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The Cloned Avirulence Gene *avrPto* Induces Disease Resistance in Tomato Cultivars Containing the *Pto* Resistance Gene

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Resistance of tomato plants to the bacterial pathogen *Pseudomonas syringae* pv. tomato race 0 is controlled by the locus *Pto*. A bacterial avirulence gene was cloned by constructing a cosmid library from an avirulent *P. syringae* pv. tomato race, conjugating the recombinants into a strain of *P. syringae* pv. maculicola virulent on a tomato cultivar containing *Pto*, and screening for those clones that converted the normally virulent phenotype to avirulence. The cloned gene, designated *avrPto*, reduced multiplication of *P. syringae* pv. tomato transconjugants specifically on *Pto* tomato lines, as demonstrated by bacterial growth curve analyses. Analysis of F_2 populations revealed cosegregation of resistance to *P. syringae* pv. tomato transconjugants carrying *avrPto* with resistance to *P. syringae* pv. tomato race 0. Surprisingly, mutation of *avrPto* in *P. syringae* pv. tomato race 0 does not eliminate the avirulent phenotype of race 0, suggesting that additional, as yet uncharacterized, avirulence genes and/or resistance genes may contribute to specificity in the *avrPto-Pto* interaction. Genetic analysis indicates that this resistance gene(s) would be tightly linked to *Pto*. Interestingly, *P. syringae* pv. glycinea transconjugants carrying *avrPto* elicit a typical hypersensitive resistant response in the soybean cultivar Centennial, suggesting conservation of *Pto* function between two crop plants, tomato and soybean.

Genetic factors governing the interactions of pathogens with their host plants play an important role in determining disease resistance or susceptibility. Genetic analysis of resistance in hosts and avirulence in pathogens has demonstrated that resistant hosts often contain single loci that specify resistance against pathogens containing complementary avirulence genes (7). This genetic pattern is the basis of the gene-for-gene hypothesis, which states that disease resistance is expressed only when the corresponding gene pairs are present in the two interacting partners (8). Mutation or lack of either member of the gene pair usually results in a susceptible response (2, 13–15, 34, 41).

Progress toward understanding the molecular basis of bacterial disease resistance has been facilitated by the molecular cloning and characterization of several bacterial avirulence genes (9, 12, 27, 35–37, 39, 40). Nucleotide sequencing of these avirulence genes has shown that avirulence activity can be accounted for by a single open reading frame and that most avirulence genes encode putatively hydrophilic proteins (2, 32, 37). Furthermore, some avirulence gene products have sequences in common (32, 37), but for the most part avirulence genes encode dissimilar products with no sequence homology to known proteins.

Elucidation of the molecular basis of recognitional specificity and the expression of disease resistance ultimately will involve the cloning and characterization of a resistance-avirulence gene pair that genetically defines resistance. We have chosen to develop bacterial speck disease of tomato as a model system for this purpose because classical and molecular genetic techniques can be applied to both the host (*Lycopersicon esculentum*) and the pathogen (*Pseudomonas syringae* pv. tomato). Genetic analysis of disease resistance in this system has revealed that resistance to race 0 strains of *P. syringae* pv. tomato is controlled by a single resistance locus, *Pto* (29). *Pto* was derived from the sexually compatible wild species *Lycopersicon pimpinellifolium* (28) and maps to the short arm of chromosome 5 at map position 30 (30). Our initial goals were to determine whether an avirulence gene in *P. syringae* pv. tomato race 0 strains was responsible for limiting disease on *Pto*-containing tomato cultivars and whether this avirulence gene acted in the same manner as avirulence genes isolated from other pathogens. In this article, we report the cloning of such an avirulence gene, designated *avrPto*, from *P. syringae* pv. tomato race 0 and demonstrate that resistance to normally virulent strains of *P. syringae* pv. tomato containing *avrPto* segregates with the resistance locus, *Pto*. We also show that transconjugants of the soybean pathogen *P. syringae* pv. glycinea carrying *avrPto* can induce a host-specific hypersensitive response in soybean plants, suggesting functional conservation of *Pto* between soybean and tomato plants. Deletion of the *avrPto* gene from *P. syringae* pv. tomato race 0 does not eliminate the avirulent phenotype of race 0 on *Pto* tomato plants, suggesting that additional genetic factors may control resistance of tomato plants to bacterial speck disease.

MATERIALS AND METHODS

Bacterial strains, media, and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* DH5α (Bethesda Research Laboratories) was used for all cosmid maintenance and plasmid construction. *P. syringae* pv. maculicola 4326, originally isolated from a radish plant, was used as the recipient for *P. syringae* pv. tomato clones. *P. syringae* pv. tomato strain T1 was used as a representative *P. syringae* pv. tomato race 1 strain and in all conjugations and inoculations, unless otherwise specified. The *P. syringae* pv. glycinea race 0 strain Cx carries a mutated copy of *avrC* (34). All *Pseudomonas* strains were grown at 28°C on King's B (KB) medium (19). *E. coli* strains were grown at 37°C on Luria medium (26).

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TABLE 1. Bacterial strains and plasmids used in this study

Bacterium or plasmid	Strain(s) or relevant characteristic(s)	Source or reference[a]
P. solanacearum	82 R	L. Sequeira
P. fluorescens	A526	S. Lindow
P. syringae pathovars		
phaseolicola	3121	N. Panopoulos
tabaci	11528	N. Panopoulos
lachrymans	PL20	N. Keen
pisi	202-12-2	N. Keen
coronafaciens	339T (Rif^r), 2304 (Rif^r)	S. Lindow
glycinea	Race 0 Cx (*avrC* Km^r Rif^r)	34
	Races 4, 5, 6 (Rif^r)	N. Keen
maculicola	4326, 2744, 795, 4981	ICMP
tomato		
Race 0	JL1065, JL1118, JL1006	J. Lindeman
	3435, 2844	ICMP
	DC3000	D. Cuppels
Race 1	T1	G. Bonn
	Pt7, Pt8	UCD
	156, 16-1, BMG13	D. Cuppels
X. campestris pv. vesicatoria	75-3	36
E. coli DH5α	F$^-$ *recA lacZ*ΔM15	Bethesda Research Laboratories
Plasmids		
pLAFR3	Tc^r pLAFR1 containing *Hae*II fragment of pUC8	34
pWB5A	Tc^r broad-host-range cloning plasmid	36
pRK404	Tc^r broad-host-range cloning plasmid	3
pDSK519	Km^r broad-host-range cloning plasmid	18
pRK2013	Km^r Tra$^+$ Mob$^+$; ColE1	6
pHP45Ω	St^r Sp^r	31
pBluescript II KS +	Ap^r	Stratagene

[a] Source information: L. Sequeira, University of Wisconsin, Madison; S. Lindow and N. Panopoulos, University of California, Berkeley; N. Keen, University of California, Riverside; ICMP, International Collection of Microorganisms from Plants, Auckland, New Zealand; J. Lindeman, Advanced Genetic Sciences, Oakland, Calif.; D. Cuppels, Agriculture Canada Research Centre, London, Ontario, Canada; G. Bonn, Harrow Research Station, Agriculture Canada; UCD, University of California, Davis.

