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Virulence of Selected Phytopathogenic Pseudomonads in *Arabidopsis thaliana*

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We are developing a model pathogenesis system that utilizes *Arabidopsis thaliana* as a host for infection by a variety of phytopathogenic *Xanthomonas* and *Pseudomonas* strains. We divided 51 different strains into four categories based on the symptoms they elicited when infiltrated into *A. thaliana* leaves at two doses, 10^6 and 10^7 cfu/ml. Highly virulent and weakly virulent strains elicited spreading water-soaked lesions within 48 hr at doses of 10^6 and 10^7 cfu/ml, respectively. Avirulent strains elicited either a hypersensitive response (dry necrotic lesion) within 12–24 hr at doses of 10^6 and 10^7 cfu/ml or pitting with mild chlorosis within 48 hr at a dose of 10^7 cfu/ml. Null strains elicited no visible symptoms at a dose of 10^7 cfu/ml. Several *Pseudomonas* strains were chosen for additional characterization. The highly virulent strains, *P. syringae* pv. *tomato* DC3000 and *P. s.* pv. *maculicola* 795, elicited spreading water-soaked lesions with chlorotic margins, multiplied 10^3- to 10^4-fold in *A. thaliana* leaves, induced a five- to 10-fold transient accumulation of mRNA corresponding to phenylalanine ammonia-lyase (PAL), and had little effect on the pH of the medium when added to *Arabidopsis* tissue culture cells. The avirulent strain, *P. cichorii* 83-1, elicited a localized, dry lesion typical of a hypersensitive response within 12–24 hr, failed to multiply in *A. thaliana* leaves, induced a 15- to 30-fold accumulation of PAL mRNA, and caused an alkalinization of the medium when added to *Arabidopsis* tissue cultures. *P. aureofaciens* strain 923 elicited a null response, did not multiply in *A. thaliana* leaves, induced a five- to sixfold accumulation of PAL mRNA, and caused an acidification of the medium when added to *Arabidopsis* tissue culture cells. To facilitate the monitoring of the induction of *A. thaliana* PAL mRNA in response to infiltration with phytopathogenic bacteria, we cloned and partially sequenced an *A. thaliana* genomic DNA sequence encoding PAL. Southern blot analysis showed that the *A. thaliana* genome contains two or three additional sequences that are at least partially homologous to the cloned PAL gene.

Additional keywords: disease resistance, plant-pathogen interactions.

Significant progress has been made in defining specific genes in phytopathogenic bacteria that are involved in both pathogenesis and the induction of active defense responses in plants (Daniels *et al.* 1988; Keen and Staskawicz 1988; Gabriel and Rolfe 1990). However, for the most part, how these bacterial genes and their corresponding products interact at the molecular level with the plant host is still undefined. Similarly, numerous studies have defined a variety of host genes that are induced during the interaction of plants with phytopathogenic microorganisms (Collinge and Slusarenko 1987; Hahlbrock and Scheel 1989; Somssich *et al.* 1989; Dixon and Lamb 1990). However, the lack of mutant plants that are defective in the expression of these putative defense genes has made it difficult to clearly demonstrate an active role for these genes in conferring resistance. Furthermore, little is known about the mechanisms involved in the activation of these defense-related genes.

Because plant-bacterial interactions involve alterations in gene expression in both the bacteria and the host plant, it would be desirable to be able to genetically manipulate both the bacterial pathogen and its host plant. Toward this end, we have been developing a model pathogenesis system that utilizes *Arabidopsis thaliana* (L.) Heynh as a host for infection by several phytopathogenic *Pseudomonas* species (Davis and Ausubel 1989; Davis *et al.* 1989; Schott *et al.* 1990; Dong *et al.* 1991). Our goal in using *A. thaliana* as a host for bacterial pathogens is to take advantage of genetic analysis to help dissect the process by which plants perceive the presence of a pathogen. The use of *A. thaliana* to study plant-bacterial interactions is also being pursued by several other laboratories (Simpson and Johnson 1990; Whalen *et al.* 1991; Daniels *et al.* 1991).

The advantages that *A. thaliana* offers as an experimental system for both genetic and molecular genetic studies have been extensively reviewed (Redei 1975; Meyerowitz 1987). These advantages include a rapid generation time (6–8 wk), a small genome size (Leutwiler *et al.* 1986; Pruitt and Meyerowitz 1986), established methods for transformation by using *Agrobacterium tumefaciens* (Smith and Townsend) Conn (Valvekens *et al.* 1988), correlated physical and genetic maps (Koornneef 1987; Chang *et al.* 1988; Nam *et al.* 1989; Hauge *et al.* in press), and a method for the direct cloning of DNA sequences that correspond to deletion mutations (Straus and Ausubel 1990). These characteristics allow one to easily obtain large numbers of mutants and to clone genes that have been identified only by mutation. None of the established plant systems

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used to study disease resistance have all of these advantages.

On the other hand, because *A. thaliana* has no agronomic importance, at the time we initiated our studies very little work had been published concerning pathogenic microorganisms that infect this plant. Our initial studies with cultured cells of *Arabidopsis* demonstrated that the bacterial elicitor, α-1,4-endopolygalacturonic acid lyase (PGA lyase), induced putative defense responses previously characterized in other plant systems (Davis and Ausubel 1989). These responses included the induction of mRNAs encoding phenylalanine ammonia-lyase (PAL), 4-coumarate:CoA ligase, and β-1,3-glucanase. The results of these studies demonstrated that the response of *Arabidopsis* cells to elicitor treatment was similar to that observed in other plant cell culture systems and suggests that the regulation of defense responses in *A. thaliana* are similar to those of other plants.

To extend the *A. thaliana* pathogenesis system to the whole plant level, it was necessary to identify suitable pathogens of *A. thaliana*. We have described the characterization of two *Pseudomonas syringae* strains that elicit readily distinguishable virulent and avirulent phenotypes when infiltrated into *A. thaliana* leaves (Dong *et al.* 1991). The avirulent strain *P. s.* pv. *tomato* (Okabe) Young *et al.* MM1065 failed to multiply in *A. thaliana* leaves and induced the transient accumulation of mRNA encoding PAL, whereas the virulent strain *P. s.* pv. *maculicola* ES4326 multiplied 10^4- to 10^5-fold and did not induce significant accumulation of PAL mRNA. In this paper, we describe the identification of additional *Xanthomonas* and *Pseudomonas* strains that elicit either disease or resistance symptoms or elicit no symptoms when infiltrated into *A. thaliana* leaves. We also report the cloning and initial characterization of an *A. thaliana* PAL gene and the identification of additional PAL genes in the *A. thaliana* genome.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *Pseudomonas* and *Xanthomonas* strains used in these studies are listed in Table 1. *Pseudomonas* strains were grown in King's B (KB) medium (King *et al.* 1954) and *Xanthomonas* strains were grown in NYGB and NYGA (Turner *et al.* 1984) at 28° C. When present, streptomycin and naladixic acid were at concentrations of 50 μg/ml, and rifampicin was used at a concentration of 100 μg/ml.

Carbon source utilization and interpretation of results were according to Hildebrand *et al.* (1988). Freshly grown bacteria of each strain were re-streaked onto AB minimal medium (Chilton *et al.* 1974) that contained one of the following compounds as the sole carbon source (0.2–0.4%): glycerol, (+)tartrate, (−)tartrate, erythritol, myo-inositol, anthranilate, sucrose, glucose, or pectin.

***A. thaliana* land races and growth conditions.** A collection of *A. thaliana* land races was obtained from the *Arabidopsis* information service seed bank (F. Kranz, Botanical Institute, J.W. Goethe-University, Frankfurt, Germany). Seeds were sown in flats containing artificial soil (Metro-Mix 200, W. R. Grace, Cambridge, MA) presoaked with tap H_2O, covered with a plastic dome, and placed in growth chambers operating at 18–22° C, 50–80% RH, with a day length of 10 or 12 hr being supplied by fluorescent bulbs providing an intensity of 100–200 $\mu E \cdot m^{-2} \cdot s^{-1}$. One to 2 wk after sowing, the covers were removed and the seedlings were watered as needed.

