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Phenotypic Expression of *Pseudomonas syringae avr* Genes in *E. coli* Is Linked to the Activities of the *hrp*-Encoded Secretion System

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The specific recognition of elicitors produced by plant pathogenic bacteria carrying avirulence (*avr*) genes is postulated to initiate cellular defense responses in plants expressing corresponding resistance genes. The biochemical functions of most *avr* genes, however, are not known. A heterologous system was developed to phenotypically express *Pseudomonas syringae avr* genes in *Escherichia coli* cells that required the *P. syringae hrp* cluster. *E. coli* MC4100 transformants carrying the plasmid-borne *P. syringae* pv. *syringae* Pss61 *hrp* cluster and *P. syringae* pv. *glycinea avrB* expressed from a triple *lac*UV5 promoter gained the ability to elicit the hypersensitive response in soybean cultivars expressing *Rpg1* and in an *Arabidopsis thaliana* accession expressing *RPM1*. Inactivation of energy transducing or outer membrane components of the *hrp*-encoded secretion system blocked phenotypic expression of *avrB* in *E. coli*, but deletions abolishing harpin$_{Pss}$ production had little effect on the production of the AvrB phenotype by the *E. coli* transformants. Phenotypic expression of *avr*A, *avr*Pto, *avr*Rpm1, *avr*Rpt2, and *avr*Pph3 in *E. coli* was also shown to require the *hrp* cluster. The results indicate that generation of the Avr phenotype in *P. syringae* strains is specifically dependent on the secretion activities of the *hrp* cluster.

Plants, like most multicellular eukaryotic organisms, have inducible defense mechanisms to actively impede colonization of tissue by bacteria and other pathogens (Staskawicz et al. 1995). These cellular defense mechanisms include enhanced production of active oxygen species, release of hydrolytic enzymes, phytoalexins, cell wall modifications, and localized loss of cellular integrity (Keen 1992). The molecular events leading to the initiation of these defense mechanisms are not fully understood but appear to result from a specific recognition event between a resistant plant and the pathogen. Genetic analyses of pathogen variants, called races, with susceptible and resistant cultivars of host plant species have led to formulation of the gene-for-gene hypothesis to explain some host-pathogen recognition events (Flor 1971; Keen 1990). This hypothesis proposes that certain pathogens carry dominant "avirulence" (*avr*) genes conditioning the initiation of necrotic plant defense responses, called the hypersensitive response (HR), in cultivars or ecotypes of plant species expressing a corresponding dominant resistance (R) gene. *avr* genes have been identified in strains of plant pathogenic bacteria, fungi, viruses, and nematodes (Dangl 1994; Staskawicz et al. 1995). More recently, R genes corresponding to specific *avr* genes have been cloned from several plant species (Bent et al. 1994; Ellis et al. 1995; Grant et al. 1995; Jones et al. 1994; Martin et al. 1994; Mindrinos et al. 1994; Whitham et al. 1994).

While the genetic components functioning during gene-for-gene interactions of pathogens with resistant hosts have been characterized, the mechanism by which the gene products interact during incompatible interactions to initiate the cellular defense responses associated with disease resistance has not been elucidated. Most models propose that plant R genes specify a surveillance system that recognizes a pathogen-produced elicitor, presumably an Avr gene product or its by-product, to initiate a defense response (receptor-ligand model) (Gabriel and Rolfe 1990; Keen 1990). The deduced R-gene products characterized thus far have motifs suggestive of a function as receptors or components of a signal transduction pathway (Staskawicz et al. 1995). R-gene specific elicitors predicted by the receptor-ligand model have been identified for a few *avr* genes (*avr*4 and *avr*9 from *Cladosporium fulvum* (Joosten et al. 1994; Van der Ackerveken et al. 1992), TMV coat protein (Culver and Dawson 1991), and *Pseudomonas syringae avrD* (Keen et al. 1990; Midlands et al. 1993), but similar activities have not been detected from pathogens carrying most other *avr* genes. Of particular interest are bacteria carrying *avr* genes. At least 30 *avr* genes have now been cloned and characterized from several *P. syringae* and *Xanthomonas campestris* strains (see Dangl 1994) but,

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with the exception of *avrD*, their mechanism of action remains unknown. The deduced gene products of most *avr* genes lack features indicative of secretion or motifs suggestive of their biochemical activity. When bacterial *avr* genes other than *avrD* are expressed in *Escherichia coli* strains, the cell lysates lack elicitor activity.

Some evidence suggests that in *P. syringae* strains the gene products of *avr* genes may interact with those of *hrp* genes. The *hrp* genes control the ability of *P. syringae* strains to elicit defense reactions during incompatible interactions with nonhost plant species and contribute to pathogenicity during compatible interactions with susceptible plant species (Anderson and Mills 1985; Lindgren et al. 1986; Willis et al. 1991; Bonas 1994). Most of the *hrp* genes appear to encode components of a type III protein secretion system similar to that used by some *E. coli*, *Salmonella*, *Shigella*, and *Yersinia* strains to secrete virulence factors (Huang et al. 1995; Salmond 1994; VanGijsegem et al. 1994). At least one proteinaceous plant response elicitor, harpin$_{Pss}$, is produced by the Pss61 *hrp* gene cluster (He et al. 1993). Several *P. syringae* pv. *glycinea* race 0 mutants carrying *hrp*::Tn*5* mutations no longer elicited the HR in those soybean cultivars carrying the *Rpg1* resistance gene (Huynh et al. 1989), suggesting that these *hrp* mutants had lost the AvrB phenotype. The loss of phenotype was due in part to the regulation of *avrB* by the *hrp* regulatory system (Huynh et al. 1989; Innes et al. 1993; Salmeron and Staskawicz 1993; Shen and Keen 1993). Later studies showed this regulatory system involves HrpL, an alternative sigma factor controlling expression of the entire *hrp* regulon (Hutcheson et al. 1996; Xiao et al. 1994; Xiao and Hutcheson 1994).