Bacto Agar (Difco) was added to KB and Luria media for plate cultures. The following concentrations of antibiotics (Sigma) were used for selection: tetracycline, 10 μg/ml; rifampin, 100 μg/ml; spectinomycin, 20 μg/ml; streptomycin, 20 μg/ml; kanamycin, 25 μg/ml; cycloheximide, 50 μg/ml; nalidixic acid, 50 μg/ml; ampicillin, 50 μg/ml; and chloramphenicol, 25 μg/ml.

To mobilize clones of DNA from *E. coli* DH5α into *P. syringae* pv. tomato strains, plasmid pRK2013 was employed in triparental matings (4). Plasmids from all transconjugants were isolated and transformed into *E. coli*. Plasmids were purified from *E. coli* and analyzed by restriction endonuclease digestion to verify their identities.

Standard techniques for subcloning procedures, plasmid preparations, DNA gel blots, and agarose gel electrophoresis were used (23). DNA restriction endonuclease fragments were labeled with ^{32}P by using random primers (5). Genomic libraries of *P. syringae* pv. tomato DC3000 and JL1065 were constructed as previously described (34). *E. coli* HB101 was the recipient in the construction of *P. syringae* pv. tomato DC3000 and JL1065 libraries. The average insert size of the libraries is 29 kb. Colony hybridization of the DC3000 library was performed according to the method of Maniatis et al. (23). Exonuclease III deletion and linker addition were performed as described previously (32).

The DNA constructs used in this study are shown in Fig. 1. Cosmid clone pPt10-24 (Fig. 1A), isolated from the JL1065 library, was partially digested with the enzyme *Sau*3A, treated with bacterial alkaline phosphatase, and size fractionated on an agarose gel. Fractions enriched for fragments in the 2- to 4-kb range were electroeluted from the gel and ligated into the *Bam*HI site of pRK404 (3) to create plasmid pPtE2 (Fig. 1B). The 2.2-kb *Bam*HI-*Eco*RI fragment of pPtE2 was cloned into the *Bam*HI-*Eco*RI site of pLAFR3 (35) to form pPtE21 (Fig. 1F). Plasmids pPtE6 (Fig. 1E) and pPtE8 (Fig. 1G) contain the *Hin*dIII-*Xba*I and *Hin*dIII-*Pst*I fragments, respectively, of pPtE2 cloned into vector pDSK519 (18). Cosmid clone pPtDC5 (Fig. 1H) was isolated from a DC3000 genomic library. Plasmid pPtDC38 was constructed by first ligating the 3.8-kb *Hin*dIII-*Cla*I fragment from pPtDC5 into the *Hin*dIII-*Cla*I site of pWB5A (36) and then isolating an *Eco*RI fragment containing the entire insert into the *Eco*RI site of pDSK519 (Fig. 1J). To construct the deletion subclone pΔ*avrPto*, a 12-kb *Eco*RI fragment was isolated from pPtDC5 and cloned into the *Eco*RI site of pUC18. The resulting clone was digested with *Xba*I and treated with exonuclease III (11) to create a 2.2-kb deletion extending 1.2 kb upstream and 1.0 kb downstream of the *Xba*I site. *Xba*I linkers were added, and this fragment was ligated to an *Xba*I-tailed Ω fragment (31) carrying streptomycin and spectinomycin resistance genes (Fig. 1K).

Transposon mutagenesis. Plasmid pPtE2 was mutagenized with the TN3HoHo derivative (33) pHoKmGus (2). Cosmid clone pPtDC5 was mutagenized with the Tn*phoA* system as described elsewhere (10, 24). The mutagenized clones were introduced into wild-type *P. syringae* pv. maculicola. Transconjugants were selected on KB medium containing the appropriate antibiotics and inoculated into tomato cultivar 76R, and the phenotypes were scored for virulence.

Growth of plants, plant inoculations, and bacterial growth curves. Seeds of the nearly isogenic tomato cultivars 76S (*pto*/*pto*) and 76R (*Pto*/*Pto*), representing six backcrossed generations, were obtained from Peto Seed Company (Woodland, Calif.). Centennial soybean seeds were obtained from N. Keen (University of California, Riverside). All plants were grown from seed in a greenhouse in clay pots with standard potting soil. Experimental plants were incubated in growth chambers with 16-h photoperiods at 25°C. Reactions of plants to infection by *P. syringae* pv. tomato were determined by infiltrating bacterial suspensions of approximately 5×10^5 CFU/ml into leaves as previously described (36). Host reactions were scored after 3 days as either susceptible, typified by dark specks surrounded by chlorotic halos, or resistant, typified by the absence of specks and chlorosis at the inoculation site. Reactions of plants to infection by *P. syringae* pv. maculicola were determined by infiltrating 5×10^6 CFU/ml into leaves. These reactions were scored after 2 days as either susceptible, typified by spreading confluent necrosis, or resistant, typified by the absence of lesions. Soybean plants were infiltrated with a suspension of *P. syringae* pv. glycinea (5×10^8 CFU) and scored after 3 days. Host reactions were scored as