Inoculation procedures. Bacterial inocula were prepared from mid- to late-log phase cultures (A_{600nm} = 0.5–1.5). Bacteria were pelleted by centrifugation and resuspended in an equal volume of sterile 10 mM $MgCl_2$. Concentrations of bacteria were estimated by measuring the A_{600nm} of the bacterial suspensions and were adjusted by diluting with sterile 10 mM $MgCl_2$. Plants were used 4–6 wk after sowing before any signs of bolting were evident. Bacteria were introduced by infiltration with a syringe, without a needle, through the underside of fully expanded leaves. In some experiments, a small wound was made in the leaf with a razor blade to facilitate infiltration. After infiltration, the plants were misted with deionized H_2O, covered with a plastic dome, and returned to the growth chamber.

Bacterial growth measurements. Leaves were infiltrated completely with bacterial strains at a concentration of 10^5cfu/ml. At the indicated times, a 0.5-cm-diameter disk was excised from each leaf with a No. 1 cork borer and placed in a microtube containing 0.2 ml of sterile 10 mM $MgCl_2$. The disk was homogenized, and 0.1-ml samples from various dilutions of the homogenate were plated in duplicate on KB plates. The data are presented as colony-forming units per disk.

Alkalinization of *Arabidopis* cell cultures. *Arabidopsis* cell cultures (land race Fi-3) were maintained in Gamborg's B5 medium containing 1 mg/L of 2,4-dichlorophenoxyacetic acid as previously described (Davis and Ausubel 1989). Cells were used 6–7 days after transfer. Five milliliters of cell culture (approximately 0.3 g fresh weight cells) was placed in a sterile 16- × 150-mm culture tube. Bacterial inoculum was added to give a final A_{600nm} = 0.1 (approximately 10^7 cfu/ml). An equivalent volume of sterile 10 mM $MgCl_2$ was added to control cultures. Tubes were placed at an angle of approximately 75° in an environmental shaker operating at 24° C and 120 rpm. At the indicated times, the pH of the cultures was determined by inserting a combination pH electrode. Additional control experiments were done by adding bacteria to sterile 1-B5 medium.

Isolation of genomic PAL clones. A genomic library of *A. thaliana* (land race Landsberg erecta) constructed in λFIX (Voytas and Ausubel 1988) was screened with a bean PAL cDNA, pPAL5 (Edwards *et al.* 1985), which previously was shown to hybridize to an elicitor-induced *A. thaliana* mRNA of similar size to that of the PAL mRNA found in bean and parsley (Davis and Ausubel 1989). Phage clones that hybridized to pPAL5 were subjected to three or four rounds of purification and analyzed by restriction mapping and DNA blot hybridization. The appropriate restriction fragments were subcloned into pUC12 or into M13 derivatives for sequencing using standard procedures (Ausubel *et al.* 1991).

Genomic DNA isolation and characterization. Genomic DNA was isolated from leaves of 3-wk-old *A. thaliana*, land race Landsberg erecta, as described (Richards 1987)

and purified by CsCl-gradient centrifugation. Samples containing 5 μg of DNA were digested with restriction enzymes, fractionated by electrophoresis through a 1% agarose gel, and transferred to a nylon membrane (Gene Screen Plus, NEN Boston, MA) with the alkaline transfer protocol suggested by the manufacturer. Filters were prehybridized for 3–5 hr at 65° C in 1 M NaCl, 10% dextran sulfate (MW 500,000, sodium salt), 1% sodium dodecyl sulfate (SDS), and 100 μg/ml of salmon testes DNA. Heat-denatured, gel-purified restriction fragments from λPAL10 labeled by a random priming reaction (Boehringer Mannheim, Indianapolis, IN) were added to the prehybridization solution to a final concentration of 10^5 cpm/ml. Filters were hybridized for 20–24 hr at 65° C and washed at 65° C for 2 hr with two changes of 2× SSC (1× = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) containing 1% SDS. Washed filters were blotted dry, wrapped in plastic wrap, and analyzed by autoradiography. DNA blots were stripped of hybridized probes by placing filters in 100° C H_2O and incubating

Table 1. Pathogenicity of bacterial strains on *Arabidopsis thaliana* No-0 and Col-0

Species/pathovar	Strain	Symptoms[a]	Other hosts	Source/reference
Xanthomonas campestris pv. *campestris*	8004	++	*Brassica*	M. Daniels
X. c. pv. *campestris*	GAC137	++	*Brassica*	P. Williams
X. c. pv. *campestris*	G2-17	++	*Brassica*	P. Williams
X. c. pv. *armoraciae*	417	+/−	*Brassica*	P. Williams
X. c. pv. *armoraciae*	756	+/−	*Brassica*	P. Williams
Pseudomonas cichorii[b]	83-1	HR	*Brassica*	Williams and Keen 1966
P. cichorii	645C	HR	calathium	R. Wick
P. cichorii	71-21	HR	celery	R. Stall
P. cichorii	71-22	HR	celery	R. Stall
P. cichorii	070-1294	HR	celery	R. Stall
P. cichorii	071-2595	HR	celery	R. Stall
P. cichorii	149	−/HR	celery	R. Stall
P. cichorii[c]	B57	−	tomato	Denny 1988a
P. s. pv. *syringae*	B76	−	?	Denny 1988b
P. s. pv. *syringae*	B 15	−	?	T. Kosuge
P. s. pv. *tomato*	PST6	++	tomato	Denny 1988a
P. s. pv. *tomato*	PT16	++	tomato	Denny 1988b
P. s. pv. *tomato*	PST17	++	tomato	Denny 1988b
P. s. pv. *tomato*	PDDCC2844	++	tomato	Denny 1988a
P. s. pv. *tomato*[b]	PDDCC5034	++	cauliflower	B. Staskawicz
P. s. pv. *tomato*	DC3000	++	tomato	B. Staskawicz
P. s. pv. *tomato*	T1	+	tomato	Denny 1988a
P. s. pv. *tomato*	JL1031	+	tomato	Denny 1988b
P. s. pv. *tomato*	PT2	+	tomato	Denny 1988b
P. s. pv. *tomato*	PT6	+	tomato	Denny 1988b
P. s. pv. *tomato*	PT18	+	tomato	Denny 1988b
P. s. pv. *tomato*	PT21	+	tomato	Denny 1988b
P. s. pv. *tomato*	B191	+	tomato	Denny 1988b
P. s. pv. *tomato*	B181	−	tomato	Denny 1988b
P. s. pv. *tomato*	ATCC10862	−	tomato	Denny 1988b
P. s. pv. *tomato*	NCPPB880	−	tomato	Denny 1988a
P. s. pv. *tomato*	JL1065	−/HR	tomato	B. Staskawicz
P. s. pv. *maculicola*	#1	++	*Brassica*	Denny 1988a
P. s. pv. *maculicola*	#5	++	*Brassica*	Denny 1988a
P. s. pv. *maculicola*	#10	++	*Brassica*	Denny 1988a
P. s. pv. *maculicola*	4326	++	radish	B. Staskawicz
P. s. pv. *maculicola*	795	++	cauliflower	B. Staskawicz
P. s. pv. *maculicola*[c]	921	−	cauliflower	B. Staskawicz
P. s. pv. *pisi*	LH150	+	pea	Denny 1988a
P. s. pv. *pisi*	LH151	+	pea	Denny 1988a
P. s. pv. *pisi*	LH152	+	pea	Denny 1988a
P. s. pv. *pisi*	NK372	−	pea	Denny 1988a
P. s. pv. *pisi*	BK373	−	pea	Denny 1988a
P. s. pv. *savastanoi*	213-3 (IAA^-)	−	oleander	T. Kosuge
P. s. pv. *delphinii*	PDDCC529	−	delphinium	Denny 1988a
P. s. pv. *delphinii*	ATCC8719	−	delphinium	Denny 1988a
P. s. pv. *morsprunorum*	B60-1	−	prunus	Denny 1988a
P. s. pv. *atrofaciens*	B143	−	oats, wheat	Denny 1988a
P. s. pv. *coronafaciens*	B142	−	rye	Denny 1988a
P. aeruginosa	PAO4141	−	?	T. Kosuge
P. aureofaciens[b]	923	−	cauliflower?	B. Staskawicz

[a] Symptoms described in detail in results. ++, strongly pathogenic; +, weakly pathogenic; −, no response; HR, hypersensitive response.
[b] Species or pathovar designation listed represents a change from the original identification based on our metabolic and/or lipid analyses.
[c] Species or pathovar designation listed is believed to be incorrect based on metabolic and/or lipid analysis.

with agitation at 65° C for 3 hr.

