Microarray Analysis of the Gene Expression Profile Induced by the Endophytic Plant Growth-Promoting Rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*

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Submitted 21 June 2004. Accepted 6 January 2005.

Pseudomonas fluorescens FPT9601-T5 was originally identified as an endophytic plant growth-promoting rhizobacteria (PGPR) on tomato. To perform a molecular dissection of physiological and biochemical changes occurring in the host triggered by *P. fluorescens* FPT9601-T5 colonization, the model plant *Arabidopsis* was used in this study. Root colonization of *Arabidopsis* with *P. fluorescens* FPT9601-T5 promoted plant growth later than three weeks after inoculation and partially suppressed disease symptoms caused by *Pseudomonas syringae* pv. *tomato* DC3000, indicating that *P. fluorescens* FPT9601-T5 acted as a PGPR on *Arabidopsis*. To obtain a global view on transcript modification during the *Arabidopsis*-FPT9601-T5 interaction, we performed microarray analysis using Affymetrix Genechip probe arrays representing approximately 22,800 genes. The results showed that 95 and 105 genes were up- or down-regulated, respectively, more than twofold in FPT9601-T5-treated *Arabidopsis* plants as compared with control plants. Those up-regulated included genes involved in metabolism, signal transduction, and stress response. Noteworthy, upon FPT9601-T5 colonization, putative auxin-regulated genes and nodulin-like genes were up-regulated, and some ethylene-responsive genes were down-regulated. Our results suggest that *P. fluorescens* FPT9601-T5 triggered plant responses in a manner similar to known PGPR and, at least in some aspects, to rhizobia.

Disease management through biological agents has attracted attention as an alternative strategy that is ecologically mild and environmentally safe. It has been shown that selected rhizobacteria enhance plant growth and yield with the ability to control soilborne pathogens (Glick 1994; Timmusk and Wagner 1999). A group of bacteria that display such effects are called plant growth-promoting rhizobacteria (PGPR). PGPR exhibit biocontrol activity by antagonistic interactions between the biocontrol agent and the pathogen as well as by stimulation of the host defense mechanism. The antagonistic interaction can involve the production of various antibiotic compounds or iron chelators that inhibit the growth of soilborne pathogens and competition for nutrients and colonization sites at the root surface between the pathogen and biocontrol agent (Bakker et

al. 2003; Handelsman and Stabb 1996; Schippers 1992; Thomashow and Weller 1995).

P. fluorescens FPT9601-T5 is a commercial PGPR in Japan (Taki Chemical, Kakogawa), which was originally isolated from tomato rhizosphere. The colonization of tomato roots by the PGPR enhances plant growth and suppresses soilborne diseases such as tomato bacterial wilt caused by *Ralstonia solanacearum* (Aino et al. 1997). It has also been shown that *P. fluorescens* FPT9601-T5 acts as a biocontrol agent of soilborne diseases on a wide range of vegetable plants, including eggplant, green pepper, cucumber, squash, and Chinese cabbage (M. Aimo, Y. Maekawa, and S. Mayama, *unpublished data*). Production of two antibiotics (DAPG [2,4-diacetylphloroglucinol] and hydrogen cyanide), uncharacterized siderophores, and proteases by the PGPR has been found and has been shown to correlate with the growth inhibition against *R. solanacearum* in vitro (Zhang et al. 2000). Ultramicroscopic and laser-microscopic observation revealed that *P. fluorescens* FPT9601-T5 was found in the intercellular space of tomato roots as well as on the root surface. Therefore, this bacterium seems to endophytically colonize the roots of tomato in addition to surface colonization.

In several plant-PGPR combinations, colonization of roots with PGPR leads to a state of induced resistance not only in the colonized roots but also in the whole plant. This phenomenon is designated as induced systemic resistance (ISR). ISR has been reported in various plant species (e.g., *Arabidopsis thaliana*, common bean, carnation, cucumber, tobacco, tomato) that are colonized mostly by fluorescent *Pseudomonads* (Van Loon et al. 1998). The mechanisms leading to ISR seem to be highly dependent on the bacterial partners. *P. fluorescens* WCS417r, one of the most frequently studied PGPR on *Arabidopsis*, elicited ISR via a salicylic acid (SA)-independent pathway without pathogenesis-related (PR) gene activation (Pieterse et al. 1996, 1998; Van Wees et al. 1997). *P. fluorescens* WCS417r-induced ISR, however, required responsiveness to the plant hormones jasmonic acid (JA) and ethylene (ET) (Pieterse et al. 1998). Similarly, another *P. fluorescens* strain, CHA0, was shown to induce ISR in a SA-independent and JA-dependent manner. However, *P. fluorescens* CHA0 triggered ISR even in certain *Arabidopsis* ethylene-insensitive mutants (*ein2-1* and *etr1-1*), suggesting that the signaling pathway triggered by *P. fluorescens* CHA0 was at least partly different from that triggered by *P. fluorescens* WCS417r. In addition, a crucial role of DAPG in *P. fluorescens* CHA0-mediated ISR induction was shown, using its DAPG-deficient mutants (Iavicoli et al. 2003). In contrast to the above two PGPRs,

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some rhizobacteria have been shown to induce systemic resistance via a SA-dependent pathway. *P. aeruginosa* 7NSK2 induced systemic resistance in tobacco in a SA-dependent manner without inducing PR1a expression (De Meyer et al. 1999). In addition, systemic resistance induced by *Paenibacillus polymyxa* was associated with up-regulation of SA-, JA-, and ET-responsive genes (Timmusk and Wagner 1999), indicating involvement of signaling through SA-, JA-, and ET in *P. polymyxa*-mediated systemic resistance. To know signaling pathways that were commonly involved in those phenomena induced by different species of PGPRs, comparison of genome-wide gene expression profiles rather than examination of a limited number of known genes could be a better approach.

Besides plant-PGPR interactions, root nodulation is a well-known symbiotic phenomenon between plants and microorganisms in the rhizosphere, in which host plants provide the carbon source and energy for nitrogen-fixing bacteroids and, in turn, utilize the fixed nitrogen. A group of soil bacteria that have the ability to produce nodules on the roots of leguminous plants are collectively called rhizobia (Van Rhijn and Vanderleyden 1995). This symbiotic relationship requires a series of highly regulated and coordinated events in which signal molecules from both leguminous plants and rhizobia play important roles for recognizing each other. The infection of legume roots by rhizobia induces the expression of a set of host genes, termed nodulin during nodule formation. An interesting common feature between endophytic PGPR and rhizobia is that their infection into root tissue does not provoke plant defense reactions normally induced by invading microorganisms (Hungria and Stacey 1997). Therefore, plant responses that are commonly observed in interactions with the different types of symbiotic microorganisms are of great interest, since the responses may be related to a fundamental process for establishing symbiotic relationship between plants and microorganisms.