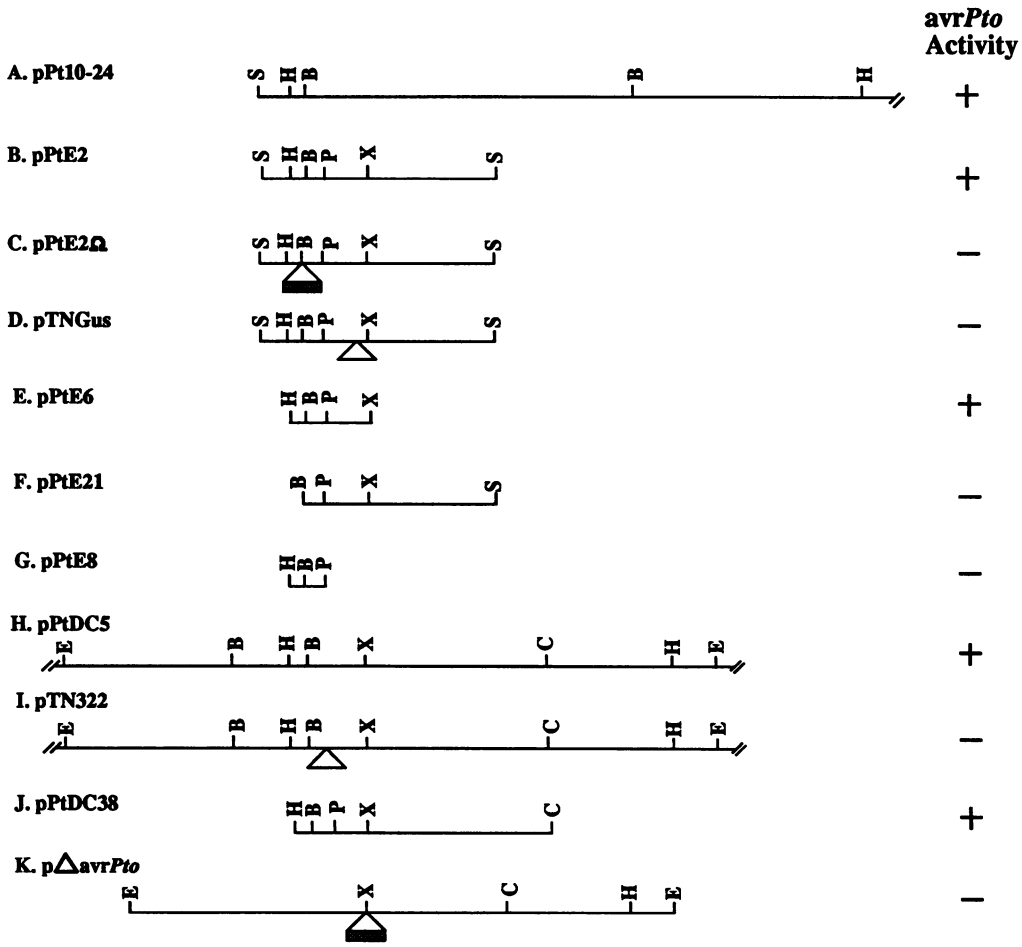

FIG. 1. Restriction map of constructs containing *avrPto* and their activities. (A) Clone pPt10-24 contains a 25-kb *Sau*3A fragment isolated from *P. syringae* pv. tomato strain JL1065 in pLAFR3. Only the region containing the 6-kb *Hin*dIII fragment is shown. (B) Clone pPtE2 contains a 2.6-kb *Sau*3A fragment from clone pPt10-24 in pRK404. (C) The shaded rectangle shows the location of the Ω fragment insertions in pPtE2Ω. (D) The open triangle shows the site of insertion TNGus in pTNGus. This construct was used to make the site-directed gene replacement strain 1065Gus (see text for details). (E) Clone pPtE6 contains the 0.75-kb *Hin*dIII-*Xba*I fragment from clone pPtE2 in pDSK519. (F) Clone pPtE21 contains the 2.2-kb *Bam*HI-*Eco*RI fragment from pPtE2 in pLAFR3. (G) Clone pPtE8 contains the *Hin*dIII-*Pst*I fragment from clone pPtE2 in pDSK519. (H) Clone pPtDC5 contains a 25-kb *Sau*3A fragment isolated from *P. syringae* pv. tomato race 0 strain DC3000. Only a partial map is shown. (I) The open triangle shows the insertion of Tn*PhoA* in plasmid pTN322. This construct was used to make the site-directed gene replacement strain DC3000TN322 (see text for details). (J) Clone pPtDC38 contains the 3.8-kb *Hin*dIII-*Cla*I fragment from pPtDC5 in pDSK519. (K) Clone pΔ*avrPto* is a pLAFR3 construct containing the *Eco*RI fragment from pDC5 with a 2.5-kb deletion at the *Xba*I site and insertion of a 2-kb Ω fragment (shaded rectangle). This construct was used to create the deletion exchange mutant DC3000Δ*avrPto* (see text for details). Avirulence activity was tested by conjugating the clones individually into *P. syringae* pv. maculicola or pv. tomato, inoculating the transconjugants into tomato cultivar 76R, and scoring the resulting reactions as described in Materials and Methods. +, avirulent; −, virulent; B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sau*3A; X, *Xba*I.

either susceptible, typified by spreading water-soaking, or hypersensitive, typified by necrosis at the inoculation site. The reactions of the *P. syringae* pv. tomato, *P. syringae* pv. maculicola, and *P. syringae* pv. glycinea strains inoculated into 76R and 76S are shown in Table 2.

Time course studies of bacterial growth were carried out with 4-week-old tomato and 2-week-old soybean cultivars. Leaves were vacuum infiltrated with bacterial suspensions of 5×10^4 to 2×10^6 CFU/ml. Infected plants were incubated as described above. Bacterial populations in leaves were sampled by taking three leaf disks with a no. 3 cork borer, macerating the disks in 10 mM $MgSO_4$, and plating appropriate dilutions on KB agar containing rifampin, cycloheximide, spectinomycin, streptomycin, and/or kanamycin. Bacterial population size was examined for 4 to 6 days after infiltration.

Construction of site-directed gene replacement strains. The deletion subclone pΔ*avrPto* and the transposon-mutagenized clones pTNGus and pTN322 were introduced into wild-type *P. syringae* pv. tomato strains by pRK2013-assisted mobilization. Transconjugants were selected on KB medium containing rifampin and tetracycline. The resolution of *cis* merodiploids was accomplished by over 100 generations of growth in KB medium containing the appropriate antibiotics. Double crossover mutants (DC3000Δ*avrPto*, 1065Gus, and DC3000TN322 [Table 2]) were identified by selection for retention of the marker carried on the mutated copy of *avrPto* and screening for loss of the plasmid. Verification of the gene replacement was obtained by DNA gel blot analysis (data not shown).

Genetic analysis to study inheritance of resistance in tomato plants. In order to examine the inheritance of resistance to *P.*

syringae pv. tomato, an F_2 population segregating for the *Pto* locus was scored for reaction to *P. syringae* pv. tomato race 1, race 1 strain T1(pPtE6), and the race 0 strains DC3000 and DC3000Δ*avrPto* (n = 742). Resistance or susceptibility was scored 4 days after inoculation. At the inoculum concentration used, the homozygous and heterozygous resistant plants (*Pto*/*Pto* and *Pto*/*pto*) both had resistant phenotypes, whereas the homozygous susceptible plants (*pto*/*pto*) showed lesions characteristic of the disease. A subset (n = 144) of this F_2 population was inoculated with both race 1 strain T1(pPtE2) and race 1 strain T1(pPtDC38) in addition to the strains listed above, in order to confirm that resistance to the JL1065-derived clone (pPtE2) cosegregated with resistance to the DC3000-derived clone (pPtDC38).