RNA isolation and blot analysis. Two or three leaves were harvested from each of three or four different plants receiving the same treatment (approximately 0.2 g total fresh weight) and frozen with liquid nitrogen. Total RNA was isolated using a modification of published procedures (Davis and Ausubel 1989). Frozen leaves were ground to a fine powder with a mortar and pestle precooled with liquid nitrogen. The powder was transferred to a 1.5-ml microtube containing 0.55 ml of extraction buffer (0.2 M Tris-HCl, pH 9, 0.4 M LiCl, 25 mM EDTA, and 1% SDS) and 0.55 ml of phenol saturated with H_2O. Samples were vortexed vigorously for 10 s and placed on ice. Samples were vortexed periodically until all the samples had been prepared. Samples were centrifuged at high speed for 2 min in a microcentrifuge; the supernatant was recovered and extracted twice with an equal volume of phenol followed by extraction with an equal volume of chloroform. RNA was precipitated by adding one-third vol of diethyl pyrocarbonate (DEPC)-treated 8 M LiCl (usually about 0.25 ml) and leaving on ice for 3 hr. Precipitated RNA was pelleted by centrifugation, dissolved in 0.3 ml of DEPC-treated H_2O, and ethanol-precipitated in the presence of 0.3 M sodium acetate, pH 5.2. RNA was collected by centrifugation, dried under vacuum, dissolved in 50 ml of DEPC-treated H_2O, and quantitated spectrophotometrically by measuring the A_{260nm}.

RNA blots were prepared as described by Davis and Ausubel (1989) or as described by Seed and Arulfo (1987). The latter method was found to be preferable because it provided superior visualization of the ethidium bromide-stained rRNA bands before and after transfer to nylon membranes. Hybridizations, autoradiography, and the determination of relative mRNA levels by scanning densitometry were conducted as previously described (Davis and Ausubel 1989). RNA samples from each experiment were analyzed in at least two independent blots and each experiment was repeated at least twice.

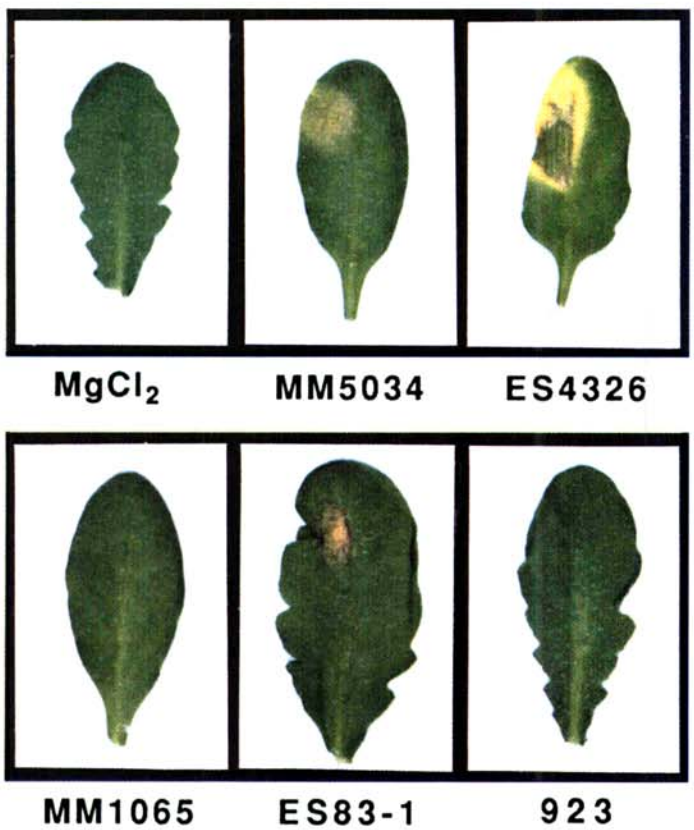

Fig. 1. Phenotypes of *Arabidopsis thaliana* leaves (land race Col-0) infiltrated with phytopathogenic pseudomonads. Leaves were infiltrated with either 10 mM $MgCl_2$ or 10^6 cfu/ml of bacteria and photographed 2 days after infiltration. MM5034 = *Pseudomonas syringae* pv. *tomato* MM5034; ES4326 = *P. s.* pv. *maculicola* ES4326; MM1065 = *P. s.* pv. *tomato* MM1065; ES83-1 = *P. cichorii* ES83-1; and 923 = *P. aureofaciens* 923.

RESULTS

Taxonomy of *Pseudomonas* strains. During our initial experiments, we observed that some of the bacterial strains provided to us as isolates of *P. s.* pv. *maculicola* differed greatly in colony morphology and growth characteristics. Because one of our goals was to attempt to identify closely related strains that differed in their virulence on *A. thaliana*, we reexamined the classification of most of the *Pseudomonas* strains listed in Table 1, including all of the strains that exhibited anomalous colony morphology or failed to elicit a hypersensitive response on tobacco. Several strains were found to have metabolic characteristics inconsistent with their original pathovar designations. *P. s.* pv. *maculicola* and pv. *tomato* strains utilize (−)tartrate but not (+)tartrate as the sole carbon source. Strains 83-1 and 921 utilized (+)tartrate but not (−)tartrate, and strain 923 did not utilize either isomer of tartrate. Fatty acid analysis of strain 83-1 indicated it is *P. cichorii*, and metabolic characteristics and fatty acid analysis of strain 923 indicated it is *P. aureofaciens* (C. Hodge and R. Stall, personal communication). Strain 921 has not been further characterized. In addition, strain B57 has been determined not to be *P. cichorii* based on its ability to grow on (−)tartrate but not (+)tartrate. Other characteristics of strain B57 are consistent with it being *P. s.* pv. *aptata*. The results of carbon source utilization studies, host range determination, and fatty acid analysis led to the reclassification of several strains as indicated in Table 1.

Characterization of the virulence of phytopathogenic bacteria in *A. thaliana*. A variety of *Xanthomonas* and *Pseudomonas* strains that were known to infect plants in the *Cruciferae* family were screened for virulence on *A. thaliana*, land races Columbia (Col-0) and Nossen (No-0; Table 1). All of the strains listed in Table 1 were initially screened by infiltrating *A. thaliana* leaves with approximately 10 μl of a bacterial suspension at two different titers (10^6 and 10^7 cfu/ml). Each strain was tested in three independent experiments in the *A. thaliana* land race No-0 and at least once in land race Col-0. In all cases, the Col-0 and No-0 land races responded similarly. The strains were divided into four different groups on the basis of the responses that they elicited (Table 1). A strain was classified as highly virulent if it elicited a spreading water-soaked lesion, usually with chlorotic margins, approximately 48 hr after infiltration at a dose of 10^6 cfu/ml. A strain was classified as mildly virulent if it elicited a

water-soaked lesion at a dose of 10^7 cfu/ml, but either failed to elicit a disease response or intermittently elicited a disease response at a dose of 10^6 cfu/ml. A strain was classified as avirulent if it elicited a dry necrotic lesion within 12–24 hr (hypersensitive response) at either 10^6 or 10^7 cfu/ml doses or if it elicited many small isolated dry lesions (pitting) accompanied by mild chlorosis within 48 hr at a dose of 10^7 cfu/ml. A strain was classified as eliciting a null response if no symptoms were visible 48 hr after infiltration at a dose of 10^7 cfu/ml.