These observations suggest that the *hrp* genes might be required for phenotypic expression of *avr* genes. To test this hypothesis, a heterologous system employing *E. coli* transformants was used to determine the genetic components necessary for phenotypic expression of several bacterial *avr* genes. Because *E. coli* is not a plant pathogen, it has been useful for the reconstruction of functional genetic systems important for virulence and regulation in plants (He et al. 1993; Heu and Hutcheson 1993; Huang et al. 1988; Lidell and Hutcheson 1994; Xiao et al. 1994; Xiao and Hutcheson 1994). Here we report phenotypic expression of six diverse *P. syringae avr* genes in *E. coli* transformants carrying the *P. syringae hrp* cluster and demonstrate that this activity requires the secretion activities of the *P. syringae hrp* cluster but not harpin production. A preliminary report of this work has been presented (Hutcheson et al. 1996).

RESULTS

Phenotypic expression of *avrB* in *E. coli*.

To examine the relationship between the *hrp* genes and *avr* genes, a set of plasmid-borne constructs expressing the *P. syringae hrp* genes [(pHIR11-2096)(pYXL2B); Table 1] or the *avrB* gene (pAVRB-600) were transformed singly or in combination into *E. coli* MC4100. The plasmid pYXL2B carries a constitutively expressed *hrpL* gene and was included in these strains to enhance expression of the *P. syringae hrp* genes in

Table 1. Bacterial strains and plasmids used in this study[a]

Strain or plasmid	Relevant characteristics	Source
Escherichia coli		
MC4100	F′ *ara*D139 Δ(*arg*F-*lac*ZYA)U169 *rps*L150 *rel*A1 f*lb*-5301 *pts*F25 *deo*C1	Casadaban 1976
Pseudomonas syringae pv. *syringae*		
Pss61	Nx^r	Baker et al. 1987
pv. *glycinea*		
Race 4	Rif^r	Keen and Buzzell 1991
pDSK600	Sp^r, incQ	Murillo et al. 1994
pDSK603	Sp^r, incQ	Murillo et al. 1994
pAVRB-600	1.3-kb *Xba*I/*Kpn*I fragment isolated from pAVRB-1 and ligated into pDSK600	Tamaki et al. 1988; this work
pAVRA-600	3-kb *Sal*I fragment carrying *avr*A ligated as a *Kpn*I/*Sac*I fragment from a pBluescript intermediate into pDSK600	Staskawicz et al. 1984; this work
pAVRPTO-600	0.8-kb *Hin*dIII/*Sac*I fragment carrying *avr*Pto cloned into pDSK600	Ronald et al. 1992; this work
pAVRRPT2-600	1.3-kb *Hin*dIII fragment carrying *avr*Rpt2 ligated into pDSK600	Innes et al. 1993; This work
pAVRRPM6-603	2-kb *Eco*RI/*Hin*dIII fragment carrying *avr*Rpm6 isolated from K4812.3 cloned into pDSK603	Debener et al. 1991; This work
pAVRPPH3-600	2-kb *Pvu*II fragment derived from a 3.4-kb *Bam*HI fragment carrying *avr*Pph3 cloned into the *Sma*I site of pDSK600	Jenner et al. 1991; This work
pYXL2B	Ap^r, colE1, 1-kb *Ssp*I-*Hin*cII fragment carrying *hrpL* cloned into pBluescriptII SK$^+$	Xiao et al. 1994
pHIR11	Tc^r, incP1, Pss61 *hrp/hrm*A gene cluster cloned into pLAFR3	Huang et al. 1988
pHIR11-2082	Tc^r Kn^r, pHIR11 derivative carrying a *hrp*J4::Tn*pho*A insertion, secretion$^-$	Huang et al. 1991; Lidell and Hutcheson 1994
pHIR11-2084	Tc^r Kn^r, pHIR11 derivative carrying a *hrp*H2::Tn*pho*A insertion, secretion$^-$	He et al. 1993; Huang et al. 1991
pHIR11-2092	Tc^r Kn^r, pHIR11 derivative carrying a *hrp*Z3::Tn*pho*A insertion, Harpin$^-$	He et al. 1993; Huang et al. 1991
pHIR11-2093	Tc^r Kn^r, pHIR11 derivative carrying a *hrp*Z2::Tn*pho*A insertion	Huang et al. 1991
pHIR11-2096	Tc^r Kn^r, pHIR11::Tn*pho*A derivative carrying a wild-type *hrp/hrm*A cluster	Huang et al. 1991
pHIR11-7000	pHIR11 derivative carrying a *Hin*dIII to *Xba*I substitution causing a *hrp*Z2::12bp mutation	This work
pHIR11-7024	pHIR11-7000 derivative carrying a 0.8-kb Δ*hrp*Z2 mutation	This work
pHIR11-7025	pHIR11-7000 derivative carrying a 0.85-kb Δ*hrp*Z2 mutation	This work

[a] Ap, ampicillin; Kn, kanamycin; Rif, rifampicin; Tc, tetracycline; Sp, spectinomycin, Nx, nalidixic acid.