RESULTS

Identification and isolation of a race-specific avirulence gene from *P. syringae* pv. tomato. To isolate a gene from *P. syringae* pv. tomato race 0 responsible for its avirulent phenotype on *Pto* tomatoes, 383 independent pLAFR3 cosmid clones, each containing approximately 25 to 30 kb of *P. syringae* pv. tomato race 0 strain JL1065 DNA, were individually conjugated by triparental matings to *P. syringae* pv. maculicola. A virulent *P. syringae* pv. maculicola strain was used as a recipient in place of a *P. syringae* pv. tomato strain because of the higher conjugation frequencies observed for *P. syringae* pv. maculicola strains (2b). The transconjugants were inoculated on the tomato cultivars 76S (*pto*/*pto*) and 76R (*Pto*/*Pto*) (Table 2). A cosmid clone, pPt10-24, that specifically converted *P. syringae* pv. maculicola to avirulence only on cultivar 76R was identified. A restriction enzyme map of cosmid clone pPt10-24 is shown in Fig. 1A.

To further localize the avirulence activity, 80 *Sau*3A subclones of pPt10-24 in plasmid pRK404 (3) were introduced into *P. syringae* pv. maculicola, and the transconjugants were inoculated into 76R. One subclone with a 2.6-kb insert (pPtE2 [Fig. 1B]) that conferred avirulence activity to two *P. syringae* pv. maculicola and five *P. syringae* pv. tomato race 1 strains was identified (Table 2). Further subcloning localized the *avrPto* activity to a 0.75-kb *Hin*dIII-*Xba*I fragment (pPtE6 [Fig. 1E and Table 2]). The *Bam*HI-*Sau*3A (pPtE21 [Fig. 1F]) and the *Hin*dIII-*Pst*I (pPtE8 [Fig. 1G]) DNA fragments lacked *avrPto* activity. The localization of *avrPto* was confirmed by transposon mutagenesis. Transposon insertions that inactivated *avrPto*, TNGus and TN322, mapped to the *Hin*dIII-*Xba*I fragment of *avrPto* (Fig. 1D and I).

***avrPto* reduces growth of bacteria in the *Pto*-containing tomato cultivar.** To quantify the effects of *avrPto* on growth of *P. syringae* pv. tomato strains in tomato plants, we performed bacterial growth curve experiments (Fig. 2). The virulent *P. syringae* pv. tomato race 1 grew 10^5-fold in 4 days in tomatoes containing and lacking the *Pto* gene. On a multicopy plasmid, pDSK519, *avrPto* reduced overall growth of the pathogen *P. syringae* pv. tomato race 1 approximately 10^5-fold in 76R but only slightly reduced growth of race 1 in the *pto*/*pto* cultivar 76S (pPtE6 [Fig. 2]). Addition of pPtE6 to the race 0 strain DC3000 also decreased its ability to grow in 76R by 10^3-fold (data not shown). *P. syringae* pv. tomato race 1 transconjugants carrying pPtE2Ω (Fig. 1C), a mutagenized copy of *avrPto*, grew as well as wild-type *P. syringae* pv. tomato race 1 in both 76S and 76R (data not shown). These results demonstrate that a reduced ability of *P. syringae* pv. tomato to grow in 76R depends specifically on the presence of a functional copy of the *avrPto* gene and that lack of lesion development in strains containing *avrPto* is reflected in a reduction in bacterial growth.

TABLE 2. Reactions of *P. syringae* strains on tomato and soybean cultivars

Pathovar and race	Strain	Reaction[a] with indicated cultivar		
		Tomato		Soybean cultivar Centennial
		76S (*pto*/*pto*)	76R (*Pto*/*Pto*)	
P. syringae pv. tomato				
Race 0	JL1065	+	−	
	1065Gus	+	−	
	DC3000	+	−	
	DC3000TN322	+	−	
	DC3000Δ*avrPto*	+	−	
Race 1	T1	+	+	
	T1(pPtE2)	+	−	
	T1(pPtE21)	+	+	
	T1(pPtE2Ω)	+	+	
	T1(pPtE6)	+	−	
	T1(pPtE8)	+	+	
	T1(pPtDC38)	+	−	
P. syringae pv. maculicola	4981	+	−	
	4326	+	+	
	4326(pPt10-24)	+	−	
	4326(pPtE2)	+	−	
	4326(pPtE21)	+	+	
	4326(pPtE2Ω)	+	+	
	4336(pPtE6)	+	−	
	4326(pPtE8)	+	+	
	4326(pPtDC5)	+	−	
	4326(pPtDC38)	+	−	
	4326(pTN322)	+	+	
	4326(pTNGus)	+	+	
	4326(pΔ*avrPto*)	+	+	
P. syringae pv. glycinea race 0	Cx			+
	Cx(pPtE2)			HR
	Cx(pPtE2Ω)			+

[a] +, disease; −, no disease; HR, hypersensitive response.

Conservation of *avrPto* among *P. syringae* strains. To determine whether *avrPto* is conserved among *P. syringae* pv. tomato race 0 strains, we hybridized DNA from 12 geographically diverse *P. syringae* pv. tomato strains with *avrPto*. The 2.2-kb *Bam*HI-*Sau*3A fragment of *avrPto* (Fig. 1F) hybridized to DNA from 14 *P. syringae* pv. tomato race 0 strains, 6 of which are shown in Fig. 3. DNA from *P. syringae* pv. tomato strain 2844 had less homology to the probe as evidenced by the weaker signal in that lane. None of the 12 race 1 strains tested, 6 of which are shown in Fig. 3, contained DNA homologous to the probe. Therefore, in the 26 *P. syringae* pv. tomato strains tested, there is a strict correlation between avirulence and the presence of *avrPto*-hybridizing DNA as well as between virulence and the absence of homology.

To test whether the *avrPto*-hybridizing band of *P. syringae* pv. tomato race 0 strain DC3000 (Fig. 3) represents a functional *avrPto* gene, one cosmid clone (pPtDC5 [Fig. 1H]) was isolated from a DC3000 library by colony hybridization to the 2.2-kb *Bam*HI-*Sau*3A *avrPto* DNA fragment.