As indicated in Table 1, three *X. campestris* pv. *campestris* strains, five *P. s.* pv. *maculicola* strains, and six *P. s.* pv. *tomato* strains were highly virulent on *A. thaliana* (land races No-0 and Col-0). Two *X. c.* pv. *armoraciae* strains, seven *P. s.* pv. *tomato* strains, and three *P. s.* pv. *pisi* strains were mildly virulent. One *P. s.* pv. *tomato* strain and seven *P. cichorii* strains were avirulent. Fifteen *P. syringae* strains representing pathovars *atrofaciens*, *coronafaciens*, *delphinii*, *morsprunorum*, *pisi*, *savastanoi*, *syringae*, and *tomato*, one strain of *P. aureofaciens*, and two unclassified *Pseudomonas* strains failed to elicit any response (null phenotype).

Initial studies with the three *X. c.* pv. *campestris* strains showed that they were all equally virulent and that identical symptoms were observed when these three strains were infiltrated at a titer of 10^6 cfu/ml into leaves of 15 different *A. thaliana* land races (land races Aa-0, Ag0, Ba-1, Bor-0, Co-4, Col-0, Cvi-0, Fi-3, Kas-1, L.e., Ll,-0, Mh-0, Ms-0, Mv-0, and No-0). Each of these strains multiplied 10^2- to 10^3-fold over 4 days in *A. thaliana* (land race Col-0) leaves when infiltrated at 10^6 cfu/ml (data not shown).

We decided to limit further studies to *Pseudomonas* strains, and based on the data in Table 1, chose several strains representing each of the four infection phenotypes for further analysis. These included four highly virulent strains, *P. s.* pv. *maculicola* 4326 and 795 and *P. s.* pv. *tomato* DC3000 and 5034; two avirulent strains, *P. cichorii* 83-1, which elicited a strong hypersensitive response, and *P. s.* pv. *tomato* JL1065, which elicited chlorotic pitting, and one null strain, *P. aureofaciens* 923. We isolated spontaneous streptomycin-resistant derivatives of *P. s.* pv. *tomato* 5034, *P. s.* pv. *maculicola* 4326, and *P. cichorii* 83-1 (MM5034, ES4326, and ES83-1, respectively) and a spontaneous nalidixic acid-resistant derivative of *P. s.* pv. *tomato* JL1065 (MM1065) to facilitate the monitoring of bacterial growth in *A. thaliana* leaves. The parental and drug resistant derivatives elicited identical pathogenicity symptoms. Strain *P. s.* pv. *tomato* DC3000 is rifampicin-resistant. Detailed studies of two of the strains, *P. s.* pv. *maculicola* ES4326 and *P. s.* pv. *tomato* MM1065, have been published (Dong *et al.* 1991). The data in this paper concerning *P. s.* pv. *maculicola* ES4326 and *P. s.* pv. *tomato* MM1065 were obtained in independent experiments and are reported here for purposes of comparison.

Photographs of the symptoms elicited by the virulent strains *P. s.* pv. *maculicola* ES4326 and *P. s.* pv. *tomato* DC3000 and the avirulent strain *P. s.* pv. *tomato* MM1065 have been published previously (Dong *et al.* 1991). Figure 1 shows the symptoms elicited at 48 hr by the virulent *P. s.* pv. *maculicola* strains MM5034 and ES4326; the avirulent strains *P. s.* pv. *tomato* MM1065 and *P. cichorii* ES83-1; and the null strain *P. aureofaciens* 923 after infiltration of *A. thaliana* (land race Col-0) leaves at a titer of 10^6 cfu/ml (approximately 10^4 cells per leaf). Strains *P. s.* pv. *maculicola* ES4326 and *P. s.* pv. *tomato* MM5034 both elicited water-soaked lesions, although the symptoms caused by *P. s.* pv. *tomato* MM5034 were usually less severe. Strain *P. s.* pv. *maculicola* 795 elicited a water-soaked lesion similar to the ones elicited by *P. s.* pv. *maculicola* ES4326 and *P. s.* pv. *tomato* DC3000 (data not shown). All of these strains also elicited water-soaked lesions when infiltrated at a titer of 10^5 cfu/ml, although symptoms took 12–24 hr longer to appear. Essentially the same phenotypes were observed when these strains were infiltrated into *A. thaliana* land races No-0 and Landsberg erecta.

In contrast to the virulent strains, infiltration with the avirulent strain *P. cichorii* ES83-1 at a titer of 10^6 cfu/

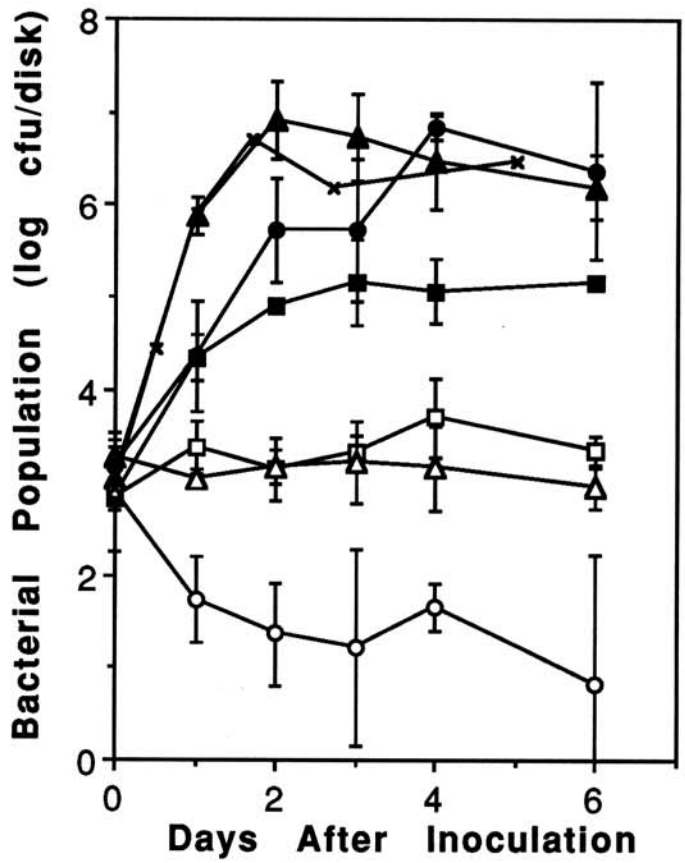

Fig. 2. Growth of *Pseudomonas* strains infiltrated into leaves of *Arabidopsis thaliana* (land race Col-0). Bacteria were infiltrated with a syringe into leaves at a concentration of 10^5 cfu/ml. At the indicated times, leaf disks were isolated from infiltrated areas, homogenized in 10 mM $MgCl_2$ and serial dilutions of the homogenate plated on King's B medium (or KB) containing the appropriate antibiotic. The data presented for all the strains except *Pseudomonas syringae* pv. *maculicola* 795 represent the average ± SD of three replicate disks obtained in a representative experiment. Similar results were obtained in two other identical experiments. The data shown for *P. s.* pv. *maculicola* 795 are the averages of duplicate disks obtained in a single experiment. *P. s.* pv. *maculicola* 795 (X); *P. s.* pv. *maculicola* ES4326 (▲); *P. s.* pv. *tomato* MM5034 (■); *P. s.* pv. *tomato* DC3000 (●); *P. s.* pv. *tomato* MM1065 (○); *P. cichorii* ES83-1 (□); *P. aureofaciens* 923 (△).

ml, caused rapid tissue collapse (12–24 hr) and the appearance of a brown dry lesion typical of a hypersensitive response (Fig. 1). These lesions were always limited to the infiltrated area and were not associated with significant amounts of chlorosis. No symptoms were elicited when the titer was decreased to 10^6 cfu/ml. Interestingly, unlike *P. cichorii* ES83-1, the other avirulent strain, *P. s.* pv. *tomato* MM1065, elicited no symptoms when infiltrated at a titer of 10^6 cfu/ml; chlorosis with pitting only occurred at a titer of 10^7 cfu/ml, and these symptoms did not appear until 48–72 hr after infiltration (Dong *et al.* 1991). Strain *P. aureofaciens* 923, like *P. s.* pv. *tomato* MM1065, did not elicit symptoms when infiltrated at 10^6 cfu/ml (Fig. 1); however, unlike *P. s.* pv. *tomato* MM1065, no symptoms were obtained with *P. aureofaciens* 923, even when the titer was increased to 5×10^7 cfu/ml.