In this study, we performed microarray analysis using the model plant *Arabidopsis* to gain insights into physiological and biochemical changes in the host plant induced by the endophytic PGPR *P. fluorescens* FPT9601-T5. The results showed that approximately 200 out of 22,810 *Arabidopsis* genes on the microarray were identified as expressed differently (twofold change as the threshold) in FPT9601-T5-treated *Arabidopsis* plants. The majority of these genes fell into functional categories, such as basic metabolisms, signal transduction, and stress (defense) response. Predicted physiological changes in the host plant by FPT9601-T5 colonization are discussed.

RESULTS

P. fluorescens FPT9601-T5 promoted *Arabidopsis* growth and partially suppressed disease symptoms in leaves infected with *P. syringae* pv. *tomato* DC 3000.

The model plant *Arabidopsis* provides many advantages for genetic dissection, including its small size, rapid life cycle, public databases for the genome sequence and gene information, and a rich source for mutants. In this study, we attempted to use *Arabidopsis* to dissect the molecular mechanisms involved in growth promotion and systemic resistance induced by *P. fluorescens* FPT9601-T5. We first examined the colonization of *P. fluorescens* FPT9601-T5 on *Arabidopsis* (Col-1). The density of the FPT9601-T5 strain endophytically colonized in *Arabidopsis* roots was monitored by taking advantage of the fact that the bacterium had both ampicillin (Amp) and streptomycin (Str)-resistant phenotypes. Five weeks after inoculation with *P. fluorescens* FPT9601-T5, bacterial population in *Arabidopsis* root tissues was assessed using media supplied with ampicillin and streptomycin. The results showed that Amp⁺ and Str-resistant bacteria was recovered from the roots treated with *P. fluorescens* FPT9601-T5 at the density of $2.4 \times 10^5 \pm 1.0 \times 10^5$ CFU/g, but no bacteria was recovered from roots of control untreated plants, indicating that the FPT9601-T5 strain grew in *Arabidopsis* roots. When a green fluorescent protein (GFP)-tagged FPT9601-T5 strain was used for inoculating *Arabidopsis*, blight GFP fluorescence indicating bacteria colonies was detected on the root surface under a fluorescence microscopy (data not shown). Therefore, the FPT9601-T5 strain had the capacity of surface colonization on *Arabidopsis* roots as well as endophytic colonization.

Next, we assessed the plant growth promoting activity of *P. fluorescens* FPT9601-T5 on *Arabidopsis*. *Arabidopsis* plants inoculated with *P. fluorescens* FPT9601-T5 showed slower growth than control plants one week after inoculation (Fig. 1). However, later than four weeks after inoculation, slightly more growth was observed with FPT9601-T5-treated *Arabidopsis*

Table 1. Effects of *Pseudomonas fluorescens* FPT9601-T5 colonization on growth of *Arabidopsis* plants

Treatment	Number of leaflets	Fresh weight (g/leaflet)
FPT9601-T5	25.18 ± 5.55	0.725 ± 0.18 ^a
Control	22.82 ± 2.48	0.559 ± 0.12

^a Significant difference by *t* test (5%).

Fig. 1. Effects of *Pseudomonas fluorescens* FPT9601-T5 colonization on growth of *Arabidopsis* plants. Photographs of bacterized *Arabidopsis* plants with *P. fluorescens* FPT9601-T5 at one and five weeks after inoculation.

plants as compared with control plants (Fig. 1). When the number of leaflets and the fresh weight of *Arabidopsis* shoots were measured five weeks after *P. fluorescens* FPT9601-T5 treatment, about three more leaflets and significantly greater fresh weight (an average of 0.76 g/shoot) than in control plants (0.50 g/shoot) were observed in the treated plants (Table 1).

Fig. 2. *Pseudomonas fluorescens* FPT9601-T5 colonization triggered partially effective systemic resistance against challenge infection of *P. syringae* pv. *tomato* DC3000 in *Arabidopsis* plants. **A**, Based on the area with visible symptoms in a leaflet, the following five disease grades were used to assess the severity of *P. syringae* infection in *Arabidopsis*: 0, no symptom; 1 = symptoms in $\leq 10\%$; 2 = 10 to 50%; 3 = $> 50\%$; and 4 = 100%. **B**, The disease index was calculated with the following equation: disease index (%) = $[(N0 \times 0 + N1 \times 1 + N2 \times 2 + N3 \times 3 + N4 \times 4) / (N0 + N1 + N2 + N3 + N4)] \times 100$; N = number of leaflets at each stage of disease grade. The disease index was scored 2, 3, 4, and 5 days after challenge-inoculation with at least five *Arabidopsis* plants.

Those results suggested that *P. fluorescens* FPT9601-T5 behaved as a PGPR on *Arabidopsis*, even though the bacterium suppressed plant growth in early stages of colonization. Interestingly, similar phenomena, suppression of plant growth in early stages and promotion of it in later stages, were also observed in tomato plants infected with *P. fluorescens* FPT9601-T5 (data not shown). This may also correspond to growth suppression observed in *Arabidopsis* infected with *P. thivervalensis* MLG45 (Cartieaux et al. 2003).

Finally, we tested whether *P. fluorescens* FPT9601-T5 induced systemic resistance in *Arabidopsis*. Shoots of *Arabidopsis* plants whose roots had been inoculated with *P. fluorescens* FPT9601-T5 were challenge-inoculated with *Pseudomonas syringae* pv. *tomato* DC3000. Typically, *Arabidopsis* shoots develop bacteria speck disease symptoms consisting of necrotic or water-soaked spots surrounded by extensive chlorosis (Fig. 2A). We scored disease severity by determining the area of leaves with the symptoms. Figure 2B shows that pretreatment of the FPT9601-T5 strain significantly reduced disease symptoms two and three days after challenge-inoculation. At four days after treatment, however, less difference in disease severity was observed between FPT9601-T5-treated and control plants, and no significant difference was found between the treated- and control plants later than five days after treatment (Fig. 2B). These results indicated that *P. fluorescens* FPT9601-T5 had the ability to induce systemic resistance that was partially effective against challenge infection with *P. syringae* pv. *tomato* DC3000.

Microarray analysis of gene expression in *Arabidopsis* after inoculation with PGPR FPT9601-T5.

To obtain a global picture of gene expression changes triggered by colonization of *P. fluorescens* FPT9601-T5 in *Arabidopsis*, two independent microarray analyses were performed. To reduce experimental variation, three sets of nine shoots were harvested from *P. fluorescens* FPT9601-T5-treated and untreated plants, respectively, at three weeks after the treatment. Then, total RNA was prepared from the replicates of the pooled samples. RNA was analyzed using the Affymetrix GeneChip *Arabidopsis* array ATH1 containing 22, 810 *Arabidopsis* probe sets. The Affymetrix Microarray Suite software (MAS version 5.0) was applied for analyzing the reliability of signals for each probe set (Wang et al. 2003). Changes in expression

Fig. 3. A scatter plot of NetAffx microarray analysis with mRNA from *Pseudomonas fluorescens* T5-treated and untreated *Arabidopsis* plants.

of all the probe sets on the microarray are depicted in Figure 3. In this study, 14,587 and 13,085 transcripts were reliably detected in the first and second microarray analyses, respectively. Changes in RNA levels in response to *P. fluorescens* FPT9601-T5 (i.e., induction or repression) were assessed using Wilcoxon's signal rank tests as described (Affymetrix Statistical Algorithms Reference Guide).

