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The Plant Phenolic Compound *p*-Coumaric Acid Represses Gene Expression in the *Dickeya dadantii* Type III Secretion System▽†

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The type III secretion system (T3SS) is a major virulence factor in many gram-negative bacterial pathogens. This secretion system translocates effectors directly into the cytosol of eukaryotic host cells, where the effector proteins facilitate bacterial pathogenesis by interfering with host cell signal transduction and other cellular processes. Plants defend themselves against bacterial pathogens by recognizing either the type 3 effectors or their actions and initiating a cascade of defense responses that often results in programmed cell death of the plant cell being attacked. Here we show that a plant phenolic compound, *p*-coumaric acid (PCA), represses the expression of T3SS genes of the plant pathogen *Dickeya dadantii*, suggesting that plants can also defend against bacterial pathogens by manipulating the expression of the T3SS. PCA repressed the expression of T3SS regulatory genes through the HrpX/Y two-component system, a core regulator of the T3SS, rather than through the global regulator GacS/A, which indirectly regulates the T3SS. A further analysis of several PCA analogs suggests that the *para* positioning of the hydroxyl group in the phenyl ring and the double bond of PCA may be important for its biological activity.

The enterobacterial plant pathogen *Dickeya dadantii* (formerly named *Erwinia chrysanthemi*) causes soft-rot, wilt, and blight diseases on a wide range of plant species (6). The family *Enterobacteriaceae* includes several other genera of plant pathogens, such as *Erwinia*, *Pantoea*, and *Pectobacterium*, and important animal pathogens, such as *Escherichia coli*, *Salmonella* spp., and *Yersinia* spp. Most of the enterobacterial pathogens, including *D. dadantii*, encode at least one type III secretion system (T3SS) (12), which is a protein secretion system capable of translocating virulence proteins directly into host cells (4, 13). Many bacterial species use a two-component system (TCS) to sense their environment and regulate genes in response to environmental changes. At least two TCSs regulate the *D. dadantii* 3937 T3SS, including the global regulatory system GacS/A and a TCS encoded by genes in the center of the T3SS gene cluster, HrpX/Y. In the GacS/A-*rsmB*-RsmA-HrpL regulatory pathway, GacS/A upregulates *hrpL* mRNA by upregulating *rsmB* and thereby inactivating RsmA, which would otherwise promote the degradation of *hrpL* mRNA (1, 22) (Fig. 1). GacS/A also induces the production of pectate lyases in *D. dadantii* 3937 (22). In the HrpX/Y-HrpS-HrpL regulatory pathway, the TCS HrpX/HrpY activates *hrpS*, which encodes an enhancer protein (Fig. 1) (25). HrpS interacts with a σ^{54} RNA polymerase holoenzyme and initiates the transcription of *hrpL*. HrpL is an alternative sigma factor that is required for the expression of genes encoding the T3SS effectors and structural components such as the units of the needle, the needle extension, and the translocon.

The T3SS is an attractive target for development of antimicrobial compounds since it is present mainly in pathogenic gram-negative bacteria and is often required for virulence by these species (5, 18). We recently discovered that the phenolic compounds *trans*-cinnamic acid (TCA) and *o*-coumaric acid (OCA) induce the expression of *D. dadantii* T3SS genes *hrpA* and *hrpN* through the *rsmB*-RsmA pathway (21). To identify potential T3SS repressors, analogs and isomers of TCA and OCA were screened for effects on *D. dadantii* 3937 *hrpA* expression. An isomer of OCA, *p*-coumaric acid (PCA), that repressed the expression of T3SS genes of *D. dadantii* 3937 is identified in this study. Based on the chemical structures and inhibitory effect of PCA and several analogs of PCA on T3SS gene expression, the structure-activity relationship (SAR) and potential active sites of PCA are identified. To our knowledge, no mechanism for SAR studies on the inhibitory activity of small molecules against T3SS gene expression has ever been reported. Finally, the regulators responsible for the repression of T3SS gene expression by PCA are elucidated.