Figure 2 shows that the virulent strains *P. s.* pv. *tomato* DC3000, *P. s.* pv. *maculicola* 795, *P. s.* pv. *maculicola* 4326, and *P. s.* pv. *tomato* MM5034 multiplied 10^3- to 10^4-fold in *A. thaliana* (land race Col-0) leaves when approximately 10 μl was infiltrated at a titer of 10^5 cfu/ml (approximately 10^3 cells per leaf). Similar results were obtained previously for *P. s.* pv. *maculicola* ES4326 (Dong *et al.* 1991) and *P. s.* pv. *tomato* DC3000 (Whalen *et al.* 1991). In contrast, titers of the avirulent strains *P. s.* pv. *tomato* MM1065 and *P. cichorii* ES83-1 and the null strain *P. aureofaciens* 923 did not increase significantly. The titer of *P. s.* pv. *tomato* strain MM1065 actually declined during the 6-day experimental period (Fig. 2).

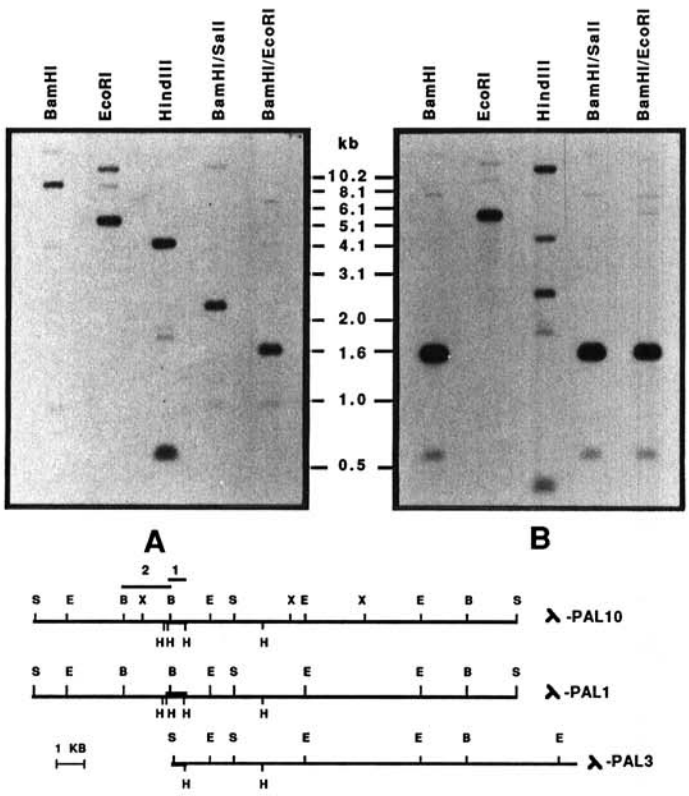

Fig. 3. Restriction maps of three independently isolated genomic clones of *Arabidopsis thaliana* PAL genes and DNA blot analysis of *A. thaliana* genomic DNA. The shaded regions represent the smallest restriction fragment that hybridizes to pPAL5. The restriction fragments designated as 1 and 2 were used as probes for genomic DNA blot analysis (**A** and **B**, respectively). Genomic DNA (5 μg) from *A. thaliana* (land race Landsberg erecta) was digested with restriction enzymes (S, *Sal*I; E, *Eco*RI; X, *Xba*I; B, *Bam*HI), fractionated by agarose gel electrophoresis, and blotted onto a nylon filter (Gene Screen Plus, NEN). Probes were labeled by using a random primer reaction. The filter was washed twice for 60 min with $2 \times$ SCC ($1 \times$ SSC = 0.15 M sodium chloride, 0.015 M sodium citrate) containing 1% sodium dodecyl sulfate (SDS) at 65° C. The data shown were obtained by using the same filter that had been stripped with boiling H_2O before being reprobed. *Xba*I sites were not determined for clones λPAL1 and λPAL3.

Isolation of an *A. thaliana* PAL gene. Based on previous studies demonstrating that the induction of PAL gene expression was generally associated with disease resistance (reviewed by Dixon 1986; Hahlbrock and Scheel 1989) and our previous work demonstrating that PAL enzyme activity and mRNA levels were induced by an elicitor in cultured *Arabidopsis* cells (Davis and Ausubel 1988), we predicted that it should be possible to distinguish the response of *A. thaliana* at the molecular level to infection with virulent or avirulent bacterial strains by monitoring the induction of PAL mRNA during infection. We sought, therefore, to clone an *A. thaliana* PAL gene that could be used as a probe for RNA blot analyses. Approximately 40,000 plaques of an *A. thaliana* (land race Landsberg erecta) genomic library (Voytas and Ausubel 1989) were screened with a bean PAL cDNA (pPAL5). Ten putative PAL clones were identified and three randomly chosen clones were purified and analyzed by restriction site mapping and DNA blot analysis. Figure 3 shows that the three λFIX clones that cross-hybridized to the bean PAL cDNA contain sequences from the same region of the *A. thaliana* genome, and therefore appeared to contain the same PAL gene. Preliminary sequence analysis of the ends of several subclones isolated from λPAL10 indicated that this clone contains open reading frames that have sequences that are approximately 85% similar to the protein-coding sequences reported for one of the bean PAL genes (gPAL2; Cramer *et al.* 1989) and that this clone includes the entire coding sequence for the *A. thaliana* PAL gene (data not shown). A detailed analysis of the DNA sequence of this PAL gene will be published elsewhere.

Genomic DNA blot analysis (Fig. 3) using either the 0.52-kb *Hin*dIII (probe 1) or the 1.5-kb *Bam*HI (probe 2) fragments from λPAL10 as probes demonstrated that there may be more than one PAL gene present in *A. thaliana*. When the blots were washed by using conditions that allowed approximately 16% mismatch, fragments in addition to the expected fragments hybridized to the probe, suggesting that there may be a total of three or four PAL genes in the *A. thaliana* genome. When the blots were washed at a higher stringency that allowed only 8% mismatch, only the expected fragments hybridized to the probes.

Induction of PAL mRNA in leaves infiltrated with *Pseudomonas* strains. We previously showed that the avirulent strain, *P. s.* pv. *tomato* MM1065, increased the level of mRNA in *A. thaliana* leaves, whereas the virulent strain *P. s.* pv. *maculicola* ES4326 failed to induce significant amounts of PAL mRNA (Dong *et al.* 1991). To determine if the induction of PAL mRNA was generally associated with a resistance response in *A. thaliana*, RNA blots of total RNA isolated from leaves infiltrated with virulent

and avirulent *Pseudomonas* strains were prepared and probed with the 0.52-kb *Hin*dIII subclone of the *A. thaliana* PAL gene in λPAL10 (probe 1). A representative blot is shown in Figure 4. In uninoculated leaves, a low level of PAL mRNA was evident (data not shown). Infiltration with 10 mM $MgCl_2$ (mock inoculation control) caused approximately a twofold induction of PAL mRNA. Infiltration with the virulent strains *P. s.* pv. *maculicola* 4326 and *P. s.* pv. *maculicola* 795 (data not shown) increased PAL mRNA levels three- to fivefold 6 hr after infiltration, and the virulent strain *P. s.* pv. *tomato* DC3000 induced PAL mRNA five- to 10-fold 6 hr after infiltration. The avirulent strains *P. cichorii* 83-1 and *P. s.* pv. *tomato* 1065 caused a 15- to 30-fold increase in PAL mRNA levels at 6 hr post-infiltration. The null strain, *P. aureofaciens* 923, caused approximately a five- to sixfold increase in PAL mRNA levels. The levels of PAL mRNA increased by these avirulent bacterial strains decreased significantly by 12 hr post-infiltration. The same results were observed in three other independent experiments.