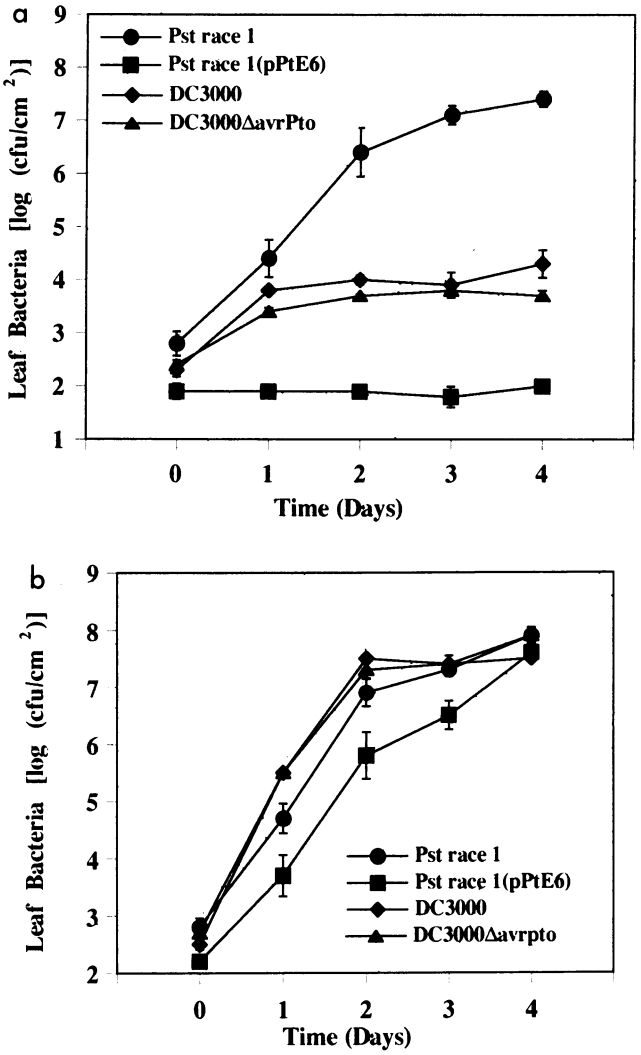

FIG. 2. Growth of *P. syringae* pv. tomato (Pst) strains in tomato cultivars 76R (*Pto*/*Pto*) (a) and 76S (*pto*/*pto*) (b). Values are means from three samples, each consisting of three leaf disks. Error bars show the standard error.

A 3.8-kb *Hin*dIII-*Cla*I DNA fragment from pPtDC5 that contained a region homologous to the probe was subcloned into vector pDSK519 (pPtDC38 [Fig. 1J]). pPtDC38 conferred avirulence to *P. syringae* pv. maculicola, indicating that the *avrPto* function is conserved between at least two *P. syringae* pv. tomato race 0 strains, DC3000 and JL1065. Resistance in tomato plants to *P. syringae* pv. tomato (pPtDC38) cosegregated with resistance to *P. syringae* pv. tomato(pPtE2) (resistant/susceptible ratio = 104:40; χ^2 = 0.59, $P = 0.40$, $n = 144$), confirming that these two subclones carry the same gene, *avrPto*. The restriction maps of pPtE2 and pPtDC38 are highly similar over the 1-kb internal region of *avrPto*, indicating a high degree of structural homology between these two alleles (Fig. 1B and J).

To determine the conservation of *avrPto* among other pathogens, including *P. syringae* pathovars, DNA gel blot analysis was performed (Fig. 4). Strains with sequences homologous to *avrPto* include *P. syringae* pv. maculicola 795 and 4981, *P. syringae* pv. phaseolicola, *P. syringae* pv. pisi, and *P. syringae* pv. coronafaciens, which are radish,

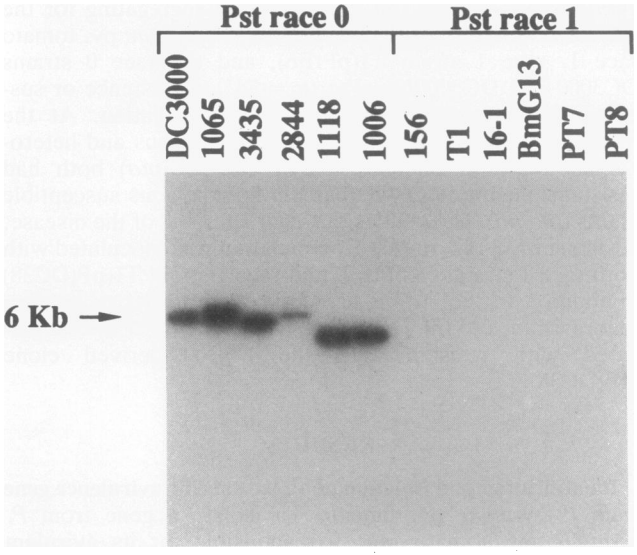

FIG. 3. Genomic DNA gel blots of total DNAs isolated from different *P. syringae* pv. tomato (Pst) strains. Approximately 2 μg of *Hin*dIII-digested DNA was loaded per lane and separated on a 0.75% agarose gel. The blot was hybridized to the 2.2-kb *Bam*HI-*Sau*3A fragment of *avrPto* (see Fig. 1F). The arrow labeled 6 kb indicates the size of the hybridizing fragments in the first four lanes.

bean, pea, and oat pathogens. *P. syringae* pv. maculicola 795 and 4981 are avirulent on *Pto* tomatoes (Table 2). *P. syringae* pv. maculicola strains 4326 and 2744, which cause disease on 76R, lack homology to *avrPto*, as do *P. syringae* pv.

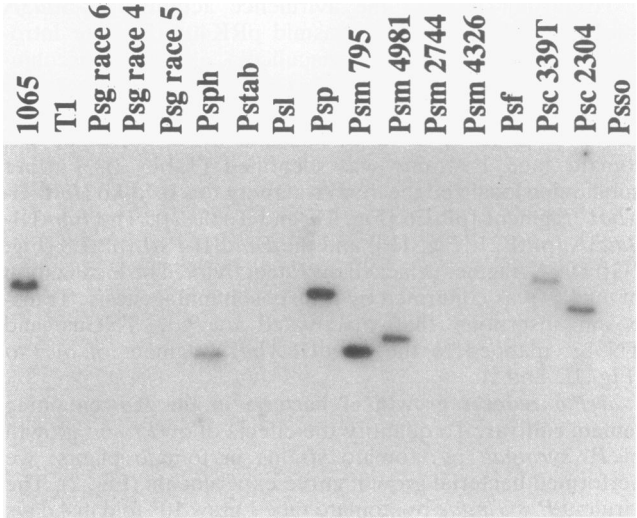

FIG. 4. Genomic DNA blot of total DNAs isolated from different *Pseudomonas* species and pathovars and hybridized to *avrPto* as described in the legend to Fig. 3. From left to right, the DNA was extracted from *P. syringae* pv. tomato race 0 strain JL1065, *P. syringae* pv. tomato race 1 strain T1, *P. syringae* pv. glycinea (Psg) race 6, *P. syringae* pv. glycinea race 4, *P. syringae* pv. glycinea race 5, *P. syringae* pv. phaseolicola (Psph), *P. syringae* pv. tabaci (Pstab), *P. syringae* pv. lachrymans (Psl), *P. syringae* pv. pisi (Psp), *P. syringae* pv. maculicola (Psm) 795, *P. syringae* pv. maculicola 4981, *P. syringae* pv. maculicola 2744, *P. syringae* pv. maculicola 4326, *P. fluorescens* (Psf), *P. syringae* pv. coronafaciens (Psc) 339T, *P. syringae* pv. coronafaciens 2304, and *P. solanacearum* (Psso).

glycinea, *P. syringae* pv. lachrymans, *P. syringae* pv. tabaci, and *P. solanacearum*, which are pathogens of soybeans, cucurbits, tobacco, and solanaceous plants, respectively. DNA from *Xanthomonas campestris* pv. vesicatoria, a pathogen of tomato plants, and *P. fluorescens*, a saprophyte, also lacks homology to the probe (Table 1 and Fig. 4). These results demonstrate that *avrPto* is present in a wide variety of pathogens but not in strains that cause disease on the *Pto* tomato cultivar 76R.

