR is dependent on activation of the host plant's physical or chemical barriers. Induced systemic resistance develops systemically following colonization of plant roots by PGPR (Wei *et al.*, 1991). By

contrast to PGPR, incompatible pathogens trigger systemic acquired resistance (SAR) following the hypersensitive response (HR), which is a plant defense mechanism that induces rapid, localized cell death at the infection site of pathogens, thereby interfering with disease progress (Heath, 2000).

Salicylic acid (SA) is one of the key chemical signals produced in response to pathogen attack on resistant plants and is required for the induction of SAR (Dempsey *et al.*, 1999). Production of SA and induction of SAR are most often exhibited following the HR. Activation of the HR is governed by resistance genes encoding receptors that recognize specific pathogens (Staskawicz *et al.*, 1995). The subsequent induction of SAR results from a complex signal transduction process (Pickett & Poppy, 2001) and leads to accumulation of pathogenesis-related (PR)-proteins. Recently, an SA analogue, benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH), was commercialized by Syngenta under the name of Bion in Europe and Actigard in USA (Tally *et al.*, 1999). We used this chemical as a positive control in our experiment.

Several approaches to defining the signal pathway for ISR have been undertaken. Induced systemic resistance mediated by *Pseudomonas fluorescens* WCS417 in *Arabidopsis* and by *Serratia marcescens* 90–166 in tobacco was shown to be independent of SA accumulation (Pieterse *et al.*, 1996; Press *et al.*, 1997). By contrast, *Pseudomonas aeruginosa* 7NSK2 elicited ISR against *Tobacco mosaic virus* in tobacco and *Botrytis cinerea* on tomato via an SA-dependent pathway (De Meyer & Hofte, 1997, 1999). However, induced resistance by the same strain against *Pseudomonas syringae* on *Arabidopsis* was SA-independent (Ran, 2002). Using mutant lines of *Arabidopsis* and strain WCS417r, van Loon *et al.* (1998) and Pieterse *et al.* (2002) proposed a model pathway for signal transduction in PGPR-mediated ISR. In the proposed pathway, ISR caused by PGPR is dependent on jasmonic acid (JA), ethylene, and the regulatory gene *NPR1*, while it is independent of SA and does not result in accumulation of PR-proteins. The studies of induced resistance related signaling pathways have used the following signaling mutants of *Arabidopsis*: *jar1* or *fad3-2 fad7-2 fad8* for jasmonic acid; *ein2* or *etr1* for ethylene; and *npr1* for the regulatory gene *NPR1* (van Loon *et al.*, 1998; Vijayan *et al.*, 1998; Kus *et al.*, 2002; Pieterse *et al.*, 2002).

The objectives of this study were (1) to determine whether PGPR strains that have been reported to induce resistance against several plant pathogens on cucumber, tomato, and tobacco in the greenhouse and field protect *A. thaliana* against *P. syringae*, (2) determine if induced systemic protection by PGPR depends on the pathogens used to challenge plants and (3) determine if signal pathways of plants treated with our PGPR are the same as the model proposed by van Loon *et al.* (1998) and Pieterse *et al.* (2002) by using plant signaling defective mutants such as NahG for SA, *fad3-2 fad7-2 fad8* for jasmonic acid, *ein2* for ethylene, and *npr1* for the regulatory gene *NPR1*.

Materials and Methods

PGPR strains and inoculum preparation

Nine different PGPR strains were used: *S. marcescens* 90–166, *Bacillus pumilus* SE34, *P. fluorescens* 89B61, *Bacillus pasteurii* C9, *Paenibacillus polymyxa* E681, *Bacillus subtilis* GB03, *Bacillus amyloliquefaciens* IN937a, *Enterobacter cloacae* JM-22, and *Bacillus pumilus* T4. These strains had previously induced systemic protection in tobacco, pepper, cucumber and tomato against several diseases (Wei *et al.*, 1991, 1996; Kloepper, 1996; Raupach *et al.*, 1996; Zehnder *et al.*, 1999; Yan *et al.*, 2002; Zhang *et al.*, 2002). Pathogens used were *P. syringae* pv. tomato DC3000 and *P. syringae* pv. maculicola ES4326 (kindly provided by B. J. Staskawicz, University of California, Berkeley, CA, USA) (Kus *et al.*, 2002).