MATERIALS AND METHODS

Bacterial strains, plasmids, and chemicals. The bacterial strains and plasmids used in this study are listed in Table 1. *D. dadantii* was grown in Luria-Bertani or T3SS-inducing minimal medium (MM) at 28°C (23). Ampicillin at 100 μg/ml was used in this work. Chemical structures of OCA, PCA, TCA, and related phenolic compounds used in this study are shown in Fig. 2.

Flow cytometry analysis. Promoter activity of *hrpA*, *hrpL*, *hrpN*, and *hrpS* was determined in a FACSCalibur flow cytometer (BD Biosciences, CA) as described previously (14). The bacterial cells carrying the promoter reporter plasmid were

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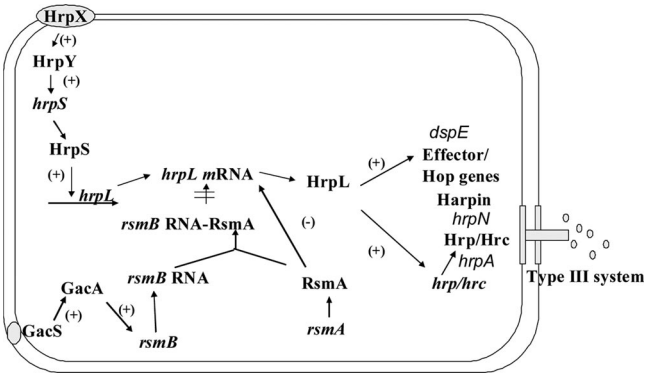

FIG. 1. Regulatory network controlling the *D. dadantii* T3SS. The *D. dadantii* T3SS is regulated by the HrpX/HrpY-HrpS-HrpL and the GacS/GacA-*rsmB*-HrpL regulatory pathways. The TCS HrpX/HrpY activates *hrpS*, which encodes a σ^{54} enhancer. HrpS is required for expression of the alternative sigma factor gene *hrpL*. HrpL activates expression of genes encoding the T3SS apparatus and its secreted substrates. RsmA is a small RNA-binding protein that acts by lowering the half-life of *hrpL* mRNA. GacS/GacA upregulates the expression of *rsmB*, which increases the mRNA level of *hrpL* by sequestering RsmA. +, positive regulation; −, negative regulation.

grown in Luria-Bertani broth at 28°C overnight and transferred to appropriate media.

qRT-PCR analysis. Total RNA from the bacterial cells was isolated by using the Tri reagent method (Sigma, MO) and treated with Turbo DNA-free DNase kits (Ambion, TX). The cDNA levels of target genes in different samples were quantified by quantitative reverse transcription-PCR (qRT-PCR) using Real Master Mix (Eppendorf, Westbury, NY) as described previously (14). qRT-PCR data were analyzed with the Relative Expression Software Tool as described previously (15), with *rplU* as an endogenous control for data analysis (9).

Western blot analysis. Wild-type *D. dadantii* 3937 was grown at 28°C in MM or MM supplemented with different amounts of PCA for 24 h. Proteins were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and analyzed by Western blotting. Polyclonal immunoglobulin Y antibody against HrpN prepared from chicken was used as the probe and was preabsorbed with *E. coli* DH5α and a HrpN mutant. An Immuno-Star AP chemiluminescent substrate (Bio-Rad, CA) was used to detect an anti-chicken secondary antibody (GeneTex, TX).

RESULTS AND DISCUSSION

Screening for T3SS inhibitor. To identify potential T3SS repressors, 29 analogs and isomers of TCA and OCA were screened for effects on *D. dadantii* 3937 *hrpA* expression (Table 2 and Fig. 2). *hrpA* encodes the T3SS pilus required for protein translocation into plant cells, and this gene is the first in a multigene operon that encodes T3SS structural and secreted proteins. A reporter plasmid, phrpA, which contains a transcriptional fusion of the *hrpA* promoter controlling expression of green fluorescent protein (GFP) was used to measure the effects of the OCA and TCA analogs and isomers on T3SS gene expression (22). Expression of T3SS genes of phytobacteria is induced in T3SS-inducing MM, which is considered to mimic plant apoplastic conditions, such as a low level of nutrients (7, 14, 17, 19). Bacterial cells containing phrpA were grown in the T3SS-inducing MM supplemented with 100 μM of each compound. GFP intensity, which is a measure of *hrpA* promoter activity, was assayed by flow cytometry. Among the OCA/TCA analogs screened, PCA (Fig. 2, compound 4) showed the strongest inhibition on T3SS gene expression (Table 2).