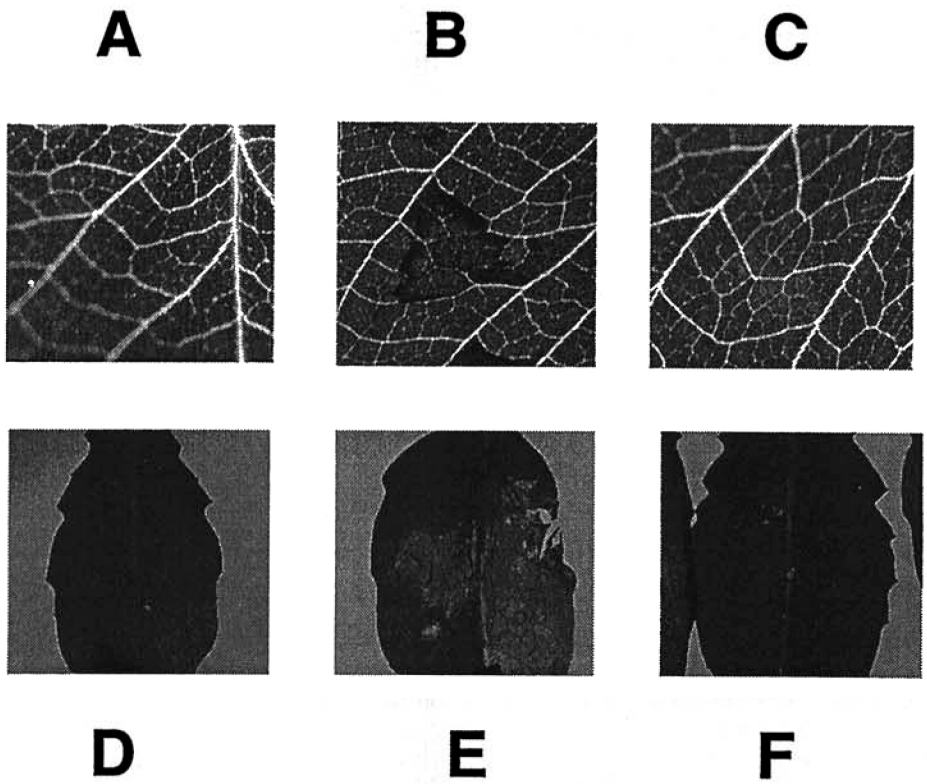


Fig. 1. Plant responses to *Escherichia coli* MC4100 transformants carrying the *Pseudomonas syringae* Pss61 *hrp/hrmA* gene cluster and the *P. syringae* pv. *glycinea avrB* gene. Cells from overnight cultures of the indicated bacteria grown in KB medium were harvested, resuspended in M63 fructose minimal salts medium, and cultured for an additional 6 h. Cells were harvested, resuspended in H_2O and the bacterial concentration was adjusted to 1×10^9 cells/ml. The bacteria were immediately inoculated into primary leaves of 10-day-old soybean plants or fully expanded leaves of approximately 4-week-old. *Arabidopsis thaliana* plants. Responses were photographed after 24 h. These experiments were repeated at least 10 times with similar results. A representative infiltration site is shown for each interaction. **A,** Soybean cv. merit inoculated with MC4100 (pHIR11-2096)(pYXL2B). **B,** Soybean cv. Merit inoculated with MC4100 (pHIR11-2096)(pYXL2B)(pAVRB-600). **C,** Soybean cv. Acme inoculated with MC4100 (pHIR11-2096) (pYXL2B) (pAVRB-600). **D,**. *A. thaliana* accession Col-0 inoculated with MC4100 (pHIR11-2096)(pYXL2B); **E,** *A. thaliana* accession Col-0 inoculated with MC4100 (pHIR11-2096)(pYXL2B)(pAVRB-600). **F,** *A. thaliana* mutant Col-0(rps3-1) inoculated with MC4100 (pHIR11-2096)(pYXL2B)(pAVRB-600).

E. coli (Xiao et al. 1994). The soybean cultivars tested were found to be insensitive to *E. coli* MC4100 transformants carrying pHIR11-2096 and pYXL2B or pAVRB-600 alone. A null response was detected when an inoculum of 10^9 cells/ml was infiltrated, irrespective of the soybean cultivar inoculated (Fig. 1A). In contrast, MC4100 transformants carrying both the Pss61 *hrp* cluster and the plasmid-borne *avrB* gene were capable of eliciting the HR (Fig. 1B), but only in those soybean cultivars carrying *Rpg1*, the resistance gene that mediates soybean responses to *P. syringae* strains carrying *avrB* (Merit, Harosoy, Norchief; Table 2). When inoculated into leaves of soybean cultivars which lack *Rpg1* (Acme, Flambeau, and Centennial), a null response similar to that produced by MC4100 (pHIR11-2096)(pYXL2B) was observed (Fig. 1C). To confirm the specificity of these plant responses to *E. coli* cells carrying the *hrp* genes and *avrB*, 11 additional soybean cultivars (Dunfield, Hawkeye, Jogun, Clark, Blackhawk, PI54610, Mukden, Norin, S100, OX615, and Vance) whose responses to *P. syringae* strains had not been published were screened in a blind experiment for their response to the *E. coli* transformants. Only those soybean cultivars that reacted hypersensitively to MC4100 carrying the *hrp* cluster and *avrB* (Hawkeye, Blackhawk, Mukden, and S100) developed a typical HR when inoculated with *P. syringae* pv. *glycinea* race 4 (pAVRB-600).