A detailed time course for the induction of PAL mRNA in infiltrated leaves was determined by using the strains

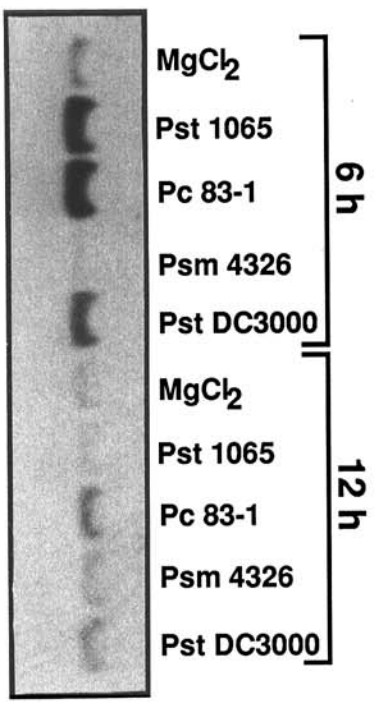

Fig. 4. Induction of PAL mRNA in *Arabidopsis thaliana* leaves infiltrated with *Pseudomonas* strains. Leaves of 4- to 5-wk-old *A. thaliana* plants (land race Col-0) were infiltrated with virulent (*Pseudomonas syringae* pv. *tomato* DC3000 and *P. s.* pv. *maculicola* 4326) or avirulent (*P. cichorii* 83-1 and *P. s.* pv. *tomato* 1065) strains at a dose of 10^7 cfu/ml. Leaves were harvested at 6 or 12 hr after infiltration. Total RNA was isolated, and 10-μg samples were analyzed by standard RNA blot hybridization methods using the 0.52-kb *Hin*dIII fragment (probe 1, Fig. 3) radiolabeled by a random primer reaction as probe. $MgCl_2$ represents RNA from leaves infiltrated with 10 mM $MgCl_2$, which is the solution used to suspend the bacteria before infiltration, Pst = *P. s.* pv. *tomato*, Pc = *P. cichorii*, and Psm = *P. s.* pv. *maculicola*. The size of the hybridizing mRNA is approximately 2.8 kb, the expected size for a PAL mRNA. Staining of the rRNA bands with ethidium bromide indicated that equal amounts of RNA were present in each lane (data not shown). Similar results were obtained in two additional experiments.

P. cichorii 83-1, *P. s.* pv. *maculicola* 4326, and *P. aureofaciens* 923. In the representative experiment shown in Figure 5, PAL mRNA was rapidly induced by the avirulent strain *P. cichorii* 83-1 with a maximum induction of approximately 25-fold at 6 hr after infiltration. PAL mRNA levels declined thereafter, reaching control levels within 12 hr after infiltration. In contrast, the pathogenic strain *P. s.* pv. *maculicola* 4326 induced PAL mRNA only twofold, with the induction kinetics being somewhat delayed when compared to that observed with *P. cichorii* 83-1. Similar results were previously reported for *P. s.* pv. *maculicola* ES4326 (Dong *et al.* 1991). *P. aureofaciens* 923, induced a sixfold induction of PAL mRNA, with induction kinetics similar to that observed with strain *P. cichorii* 83-1.

Alkalinization of *Arabidopsis* cell cultures. Previous studies have demonstrated that *Pseudomonas* strains that induce a hypersensitive response in intact plant tissue also cause the activation of K+/H+ exchange across the plasma membrane of cultured plant cells, which results in an increase of the pH of the culture medium (Atkinson *et al.* 1985; Baker *et al.* 1987). To identify a second biochemical characteristic that would distinguish virulent from avirulent *Pseudomonas* interactions with *A. thaliana*, we examined the alkalinization of cultured *Arabidopsis* cells

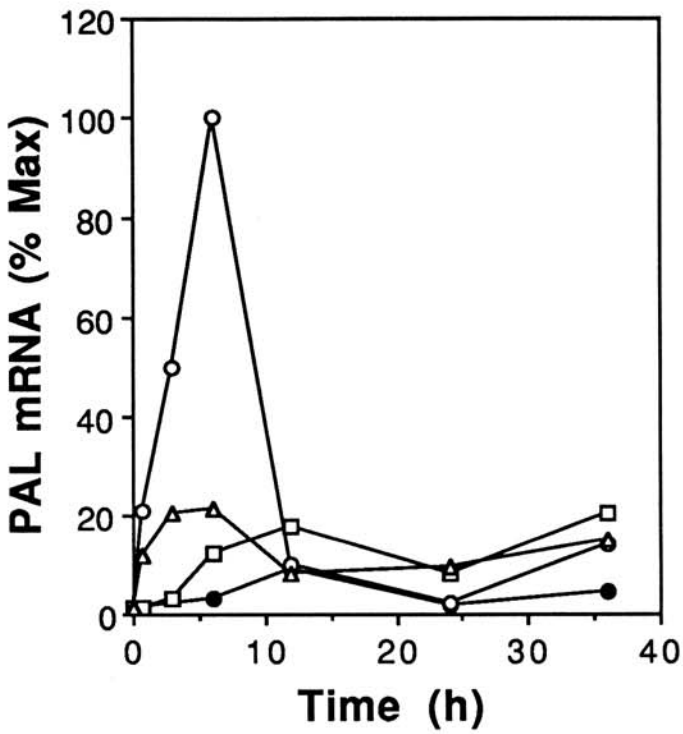

Fig. 5. Induction kinetics of PAL mRNA in leaves of *Arabidopsis thaliana* (land race Col-0) infiltrated with virulent and avirulent *Pseudomonas*. Leaves were infiltrated with either the virulent strain *P. s.* pv. *maculicola* 4326 (■) or avirulent strains *P. cichorii* 83-1 (○) and *P. aureofaciens* 923 (△) at a dose of 10^7 cfu/ml. Control leaves were infiltrated with 10 mM $MgCl_2$ (●). Leaves were harvested at indicated times after infiltration and total RNA was isolated. Ten micrograms of RNA from each sample was analyzed by RNA blot hybridization as described in Figure 4. Relative RNA levels were quantitated by scanning densitometry of films obtained from several different exposures of the blot. The data are presented as the percentage of the maximum observed in the experiment. Staining of the rRNA bands with ethidium bromide indicated that equal amounts of RNA were present in each lane (data not shown).

treated with selected *Pseudomonas* strains (Fig. 6A). The avirulent strain *P. cichorii* 83-1, which caused the strongest phenotypic hypersensitive response of the strains tested, induced an increase of approximately 1–1.5 pH units in 5 hr. The avirulent strain *P. s.* pv. *tomato* 1065, which caused a weak hypersensitive response, induced an increase of approximately 0.3–0.6 pH units in 5 hr. The virulent strains *P. s.* pv. *tomato* DC3000, *P. s.* pv. *maculicola* 4326, and *P. s.* pv. *tomato* 5034, or mock inoculation with 10 mM $MgCl_2$, did not have significant effect on the pH of the culture medium. The null strain, *P. aureofaciens* 923, caused an acidification of approximately 0.75 pH units in 5 hr; however, this acidification was most likely not dependent on the presence of plant cells. Control experiments in which bacteria were inoculated into culture medium without plant cells demonstrated that all the bacterial strains tested caused a similar acidification of the medium except for *P. aureofaciens*, which caused a larger and more rapid acidification of the medium (Fig. 6B).

DISCUSSION

The results presented in this paper further the development of a model pathogenesis system that utilizes *A. thaliana* as the host for infection by several *Pseudomonas* species. The initial step in establishing this system was to identify specific bacterial strains that reproducibly elicited disease or resistance phenotypes when infiltrated into leaves of *A. thaliana*. We found that many *Pseudomonas* strains that had previously been shown to be crucifer pathogens proved to be pathogenic or to elicit a hypersensitive response or other resistance symptoms when infiltrated into *A. thaliana* leaves. The *Pseudomonas* strains were categorized on the basis of symptom expression, multiplication in *A. thaliana* leaves, induction of PAL mRNA, and the ability to cause an alkalinization of the medium in cultured *Arabidopsis* cells (Table 2). Four virulent strains studied caused spreading chlorotic lesions, multiplied 10^3- to 10^4-fold within 48 hr after infiltration, induced PAL mRNA two- to 10-fold and did not cause an alkalinization of the medium in cultured *Arabidopsis* cell cultures. In contrast, two avirulent strains did not multiply significantly in leaves, caused a 15- to 30-fold induction of PAL mRNA, and caused a significant alkalinization of the medium in *Arabidopsis* cell cultures. In addition, the avirulent strain *P. cichorii* 83-1 was found to rapidly induce a localized necrotic lesion typical of a hypersensitive response (Klement 1982).