SAR of phenolic compound analogs. OCA (Fig. 2, compound 6) and *meta*-coumaric acid (MCA) (compound 5) induced *hrpA* expression (Table 2). PCA, OCA, and MCA are regioisomers, differing only in the position of the hydroxyl group in the phenyl ring (Fig. 2). Two derivatives of PCA, *trans*-2,4-dihydroxycinnamic acid (compound 2) and *trans*-3,4-dihydroxycinnamic acid (compound 3), with an additional hydroxyl group in the *ortho* and *meta* positions of the phenyl ring, respectively, had reduced inhibitory activity against *hrpA* expression in comparison to PCA. These results indicated that the *para* positioning of the hydroxyl group in the phenyl ring is

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Characteristics[a]	Reference or source
D. dadantii strains		
3937	Wild type, *Saintpaulia* sp. (African violet) isolate	N. Hugouvieux-Cotte-Pattat
3937(pAT)	3937 containing pPROBE-AT	14
3937(phrpA)	3937 containing phrpA; Ap^r	22
3937(phrpN)	3937 containing phrpN; Ap^r	23
3937(phrpL)	3937 containing phrpL; Ap^r	23
3937(phrpS)	3937 containing phrpS; Ap^r	This work
3937(pmrp)	3937 containing pmrp; Ap^r	14
Plasmids		
pPROBE-AT	Promoter-probe vector; Ap^r	11
phrpA	pProbe-AT derivative with PCR fragment containing *hrpA* promoter region; Ap^r	22
phrpN	pProbe-AT derivative with PCR fragment containing *hrpN* promoter region; Ap^r	23
phrpL	pProbe-AT derivative with PCR fragment containing *hrpL* promoter region; Ap^r	23
phrpS	pProbe-AT derivative with PCR fragment containing 709-bp *hrpS* promoter region; Ap^r	This work
pmrp	pProbe-AT derivative with PCR fragment containing *mrp* promoter region; Ap^r	14
$p50HrpN_{0–117}$	pCPP50 derivative with fragment encoding 3937 *hrpN* lacking nucleotides 50–117	24
pCPP50::HrpN	pCPP50 derivative with fragment encoding 3937 *hrpN*	24

[a] Ap^r, ampicillin resistance.