P. fluorescens FPT9601-T5-responsive genes were identified by the following selection criteria. i) The gene expression was statistically significantly detected in at least one of the data sets; ii) the changes in gene expression occurred in the same direction in the two microarray analyses; and iii) the average

of fold changes (*P. fluorescens* FPT9601-T5-treatment/control) in expression level between the two microarray analyses was greater than 2.0 or less than -2.0. When this criterion was applied, the number of genes with increased signals was 95 and that with decreased signals was 105. We took those genes as *P. fluorescens* FPT9601-T5-regulated genes and subjected them to further analysis (Table 2).

With the information from the MIPS *Arabidopsis* database, we were able to identify or assign putative function of the *P. fluorescens* FPT9601-T5-regulated genes. The majority of the FPT9601-T5-regulated genes belonged to the following functional categories, "metabolism" (22.11% of up-regulated genes,

Table 2. Genes that were identified as expressed differently in *Arabidopsis* plants colonized by *Pseudomonas fluorescens* FPT9601-T5^a

Annotation	Fold-change ratio	Probe set number	AGI number
Up-regulated genes			
Metabolism (22.11%)			
Seed imbibition protein	3.14	246114_at	At5g20250
Putative fatty acid elongase	2.87	263443_at	At2g28630
Putative glutathione S-transferase	2.66	266746_s_at	At2g02930
β-Xylosidase	2.52	260914_at	At1g02640
Xylosidase	2.45	248622_at	At5g49360
Invertase inhibitor homolog	2.45	247246_at	At5g64620
Xyloglucan endotransglycosylase	2.38	257203_at	At3g23730
Putative xyloglucan endotransglycosylase	2.35	255433_at	At4g03210
Proline oxidase	2.33	257315_at	At3g30775
β-Galactosidase	2.33	247954_at	At5g56870
Xyloglucan endotransglycosylase-related protein XTR-7	2.28	245325_at	At4g14130
Putative endoxyloglucan glycosyltransferase	2.27	266215_at	At2g06850
Xyloglucan endo-1,4-β-D-glucanase precursor	2.22	253666_at	At4g30270
Putative glucosyltransferase	2.16	263847_at	At2g36970
Glutathione S-transferase-like protein	2.16	250083_at	At5g17220
Vegetative storage protein-like	2.13	249073_at	At5g44020
Flavanone 3-hydroxylase-like protein	2.12	249754_at	At5g24530
β-Glucosidase	2.08	262118_at	At1g02850
Putative S-adenosyl-L-methionine:trans-caffeoyl-coenzyme A 3-O-methyltransferase	2.07	260015_at	At1g67980
UDP-glucose glucosyltransferase	2.07	260799_at	At1g78270
UDP-glucose glucosyltransferase	2.02	261934_at	At1g22400
Storage Protein (1.05%)			
Similar to latex allergen	2.73	245038_at	At2g26560
Transcription (7.37%)			
Putative transcription factor	2.39	254016_at	At4g26150
NAM (no apical meristem)-like protein	2.38	263584_at	At2g17040
RING zinc finger protein-like	2.27	249306_at	At5g41400
bZIP transcription factor ATB2	2.22	253245_at	At4g34590
Putative helix-loop-helix DNA-binding protein	2.04	260070_at	At1g73830
Putative WRKY-type DNA binding protein	2.02	267246_at	At2g30250
Unknown protein similar to putative AP2 domain transcription factor	2.00	261395_at	At1g79700
Cellular transport, transport facilitation, and transport routes (4.2%)			
Unknown protein contains plant lipid transfer protein motif	3.71	256933_at	At3g22600
Ca ²⁺ /H ⁺ -exchanging protein-like	2.85	246302_at	At3g51860
Dynein light chain-like protein	2.39	246144_at	At5g20110
Major intrinsic protein (MIP)-like nodulin-26	2.10	254971_at	At4g10380
Cellular communication/signal transduction mechanism (4.2%)			
Protein kinase similar to receptor-like protein kinase RLK3	2.75	252549_at	At3g45860
Cbp1-like calcium-binding protein	2.50	249417_at	At5g39670
Unknown protein contains similarity to calcium-binding protein	2.07	260881_at	At1g21550
Protein kinase similar to wall-associated kinase 4	2.00	251621_at	At3g57700
Cell rescue, defense, and virulence (8.42%)			
Drought-induced protein-like	4.80	245523_at	At4g15910
β-1,3-glucanase 2 (BG2) (PR-2)	3.22	251625_at	At3g57260
Band 7 family protein	3.03	259272_at	At3g01290
Endochitinase isolog	2.61	260568_at	At2g43570
β-1,3-glucanase	2.50	251673_at	At3g57240
Similar to small proteins induced by heat, auxin, ethylene, and wounding	2.36	255479_at	At4g02380
Putative peroxidase ATP2a	2.33	265471_at	At2g37130
Putative cytochrome P450	2.32	267614_at	At2g26710
Interaction with the environment (systemic) (1.05%)			
GAST1 protein homolog	2.50	262947_at	At1g75750
Development (systemic) (4.2%)			
NAM/CUC2-like protein CUC2	2.56	249467_at	At5g39610
Nodulin MtN21 family protein	2.39	261576_at	At1g01070

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^a Twofold change as the threshold.

27.62% of down-regulated genes), “transcription” (7.37% of up-regulated genes, 16.19% of down-regulated genes), “cellular communication/signal transduction mechanism” (4.2% of up-regulated genes, 3.81% of down-regulated genes), “cell rescue, defense, and virulence” (8.42% of up-regulated genes, 5.71% of down-regulated genes), “development” (including systemic and biogenesis of cellular components) (7.35% of up-regulated genes), and “unclassified proteins” (including classification not yet clear-cut) (43.16% of up-regulated genes, 30.48% of down-regulated genes) (Fig. 4A).