Deletion of *avrPto* in *P. syringae* pv. tomato race 0. Previous studies have indicated that avirulence genes isolated from specific pathogens are often the sole determinants for limiting infection of corresponding cultivars (2, 34, 41). To determine whether this is the case for *avrPto*, we deleted the *avrPto* gene from strain DC3000 to form strain DC3000Δ*avrPto* as described in Materials and Methods. Surprisingly, bacterial growth curve experiments showed that DC3000Δ*avrPto* grows to levels equal to those of DC3000 in cultivars 76R (*Pto*/*Pto*) and 76S (*pto*/*pto*) (Fig. 2). DC3000Δ*avrPto* also did not cause disease in 76R (Table 2). Similarly, gene replacement strains of DC3000 and JL1065 that contained transposon insertions in the *Hin*dIII-*Xba*I fragment of *avrPto* (DC3000TN322 and 1065Gus), showed no symptoms in 76R and grew to wild-type levels in 76R and 76S (Table 2). Southern analysis indicated that only one copy of *avrPto* exists in DC3000 and 1065 and that *avrPto* has been deleted in DC3000Δ*avrPto* and insertionally mutated in DC3000TN322 and 1065Gus (data not shown). These results indicate that additional avirulence factors in DC3000 and JL1065 may contribute to resistance of *Pto* tomatoes to these strains.

The possibility that a second nonhomologous avirulence gene was present in *P. syringae* pv. tomato strain DC3000 was examined by mobilizing a library of DC3000 into *P. syringae* pv. maculicola and screening for avirulence activity. Of 611 clones tested, one clone (pDC13-19) showed avirulence activity; this clone was homologous to pDC5, which contains *avrPto*. No other clones contained host-specific avirulence activity. Although a second avirulence gene was not identified in these experiments, it is possible that such a gene exists but is not expressed in *P. syringae* pv. maculicola.

To investigate the genetics of resistance of tomato plants to DC3000Δ*avrPto*, F_2 progeny segregating for *Pto* were inoculated with the *P. syringae* pv. tomato race 1 strain T1(pPtE6) and the race 0 strain DC3000Δ*avrPto*. Among the 742 F_2 individuals scored, resistance to T1(pPtE6) and resistance to DC3000Δ*avrPto* segregated in the expected 3:1 ratio (resistant/susceptible ratio = 542:200; $\chi^2 = 1.51$, $P = 0.21$, $n = 742$). All plants resistant to *P. syringae* pv. tomato T1(pPtE6) were resistant to DC3000Δ*avrPto*, and all plants susceptible to T1(pPtE6) were susceptible to DC3000Δ*avr Pto*. These results indicate that tomato DNA contains a single locus conferring resistance to both *P. syringae* pv. tomato race 1 transconjugants containing *avrPto* and *P. syringae* pv. tomato race 0 derivatives lacking *avrPto*. If the *Pto* locus is composed of two distinct genes governing each of these resistances, then they are separated by no more than 0.13 centimorgan.

***avrPto* is recognized by soybean plants.** Previous studies indicated that avirulence genes may play roles in limiting host ranges of pathogens (21, 40, 41). We introduced *avrPto* into the soybean pathogen *P. syringae* pv. glycinea race 0 Cx to investigate whether *avrPto* plays such a role. *avrPto* converted the reaction of this strain from the appearance of normally water-soaked lesions on the soybean cultivar Centennial to localized necrosis characteristic of a hypersensitive response (20) (Table 2). Both *P. syringae* pv. glycinea race 0 Cx and *P. syringae* pv. glycinea race 0 Cx(pPtE2Ω) caused the appearance of water-soaked lesions (Table 2). Comparison of growth of *P. syringae* pv. glycinea race 0 Cx(pPtE2) with *P. syringae* pv. glycinea race 0 Cx(pPtE2Ω) in soybean plants shows that *P. syringae* pv. glycinea race 0 Cx(pPtE2) grew only 500-fold in 6 days, whereas *P. syringae* pv. glycinea race 0 transconjugants containing a mutated copy of *avrPto* grew 10^4-fold in 4 days (Fig. 5).

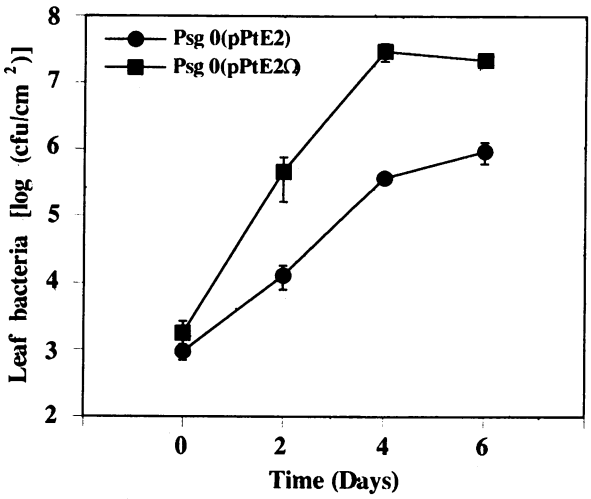

FIG. 5. Growth of *P. syringae* pv. glycinea race 0 Cx(Psg 0) strains in the soybean cultivar Centennial. Values are means from two or three samples, each consisting of one to three leaf disks. Error bars show the standard error.

DISCUSSION

Bacterial speck disease of tomato provides an excellent model system for studying both plant and bacterial genes that specify disease resistance. As a first step towards understanding the molecular basis of disease resistance, we have cloned and characterized the bacterial avirulence gene, *avrPto*, from *P. syringae* pv. tomato and demonstrated that pathogens containing *avrPto* induce disease resistance specifically in tomato cultivars containing the locus *Pto*. Isolation of *avrPto* will facilitate genetic and biochemical studies of the host resistance gene, *Pto*, that may ultimately lead to its isolation.