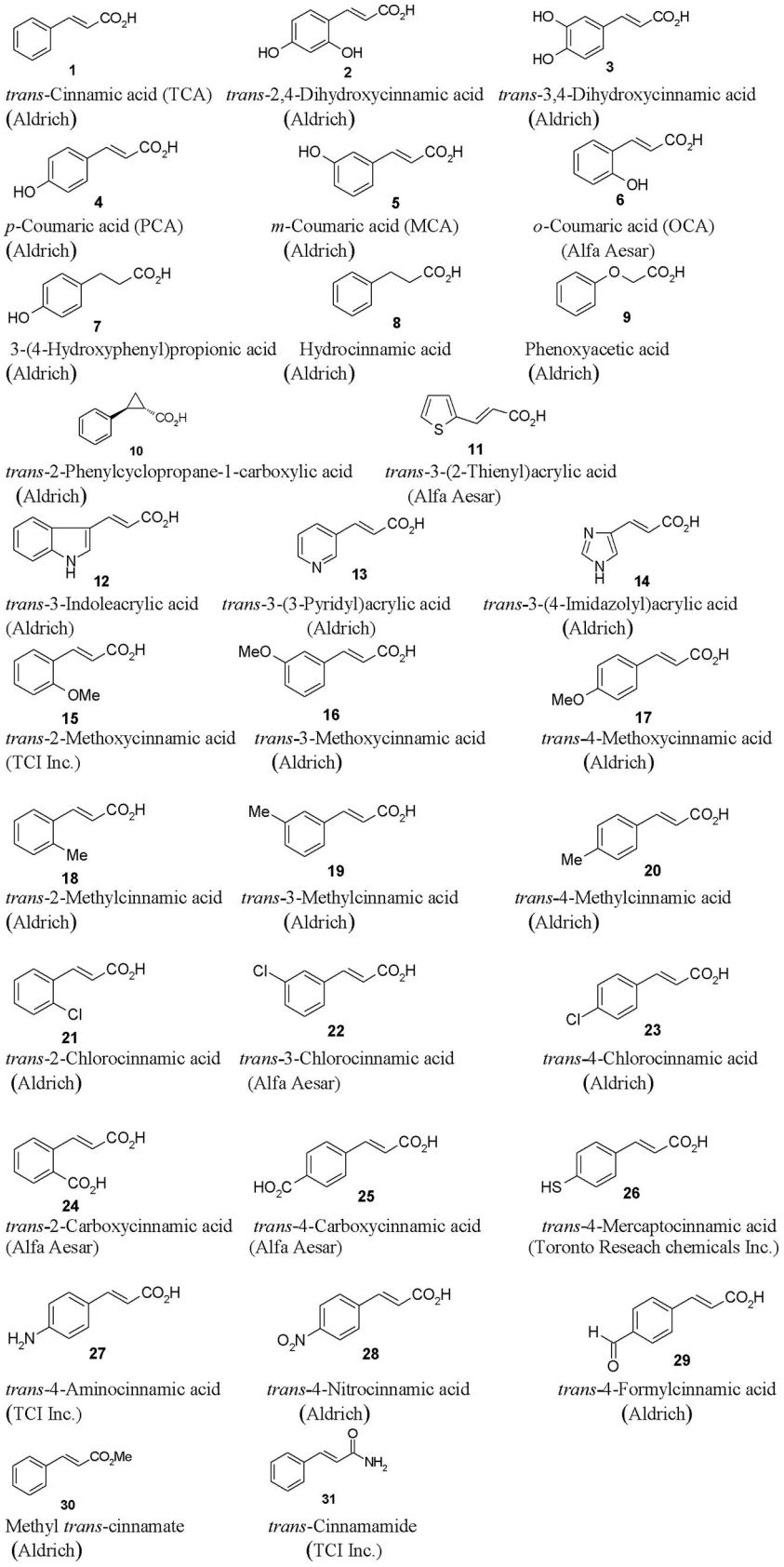

FIG. 2. Chemical structures of OCA, PCA, TCA, and related phenolic compounds.

TABLE 2. Expression of *D. dadantii* 3937 *hrpA* in MM and MM supplemented with different isomers and analogs of OCA and TCA