The majority of the up-regulated genes seem to be related to two major biological changes induced by the bacterium in the

host plant, growth promotion and elevated disease resistance. Possible genes involved in plant growth promotion are auxin-induced genes (At4g36110, At2g33830), NAM-like genes (At2g17040, At5g39610, At1g69490, At3g04070), and those involved in C-compound and carbohydrate metabolism (e.g., β -galactosidase [At5g56870], xyloglucan endotransglycosylase-related protein [At4g14130], putative endoxyloglucan glycosyltransferase [At2g06850], xyloglucan endo-1,4- β -D-glucanase [At4g30270], UDP-glucose glucosyltransferase [At1g78270]), some of which are shown to be regulated by auxin in other plant species (Catala et al. 1997; Taguchi et al. 2001). Genes related to elevated disease resistance could be

Table 2. *Continued from preceding page*

Annotation	Fold-change ratio	Probe set number	AGI number
Development (systemic) (4.2%) (<i>Continued</i>)			
Unknown protein similar to N-term half of NAC domain protein NAM	2.30	256300_at	At1g69490
NAM-like protein similar to NAM	2.16	258809_at	At3g04070
Biogenesis of cellular components (3.15%)			
Zinc finger protein ATZF1	2.16	264624_at	At1g08930
Putative pectinesterase	2.15	245052_at	At2g26440
Putative protein cim1-induced allergen	2.10	252563_at	At3g45970
Subcellular localization (1.05%)			
Nodulin-like protein	2.59	246927_s_at	At5g25260
Unclassified or unknown protein (43.16%)			
Putative protein contains similarity to carboxyl-terminal proteinase	9.19	250804_at	At5g05030
Unknown protein	5.41	262832_s_at	At1g14870
Hevein-like protein precursor (PR-4)	4.76	258791_at	At3g04720
Putative auxin-induced protein	4.35	253103_at	At4g36110
Unknown protein	4.25	258225_at	At3g15630
Hypothetical protein	3.26	255107_at	At4g08730
Hypothetical protein	3.23	264091_at	At1g79110
Nodulin-like protein			
Unknown protein	3.14	247189_at	At5g65390
Unknown protein	3.01	259211_at	At3g09020
Hypothetical protein	2.84	259385_at	At1g13470
Expressed protein	2.84	249454_at	At5g39520
Expressed protein	2.80	267591_at	At2g39705
Putative trypsin inhibitor	2.80	260551_at	At2g43510
Putative auxin-regulated protein	2.70	267461_at	At2g33830
Unknown protein	2.62	264774_at	At1g22890
Putative protein	2.62	250777_at	At5g05440
Putative protein stem-specific protein	2.38	253874_at	At4g27450
Unknown protein	2.33	259841_at	At1g52200
Unknown protein	2.33	263046_at	At2g05380
Unknown protein	2.32	264590_at	At2g17710
Unknown protein	2.30	263914_at	At2g36400
Unknown protein	2.28	258402_at	At3g15450
Unknown protein	2.23	260081_at	At1g78170
Putative protein	2.22	247474_at	At5g62280
Putative protein	2.18	247293_at	At5g64510
Putative protein	2.15	248321_at	At5g52740
Hypothetical protein	2.14	252170_at	At3g50480
Hypothetical protein	2.13	266070_at	At2g18660
Putative protein	2.13	246550_at	At5g14920
Unknown protein	2.12	259856_at	At1g68440
Putative protein	2.12	248322_at	At5g52760
Unknown protein	2.08	246989_at	At5g67350
Expressed protein	2.08	246018_at	At5g10695
Hypothetical protein	2.07	266150_s_at	At2g12290
Conglutin gamma-like protein	2.03	249923_at	At5g19120
Putative protein	2.03	249752_at	At5g24660
Unknown protein	2.02	258939_at	At3g10020
Hypothetical protein	2.02	256569_at	At3g19550
E3 ubiquitin ligase, RMA1	2.02	255381_at	At4g03510
Hypothetical protein	2.00	261456_at	At1g21050
Down-regulated genes			
Metabolism (27.62%)			
Thioesterase family protein	-4.78	245422_at	At4g17470
Putative cytochrome P450	-3.14	266246_at	At2g27690
β -Xylosidase-like protein	-2.92	250499_at	At5g09730
Nucleotide pyrophosphatase-like protein	-2.65	253697_at	At4g29700

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PR proteins (At2g43570, At3g04720, At3g57240, At3g57260), WRKY transcription factors (At2g30250), those involved in secondary metabolism (flavanone 3-hydroxylase-like protein [At5g24530], trans-caffeoyl-coenzyme A 3-O-methyltransferase [At1g67980], cytochrome P450 [At2g26710]), and stress response proteins such as glutathione S-transferase (At5g17220), drought-induced protein (At4g15910), peroxidase (At2g37130), and stress-responding small protein (At4g02380). Interestingly, four nodulin-like genes (At1g01070, At2g16660, At4g10380, and At5g25260) are up-regulated by *P. fluorescens* FPT9601-T5 treatment. Nodulin is a group of plant genes that are highly induced during nodule formation, and thus, are specifically expressed during plant-rhizobia inter-

actions. This result suggested that endophytic PGPR and rhizobia might share a common signaling pathway and mechanism to establish the relationship with the host plant.

The two largest functional categories (except for unclassified protein) of the down-regulated genes are metabolism (27.62%) and transcription (16.19%) (Fig. 4B). The down-regulated genes belonging to the metabolism category mainly include those involved in carbohydrate and secondary metabolism, and those that belong to the transcription category largely comprise two major transcription factor families, ERF (ethylene-response factor)/AP2 (At1g74930, At2g22200, At2g44840, At4g34410, At5g47220) and Myb (At1g18710, At1g74430, At5g60890, At5g67300) (Table 2). Noteworthy, some parts of signaling