P. syringae pv. *maculicola* and *P. syringae* pv. *tomato* DC3000 transformants carrying a plasmid-borne *avrB* gene also induce the HR in accessions of *A. thaliana* carrying *RPM1*, a R-gene mediating recognition of *P. syringae* strains carrying either *avr*Rpm1 or *avrB* (Grant et al. 1995). *A. thaliana* accession Col-0 and a *rpm1* mutant (Col-0 (rps3-1))(a gift of R. Innes) as well as five accessions of *A. thaliana* lacking the *RPM1* gene (Aa-O, Mt-0, Fe-1, Nd-0, and Bs-1; a gift from J. Dangl) were screened for their ability to respond to *E. coli* MC4100 carrying the *hrp* genes and *avrB*. The HR was observed when MC4100 (pHIR11-2096) (pYXL2B) (pAVRB-600) was inoculated into Col-0 (Fig. 1E). As in soybean, *E. coli* strains lacking either the plasmid-borne *avrB* gene or the Pss61 *hrp* cluster produced a null response (e.g., Fig. 1D). Neither the Col-0 (rps3-1) mutant (Fig. 1F) nor accessions lacking the *RPM1* gene (data not shown) responded hypersensitively to any of the MC4100 transformants.

Role of the *hrp*-encoded secretion system.

Since transcription of *avrB* in the construct used in these experiments was directed by vector *lac*UV5 promoters and expression of the *hrp* operons was enhanced by the presence of the plasmid-borne *hrpL* gene, it is likely that the *hrp*-dependent expression of the Avr phenotype is due to either: (i) the secretion activity of the *hrp* cluster; or (ii) the secreted

Table 2. Responses of soybean leaves to *Escherichia coli* MC4100 transformants carrying *Pseudomonas syringae hrp* and *avrB* genes

Transformant carrying[a]:	Reaction observed in soybean cultivar[b]					
	Acme	Merit	Harosoy	Norchief	Flambeau	Centennial
pHIR11-2096, pYXL2B	Null	Null	Null	Null	Null	Null
pAVRB-600	Null	Null	Null	Null	Null	Null
pHIR11-2096, pYXL2B, pAVRB-600	Null	HR	HR	HR	Null	Null

[a] *E. coli* MC4100 transformants carrying the indicated plasmids were inoculated into the primary leaves of 10-day-old soybean plants at a concentration of 1×10^9 cells/ml as described in Figure 1. Leaves were scored for responses after 24 h.

[b] Resistance phenotypes of the soybean cultivars are: Acme (rpg1); Merit (Rpg1); Harosoy (Rpg1); Norchief (Rpg1); Flambeau (rpg1); Centennial (rpg1); HR, necrotic reaction typical of the hypersensitive response observed after 24 h.; Null, no reaction observed up to 48 h after inoculation.

harpin product produced by the *hrp*Z operon. To test the role of the secretion activity of the *hrp* cluster in the production of the *avrB* phenotype, experiments were conducted using pHIR11 derivatives carrying *hrp*J4::Tn*phoA* (pHIR11-2082) or *hrp*H2::Tn*phoA* (pHIR11-2084) mutations (Huang et al. 1991). The *hrp*J4 gene product has been deduced to be an ATPase associated with the inner membrane and is a necessary component of the *hrp*-encoded secretion system (Lidell and Hutcheson 1994). The *hrp*H2 gene product is an outer membrane protein that is also essential for the *hrp*-encoded secretion system (He et al. 1993; Huang et al. 1992). These mutations were previously shown to produce secretion minus (Hsc^-) phenotype in MC4100 transformants (He et al. 1993; Huang, He et al. 1992; Lidell and Hutcheson 1994). Phenotypic expression of *avrB* was lost in any of the MC4100 (pAVRB-600)(pYXL2B) transformants carrying the Hsc^- pHIR11::Tn*phoA* derivatives (Table 3).

Table 3. Role of the *hrp*-encoded secretion system in the phenotypic expression of *avrB* in MC4100 transformants

pHIR11 derivative[a]	*hrp* Mutation[b]	Reaction of inoculated plant host[c]				
		Tobacco	Merit	Acme	Col-0	Col-0 (rps3-1)
2096	Wild type	HR	HR	Null	HR	Null
2082	*hrp*J4	Null	Null	Null	Null	Null
2084	*hrp*H2	Null	Null	Null	Null	Null

[a] *E. coli* MC4100 transformants carrying pAVRB-600, pYXL2B and the indicated pHIR11 derivative

[b] Mutations were characterized previously (He et al. 1993; Huang et al. 1991; Lidell and Hutcheson 1994). The genetic nomenclature of Xiao and Hutcheson (1994) was used.

[c] Conditions were as described in Figure 1. Acme and Merit are soybean cultivars. Col-0 and Col-0 (rps3-1) are accessions of *A. thaliana*. *Nicotiana tobacum* L. (tobacco) var. Samsun was inoculated as described in Huang et al. 1988.

Requirement for harpin in the phenotypic expression of *avrB* in *E. coli*.