Phenolic compound (compound no.)[a]	Avg MFI ± SD[b] at:	
	12 h	24 h
None	78.7 ± 6.3	92.1 ± 17.1
TCA (1)	133.9 ± 12.9*	203.7 ± 16.1*
OCA (6)	115.5 ± 7.9*	225.8 ± 15.6*
MCA (5)	133.0 ± 38.2	203.3 ± 9.6*
PCA (4)	10.2 ± 0.4*	11.4 ± 1.0*
Hydrocinnamic acid (8)	200.3 ± 35.8*	213.5 ± 18.9*
Phenoxyacetic acid (9)	222.7 ± 64.3*	205.7 ± 11.8*
trans-2-Phenylcyclopropane-1-carboxylic acid (10)	67.0 ± 18.4	84.0 ± 14.3
trans-3-Indoleacrylic acid (12)	23.9 ± 1.3*	121.0 ± 6.2
trans-3-(3-Pyridyl)acrylic acid (13)	184.9 ± 35.6*	204.0 ± 16.8*
trans-2-Methylcinnamic acid (18)	157.2 ± 11.7*	342.5 ± 16.6*
trans-2-Chlorocinnamic acid (21)	166.2 ± 17.8*	319.8 ± 48.3*
Methyl *trans*-cinnamate (30)	135.8 ± 8.1*	219.2 ± 14.5*
None	101.8 ± 3.8	134.6 ± 3.3
trans-3-(2-Thienyl)acrylic acid (11)	201.9 ± 3.7*	338.2 ± 14.9*
trans-3-(4-Imidazolyl)acrylic acid (14)	118.8 ± 11.1	144.1 ± 13.7
trans-2-Methoxycinnamic acid (15)	213.9 ± 7.4*	304.3 ± 15.5*
trans-2-Carboxycinnamic acid (24)	109.0 ± 21.5	139.7 ± 17.1
3-(4-Hydroxyphenyl)propionic acid (7)	138.9 ± 10.5*	186.9 ± 3.9*
trans-Cinnamamide (31)	126.3 ± 10.2	228.5 ± 2.0*
trans-2,4-Dihydroxycinnamic acid (2)	66.6 ± 3.9*	78.6 ± 5.2*
trans-3,4-Dihydroxycinnamic acid (3)	82.3 ± 9.0	111.4 ± 14.6
None	133.3 ± 4.8	176.4 ± 6.1
trans-3-Methoxycinnamic acid (16)	114.7 ± 12.7	143.9 ± 35.9
trans-4-Methoxycinnamic acid (17)	74.5 ± 7.5*	100.2 ± 3.7*
trans-3-Methylcinnamic acid (19)	108.0 ± 8.1	136.2 ± 21.2
trans-4-Methylcinnamic acid (20)	58.9 ± 2.7*	91.1 ± 4.1*
trans-3-Chlorocinnamic acid (22)	113.3 ± 7.5	157.0 ± 6.2
trans-4-Chlorocinnamic acid (23)	54.3 ± 3.3*	81.3 ± 2.5*
trans-4-Carboxycinnamic acid (25)	114.4 ± 11.9	125.2 ± 5.5*
trans-4-Mercaptocinnamic acid (26)	117.4 ± 22.4	128.0 ± 9.3*
trans-4-Aminocinnamic acid (27)	125.9 ± 9.4	154.6 ± 1.8*
trans-4-Nitrocinnamic acid (28)	100.5 ± 3.0*	114.2 ± 13.5*
trans-4-Formylcinnamic acid (29)	105.1 ± 4.8*	137.9 ± 8.1*

[a] MM was supplemented with 100 μM of the indicated compounds. The compounds were assayed three different times, with MM only (no supplementation) as the control treatment (indicated by "none") for each set of experiments. The compound numbers are as used in Fig. 2.

[b] *D. dadantii* 3937 cells carrying GFP reporter phrpA were used in this study. The promoter activities at 12 and 24 h of bacterial growth were determined. GFP mean fluorescence intensity (MFI) was determined for gated populations of bacterial cells by flow cytometry. Values are representative of two or three experiments, and three replicates were used for each experiment. Asterisks indicate statistically significant differences in GFP MFI between bacterial cells grown in MM and MM supplemented with the different compounds ($P < 0.01$, Student's t test).

TABLE 3. Expression of T3SS genes *hrpA*, *hrpN*, *hrpS*, and *hrpL* of *D. dadantii* 3937 in MM and MM supplemented with 100 μM PCA

Reporter plasmid	Avg MFI ± SD for growth in the indicated medium[a] at:			
	12 h		24 h	
	MM	MMPCA	MM	MMPCA
phrpA	64.3 ± 0.9	10.3 ± 1.3*	150.8 ± 4.4	18.5 ± 3.5*
phrpN	39.8 ± 5.8	6.8 ± 0.8*	133.3 ± 3.2	11.3 ± 3.4*
phrpS	72.7 ± 11.3	37.9 ± 1.3*	95.0 ± 17.7	43.6 ± 2.6*
phrpL	12.8 ± 0.1	7.8 ± 0.1*	27.2 ± 1.0	11.1 ± 3.3*
pmrp	113.0 ± 7.7	124.1 ± 2.7	93.4 ± 2.6	98.9 ± 1.0
pPROBE-AT	2.1 ± 0.1	2.2 ± 0.2	13.4 ± 8.4	14.0 ± 10.1