Before use, the strains of PGPR and pathogens were stored at −80°C in tryptic soy broth (TSB) amended with 20% glycerol. The strains were removed from ultra-cold storage, streaked onto tryptic soy agar (TSA), and incubated at 28°C for 24 h to check for purity. Single colonies were transferred to TSA and incubated for 2 d. Both pathovars of *P. syringae* were grown on *Pseudomonas* Agar F (Difco, St Louis, MO, USA). For experimental use, fully grown bacteria were scraped off plates and resuspened into sterilized distilled water (SDW). The bacterial suspensions were adjusted to10^9 colony forming-units (cfu) ml^{-1} based on optical density.

Arabidopsis lines and growth conditions

Transgenic NahG (SA deficient) and mutant *npr1* (nonexpression of PR proteins) *Arabidopsis* were obtained from Dr Xinnian Dong, Duke University, Durham, NC, USA (Cao *et al.*, 1994). The mutant line *fad3-2 fad7-2 fad8* (jasmonic acid deficient) was provided by Dr John Browse, Washington State University, Pullman, WA, USA (Vijayan *et al.*, 1998). Mutant *ein2* (ethylene insensitive) was obtained from Dr Joseph R. Ecker, University of Pennsylvania, Philadelphia, PA, USA (Alonso *et al.*, 1999). All mutant and transgenic lines were derived from the parental *A. thaliana* ecotype Columbia (Col-0), which was obtained from the Ohio State University Stock Center, Columbus, OH, USA. The *Arabidopsis* seeds were surface-sterilized with 6% sodium hypochlorite (100% commercial laundry bleach) containing 0.1% Triton X-100, washed four times with SDW, and maintained at 4°C for 2 d to enhance germination. The seeds were then suspended in 0.4% low-melting-point agarose on soil-less media (Speedling, Sun City, FL, USA), hereinafter referred to as potting media. Plants were grown at 23 ± 3°C under a 12-h natural light regime in a greenhouse.

Initial screening of induced resistance of *A. thaliana* against *P. syringae* pv. tomato and *P. syringae* pv. maculicola by PGPR

Two weeks after seeding, one seedling of Col-0 was transplanted into a 10-cm square pot. Five millliters of PGPR suspension was applied to the base of plants in the potting media at 10^8–10^9 cfu g^{-1} soil at the time of transplanting. An additional PGPR treatment (booster) was applied 1 wk after transplanting. A stock solution of benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Syngenta Research, Triangle Park, NC, USA) at 0.33 mM was freshly prepared in SDW for each experiment. The BTH, a chemical inducer, was used as a positive control. Control treatments consisted of SDW. One week after booster treatment, freshly prepared suspensions of *P. syringae* pv. tomato and *P. syringae* pv. maculicola suspensions in SDW containing 200 µl l^{-1} Tween-20 (Sigma, St Louis, MO, USA) were sprayed onto the leaves. Inoculated plants were placed in a dew chamber (100% humidity) under darkness for 2 d at 27°C and were then transferred to a greenhouse. Seven days after pathogen challenge, disease severity was measured by two methods. First, the 'percentage disease' was measured by recording the per cent of total plant leaf surface showing symptoms for each plant from 0 = no symptoms to 100 = most severe with necrotic symptoms. Second, the number of symptomatic leaves per plant was counted. This experiment was designed as a randomized complete block (RCB) with 12 replications and one plant per replication. The experiment was conducted three times.

Spatial separation of PGPR and pathogens

To confirm spatial separation of PGPR and pathogens, one antibiotic-resistant mutant of each strain was used. Spontaneous rifampicin-resistant mutants were screened by growing colonies on TSA amended with 100 µg ml^{-1} rifampicin (rif-TSA). Isolated colonies with similar growth rates as the wild-type strains were stabilized by growing on rif-TSA for several generations. The rif-mutants of each strain were applied to *Arabidopsis* seedlings in the potting media as described previously. Four weeks after treatment with rif-resistant PGPR strains, three leaves on each plant were removed and ground with a sterile mortar and a pestle. The dilution plating method was used to isolate rif-resistant colonies on TSA amended with 100 µg ml^{-1} rif for selection of rif-resistant and 100 µg ml^{-1} cycloheximide for inhibition of fungal growth. The cfu were counted 48 h after incubation at 27°C.