One null strain studied in detail was classified as *P. aureofaciens* 923, a plant-associated saprophyte. It was not

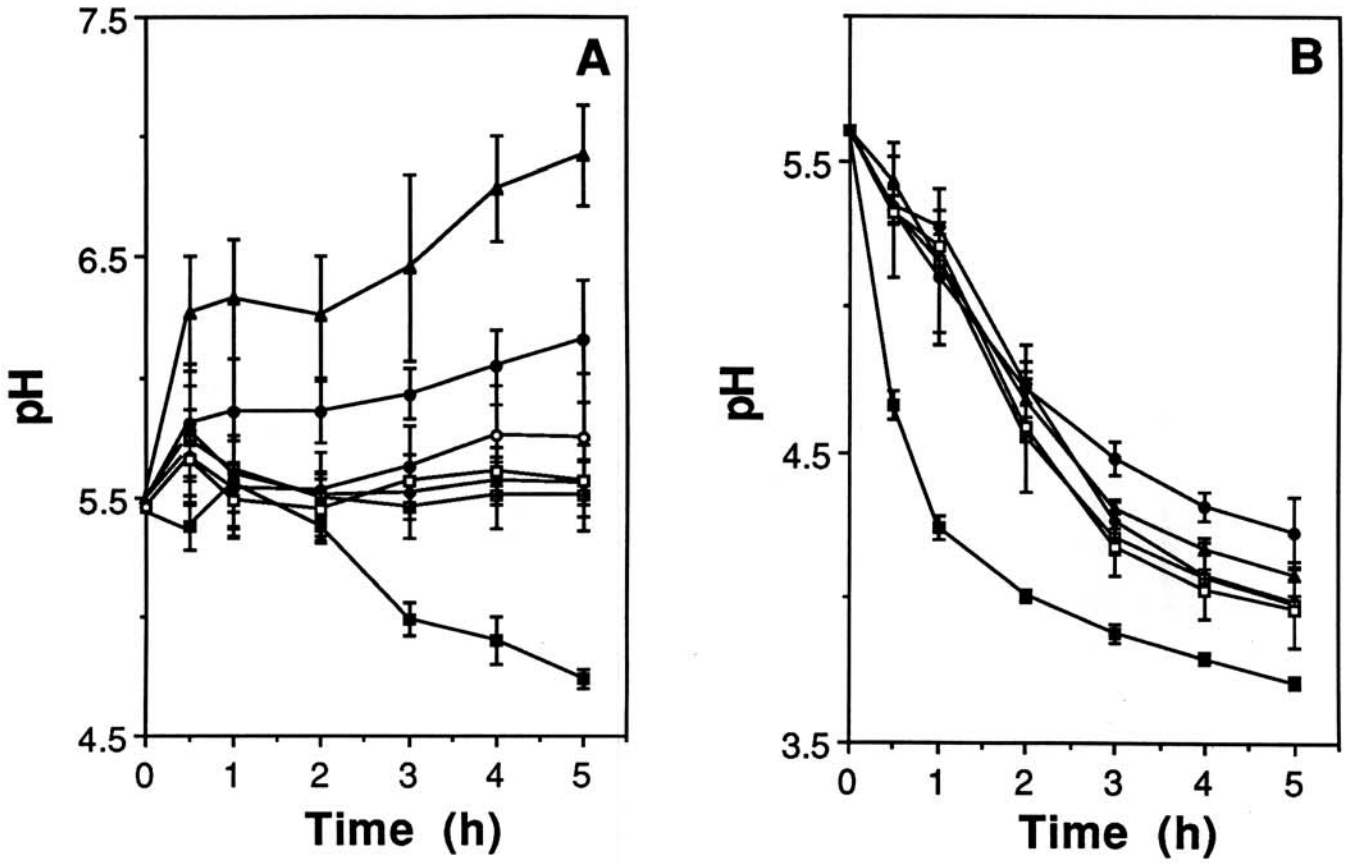

Fig. 6. Effect of inoculation of *Pseudomonas* on the pH of the culture medium in *Arabidopsis* cell cultures. Bacteria were added to *Arabidopsis* cell cultures (land race Fi-3) to a final concentration of 10^7 cfu/ml, and at the indicated times, the pH was measured using a combination pH electrode. Results are the average ± SD of three to five separate experiments. **A,** pH of cultures inoculated with 10 mM $MgCl_2$ (○); the virulent strains *P. s.* pv. *maculicola* 4326 (◇), *P. s.* pv. *tomato* DC3000 (⊞), *P. s.* pv. *tomato* 5034 (□); the avirulent strains *P. s.* pv. *tomato* 1065 (●) and *P. cichorii* 83-1 (▲); and the null strain *P. aureofaciens* 923 (■). **B,** pH of control experiments in which bacteria were inoculated into sterile 1-B5 medium; symbols are the same as in A.

clear whether the failure of this null strain and the other null strains that we identified (Table 1) to elicit symptoms was due to the fact that they induced a particularly strong defense response or simply lacked important virulence factors. Strain *P. aureofaciens* 923 failed to elicit any symptoms even when infiltrated at the relatively high titer of 5×10^7 cfu/ml, but induced the activation of PAL mRNA with the same kinetics as the avirulent strains *P. s.* pv. *tomato* 1065 and *P. cichorii* 83-1. However, the level of the PAL mRNA increase was no more than that induced by the virulent strain *P. s.* pv. *tomato* DC3000. Strain *P. aureofaciens* 923 also failed to cause the alkalinization of *Arabidopsis* tissue culture medium, but this may have been due to the fact that this strain caused a significant acidification of the culture medium in the absence of plant cells.

We previously reported that a *P. s.* pv. *tomato* strain MM1065 elicited a resistance response when infiltrated into *A. thaliana* leaves, failed to multiply *in planta*, and induced PAL mRNA, whereas a *P. s.* pv. *maculicola* strain, ES4326, elicited disease symptoms, multiplied at least 10^4-fold *in planta*, and did not induce PAL mRNA significantly (Dong *et al.* 1991). The significance of the results reported in this paper is that these initial results appear to apply generally to six different virulent and avirulent *Pseudomonas* strains. That is, in this current series of experiments, two *Pseudomonas* strains that elicited a visible defense response failed to multiply in leaves and induced PAL mRNA 15- to 30-fold. Four *Pseudomonas* strains that elicited a water-soaked lesion, on the other hand, multiplied 10^3- to 10^4-fold in leaves and induced PAL mRNA three- to 10-fold. The two highly virulent strains that induced relatively little PAL mRNA, 4326 and 795, are both *P. s.* pv. *maculicola* isolates, whereas the virulent strain DC3000 that induces PAL mRNA as much as 10-fold is *P. s.* pv. *tomato*. Preliminary data suggest that *P. s.* pv. *tomato* strains in general may induce higher levels of PAL mRNA compared to the *P. s.* pv. *maculicola* strains (K. R. Davis, unpublished).

The strong chlorosis caused by several of the virulent strains, particularly *P. s.* pv. *tomato* DC3000, may be due to the production of the phytotoxin coronatine (Mitchell 1984). Studies have shown that Tn*5*-mutants of strain DC3000 that do not produce toxin do not cause typical chlorotic lesions when inoculated on tomato (Moore *et al.* 1989). Thus, it is possible that *P. s.* pv. *tomato* DC3000 and other toxin-producing strains are pathogenic on *A. thaliana* primarily because of the production of a nonhost-specific toxin that suppresses the plants ability to mount an effective defense. Further studies are required to determine the role of toxin production in the virulence of these strains on *A. thaliana*. However, the observation that *P. s.* pv. *tomato* DC3000, containing a putative avirulence gene, is significantly less virulent on *A. thaliana* compared to the wild type strain (Whalen *et al.* 1991) indicates that *A. thaliana* can respond to DC3000 when an appropriate signal is present and that coronatine does not compromise the ability of *A. thaliana* to exhibit some resistance to this strain.