Other workers have shown that the activity of a cloned avirulence gene is not restricted to the pathogen from which it was isolated (21, 40, 41). The work reported in this article extends this observation to include the avirulence gene *avrPto*. *P. syringae* pv. glycinea transconjugants carrying the *avrPto* subclone induced a hypersensitive response on the soybean cultivar Centennial. These results support the idea (21) that the presence of resistance genes in nonhost plants can confer resistance to certain avirulent *P. syringae* pathovars such as *P. syringae* pv. tomato. In some cases, these resistance genes have been shown to segregate as single dominant genes (16, 41). Similarly, the soybean cultivar Centennial may contain the disease resistance gene *Pto* or a functionally equivalent gene. It is likely that *avrPto* produces directly or indirectly a biochemical factor responsible for limiting growth of both pathogens in the resistant hosts.

The insecticide fenthion elicits necrotic specks only on tomato cultivars containing the *Pto* resistance gene. As shown in field studies (22) and confirmed in our laboratory (2a), recombinants between *Pto* and the gene encoding fenthion sensitivity have not been identified, suggesting that *Pto* resistance and fenthion sensitivity are encoded by the same locus. This necrotic reaction is similar to the hypersensitive response observed in the tomato cultivar 76R and the soybean cultivar Centennial when plants are inoculated with high concentrations of avirulent *P. syringae* pv. tomato strains (reference 33a and this article, respectively). We have also observed a copy effect of *avrPto* on growth of *P. syringae* pv. tomato race 0, *P. syringae* pv. tomato race 1, and *P. syringae* pv. glycinea race 0 strains in the tomato cultivar 76R and the soybean cultivar Centennial (unpublished results). Keen et al. (17) have demonstrated that expression of the avirulence gene *avrD* in *P. syringae* pathovars and in *E. coli* caused these bacteria to produce a diffusible, low-molecular-weight molecule that can elicit a host-specific hypersensitive response on resistant but not susceptible soybean cultivars. The production of an elicitor by an avirulence gene protein has thus far been limited to *avrD*. Studies of the expression of the cloned avirulence gene *avrPto* may lead to isolation of a molecule that induces a host-specific hypersensitive response in tomato and soybean plants. If such a small molecule is indeed produced, it may act like fenthion to induce *Pto*-specific necrosis.

A surprising result of our work is that strains containing a mutated or deleted copy of *avrPto* grew to wild-type levels in the susceptible and resistant tomato hosts. In all other cases studied, mutation of the avirulence gene in the wild-type strain results in increased growth of the mutated strain in a cultivar containing a single resistance locus (2, 15, 34, 41). It is possible that the *Pto* locus encodes a host gene product(s) corresponding to two avirulence gene products present in *P. syringae* pv. tomato race 0 strains but not in *P. syringae* pv. tomato race 1 or *P. syringae* pv. maculicola. Similarly, a single locus in wheat appears to be involved in specific interactions with two wheat rust pathogens (25, 38), implying that the same host gene product may recognize products of avirulence alleles of the two pathogens. In the case of the tomato-*P. syringae* pv. tomato interaction, a recombinant between two hypothetical resistance genes was not identified among 742 F_2 progeny analyzed, indicating that if there are two avirulence genes, they interact with the single host gene, *Pto*, or with two resistance genes at the *Pto* locus less than 0.13 map unit apart. Alternatively, *P. syringae* pv. tomato race 0 strains may lack a *Pto*-specific virulence function present in *P. syringae* pv. tomato race 1. To differentiate between these two possibilities, we are attempting to isolate a second avirulence gene from *P. syringae* pv. tomato race 0 and test whether the two *P. syringae* pv. tomato race 0 avirulence genes interact with the same or different resistance genes in 76R. We are also trying to identify a *Pto*-specific virulence function from *P. syringae* pv. tomato race 1 by complementation in DC3000Δ*avrPto*.

In an attempt to characterize single gene resistance in plants, many laboratories have undertaken mutagenesis experiments directed at inactivating the host resistance gene (1, 13, 14). These strategies are based on the assumption that a single mutation in the host resistance gene will result in loss of resistance when host plants are inoculated with a naturally occurring avirulent strain of the pathogen. While this assumption is true in some cases (13), we have shown that the *avrPto-Pto* interaction is not governed by a simple gene-for-gene relationship. Consequently, screening a mutagenized tomato population with *P. syringae* pv. tomato race 0 (a strain possibly containing two avirulence genes) may require two independent mutation events at the *Pto* locus to obtain a susceptible mutant. The cloning of *avrPto* has enabled us to screen for loss of *Pto* resistance using a genetically defined pathogen. We expect that only a single host mutation will be required to render the plant susceptible to a pathogen carrying the single avirulence gene *avrPto*.

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REFERENCES

1. **Bennetzen, J. L., M.-M. Qin, S. Ingles, and A. E. Ellingboe.** 1988. Allele-specific and mutator-asscIated instability at the *Rp1* disease resistance locus of maize. Nature (London) **332:**369–370.
2. **Bonas, U., R. E. Stall, and B. J. Staskawicz.** 1989. Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Gen. Genet. **218:**127–136.
2a. **Carland, F. M.** Unpublished data.
2b. **Dahlbeck, D.** Unpublished data.
3. **Ditta, G., T. Schmidhauser, E. Yakobson, P. Lu, X.-W. Liang, D. R. Finlay, D. Guiney, and D. R. Helinski.** 1985. Plasmids related to the broad host range vector, pRK290, useful for gene cloning and monitoring gene expression. Plasmid **13:**149–153.
4. **Ditta, G., S. Stanfield, D. Corbin, and D. R. Helinski.** 1980. Broad host range DNA cloning system for Gram-negative bacteria: construction of a gene bank of *Rhizobium meliloti*. Proc. Natl. Acad. Sci. USA **77:**7347–7351.
5. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6–13.
6. **Figurski, D., and D. R. Helinski.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided *in trans*. Proc. Natl. Acad. Sci. USA **76:**1648–1652.
7. **Flor, H. H.** 1935. Physiologic specialization of *Melampsora Lini* on *Linum Usitatissimum*. J. Agric. Res. **51:**819–836.
8. **Flor, H. H.** 1956. The complementary genetic systems in flax and flax rust. Adv. Genet. **8:**29–54.
9. **Gabriel, D. W., A. Burges, and G. R. Lazo.** 1986. Gene-for-gene interactions of five cloned avirulence genes from *Xanthomonas campestris* pv. *malvacearum* with specific resistance genes in cotton. Proc. Natl. Acad. Sci. USA **83:**6415–6419.
10. **Gutierrez, C., J. Barondess, C. Manoil, and J. Beckwith.** 1987. The use of transposon Tn*phoA* to detect genes for cell envelope proteins subject to a common regulatory stimulus: analysis of osmotically regulated genes in *Escherichia coli*. J. Mol. Biol. **195:**289–297.
11. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28:**351–359.
12. **Hitchin, F. E., C. Jenner, S. Harper, J. Mansfield, C. Barber, and M. Daniels.** 1989. Determinant of cultivar specific avirulence cloned from *Pseudomonas syringae* pv. *phaseolicola* race 3. Physiol. Mol. Plant Pathol. **34:**309–322.
13. **Johal, G. S., and S. P. Briggs.** 1991. Molecular cloning of a disease resistance gene from maize. J. Cell. Biochem. Suppl. **15A:**144.
14. **Jorgensen, J. H.** 1988. Genetic analysis of barley mutants with modifications of powdery mildew resistance gene *Ml-a12*. Genome **30:**129–132.
15. **Kearney, B., and B. J. Staskawicz.** 1990. Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene, *avrBs2*. Nature (London) **346:**385–386.
16. **Keen, N. T., and R. I. Buzzell.** 1991. New disease resistance genes in soybean against *Pseudomonas syringae* pv. *glycinea*; evidence that one of them interacts with a bacterial elicitor.