Induced resistance on NahG transgenic plants by PGPR

Among PGPR strains in the initial screening, four strains – 90-166, SE34, 89B61, and T4 – were selected for further study based on consistent elicitation of ISR. To determine the role of SA in ISR, protection against *P. syringae* pv. tomato and *P. syringae* pv. maculicola was assessed on NahG plants. This experiment was designed as a randomized complete block (RCB) with 12 replications and one plant per replication. The experiment was repeated three times.

Induced resistance on *npr1*, *fad3-2 fad7-2 fad8* and *ein2* plants by PGPR

To test if PGPR elicit ISR via signaling pathways that are different from the model proposed by van Loon *et al.* (1998) and Pieterse *et al.* (2002), protection was assessed on *npr1, fad3-2 fad7-2 fad8*, and *ein2* against *P. syringae* pv. tomato and *P. syringae* pv. maculicola. The effect of PGPR on growth of *Arabidopsis* challenged with the two pathogens was also assessed by measuring foliar fresh weight 3 wk after PGPR inoculation. This experiment was designed as a randomized complete block (RCB) with 12 replications and one plant per replication. The experiment was repeated three times.

Data analysis

Data were subjected to analysis of variance using JMP software (SAS Institute Inc., Cary, NC, USA). Significance of PGPR treatment effects was determined by the magnitude of the *F*-value at $P = 0.05$. When a significant *F*-value was obtained for treatments, separation of means was accomplished using Fisher's protected least significant difference (LSD) at $P = 0.05$. Results of repeated trials of each experiment outlined above were similar. Hence, one representative trial of each experiment is reported in the Results section.

Results

Initial screening of induced resistance of *A. thaliana* against *P. syringae* pv. tomato and *P. syringae* pv. maculicola by PGPR

Disease severity was decreased by six of the nine PGPR strains against *P. syringae* pv. tomato and by seven strains against *P. syringae* pv. maculicola in *Arabidopsis* Col-0 (Table 1). Six strains (90-166, SE34, 89B61, C9, JM22 and T4) elicited systemic protection against both pathovars (Table 1). Strains GB03 and IN937a did not protect plants against both pathovars, although the two PGPR strains have been reported to induce resistance in cucumber and tomato (Kloepper *et al.*, 1996; Raupach *et al.*, 1996; Zender *et al.*, 1999).

Spatial separation of PGPR and pathogens

To exclude direct contact between PGPR strains and pathogen, we confirmed that none of the PGPR strains were detected on the rosette leaves where inoculated with pathogen. No

Table 1 Induced resistance of *Arabidopsis thaliana* against *Pseudomonas syringae* pv. tomato and *P. syringae* pv. maculicola by plant growth-promoting rhizobacteria (PGPR)

	Disease severity measured as percentage disease (%)[b]	
Treatment[a]	Psm[c]	Pst[c]
90-166	52f	40bc
SE34	63cde	41bc
89B61	50fg	45b
C9	52ef	40b
E681	50fg	71a
GB03	70bcd	73a
IN937a	75abc	71a
JM22	39gh	26c
T4	59def	36bc
BTH	28h	27c
Control	78ab	65a

Numbers represent mean of 12 replications per treatment, one seedling per replication. [a]PGPR were inoculated in the potting media, and a 2-wk-old seedling of Col-0 and NahG transgenic *A. thaliana* was transplanted into the media. two milliliters of 0.33 mM benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) solution was applied by drenching. [b]Percentage disease was measured by recording the per cent of total plant leaf surface showing symptoms for each plant.
[c]Pst, *P. syringae* pv. tomato DC3000; Psm, *P. syringae* pv. maculicola ES4326. Pst and Psm were sprayed onto leaves until run-off, 1 wk after PGPR treatment. Different letters indicate significant differences among means using Fisher's protected LSD test at $P = 0.05$.