[a] The promoter activities were compared at 12 and 24 h of bacterial growth in PCA. GFP mean fluorescence intensity (MFI) was determined for gated populations of bacterial cells by flow cytometry. Values are representative of two experiments, and three replicates were used for each experiment. Asterisks indicate statistically significant differences in GFP intensity between bacterial cells grown in MM and MM supplemented with 100 μM PCA (MMPCA) ($P < 0.01$, Student's t test).

important for the biological activity of PCA. Similarly, chloro-, methyl-, and methoxycinnamic acids demonstrated the same activity pattern, with the *para* isomers *trans*-4-chlorocinnamic acid (compound 23), *trans*-4-methylcinnamic acid (compound 20), and *trans*-4-methoxycinnamic acid (compound 17) inhibiting *hrpA* expression but not the *ortho* isomers (compounds 21, 18, and 15) and the *meta* isomers (compounds 22, 19, and 16) (Table 2). However, when the hydroxyl group in PCA is replaced by a carboxyl, mercapto, amino, nitro, or formyl group, the resulting cinnamic acid derivatives, *trans*-4-carboxycinnamic acid (compound 25), *trans*-4-mercaptocinnamic acid (compound 26), *trans*-4-aminocinnamic acid (compound 27), *trans*-4-nitrocinnamic acid (28), and *trans*-4-formylcinnamic acid (compound 29), respectively, exhibited a reduced inhibitory effect on *hrpA* (Table 2).

Along with the position of the hydroxyl group in the phenyl ring, the double bond in PCA is essential for its inhibitory activity. For example, if the double bond is reduced, the resulting 3-(4-hydroxyphenyl)propionic acid (compound **7**) did not inhibit *hrpA* expression. Replacing the carboxylic acid group with an amide or methyl ester did not change the activity of TCA (compound 1) to induce T3SS gene expression, as both methyl *trans*-cinnamate (compound 30) and *trans*-cinnamamide (compound 31) demonstrated strong inducing activity. Finally, *trans*-3-indoleacrylic acid (compound 12) had an inhibitory effect on *hrpA* expression at 12 h of bacterial growth but not at 24 h (Table 2). However, when other heterocyclic rings, such as pyridine, imidazole, and thiophene, are introduced in the same position, the resulting acrylic acids, *trans*-3-(3-pyridyl)acrylic acid (compound 13), *trans*-3-(4-imidazolyl)acrylic acid (compound 14), and *trans*-3-(2-thienyl)acrylic acid (compound 11), respectively, did not have any inhibitory activity.

Inhibitory effect of PCA on T3SS gene expression. To confirm the inhibitory effect of PCA on the *D. dadantii* T3SS, the expression of additional T3SS genes was examined. *hrpN* encodes a T3SS harpin, and *hrpN* promoter activity was reduced in MM supplemented with 100 μM PCA in comparison to that in unamended MM (Table 3). The promoter of a conserved ATPase gene, *mrp*, was used as a reference gene (14). Similar levels of *mrp* expression were observed in *D. dadantii* 3937(pmrp) when the bacterial cells were grown in MM or MM supplemented with 100 μM PCA, thus showing that the effect of PCA was specific to the T3SS (Table 3).

The effect of PCA on T3SS gene transcription was directly measured by qRT-PCR. Compared to that in *D. dadantii* 3937 grown in unamended MM, there was significantly less *hrpA*, *hrpN*, and T3SS effector *dspE* mRNA in cells grown in MM supplemented with 100 μM PCA (Fig. 3). Since PCA represses the expression of several T3SS genes such as *hrpN*, we examined the effect of PCA on HrpN protein production. Less HrpN was detected in protein extracts from *D. dadantii* 3937 grown in MM supplemented with 100 μM PCA than in those