Assessment of the role of harpin in production of the AvrB phenotype is complicated by the genetic organization of the *hrp* cluster. $Harpin_{Pss}$ is the gene product of the second gene of the *hrp*Z operon (Huang et al. 1995; Xiao and Hutcheson 1994). At least four genes transcribed after *hrp*Z2 encoded gene products which appear to be part of the *hrp*-encoded protein secretion system (Huang et al. 1995; Preston et al. 1995). In addition, deletion derivatives of *hrp*Z2 retaining either the N or C halves of the gene product still exhibit HR-eliciting activity when inoculated into tobacco (Alfano et al. 1995), thereby limiting the type of mutations that can be used in these experiments. Initial experiments employed constructs carrying Tn*phoA* mutations that had been originally mapped to the complementation group encoding for $harpin_{Pss}$ (pHIR11-2092; -2093; Fig. 2) (Huang et al. 1991) and had been reported to suppress harpin production (He et al. 1993). MC4100 transformants carrying either pHIR11::Tn*phoA* derivative and *avrB* failed to elicit the HR in *A. thaliana* Col-0 or soybean cv. Merit (data not shown), suggesting that harpin may be necessary for generation of the AvrB phenotype. These mutations, however, could have a polar effect on downstream genes encoding apparent components of the *hrp*-encoded secretion system (Huang et al. 1995; Preston et al. 1995).

To clarify the role of $harpin_{Pss}$ in the phenotypic expression of *avrB*, Δ*hrp*Z2 mutations were constructed directly in pHIR11. A short oligonucleotide was used to convert the *Hin*dIII site of pHIR11 that is internal to *hrp*Z2 into a unique *Xba*I site. The resulting pHIR11 derivative (pHIR11-7000) carried a 12-bp insertion which did not affect the Harpin phenotype. MC4100 (pYXL2B)(pHIR11-7000) retained the ability to elicit an HR in tobacco. Similarly, MC4100 (pYXL2B) (pHIR11-7000)(pAVRB-600) was able to elicit the HR in *A. thaliana* Col-0 but not in Col-0(rps3-1) (Fig. 2). Deletions were generated by *Bal*31 digestion of *Xba*I-digested pHIR11-7000 and characterized by PCR and sequence analysis. MC4100 (pYXL2B)(pAVRB-600) tranformed with pHIR11-7000 derivatives carrying nearly complete *hrp*Z2 deletions (pHIR11-7024 or pHIR11-7025) retained the ability to elicit the HR when inoculated into *N. tabacum*, *A. thaliana* Col-0, or soybean cv. Merit leaves (Fig. 2). Similar to the observations reported above, the transformants failed to elicit a response in the *A. thaliana* Col-0 (rps3-1) mutant or soybean cv. Acme and transformants carrying either of the mutant *hrp* clusters alone did not elicit a response in *A. thaliana* or soybean leaves (data not shown), indicating that the observed response is due to expression of *avrB*. Surprisingly, MC4100 (pYXL2B) transformants carrying either pHIR11-7024 or pHIR11-7025 retained the ability to elicit the HR in tobacco. Sequence analysis showed that the deletion constructed in pHIR11-7024 begins at +40 bp relative to the translational start codon, whereas the deletion characterized in pHIR11-7025 abolished the translational start for *hrp*Z2. Because two 8-bp *Xba*I linkers were incorporated into the pHIR11-7024 during construction, this deletion results in a frameshift that likely abolishes translation of the native C-terminus of $harpin_{Pss}$. No in-frame translational initiation sites were apparent in retained portions of *hrpZ2* in either mutant. Transformants carrying either pHIR11 derivative are therefore unlikely to produce a $harpin_{Pss}$-derived elicitor. The ability to detect a response in the absence of

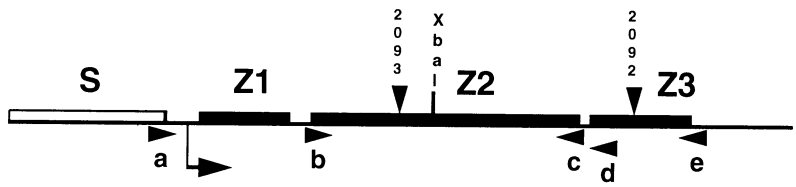
harpin$_{Pss}$ production indicates that harpin is not an obligate component needed for phenotypic expression of *avrB* or elicitation of the HR in tobacco.

Hrp-dependent expression of other *avr* genes in *E. coli*.

Since R-gene dependent responses to *E. coli* strains carrying the Pss61 *hrp* cluster and *avrB* were detected in soybean and *A. thaliana*, we tested whether other *P. syringae avr* genes exhibit a similar dependence on the *hrp* cluster. Accordingly, *avrA* isolated from *P. syringae* pv. *glycinea* race 6, *avr*Pto and *avr*Rpt2 isolated from *P. syringae* pv. *tomato*, *avr*Rpm1 isolated from *P. syringae* pv. *maculicola*, and *avr*Pph3 isolated from *P. syringae* pv. *phaseolicola* were screened for activity in MC4100 (pHIR11-2096)(pYXL2B). Phenotypic expression of these *avr* genes was detected in *E. coli* transformants carrying the Pss61 *hrp* cluster. Soybean cultivars that reacted hypersensitively to *P. syringae* pv. *glycinea* race 4 transformants carrying these *avr* genes also exhibited the HR when inoculated with *E. coli* transformants carrying the same *avr* gene in addition to the *hrp* gene cluster (Table 4). As reported previously (Keen et al. 1990; Midlands et al. 1993), phenotypic expression of *avrD* in *E. coli* was not dependent on the *hrp* cluster since a similar HR was elicited by *E. coli* transformants carrying only *avrD* (data not shown).