Table 2 Induced resistance on NahG transgenic and Col-0 *Arabidopsis thaliana* against *Pseudomonas syringae* pv. tomato and *P. syringae* pv. maculicola by plant growth-promoting rhizobacteria (PGPR)

	Disease severity measured as percentage disease (%)[b]			
	Psm[c]		Pst[c]	
Treatments[a]	Col-0	NahG	Col-0	NahG
90-166	13c	40c	56b	49b
SE34	13c	60b	46b	64ab
89B61	10c	21d	55b	53b
T4	26b	43c	55b	58b
BTH	13c	14c	15c	23c
Control	40a	81a	83a	83a

Numbers represent mean of 12 replications per treatment, one seedling per replication. [a]PGPR were inoculated in the soilless mixture at 3 wk-old seedling of Col-0 and NahG transgenic *A. thaliana* transplanted in the soilless media. 2 mL of 0.33 mM benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) solution was applied by drenching. [b]Percentage disease was measured by recording the percent of total plant leaf surface showing symptoms for each plant. [c]Pst, *P. syringae* pv. tomato DC3000; Psm, *P. syringae* pv. maculicola ES4326. Pst and Psm were sprayed onto leaves until run-off, 1 wk after PGPR treatment. Different letters indicate significant differences among means using Fisher's protected LSD test at $P = 0.05$.

rif-mutants of any strain were detected on *Arabidopsis* leaves (data not shown).

Induced resistance on NahG transgenic plant by PGPR

Among PGPR strains in the initial screening, four strains (90-166, SE34, 89B61 and T4) were selected for further studies because of their consistent induced resistance capacity (data not shown). In the NahG transgenic line, all four strains significantly reduced disease severity of *P. syringae* pv. maculicola and three strains significantly reduced disease severity against *P. syringae* pv. tomato, as measured by the percentage disease scale, compared with the control (Table 2). The BTH treatment protected both Col-0 and NahG plants.

Induced resistance on *npr1*, *fad3-2 fad7-2 fad8*, and *ein2* plants by PGPR

All four strains consistently elicited ISR on Col-0 with both methods of assessing disease severity (percentage disease and number of symptomatic leaves) (Figs 1 and 2). To determine signaling pathway of ISR elicited by the four selected PGPR