Table 2. Continued from preceding page

Annotation	Fold-change ratio	Probe set number	AGI number
Metabolism (27.62%) (Continued)			
Hydroperoxide lyase (HPOL)-like protein	-2.60	245253_at	At4g15440
AMP-binding protein	-2.48	262698_at	At1g75960
Xyloglucan endo-transglycosylase	-2.44	252607_at	At3g44990
Anthranilate <i>N</i> -hydroxycinnamoyl/benzoyltransferase-like protein	-2.38	247040_at	At5g67150
Hypothetical protein	-2.36	259518_at	At1g20510
Leucoanthocyanidin dioxygenase-like protein	-2.32	250793_at	At5g05600
Cellulose synthase-like protein	-2.26	245465_at	At4g16590
Adenylosuccinate lyase-like protein	-2.22	256461_s_at	At4g18440
Polygalacturonase	-2.20	264931_at	At1g60590
β -Glucosidase	-2.17	264270_at	At1g60260
β -Fructosidase	-2.16	260969_at	At1g12240
Cinnamoyl CoA reductase-like protein	-2.14	250149_at	At5g14700
Putative aspartate kinase-homoserine dehydrogenase	-2.13	263696_at	At1g31230
2-Dehydro-3-deoxyphosphoheptonate aldolase	-2.12	252831_at	At4g39980
6-Phosphogluconolactonase-like protein	-2.07	249732_at	At5g24420
Putative cellulose synthase celA	-2.07	246494_at	At5g16190
Similar to flavin-containing monooxygenase	-2.07	265121_at	At1g62560
Putative dihydrofolate reductase	-2.06	254158_at	At4g24380
Putative indole-3-acetate β -glucosyltransferase	-2.05	264873_at	At1g24100
3-Keto-acyl-CoA thiolase 2	-2.04	248625_at	At5g48880
Putative flavonol sulfotransferase	-2.02	260385_at	At1g74090
Putative glucosyltransferase	-2.02	263477_at	At2g31790
Neutral invertase	-2.01	258507_at	At3g06500
β -Galactosidase-like protein	-2.01	253090_at	At4g36360
β -Glucosidase	-2.00	264271_at	At1g60270
Energy (2.86%)			
BCS1 protein-like protein	-2.28	252131_at	At3g50930
Cytochrome b6-f complex, subunit V	-2.26	244966_at	petG
12-Oxophytodienoate-10,11-reductase	-2.14	265530_at	At2g06050
Storage protein (2.86%)			
Vegetative storage protein Vsp1	-2.87	245928_s_at	At5g24780
Putative storage protein	-2.54	254150_at	At4g24350
Putative storage protein	-2.06	254163_s_at	At4g24340
Cell cycle and DNA processing(0.95%)			
3-Methyladenine DNA glycosylase	-2.14	256457_at	At1g75230
Transcription (16.19%)			
RGA-like protein	-4.76	246432_at	At5g17490
Similar to transcriptional activator CBF1	-4.37	255937_at	At1g12610
Putative ethylene response element binding protein (EREBP)	-3.55	266821_at	At2g44840
Ethylene responsive element binding factor 1	-3.50	245252_at	At4g17500
Protein kinase	-3.13	261713_at	At1g32640
Ethylene-responsive element binding protein homolog	-2.83	253259_at	At4g34410
Putative bHLH transcription factor	-2.63	266456_at	At2g22770
(EREBP-like) AP2 domain transcription factor	-2.49	263486_at	At2g22200
RNA polymerase β subunit	-2.48	244999_at	rpoB
Ethylene responsive element binding factor 2 (ATERF2)	-2.38	248794_at	At5g47220
Putative CHP-rich zinc finger protein	-2.32	255619_s_at	At4g01350
Myb-related protein	-2.31	246987_at	At5g67300
Myb transcription factor homolog (ATR1)	-2.28	247600_at	At5g60890
Myb-related transcription factor mixta	-2.20	261431_at	At1g18710
RNA polymerase β' subunit-2	-2.19	244997_at	rpoC2
Putative MYB family transcription factor	-2.12	260237_at	At1g74430
AP2 domain containing protein	-2.05	262211_at	At1g74930
Protein synthesis (2.86%)			
25S rRNA	-4.11	AFFX-Athal-25SrRNA	
Rubisco subunit binding-protein β subunit	-2.80	265076_at	At1g55490

Continued on next page

pathways related to plant defense seem to be suppressed during the *Arabidopsis*-*P. fluorescens* FPT9601-T5 interaction. For instance, genes involved in the ethylene signaling pathway, as mentioned above, and those involved in antimicrobial substance synthesis such as cinnamoyl CoA reductaselike protein (At5g14700) and anthranilate *N*-hydroxycinnamoyl/benzoyl-transferaselike protein (At5g67150) were down-regulated.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis of representative genes.

Since cross-hybridization from closely related members of the gene families may be a confounding factor in microarray experiments, RT-PCR analysis was carried out to verify the

array hybridization data. RT-PCR assays were performed on 24 randomly selected genes, including up- and down-regulated genes identified in the microarray analysis. These RT-PCR experiments used RNA from untreated and treated plants that were used for independent biological experiments from the microarray analysis. The PCR reaction with 25 cycles of amplification was analyzed by gel electrophoresis. The results of RT-PCR analysis mostly coincided with the expression profiles obtained through the microarray hybridization experiments. Thus the increased and decreased expression patterns of the microarray analysis were supported by RT-PCR analyses. Examples in the RT-PCR analyses are shown in Figure 5.

Table 2. Continued from preceding page

Annotation	Fold-change ratio	Probe set number	AGI number
Protein synthesis (2.86%) (Continued)			
Similar to elongation factor G	-2.01	262645_at	At1g62750
Protein with binding function or cofactor requirement (0.95%)			
Nodulin-like protein	-2.29	267044_at	At2g34350
Cellular transport, transport facilitation, and transport routes (0.95%)			
Putative proline transporter	-2.21	263918_at	At2g36590
Cellular communication/signal transduction mechanism (3.81%)			
Receptor-like kinase	-3.20	265030_at	At1g61610
Putative protein kinase	-2.54	267058_at	At2g32510
Inositol 1,3,4-trisphosphate 5/6-kinase-like protein	-2.12	255132_at	At4g08170
Receptor lectin kinase 3	-2.10	251482_s_at	At3g59740
Cell rescue, defense, and virulence (5.71%)			
Putative lipoxygenase	-4.59	260399_at	At1g72520
KED-like protein	-3.81	247649_at	At5g60030
Cytochrome P450	-2.71	248353_at	At5g52320
Putative cytochrome P450	-2.31	264052_at	At2g22330
Cytochrome P450-like protein	-2.01	252827_at	At4g39950
Polygalacturonase inhibiting protein	-2.01	250669_at	At5g06870
Transposable elements, viral, and plasmid proteins (1.9%)			
Putative polyprotein	-2.68	255333_at	At4g04410
En/Spm-like transposon protein	-2.49	262448_at	At1g49450
Subcellular localization (2.86%)			
Putative annexin	-3.06	266418_at	At2g38750
Putative annexin	-2.51	266419_at	At2g38760
Mucin-like protein	-2.40	252114_at	At3g51450
Unclassified or unknown protein (30.48%)			
Hypothetical protein	-9.50	266901_at	At2g34600
Hypothetical protein	-3.95	255604_at	At4g01080
Unknown protein	-3.52	256017_at	At1g19180
Unknown protein	-3.41	261033_at	At1g17380
Hypothetical protein	-3.11	264289_at	At1g61890
Putative protein	-2.93	250960_at	At5g02940
Putative copper amine oxidase	-2.86	246573_at	At1g31680
Putative protein	-2.79	247933_at	At5g56980
Unknown protein contains similarity to chlorophyllase	-2.69	255786_at	At1g19670
Hypothetical protein	-2.60	262661_s_at	At1g14250
Hypothetical protein	-2.49	256655_at	At3g18890
Putative protein	-2.44	253849_at	At4g28080
Expressed protein	-2.43	262226_at	At1g53885
Unknown protein	-2.34	256096_at	At1g13650
Unknown protein	-2.34	260429_at	At1g72450
Hypothetical protein	-2.32	260205_at	At1g70700
Expressed protein	-2.31	267177_at	At2g37580
Expressed protein	-2.28	253758_at	At4g29060
Hypothetical protein	-2.28	251083_at	At5g01590
Putative copper amine oxidase	-2.21	246603_at	At1g31690
Hypothetical protein	-2.19	259885_at	At1g76380
Expressed protein	-2.19	253832_at	At4g27654
Unknown protein	-2.16	262171_at	At1g74950
Putative membrane-associated salt-inducible protein	-2.16	253209_at	At4g34830
Putative monodehydroascorbate reductase	-2.14	260325_at	At1g63940
Putative protein	-2.12	248091_at	At5g55120
Unknown protein	-2.11	262577_at	At1g15290
Hypothetical protein	-2.09	267344_at	At2g44230
Unknown protein	-2.04	263972_at	At2g42760
Hypothetical protein	-2.01	258997_at	At3g01810
Unknown protein	-2.01	257717_at	At3g18390
Putative trypsin inhibitor	-2.00	260547_at	At2g43550