DISCUSSION

The biochemical mechanism by which the phenotype of *P. syringae avr* genes is produced has been a long-standing question. The results presented here demonstrate that the activities encoded by the *P. syringae hrp* gene cluster are required for the generation of several *P. syringae* Avr phenotypes. Most *avr* genes have been phenotypically silent when expressed in *E. coli* strains. By using a plasmid-borne construct to express a *P. syringae hrp/hrmA* gene cluster in *E. coli* together with an *avr* gene, phenotypic expression of the



Derivative	Deletion	Position	Tobacco HR	AvrB Phenotype Soybean	AvrB Phenotype Arabidopsis
pHIR11-7000	(+12 bp)		+	+	+
pHIR11-7024	807 bp		+	+	+
pHIR11-7025	878 bp		+	+	+

Fig. 2. Location and phenotype of *hrpZ2* deletions. Open reading frames identified within the *hrpZ* operon (Huang et al. 1995) are shown as boxes and labeled using the nomenclature of Xiao and Hutcheson (1994). Approximate position of Tn*phoA* insertions 2092 and 2093 as determined by PCR-based mapping are indicated. The horizontal arrowheads labeled with letters indicate the approximate location of sequences used to construct the PCR primers used in the characterization of deletion derivatives. The bent arrow indicates the position of the *hrpZ* promoter. The *Hin*dIII site internal to *hrpZ2* was converted to the shown *Xba*I site as described in Materials and Methods. Deletions were created by *Bal*31 digestion of the *Xba*I-digested plasmid. Deletions were estimated by PCR employing paired primers a/d, a/c, and b/d and confirmed by sequence analysis. Deletions are shown positioned relative to the *Xba*I site. The 7024 deletion extended to +40 bp relative to the initiation codon of *hrpZ2* and continued to position -176 relative to the stop codon. The 7025 deletion begins in the intergenic region between *hrpZ1* and *hrpZ2* at position -29 relative to the translational start codon of *hrpZ2* and terminates at position -184 relative to the stop codon. No subsequent in-frame translational initiation sites are apparent in the retained portions of the gene. The indicated pHIR11 derivative was transformed into MC4100 (pYXL2B)(pAVRB-600) and transformants screened in tobacco, *A. thaliana* (Col-0 and Col-0(rps3-1)) and soybean (cvs. Merit and Acme) for the ability to elicit the HR. Plant responses were determined as described in Figure 1. The AvrB phenotype was scored as "+" if the HR was observed in soybean cv. Merit and *A. thaliana* Col-0 but not in soybean cv. Acme and *A. thaliana* Col-0(rps3-1). The responses detected in *A. thaliana* Col-0 leaves to either of the deletion mutants were slower to develop than that induced by transformants carrying the wild-type *hrp* cluster and only 50% of the infiltrated tissue responded. No differences in responses relative to the wild-type cluster were noted in soybean leaves. *E. coli* transformants carrying pHIR11-7024 or pHIR11-7025 exhibited a HR$^+$ phenotype in tobacco irrespective of whether pAVRB-600 was present in the strain.

Table 4. Response of soybean cultivars to *Escherichia coli* MC4100 carrying the *Pseudomonas syringae hrp* cluster and *avr* genes

	Reaction observed in soybean cultivar[b]					
Cloned *avr* gene[a]	Acme	Merit	Harosoy	Norchief	Flambeau	Centennial
*avr*A	HR (I)	HR (I)	HR (I)	Null (C)	Null (C)	HR (I)
*avr*Rpm1	HR (I)	Null (C)	Null (C)	HR (I)	HR (I)	HR (I)
*avr*Pto	Null[c] (C)	Null[c] (C)	Null[c] (C)	Null (C)	HR (I)	HR (I)
*avr*Rpt2	Null (C)	Null (C)	HR (I)	Null (C)	HR (I)	HR (I)
*avr*Pph3	Null (C)	HR (I)	Null (C)	HR (I)	HR (I)	HR (I)

[a] *E. coli* MC4100 (pHIR11-2096)(pYXL2B) transformants carrying the indicated *avr* gene. The *avr* genes were cloned into pDSK600 except *avr*Rpm1 which was cloned into pDSK603.

[b] Plants were inoculated as in Figure 1. Plant reactions were scored as described in Table 1; responses in parentheses indicate the responses elicited by *P. syringae* pv. *glycinea* race 4 (PsgR4) transformants carrying the indicated *avr* gene; (C), compatible interaction resulting in disease symptoms; (I), Incompatible interaction causing a hypersensitive response (HR).

[c] Variable response of an intermediate nature.

avr gene could now be detected. For the 17 soybean cultivars screened, only cultivars carrying the *Rpg1* allele responded hypersensitively to MC4100 with the *P. syringae hrp* cluster and *avrB*. Similarly, the reactivity of *A. thaliana* to MC4100 transformants carrying *avrB* and the *hrp* cluster was linked to *RPM1*. The specificity of these plant responses to *E. coli* transformants is thus consistent with the phenotypic expression of *avrB* in this heterologous system. For the other *avr* genes tested, the responses of the soybean cultivars screened to *P. syringae* pv. *glycinea* race 4 and the *E. coli* transformants were identical, suggesting that the *E. coli* system was also expressing the phenotype of these *avr* genes with fidelity. The *hrp*-dependent expression of *P. syringae avr* genes in *E. coli* thus provides a powerful new system for studying gene-for-gene recognition events in plants. These results, coupled with those of Huynh et al. (1989), demonstrate that the phenotypic expression of several *P. syringae avr* genes is directly dependent on the *hrp* cluster.