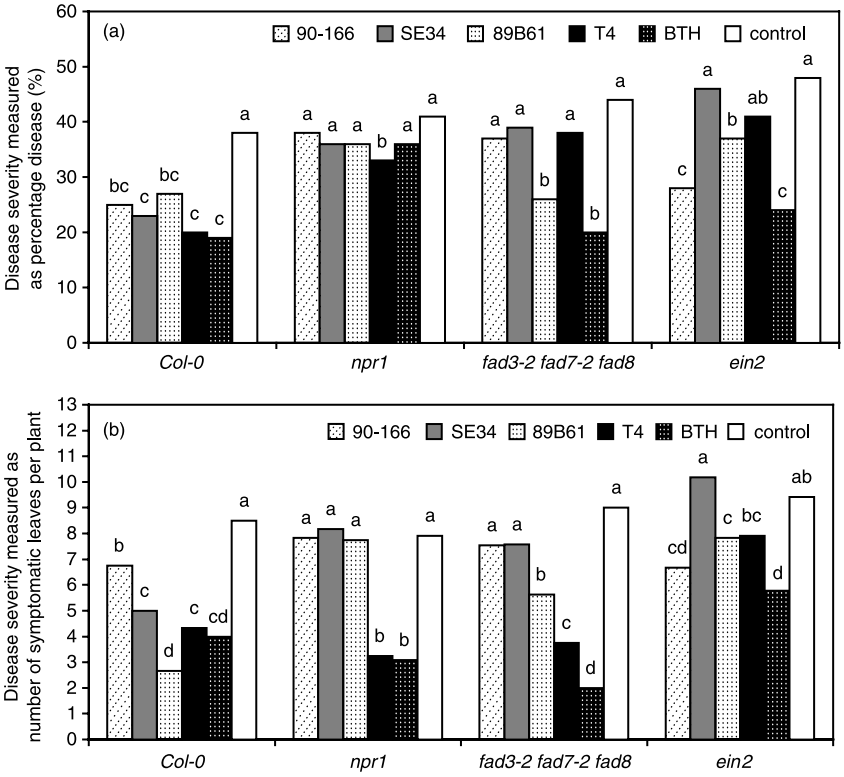

Fig. 1 Induced systemic resistance of *Arabidopsis thaliana* against *Pseudomonas syringae* pv. maculicola by selected plant growth-promoting rhizobacteria (PGPR) strains 1 wk after challenge. (a) percentage disease where 0 = no symptoms; 100 = most severe necrotic symptoms; (b) number of symptomatic leaves per plant. Numbers represent means of 12 replications per treatment, one seedling per replication. The PGPR were inoculated in the potting media containing 3-wk-old seedlings of Col-0, *npr1-1, fad3-2 fad7-2 fad8* and NahG transgenic *Arabidopsis* lines. Two milliliters of 0.33 mM benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) solution was applied by drenching. Disease severity was measured by percentage disease and number of symptomatic leaves per plant. *Pseudomonas syringae* pv. maculicola ES4326 was sprayed onto leaves until run-off, 1 wk after PGPR treatment. Different letters indicate significant differences using Fisher's protected LSD test at $P = 0.05$. The experiment was conducted three times. Results of repeated trials of each experiment outlined were similar. Hence, one representative trial of each experiment is reported here.

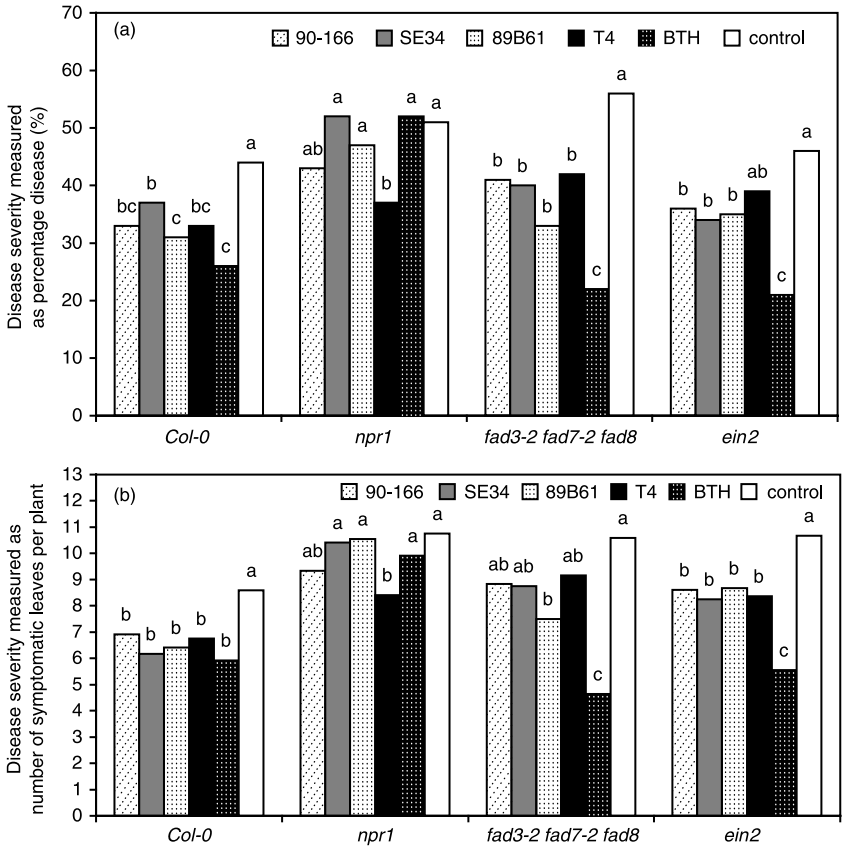

Fig. 2 Induced systemic resistance of *Arabidopsis thaliana* against *Pseudomonas syringae* pv. tomato by selected plant growth-promoting rhizobacteria (PGPR) strains 1 wk after challenge. (a) percentage disease where 0 = no symptoms; 100 = most severe necrotic symptoms; (b) number of symptomatic leaves per plant. Numbers represent mean of 12 replications per treatment, one seedling per replication. The PGPR were inoculated in the potting media containing 3-wk-old seedlings of Col-0, *npr1-1*, *fad3-2 fad7-2 fad8*, and NahG transgenic *Arabidopsis* lines. Two milliliters of 0.33 mm benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) solution was applied by drenching. Disease severity was measured by percentage disease and number of symptomatic leaves per plant. *Pseudomonas syringae* pv. tomato DC3000 was sprayed onto leaves until run-off, 1 wk after PGPR treatment. Different letters indicate significant differences using Fisher's protected LSD test at $P = 0.05$. This experiment was conducted three times. Results of repeated trials of each experiment outlined were similar. Hence, one representative trial of each experiment is reported here.