DISCUSSION

Gene expression profiling through the use of microarrays has been recognized as a powerful approach to obtain a global view on gene expression and physiological processes involved in response to a particular stimulus (Maleck et al. 2000; Schenk et al. 2000). Based on genome-wide sequence information, oligonucleotide-based microarrays that provide the advantage of greater specificity and improved quantitative accuracy over spotted cDNA microarrays, have been developed especially in model organisms. One of the model organisms, *Arabidopsis*, offers an excellent experiment system in plant biology (Meinke et al. 1998). Using the Affymetrix *Arabidopsis* GeneChip probe array, we performed a global analysis of transcript modification associated with colonization by the endophytic PGPR, *P. fluorescens* FPT9601-T5. *P. fluorescens* FPT9601-T5 is commercially available as a biological control agent in Japan (Taki Chemical). The endophytic PGPR is currently registered as a biological pesticide only against tomato bacterial wilt disease caused by *R. solanacearum*, but the effectiveness of the bacterium for controlling other soilborne diseases on a wide range of vegetable plants has already been shown at the experimental scale (M. Aimo, Y. Maekawa, and S. Mayama, unpublished data).

Similarity in gene expression profiles of host plants treated with different types of rhizobacteria.

Cartieaux and associates (2003) conducted microarray analysis of *Arabidopsis* colonized by the PGPR *P. thivervalensis* MLG45 and reported transcript modifications of 63 genes in shoots and of nine genes in roots by the bacterial colonization. An increase of defense-related transcripts and a repression of photosynthesis-related transcripts by the colonization were reported as characteristic changes. Interestingly, some of the up-regulated genes identified with *P. thivervalensis* MLG45 infection were also activated in the present microarray analysis with *P. fluorescens* FPT9601-T5. For instance, putative auxin-regulated protein (At2g33830), xyloglucan endo-1,4-β-D-glucanase precursor (At3g30270) and heat, auxin, ethylene, and wounding-induced small protein (At4g02380), all of which were reported to be related to auxin response, were commonly up-regulated. Plant hormone plays important roles in plant devel-

opment (Simon and Chua 1999). Auxin is implicated in a variety of plant developmental processes, including lateral root development and elongation of the hypocotyls. In tomato, the activation of the Cel7 endo-1, 4-β-D-glucanase was correlated with elongation of the hypocotyls induced by auxin treatment (Catala et al. 1997). Therefore, those genes may be involved in plant growth-promoting activity of the bacteria. Activation of β-galactosidase (At5g56870), seed imbibition protein (At5g20250), and conglutin gammalike protein (At5g19120) were also identified in both the microarray analyses with *P. thivervalensis* and *P. fluorescens* FPT9601-T5.

Recently, Verhagen and associates (2004) have reported transcriptome analysis of *Arabidopsis* plants colonized with the well-studied *P. fluorescens* strain WCS417r. In the analysis, 97 genes were identified as WCS417r-responsive genes in roots, but no detectable transcriptional changes were found in shoots, suggesting that systemic changes in gene expression were not induced throughout *Arabidopsis* plants upon local colonization of *P. fluorescens* WCS417r. Even though our microarray analysis was performed with mRNA from shoots, five genes were shown to be commonly responsive to the coloniza-

ProbeSet ID	MIPS code	RT-PCR		Signal Ratio Log ₂ (T5/C)
		C	T5	
257832_at	At3g26740			0.5
246114_at	At5g20250			1.9
265478_at	At2g15890			1.2
251341_at	At3g60770			-0.7
Actin	At3g46520			

Fig. 5. Reverse transcription-polymerase chain reaction analysis of mRNA expression of randomly selected genes based on the microarray results. C = mRNA from control plants; T5 = mRNA from *Pseudomonas fluorescens* T5-treated plants.

Fig. 4. Pie charts showing the percentage of A, up-regulated and B, down-regulated genes in each of the functional categories. Biogenesis = biogenesis of cellular components; Cell cycle = cell cycle and DNA processing; Cellular communication = cellular communication/signal transduction mechanism; Cellular transport = cellular transport/transport function and transport routes; Development = development (systemic); Interaction with the environment = interaction with the environment (systemic); Protein with binding function = protein with binding function or cofactor requirement; Transposable elements = transposable elements/viral and plasmid proteins.

tion of two different *P. fluorescens* strains, WCS417r and FPT9601-T5. Those include up-regulation of two genes, drought-induced protein (At4g15910) and GAST1 protein homolog (AT1g75750), and down-regulation of three genes, ethylene-responsive element binding factor 1 and 2 (At4g17500, At5g47220) and myb-related protein (At5g67300). It is noteworthy that transcriptional changes induced by *P. fluorescens* FPT9601-T5 have some overlap with those induced by either *P. fluorescens* WCS417r or *P. thivervalensis* MLG45, whereas little overlap was observed between transcriptional changes induced by *P. fluorescens* WCS417r and *P. thivervalensis* MLG45. Therefore, *P. fluorescens* FPT9601-T5 may possess intermediate characteristics between *P. fluorescens* WCS417r and *P. thivervalensis* MLG45.

Our microarray analysis also suggested intriguing similarity between different types of rhizobacteria, endophytic PGPR, and rhizobia. Infection of leguminous plants with rhizobia induces up-regulation of a series of characteristic genes, designated nodulin genes. Even though biological roles of most nodulin genes still remain largely unknown, evidence is accumulating on involvement of certain nodulin genes in metabolite fluxes and morphological development of the host plants during legume-rhizobia interactions (Charon et al. 1997; Guenther et al. 2003). In the present study, four nodulin-like genes (At1g01070, At2g16660, At4g10380, At5g25260) were up-regulated upon infection with FPT9601-T5 in *Arabidopsis*. Three of the four nodulin-like genes (At1g01070, At2g16660, At4g10380) possess predicted transmembrane domains and motifs similar to transporter proteins. Interestingly, according to the information provided by MIPS, the molecular function of one of the nodulin-like genes (At2g16660) was predicted to be "endomembrane system" or "mating-type factor pheromone receptor activity" with amino acid sequence similarity to yeast G-protein-coupled receptor STE3. Therefore, the nodulin-like gene could be involved in signal perception or secretion during *Arabidopsis*-*P. fluorescens* FPT9601-T5 interactions, or those nodulin-like genes may be involved in enhancement of plant mineral and nutrient uptake induced by PGPR colonization as described previously (Bashan et al. 1989; Kapulnik 1996). It was also reported that some nodulin genes were activated in roots infected with another type of symbiotic microorganisms, vesicular arbuscular mycorrhizal fungi (Van Rhijn et al. 1997). In addition, endoparasitic nematode infection induced some nodulin genes in *Medicago truncatula*, even though the number of the induced nodulin genes was limited (Favery et al. 2002). Thus, it might be possible that those endophytic microorganisms may have, at least in part, overlapping regulatory pathways to establish relationship between the host plants. Noteworthy, FPT9601-T5 colonization led to down-regulation of *Arabidopsis* genes involved in the ethylene signaling pathway as mentioned above. This may implicate another aspect of similarity between rhizobia and *P. fluorescens* FPT9601-T5, since certain rhizobia were shown to reduce plant ethylene levels during nodule formation (Ma et al. 2002).