Both the regulatory components and the secretion activities associated with the Pss61 *hrp/hrmA* gene cluster were found to be required for phenotypic expression of *P. syringae avr* genes in *E. coli*. It was previously established that several *avr* genes are regulated by the HrpL-linked regulatory system and all *P. syringae avr* genes characterized to date carry a HrpL-dependent promoter consensus sequence (Innes et al. 1993; Salmeron and Staskawicz 1993; Shen and Keen 1993; Xiao and Hutcheson 1994). Inactivation of genes encoding energy-transducing (*hrp*J4) or outer membrane components (*hrp*H2) of the *hrp*-encoded protein secretion system abolished phenotypic expression of *avrB* in the *E. coli* expression system. The deduced gene products of these genes are conserved in each of the Type III secretion systems characterized thus far from pathogenic bacteria and are thought to be essential components of the type III secretion apparatus (Huang et al. 1995; Huang et al. 1993; Lidell and Hutcheson 1994; VanGijsegem et al. 1995). These mutations had previously been shown to abolish *hrp*-dependent secretion of HR-eliciting factors active in tobacco (He et al. 1993; Lidell and Hutcheson 1994). Therefore the *hrp*-encoded secretion apparatus must play an essential role in production of the AvrB phenotype.

Involvement of the *hrp*-encoded protein secretion system in production of the *avr* phenotype in *P. syringae* strains argues that at least one secreted protein is necessary to generate the Avr-linked elicitor activity. *P. syringae* strains do not typically invade plant cells during early phases of pathogenesis, and thus an extracellular signal must be involved in the recognition process. In addition, many of the resistance gene products characterized thus far from plant species lack obvious features indicative of secretion, suggesting they may be cytoplasmic proteins (Dangl 1995). Therefore, either the plant cells have a mechanism for uptake of an elicitor from the medium or the pathogen physically introduces the elicitor into the plant cell. Type III protein secretion systems have been found in a number of enteric bacteria pathogenic to mammals (see Hutcheson et al. 1996; VanGijsegem et al. 1994; VanGijsegem et al. 1995) and have been associated with production of filamentous structures or the apparent direct injection of secreted proteins into mammalian cells (Parsot et al. 1995; Rosqvist et al. 1994). Pathogenesis by these mammalian pathogens requires contact between the bacterium and the affected host cell (Cornelis 1994; Finlay 1994; Jarvis et al. 1995; Parsot 1994). Contact between bacteria and plant cells is also thought to be a key step in the initiation of the HR during incompatible interactions of *P. syringae* strains with resistant hosts (Klement 1977). Since extracts of bacteria expressing various *avr* genes lack elicitor activity when infiltrated into tissue (see Dangl 1994; M C. Lidell and S. W. Hutcheson, unpublished results), it may be that the *hrp*-encoded secretion system functions to directly introduce the *avr*-encoded elicitor into the plant cell. It remains to be determined, however, whether the *hrp*-encoded secretion system functions in a manner analogous to that of mammalian pathogens. Attempts to detect secretion of an *avr* gene product have been unsuccessful thus far (Brown et al. 1993; Dangl 1994).

Although harpin$_{Pss}$ had previously been reported to function as an elicitor of the HR in tobacco (He et al. 1993), the deletion analyses indicate that harpin production is not required for generation of the *avrB* phenotype or for the initiation of the HR in tobacco. These deletions were constructed in a manner to minimize the possibility of second-site mutations that might occur during alternative mutagenesis strategies, such as marker exchange. Transformants expressing *avrB* and mutant *hrp* clusters in which the *hrp*Z2 gene had been specifically deleted elicited a clearly detectable HR in tobacco, *A. thaliana* Col-0, or soybean cv. Merit tissue. It may be that other HR elicitors active in tobacco are encoded by the Pss61 *hrp/hrmA* gene cluster as suggested by Alfano et al. (1995). The ability to generate the *avrB* phenotype in the absence of the *hrp*Z2 gene product indicates that either the AvrB gene product itself or in combination with another secreted Hrp gene product elicits the plant response. Since the HR elicited by transformants carrying the Δ*hrp*Z2 derivatives in *A. thaliana* Col-0 was more limited in area and slower to develop than that elicited by transformants carrying the wild-type *hrp* cluster, it may be that harpin has an accessory role in the production of the Avr phenotype of the transformant.

The various *avr* genes tested in this study exhibit little similarity other than their common regulation by *hrpL*. This suggests that several *P. syringae avr* genes, with the exception of *avrD*, will require the secretion activities of the *hrp* cluster for phenotypic expression. Preliminary results suggest that phenotypic expression of *P. syringae* pv. *glycinea avrC* is also dependent on the *hrp* cluster. An interesting question remains as to whether the *avr* genes of *Xanthomonas campestris* strains will also be dependent on the resident *hrp* cluster for phenotype as originally suggested by Fenselau (1992). *X. campestris* strains carry an analogous cluster to the *P. syringae hrp* genes which is also thought to form a protein secretion system (Bonas 1994; Fenselau et al. 1992). The proteins secreted by this putative secretion system have not been identified. It is clear that this system is essential for the pathogenicity of *X. campestris* strains as in *P. syringae* strains, and thus, is likely to function in an analogous manner. In contrast to *P. syringae avr* genes, at least one family of *X. campestris avr* genes, those related to *avrBs3*, are regulated independently of the resident *hrp* cluster (Knoop et al. 1991). Curiously, some members of this protein family also carry eukaryotic nuclear localization signals (Yang and Gabriel 1995). Further work will be necessary to establish the mechanism by which *hrp* gene products generate the *avr* phenotype in these bacteria.