Table 3 Summary of induced resistance elicited by several plant growth-promoting rhizobacteria (PGPR) strains in *Arabidopsis thaliana*

PGPR Strains	Salicylic acid Psm[a]	Pst[a]	NPR1 Psm	Pst	Jasmonic acid Psm	Pst	Ethylene Psm	Pst
90-166	–[b]	–	+ +	+ +	+ +	±	–	–
SE34	–	+ +	+ +	+ +	+ +	±	+ +	–
89B61	–	–	+ +	+ +	–	–	–	–
T4	–	–	–	–	–	±	+ +	±
BTH	–	–	+ +	+ +	±	–	–	–

[a]Pst, *Pseudomonas syringae* pv. tomato DC3000; Psm, *P. syringae* pv. maculicola ES4326. [b] – , independent of this signal based on both disease severity measurements; + +, dependent on this signal based on both disease severity measurements; ±, either one independent of this signal from two disease severity measurements.

strains, ISR capacity of these PGPR strains was evaluated in the three signaling *Arabidopsis* mutants, which are *npr1* for a regulatory gene *NPR1*, *fad3-2 fad7-2 fad8* for jasmonic acid signaling and *ein2* for ethylene signaling. Strain SE34 did not elicit ISR in *npr1* or *ein2* plants as determined by both methods of assessing disease severity. Strain T4 caused reduction in disease by both pathovars in *npr1 and* the wild-type Col-0. Protection by the other three strains, 90-166, SE34 and 89B61 varied, depending on mutant lines, method of assessing disease severity and *P. syringae* pathovars. Plants treated with strain SE34 showed reduction of both percentage disease and number of symptomatic leaves only on *ein2* against *P. syringae* pv. tomato (Fig. 2). Strain 90-166 reduced both percentage disease and number of symptomatic leaves per plant against both *P. syringae* pathovars on *ein2* plants (Figs 1 and 2) but only percentage disease against *P. syringae* pv. tomato on the *fad3-2 fad7-2 fad8* plants (Fig. 2). Strain 89B61 caused a reduction of both percentage disease and number of symptomatic leaves per plant in *ein2* and *fad3-2 fad7-2 fad8* plants with both pathovars (Figs 1 and 2). The BTH treatment reduced both percentage disease and number of symptomatic leaves per plant with both pathovars in *ein2* and *fad3-2 fad7-2 fad8* plants, but in *npr1* plants it only reduced the number of symptomatic leaves with *P. syringae* pv. maculicola (Fig. 1) (Table 3).

Discussion

Research into how PGPR induce systemic disease resistance provides an understanding of how microorganisms signal physiological changes in plants. Novel signaling mechanisms are revealed by finding differences between reported models of signal transduction and plant responses to pathogens during induced resistance elicited by different microorganisms. Collectively, our results suggest potential novel signal mechanisms of ISR because our results differ from past studies and current models of induced resistance by PGPR.

The results reported here demonstrate that the level of systemic protection elicited in *Arabidopsis* by PGPR was dependent on the PGPR strain and the challenge pathogen. Six of nine PGPR strains reduced severity of *P. syringae* pv. tomato, while seven strains reduced severity of *P. syringae* pv. maculicola (Table 1). Although only one strain, E681, differed in ISR capacity with the two pathovars, this was still unexpected, because ISR is considered to be a broad-spectrum resistance against many pathogens. Our finding that PGPR strain E681 elicits ISR against one pathovar but not against another indicates some specificity in the defensive reactions elicited during ISR for this strain.