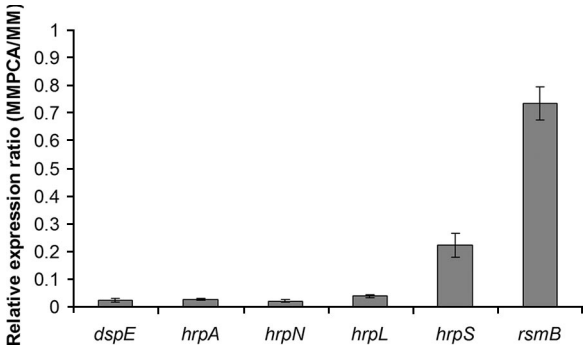

FIG. 3. Relative mRNA levels of *hrpS*, *hrpL*, *dspE*, *hrpA*, *hrpN*, and *rsmB* of *D. dadantii* 3937 in MM supplemented with 100 μM PCA compared to mRNA levels in MM without PCA as determined by qRT-PCR. There is no significant difference between MM and MM supplemented with PCA for gene *rsmB* ($P = 0.928$), but levels of gene expression of *hrpS*, *hrpL*, *dspE*, *hrpA*, and *hrpN* are significantly different between MM and MM supplemented with 100 μM PCA ($P < 0.001$). Three replicates were used in this experiment. The *P* value was calculated with the Relative Expression Software Tool as described by Pfaffl et al. (15).

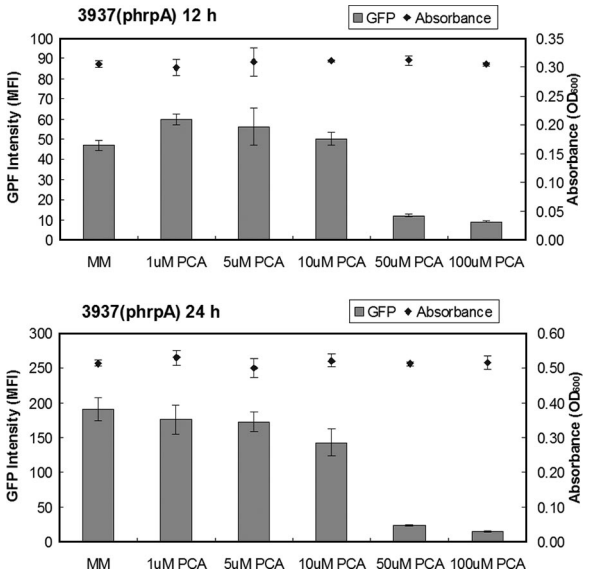

FIG. 5. Promoter activities of *hrpA* in *D. dadantii* 3937 grown in MM and MM supplemented with different amounts of PCA at 12 h and 24 h of growth. To study the effect of PCA on *hrpA* expression, 50 μl of bacterial suspension (optical density at 600 nm [OD_{600}] = 1.0) was used as the initial inoculum and added into 5 ml MM and MM supplemented with PCA. GFP intensity was determined on gated populations of bacterial cells by flow cytometry and analyzed with the Cell Quest software (BD Biosciences, San Jose, CA). The growth of *D. dadantii* 3937 in MM supplemented with different concentrations of PCA was recorded. Results from one representative experiment are shown. Three replicates were used in this experiment, and the experiment was repeated twice. MFI, mean fluorescence intensity.

from the strain grown in MM supplemented with 10 μM PCA (Fig. 4).

To determine if the repression of T3SS gene expression by PCA was due to toxicity or nutritional status of the phenolic compound, different concentrations of PCA were used to supplement MM to measure the effect of PCA on *hrpA* expression and bacterial growth. Compared to that for growth in unamended MM, the average GFP fluorescence intensity of *D. dadantii* 3937(phrpA) cells was reduced more than fourfold when 50 or 100 μM of PCA was added to the medium (Fig. 5). The addition of PCA at concentrations of 1, 5, and 10 μM did not result in a substantial reduction of GFP fluorescence intensity for *D. dadantii* 3937. No inhibition or promotion of bacterial growth was observed when PCA was added to MM (Fig. 5).

PCA inhibits T3SS through the HrpX/Y-HrpS-HrpL pathway. Since strong inhibition of *hrpA* expression by PCA in *D. dadantii* 3937 was observed, the mechanism of PCA regulation of the T3SS pathway was investigated. To determine whether PCA affects T3SS gene expression through the Gac-Rsm regulatory pathway, *rsmB* mRNA levels were quantified by qRT-PCR. No significant difference in the level of *rsmB* mRNA between *D. dadantii* 3937 cells grown in MM and cells grown in MM supplemented with 100 μM PCA was observed (Fig. 3). Thus, repression of T3SS expression by PCA does not occur through the Gac-Rsm pathway. PCA supplementation did not affect *D. dadantii* 3937 pectate lyase production, providing further support that PCA does not affect the GacS/A system (data not shown).