FPT9601-T5-induced transcriptional changes share overlap with, but appear to be distinct in, plants expressing systemic acquired resistance (SAR) or ISR.

Rhizobacterium-mediated ISR is a broad-spectrum resistance that is triggered by selected strains of nonpathogenic rhizosphere bacteria (Van Loon et al. 1998). Several approaches have been conducted to identify ISR-related gene expression (Cartieaux et al. 2003; Pieterse et al. 1996; Pieterse et al. 2000; Timmusk and Wagner 1999; van Wees et al. 1999; Verhagen et al. 2004). The expression pattern of a set of known defense-related genes was analyzed upon ISR induction in *Arabidopsis*

infected with *P. fluorescens* WCS417r. Of the many defense-related genes examined (e.g., the SA-inducible genes *PR-1*, *PR-2*, and *PR-5* and the ethylene- and JA-inducible genes *Hel*, *ChiB*, *Pdfl.2*, *Atvsp*, *Lox1*, *Lox2*, and *Pal1*), none were found to be up-regulated in *Arabidopsis* inoculated with *P. fluorescens* WCS417r. Recent transcriptome analysis on *P. fluorescens* WCS417r-ISR in *Arabidopsis* further supported this conclusion (Verhagen et al. 2004). Interestingly however, their microarray analysis revealed that augmented expression of defense-related genes, especially those regulated by ethylene and JA, were observed upon challenge-inoculation of the bacterial leaf pathogen *P. syringae* pv. *tomato* DC3000 in ISR-expressing leaves (Verhagen et al. 2004). This was consistent with their previous observation that the jasmonate-inducible gene *Atvsp* was up-regulated upon challenge-inoculation with the pathogen in ISR-expressing plants (van Wees et al. 1999). Therefore, in ISR-expressing plants, the expression of a set of defense-related genes are not really induced at that state but seem to be primed to respond upon challenge infection, therefore leading to elevated disease resistance of the plant.

In this study, *P. fluorescens* FPT9601-T5-mediated systemic resistance in *Arabidopsis* was partially effective to challenge infection by *P. syringae* DC3000. The gene expression changes to support this partially effective systemic resistance induced by *P. fluorescens* FPT9601-T5 colonization seemed to be distinct from those reported in canonical ISR induced by *P. fluorescens* WCS417r but rather to share some overlap with those observed in SAR. SAR is another type of systemic disease resistance induced after local infection of a pathogen and is typically accompanied by an increase in the level of endogenous SA and subsequent PR protein expression (Ross 1961). In our microarray analysis, up-regulation of some PR proteins (At2g43570, At3g04720, At3g57240, At3g57260) and SAR-related genes, such as caffeoyl-coenzyme A 3-O-methyltransferase (At1g67980) (Busam et al. 1997), was observed. However, it should be noted that the number of induced defense-related genes in our analysis was very limited compared with those usually observed in SAR-expressing plants (van Wees et al. 1999; Ward et al. 1991). In fact, by Northern analysis, we did not observe a local or systemic increase in the level of PR-1 mRNA accumulation in *P. fluorescens* FPT9601-T5-treated plants (data not shown).

Interestingly, some positive transcription factors of ethylene-responsive genes were down-regulated in *Arabidopsis* plants colonized by *P. fluorescens* FPT9601-T5 as well as by *P. fluorescens* WCS417r (Verhagen et al. 2004). Verhagen and associates (2004) postulated that the onset of ISR is associated with a reduction in ethylene signaling. If this is correct, the same signaling pathway associated with ISR is also likely to be primed by *P. fluorescens* FPT9601-T5 colonization. Thus, systemic induced resistance by *P. fluorescens* FPT9601-T5 may partly share signaling pathways with ISR induced by *P. fluorescens* WCS417r. In this context, it would be of interest to examine transcription profiles in *P. fluorescens* FPT9601-T5-treated *Arabidopsis* plants upon challenge infection with a pathogen. In conclusion, transcriptional changes induced by *P. fluorescens* FPT9601-T5 in the host plant have some overlap with those reported in typical SAR- or ISR-expressing plants but appear to be significantly distinct from either of them.

Genes associated with partially effective systemic resistance induced by *P. fluorescens* FPT9601-T5 in *Arabidopsis*.

The gene expression profile during systemic induced resistance by *P. fluorescens* FPT9601-T5 appeared to be somewhat puzzling, since both up- and down-regulation of possible defense-related genes were observed. Transcriptional up-regula-

tion was detected in PR proteins, stress-related transcription factors (At4g34590, at2g30250), general stress responding genes (At2g37130, At4g02380, At4g15910, At5g17220), and those possibly involved in antimicrobial substance production (At1g67980, At2g26710, At5g24530), all of which can be related to disease defense response of the plant. Conversely, transcriptional down-regulation was also observed in possible defense-related genes, including lipoxygenase (At1g72520), which is a key enzyme in the jasmonate synthesis pathway, ERF transcription factors (At1g74930, At2g22200, At2g44840, At4g34410, At5g47220), positive regulators of ethylene-responsive genes including defense-related genes, and those reported to be involved in lignin or phytoalexin synthesis, such as cinnamoyl CoA reductase (At5g14700) and anthranilate *N*-hydroxycinnamoyl/benzoyltransferase (At5g67150) (Jones et al. 2001; Yang et al. 2004). It is attractive to postulate that *P. fluorescens* FPT9601-T5 may suppress some parts of defense signaling pathways of the host plant to facilitate its endophytic colonization. Actually, symbiotic rhizobia have been shown to have several mechanisms to suppress the activation of host defense (Bartsev et al. 2004; Mithöfer 2002). This negative effect on inducing disease resistance may make FPT9601-T5-mediated systemic resistance partially effective. Of note, even though systemic resistance induced by *P. fluorescens* FPT9601-T5 is partially effective, the bacterium shows high biocontrol ability of soilborne pathogens in a variety of plants. Therefore, the ability of the bacterium to biologically control pathogens may mainly depend on direct antibiotic effects of the bacterium against pathogens in soil.

MATERIALS AND METHODS

Plant growth conditions.

Seeds of *A. thaliana* ecotype Columbia (Col-0) were surface-sterilized by 70% ethanol and NaClO and were washed with distilled water. The seeds were sown on MS medium (Murashige and Skoog 1962), were laid in 4°C for two days as dormant releasing, and then, the MS petri dishes were placed in a growth chamber at 21°C with a photoperiod of 12 h of light and 12 h of dark. After a week, plants were replanted in potting soil and were maintained in the growth chamber. Plants were watered twice a week.