Theor. Appl. Genet. **81:**133–138.
17. **Keen, N. T., S. Tamaki, D. Kobayashi, D. Gerhold, M. Stayton, H. Shen, J. Gold, H. Lorang, D. Thordal-Christensen, D. Dahlbeck, and B. J. Staskawicz.** 1990. Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. Mol. Plant Microbe Interact. **3:**112–121.
18. **Keen, N. T., D. Tamaki, D. Kobayashi, and D. Trollinger.** 1988. Improved broad-host-range plasmids for DNA cloning in gram-negative bacteria. Gene **70:**191–196.
19. **King, E. O., N. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyrocyanin and fluorescein. J. Lab. Clin. Med. **44:**301–307.
20. **Klement, Z.** 1982. Hypersensitivity, p. 149–177. *In* M. S. Mount and G. H. Lacy (ed.), Phytopathogenic procaryotes. Academic Press, Inc., New York.
21. **Kobayashi, Y., J. Stanley, S. Tamaki, and N. T. Keen.** 1989. Cloned avirulence genes from the tomato pathogen, *Pseudomonas syringae* pv. *tomato* conferring cultivar specificity on the non-host soybean. Proc. Natl. Acad. Sci. USA **86:**157–161.
22. **Laterrot, H.** 1985. Susceptibility of the *Pto* plants to Lebacyde in insecticide: a tool for breeders? Tomato Genet. Coop. Newsl. **35:**6.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Manoil, C., and J. Beckwith.** 1985. Tn*phoA*: a transposon probe for protein export signals. Proc. Natl. Acad. Sci. USA **82:**8129–8133.
25. **McIntosh, R. A.** 1977. Nature of induced mutations affecting disease reaction in wheat, p. 551–562. *In* International Atomic Energy Agency (ed.), Induced mutations against plant diseases. International Atomic Energy Agency, Vienna.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Minsavage, G. V., D. Dahlbeck, M. C. Whalen, B. Kearney, U. Bonas, B. J. Staskawicz, and R. E. Stall.** 1990. Molecular analysis of gene-for-gene relationships in *Xanthomonas campestris* pv. *vesicatoria*-pepper interactions. Mol. Plant Microbe Interact. **3:**41–47.
28. **Pilowsky, M., and D. Zutra.** 1982. Screening wild tomatoes for resistance to bacterial speck pathogen *Pseudomonas tomato*. Plant Dis. **66:**46–47.
29. **Pitblado, R. E., and B. H. MacNeill.** 1983. Genetic basis of resistance to *Pseudomonas syringae* pv. *tomato* in field tomatoes. Can. J. Plant Pathol. **5:**251–255.
30. **Pitblado, R. E., B. H. MacNeill, and E. A. Kerr.** 1984. Chromosomal identity and linkage relationships of *Pto*, a gene for resistance to *Pseudomonas syringae* pv. *tomato* in tomato. Can. J. Plant Pathol. **6:**48–53.
31. **Prentki, P., and H. M. Krisch.** 1984. In vitro insertional mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
32. **Ronald, P. C., and B. J. Staskawicz.** 1988. The avirulence gene *avrBs1* from *Xanthomonas campestris* pv. *vesicatoria* encodes a 50 kD protein. Mol. Plant Microbe Interact. **1:**191–198.
33. **Stachel, S. E., G. An, C. Flores, and E. W. Nester.** 1985. A Tn3 *lacZ* transposon for the random generation of β-galactosidase gene fusions: application to the analysis of gene expression in *Agrobacterium*. EMBO J. **4:**891–898.
33a. **Staskawicz, B. J.** Unpublished data.
34. **Staskawicz, B. J., D. Dahlbeck, N. Keen, and C. Napoli.** 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. **169:**5789–5794.
35. **Staskawicz, B. J., D. Dahlbeck, and N. T. Keen.** 1984. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max*. (L.) Merr. Proc. Natl. Acad. Sci. USA **81:**6024–6028.
36. **Swanson, J., B. Kearney, D. Dahlbeck, and B. J. Staskawicz.** 1988. Cloned avirulence gene of *Xanthomonas campestris* pv. *vesicatoria* complements spontaneous race-change mutants. Mol. Plant Microbe Interact. **1:**5–9.
37. **Tamaki, S., D. Dahlbeck, B. J. Staskawicz, and N. T. Keen.** 1988. Characterization and expression of two avirulence genes cloned from *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. **170:**4846–4854.
38. **The, T. T., and R. A. McIntosh.** 1975. Cytogenetical studies in wheat. VIII. Telocentric mapping and linkage studies involving *Sr22* and other genes in chromosome 7AL. Aust. J. Biol. Sci. **28:**531–538.
39. **Vivian, A., G. Atherton, J. Bevan, I. Crute, L. Mur, and J. Taylor.** 1989. Isolation and characterization of cloned DNA conferring specific avirulence in *Pseudomonas syringae* pv. *pisi* to pea (*Pisum sativum*) cultivars, which possess the resistance allele, *R2*. Physiol. Mol. Plant Pathol. **34:**335–344.
40. **Whalen, M. C., R. W. Innes, A. F. Bent, and B. J. Staskawicz.** 1991. Identification of a *Pseudomonas syringae* pathogen of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. Plant Cell **3:**49–59.
41. **Whalen, M. C., R. E. Stall, and B. J. Staskawicz.** 1988. Characterization of a gene from a tomato pathogen determining hypersensitive resistance in non-host species and genetic analysis of this resistance in bean. Proc. Natl. Acad. Sci. USA **85:**6743–6747.