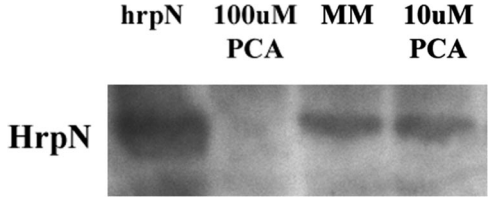

FIG. 4. HrpN protein expression of *D. dadantii* 3937 in MM and MM supplemented with PCA. Lane 1 (left lane), 3937(pCPP50::HrpN); lane 2, *D. dadantii* 3937 grown in MM supplemented with 100 μM PCA; lane 3, *D. dadantii* 3937 grown in MM; lane 4, *D. dadantii* 3937 grown in MM supplemented with 10 μM PCA. Similar amounts of proteins were loaded in each lane.

To determine if PCA represses T3SS gene expression through the HrpX/Y-HrpS-HrpL pathway, the promoter activities of *hrpS* and *hrpL* were examined and found to be reduced in MM supplemented with 100 μM PCA in comparison to those in unamended MM (Table 3). The expression of *hrpS* and *hrpL* was also confirmed by qRT-PCR. Our result showed that, compared with those for *D. dadantii* 3937 grown in MM, significantly smaller amounts of *hrpS* (relative expression ratio, 0.223; $P < 0.001$) and *hrpL* (relative expression ratio, 0.039; ($P < 0.001$) mRNA were present (Fig. 3). These results demonstrate that PCA inhibits expression of T3SS genes through the HrpX/Y-HrpS-HrpL regulatory pathway.

Phenylpropanoids are a group of secondary metabolites produced by plants from L-phenylalanine. Although the end products vary among plant species, the initial reactions of the phenylpropanoid biosynthesis pathway are conserved (2). These multiple-branch pathways can all be derived from initial steps of the following pathways. (i) TCA is produced by a deamination of L-phenylalanine by phenylalanine ammonia-lyase. (ii) Cinnamic acid 4-hydroxylase catalyzes the addition of the hydroxyl group at the *para* position of the phenyl ring of TCA, producing PCA. (iii) The carboxyl group of PCA is activated by

formation of a thioester bond with coenzyme A (CoA), a process catalyzed by 4-coumarate:CoA ligase, which gives rise to a variety of secondary compounds such as flavonoids, isoflavonoids, stilbenes, and lignin (see Fig. S1 in the supplemental material) (10, 20). Isoflavonoids and stilbenes are phytoalexins which are induced in response to microbial attack and are able to inhibit the growth of pathogens (8), while lignin is a major component of the plant cell wall and provides mechanical strength and impermeability to plant tissues (8). In addition, TCA is an important intermediate in the pathway for salicylic acid biosynthesis involved in the disease resistance of plants (16). In salicylic acid biosynthesis, TCA is converted to OCA through *ortho* hydroxylation, followed by β-oxidation to produce salicylic acid. Salicylic acid is a plant signaling molecule that triggers basal resistance locally and systemically acquired resistance against a broad spectrum of pathogens including viruses, bacteria, fungi, and oomycetes (3).

It is intriguing to see intermediates in phenylpropanoid biosynthesis either induce or repress the expression of T3SS genes of *D. dadantii* 3937. Based on the chemical structures and inhibitory effect of PCA and analogs of PCA on T3SS, the SAR and potential active sites of PCA are revealed (Table 2 and Fig. 2). Given the similarity of the T3SS regulatory systems among many plant and animal pathogens, the effects of plant phenolic compounds on *D. dadantii* 3937 unveiled here provide a new direction for development of novel antimicrobial reagents for agriculture.

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This work is dedicated to Noel T. Keen.

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