Expression profiling using microarray has recently suggested that the response of *Arabidopsis* to *P. syringae* pv. maculicola and to *P. syringae* pv. tomato is mostly similar (Tao *et al.*, 2003). This result agrees with our data. However, there are some exceptions with strain SE34 in NahG and *ein2* plants and T4 and BTH treatments in *fad3-2 fad7-2 fad8* plants (Table 3). Surprisingly, assessing ISR also depended on the method used to measure disease severity, which has not been reported previously. Previous results showed that PGPR strain *P. fluorescens* WCS417r elicited ISR in *Arabidopsis* against the bacterial leaf pathogens *P. syringae* pv. tomato (Pieterse *et al.*, 1996; van Wees *et al.*, 1997) and *Xanthomonas campestris* (*axonopodis*) pv. armoraciae (Ton *et al.*, 2002). These results were based on measuring disease severity as the proportion of leaves with symptoms. Using basically this same measure (number of leaves per plant showing symptoms) in our study, we concluded that ISR resulted in fewer cases by PGPR compared with measuring disease severity with a 0–100% scale. This finding suggests that conclusive evidence of repeatable systemic protection by PGPR might be more accurate when based on more than one method of assessing disease severity.

The role of defense signaling molecules such as SA, JA and ethylene in ISR has been studied with transgenic or insensitive mutant plants (Pieterse *et al.*, 1996; van Wees *et al.*, 1997; Yan *et al.*, 2002; Zhang *et al.*, 2002). NahG plants carry a bacterial *nahG* gene encoding salicylate hydroxylase that degrades SA to catechol, an inactive form that does not elicit SAR but is involved in nonhost resistance (Dempsey *et al.*, 1999; van Wees & Glazebrook 2003). NahG plants do not totally block salicylic acid accumulation but are enough to interfere SAR and SA-dependent induction of SAR-related genes (Dempsey *et al.*, 1999). However, the precise pathway of SA biosynthesis

and signaling is not yet clearly established (Cameron, 2000). *Sid1* and *Sid2* reported by Wildermuth *et al.* (2001) are genes that are similar to SA biosynthesis pathways from bacterial origin. Wildermuth *et al.* (2001) suggested, therefore, that the pathway is located in the plastid. These *sid* mutants have been little affect on phenylpronanoid pathway (directly associate with SA biosynthasis) than the NahG plants (Cameron, 2000). However, many scientists still use NahG transgenic plants for determining SA signaling pathways. In our studies, experiments with NahG plants showed that strains 89B61 and SE34 induced resistance in tomato against *Phytophthora infestans*, and strains 90-166, 89B61 and SE34 induced resistance in tobacco against *Peronospora tabacina*, indicating that these strains do not require SA to protect plants (Yan *et al.*, 2002; Zhang *et al.*, 2002). In this study, we confirmed these results by finding that strains 90-166, 89B61 and T4 systemically protected NahG *Arabidopsis* against *P. syringae* pv. maculicola and *P. syringae* pv. tomato (Table 2). These results are in agreement with the signal pathway model proposed by van Loon *et al.* (1998) and Pieterse *et al.* (2002). Results with strain SE34 were different. This strain did not induce resistance in NahG *Arabidopsis* against *P. syringae*. pv. tomato and it induced resistance against *P. syringae.* pv. maculicola at a reduced level compared with the other three PGPR strains. These results indicate that ISR elicited by SE34 is somehow dependent on SA-signaling pathways (Table 3). In our study, ISR elicited by strains 90-166, SE34 and 89B61 required JA and ethylene signaling pathways, based on lack of protection of JA- or ethylene-insensitive tomato lines (Yan *et al.*, 2002). These results are also in agreement with the model proposed by van Loon *et al.* (1998) and Pieterse *et al.* (2002). However, our results showing that strain 89B61 protected JA-insensitive *fad3-2 fad7-2 fad8* and ethylene-insensitive *ein2* mutants (Figs 1 and 2) and that T4 protected *npr1* are at variance with the model (Table 3). All previous reports of ISR elicited by PGPR were dependent on NPR1 (van Loon *et al.*, 1998). Our results with strain 89B61 and T4 suggest that they induced resistance via a new pathway or possibly a variation on previously reported pathways.

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