A second important result reported in this paper is that the *P. cichorii* strain studied in detail, 83-1, as well as six other *P. cichorii* strains, elicited a very rapid tissue collapse with marked browning within 12–24 hr after infiltration into *A. thaliana* leaves. This classic hypersensitive response appears to be the same as that observed on a variety of plants when infiltrated with an avirulent bacterial pathogen (Klement 1982). In contrast, the resistance response reported previously for *P. s.* pv. *tomato* MM1065 consisted of chlorosis accompanied by many small dry lesions (pitting) that appeared 48–72 hr after infiltration (Dong *et al.* 1991; Whalen *et al.* 1991). Interestingly, however, the kinetics and extent of PAL mRNA induction appear similar for both *P. cichorii* 83-1 and *P. s.* pv. *tomato* MM1065.

In conjunction with the identification of bacterial pathogens of *A. thaliana*, we cloned and partially characterized an *A. thaliana* PAL gene. A number of studies have correlated the induction of PAL expression with a resistance response (reviewed by Dixon 1986; Hahlbrock and Scheel 1989), including our previous studies demonstrating that PAL activity and steady-state mRNA levels are rapidly increased in *Arabidopis* cell cultures by elicitor treatment (Davis and Ausubel 1989). Our current and previously published results (Dong *et al.* 1991) indicate a strong correlation of rapid PAL mRNA accumulation with a resistance response and are similar to results obtained in other plant-pathogen systems. Lamb and co-workers demonstrated that PAL is induced in bean plants infected with the phytopathogenic fungus *Colletotrichum lindemuthianum* (Bell *et al.* 1986). These detailed studies of PAL

Table 2. Summary of phenotypes induced by *Pseudomonas* strains in *Arabidopsis*

Strain	Symptoms[a]	Maximum growth ($\log_{10}$)[b]	pH change in cell cultures[c]	PAL mRNA induction[d]
P. s. pv. *maculicola* ES4326	chl, ws/chl, ws, necrosis	3.88	−0.19	4
P. s. pv. *maculicola* 795	chl, ws/chl, ws, necrosis	3.00	nt	5
P. s. pv. *tomato* MM5034	chl, ws/chl, ws, necrosis	2.96	−0.18	nt
P. s. pv. *tomato* DC3000	chl, ws/chl, ws, necrosis	3.69	−0.24	10
P. s. pv. *tomato* MM1065	null/weak HR, chl	<0	+0.40	24
P. cichorii ES83-1	null/HR	0.86	+1.17	30
P. aureofaciens 923	null/null	<0	−1.01	6

[a] Symptoms 48 hr after infiltration of leaves of land race Col-0 with bacteria at 10^6/ or 10^7 cfu/ml; chl, chlorosis; ws, water-soaked lesions; null, no response; HR, hypersensitive response.
[b] Bacterial populations measured in land race Col-0 3 days post-infiltration.
[c] pH change relative to mock inoculated cell cultures 5 hr after treatment, average of three to five experiments; nt, not tested.
[d] Maximum fold-induction of PAL mRNA levels in leaves of land race Col-0 inoculated with 10^7 cfu/ml bacteria 6 hr after infiltration; nt, not tested.

expression in bean hypocotyls infected with virulent and avirulent races of *C. lindemuthianum* demonstrated that PAL mRNA was induced more rapidly during the resistance reaction compared to the susceptible response. Further studies demonstrated that the induction of PAL mRNA was due primarily to the transcriptional activation of a PAL gene (Lawton and Lamb 1987). Similarly, Habereder *et al.* (1989) have demonstrated that PAL mRNA is rapidly induced in primary roots of soybean inoculated with incompatible races of *Phytophthora megasperma* f. sp. *glycinea*. Roots inoculated with compatible races exhibited a much delayed induction of PAL mRNA. Other studies by Hahlbrock and co-workers demonstrated that PAL is activated by elicitors in cultured parsley and potato cells and during a hypersensitive response in both parsley and potato plants inoculated with avirulent phytopathogenic fungi (Kombrink and Hahlbrock 1986; Fritzemeier *et al.* 1987; Hahlbrock and Scheel 1989). Further studies of the parsley and potato systems with *in situ* hybridization methods demonstrated that PAL activation was limited to the cells surrounding the infection site, and thus was being expressed in cells that were likely to be critically involved in elaborating the defense response (Cuypers *et al.* 1988; Jahnen and Hahlbrock 1988).

Our initial sequence analysis (F. Shaheen and K. Davis, unpublished results) indicates that the PAL gene present in λPAL10 is the same as that described by Ohl *et al.* (1990). DNA blot analysis of *Arabidopsis* genomic DNA using a portion of the cloned PAL gene as a probe indicates that there are a total of three or four PAL genes in the *A. thaliana* genome. This is supported by studies in which two additional PAL genes distinct from the gene in λPAL10 were isolated (K. Davis, I. Somssich, K. Hahlbrock, unpublished results). These results are consistent with molecular analyses of PAL genes in bean and parsley that have demonstrated that in both of these plant species PAL is encoded by a small multigene family. In parsley, there are at least four PAL genes, three of which are approximately 90% similar in nucleotide sequence. These three PAL genes are activated by both elicitor and UV light in cultured parsley cells (Lois *et al.* 1989). In bean, there appears to be three divergent classes of PAL genes (Cramer *et al.* 1989). Analysis of the expression of PAL genes in bean indicated that these genes are differentially regulated during development and in response to environmental stimuli (Edwards *et al.* 1985; Liang *et al.* 1989). For example, all three PAL genes were induced by mechanical wounding; however, only PAL1 and PAL3 were induced by fungal infection (Liang *et al.* 1989). Taken together, these results suggest that the PAL gene family has diverged significantly both in structure and regulation. Studies are currently underway to determine if a similar pattern of differential regulation of PAL genes occurs in *A. thaliana*.

The results presented in this paper confirm and extend studies by other laboratories that indicate that *A. thaliana* is a good model host for studying disease resistance in plants (Tsuji and Somerville 1988; Davis *et al.* 1989; Schott *et al.* 1990; Simpson and Johnson 1990; Koch and Slusarenko 1990; Dong *et al.* 1991; Whalen *et al.* 1991). Recent work has suggested that the interaction of *A. thaliana* with bacterial pathogens may be governed by single genes present in both the host and the pathogen in a manner similar to that observed in other plant-pathogen interactions. Several putative avirulence (*avr*) genes have been isolated from avirulent *P. s.* pv. *tomato* strains, which when introduced into virulent *P. s.* pv. *maculicola* or *P. s.* pv. *tomato* strains, cause an attenuation of virulence (Dong *et al.* 1991; Whalen *et al.* 1991; M. Mindrinos and F. Ausubel, unpublished results). This attenuation of virulence by cloned *avr* genes is associated with increased levels of PAL mRNA, suggesting that the activation of phenylpropanoid biosynthesis may play a role in disease resistance in *A. thaliana* (Dong *et al.* 1991; K. Davis, A. Bent, and B. Staskawicz, unpublished results). Further studies have demonstrated that some land races of *A. thaliana* are susceptible to infection by *P. syringae* strains that are avirulent on other land races (Bent *et al.* 1991). Genetic analysis of crosses between the resistant and susceptible land races is required to determine if the resistance is due to single dominant genes. Similarly, several groups have established that many strains of *X. c.* pv. *campestris* are virulent on *A. thaliana* and cause symptoms similar to those observed on their normal hosts (Tsui and Somerville 1988; Simpson and Johnson 1990; Daniels *et al.* 1991). These researchers have also identified land races of *A. thaliana* that are differentially susceptible to specific *X. c.* pv. *campestris* strains, and Daniels *et al.* (1991) have obtained preliminary evidence that one of these *X. c.* pv. *campestris* strains contains an *avr* gene that is recognized by *A. thaliana*. These results suggest that there may be gene-for-gene interactions (Flor 1971) between *A. thaliana* and bacterial phytopathogens and that the *A. thaliana* system will be useful for studying this interaction.

ACKNOWLEDGMENTS

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