Cultivation of bacteria.

P. fluorescens was cultivated in King's B (KB) medium supplemented with streptomycin (200 ug/ml) and ampicillin (50 ug/ml) at 30°C overnight. Bacterial cells collected by centrifugation were washed twice with water and were resuspended in distilled water to a final density of 10^9 CFU/ml, for inoculating plants. *P. syringae* pv. *tomato* DC3000 (Whalen et al. 1991) was grown in KB medium supplemented with rifampicin (50 ug/ml) at 30°C for 48 h. Cells collected by centrifugation were washed twice with distilled water and were resuspended in 10 mM MgSO₄, to a final density of 10^8 CFU/ml for dipping inoculation of *Arabidopsis*.

For the detection of endophytic population of bacteria, root samples were washed with distilled water to remove attached soil and then were surface-sterilized as described above. Surface-sterilized roots (1 g) were mixed with 4 ml of phosphate buffer (0.1 M, pH 7.0) and were ground with a mortar and pestle. Ground samples were serially diluted with distilled water and spread on a plate to calculate the bacterial population.

Bioassay of plant growth promotion and ISR by *P. fluorescens* FPT9601-T5 colonization.

Arabidopsis plants were inoculated with *P. fluorescens* FPT9601-T5 by immersing the roots in the bacterial suspen-

sion for 10 min when plants were transplanted from MS medium to soil (discussed above). Plant growth was evaluated by counting the number of leaflets and weighing each one. For the plant growth assay, 11 plants were used for each treatment. To evaluate induced resistance, plants were challenge-inoculated three weeks after *P. fluorescens* FPT9601-T5 inoculation by dipping entire leaf rosettes into *P. syringae* DC3000 suspension in 10 mM MgSO₄ with 0.01% (vol/vol) Silwet L-77, as described by Kunkel and associates (1993). Based on the area with visible symptoms in an *Arabidopsis* leaflet, the following five disease grades were used: 0, no symptoms; 1, symptoms in ≤10% of the leaflet; 2, 10 to 50%; 3, >50%; and 4, 100% (Fig. 2A). The disease index was calculated with the following equation: disease index (%) = $[(N0 \times 0 + N1 \times 1 + N2 \times 2 + N3 \times 3 + N4 \times 4) / 4 \times (N0 + N1 + N2 + N3 + N4)] \times 100$, with N = number of leaflets at each stage of disease grade. At least six *Arabidopsis* plants were used for each treatment in this assay.

RNA extraction and microarray analysis.

Total RNA was extracted from shoots of FPT9601-T5-treated and untreated plants. Frozen shoots (approximately 0.5 g) were homogenized with a mortar and pestle in liquid N₂. The ground powder was transferred to a polypropylene tube, and 5 ml of TriZol reagent (Invitrogen, Tokyo) prewarmed to 80°C was added. The homogenates were shaken with vortex for 30 s, and plant debris was pelleted by centrifugation (10,000 × g) for 10 min at 4°C. Chloroform-isoamylalcohol (24:1) (2 ml) was added to the supernatant and was centrifuged for 10 min at 4°C after mixing. Total RNA was precipitated from the aqueous phase with 0.6× volume of isopropanol by centrifugation (10,000 × g) at 4°C for 10 min. The pellet was washed with 70% ethanol, dried, and then was dissolved in an appropriate volume of distilled water treated with diethyl pyrocarbonate. The RNA samples were further purified using RNeasy mini plant kit (Qiagen, Valencia, CA, U.S.A.) for microarray analysis. RNA quality was assessed by agarose gel electrophoresis and spectrophotometry.

For microarray analysis, total RNA was processed for use on Affymetrix *Arabidopsis* genome GeneChip arrays according to the manufacturer's protocol. In brief, 10 ug of total RNA was used in a reverse transcription reaction to generate first-strand cDNA, using the SuperScript choice system (Invitrogen) with oligo(dT)₂₄ primer fused to T7 RNA polymerase promoter. After second-strand synthesis, biotin-labeled target complementary RNA (cRNA) was prepared using the BioArray high-yield RNA transcript labeling kit (Enzo Biochem, New York) in the presence of biotinylated UTP and CTP. After purification and fragmentation, 15 ug of cRNA was used in a 300-μl hybridization mixture containing added hybridization controls. A total of 200 μl of the mixture was hybridized on arrays for 16 h at 45°C. Standard posthybridization wash and double-stain protocols were used on an Affymetrix GeneChip fluidics station 450. Arrays were scanned on an Affymetrix GeneChip scanner 2500.

RT-PCR.

Total RNA was isolated as described above. Total RNA used for RT-PCR verification was obtained from plants that were grown independently from those used to isolate RNA for microarray analysis. After treatment with RNase-free DNase followed by phenol extraction and ethanol precipitation, total RNA was reverse transcribed in a 20-μl reaction using ReverTra Ace (Toyobo, Tokyo) with the oligo (dT)₁₅ primer. For PCR amplification, 1 μl of 5× diluted RT mix was used as a template. The PCR reaction was carried out in 20 μl with 0.5 unit of *rTaq* DNA polymerase (Toyobo), 1.2 mM MgCl₂, and 20 pmol of

each primer. The PCR program was as follows: 10 min at 94°C, 25 cycles of 45 s at 94°C, 45 s at the optimal annealing temperature (50 to 65°C), and 45 s at 72°C, followed by 10 min at 72°C. Sets of gene-specific primers used were as follows:

At2g15890-F: TGGTTTACCAAAATCTGAACAAGA
At2g15890-R: CCGCTAGTACTATAATAGGCACA
At3g60770-F: GCGCAGCAGCAGCCGTAAGAAAGA
At3g60770-R: ACGGGTGGGAGCTTCTTGGTCTTCT
At5g20250-F: GGTACGCTTCGCAATCCCGGTTTA
At5g20250-R: CGCGTCCCAAGTGCACCATCCGAAG
At3g26740-F: AAGTAATCAGTGGATACGCTTGTTTC
At3g26740-R: ATTCTTGTGTAAACCCAAACATC
actF: GATATGGAAAAGATCTGGCATCAC
actR: TCATACTCGGCCCTTGGAGATCCAC.

ACKNOWLEDGMENTS

We are grateful to M. Aino, Hyogo Prefectural Agricultural Institute, for valuable suggestions. This work is partly supported by grants from the Japan Society for the Promotion of Science (grant numbers 99L01205 and 11695074).

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AUTHOR-RECOMMENDED INTERNET RESOURCES

- Affymetrix microarray probe set ID files: <ftp://tairpub:tairpub@ftp. Arabidopsis.org/home/tair/Microarrays/Affymetrix/>
- Affymetrix statistical algorithms reference guide: www.affymetrix.com/support/technical/technotes/statistical_reference_guide.pdf
- The MIPS *Arabidopsis* database: mips.gsf.de/proj/thal/db/index.html