MATERIALS AND METHODS

Bacteria, plasmids, and culture conditions.

Bacteria and plasmids are described in Table 1. *E. coli* MC4100 was routinely grown in LB medium at 28°C. Prior to inoculation into plants, an overnight LB broth culture was harvested, washed, and resuspended in M63 medium containing 0.2% fructose or mannitol and 50 μg of thiamine per ml. After culture for an additional 6 h, cells were harvested and resuspended in H_20 to the indicated concentration immediately prior to infiltration of plant tissue. Media were supplemented with antibiotics, when indicated, at the following concentrations (μg/ml): kanamycin, 50; ampicillin, 100; tetracycline, 20; and spectinomycin, 100.

General DNA manipulations and analysis.

Plasmid DNA was isolated and manipulated by using standard techniques. Restriction enzymes and related reagents were purchased from Gibco (Gaithersburg, MD) and were used according to the manufacturer's instructions. Transformations employed electroporation. PCR templates were released from picked colonies by heat lysis for 10 min at 95°C and PCR performed using a "hot start" and *Taq* Polymerase. After PCR, samples were fractionated by electrophoresis in 1% agarose gels. Sequence analysis of PCR-amplified templates utilized an Applied Biosystems automated nucleotide sequencer.

Construction of *hrpZ2* deletion mutants.

A short oligonucleotide consisting of the complementary sequence to the *Hin*dIII overhang and the sequence for a *Xba*I restriction site (AGCTCTCTAGAG) was used to modify pHIR11 to carry a unique *Xba*I site internal to *hrpZ2*. The oligonucleotide was ligated into pHIR11 that had been partially digested with *Hin*dIII, and the resulting plasmid was transformed into *E. coli* DH5α. The region containing *hrpZ2* from selected transformants was amplified by PCR as described above and digested with *Xba*I to identify transformants carrying the intended modification. One plasmid (pHIR11-7000) was chosen for further manipulation. The plasmid was isolated, digested with *Xba*I, and then subjected to digestion with *Bal*31 for 5, 10, 20, 30, and 40 min. The *Bal*31 digests were pooled, extracted with phenol/chloroform, and ethanol precipitated. The digested plasmid was ligated in the presence of *Xba*I linkers and transformed into MC4100 (pYXL2B) and MC4100 (pYXL2B)(pAVRB-600). The transformants were screened for deletions by using PCR. The PCR (annealing T, 53 C; 26 cycles) employed an oligonucleotide primer internal to *hrpS* (ACYGNCGYATCAAGGART) and a second primer internal to *hrpZ3* (TGAAACGAGCCCCTGTGG). Deletions were confirmed by sequence analysis. Transformants carrying pHIR11 derivatives with deletions (pHIR11-70XX) were then screened for the ability to elicit the HR in tobacco, soybean, and *A. thaliana*.

Plant response assays.

Glycine max L. Merr. cultivars were grown from seed in greenhouses in commercial potting soil and transferred to growth chambers 24 to 48 h prior to inoculation. The bacterial suspensions were infiltrated into the primary leaves of 10-day-old plants through abaxial epidermal wounds by using disposable plastic syringes (Napoli and Staskawicz 1987). An inoculum concentration of 10^9 cells per ml was used in most experiments. *Arabidopsis thaliana* accessions were grown from seed in growth chambers and leaves of mature nonflowering plants syringe-inoculated as before. Tobacco inoculations were performed as described previously (Lidell and Hutcheson 1994).

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LITERATURE CITED

Alfano, J. R., Bauer, D. W., Milos, T. M., and Collmer, A. 1995. Mutational analysis of the role of the *Pseudomonas syringae* pv. *syringae hrpZ* in elicitation of the hypersensitive response in tobacco. (Abstr.) Phytopathology 85:1158.

Anderson, D. M., and Mills, D. 1985. The use of transposon mutagenesis in the isolation of nutritional and virulence mutants in two pathovars of *Pseudomonas syringae*. Phytopathology 75:104-108.

Baker, C. J., Atkinson, M. M., and Collmer, A. 1987. Concurrent loss in Tn*5* mutants of the ability to induce the HR and host plasma membrane K^+/H^+ exchange in tobacco. Phytopathology 77:1268-1272.

Bent, A., Kunkel, B., Dahlbeck, D., Brown, K., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B. J. 1994. RPS2 of *Arabidopsis thaliana*: A leucine-rich repeat class of plant disease resistance genes. Science 265:1856-1860.

Bonas, U. 1994. *hrp* genes of phytopathogenic bacteria. Pages 79-98 in: Bacterial Pathogenesis of Plants and Animals. Vol. 192. J. L. Dangl, eds. Springer-Verlag, Berlin.

Brown, I., Mansfield, J., Irlam, I., Conrads-Strauch, J., and Bonas, U. 1993. Ultrastructure of interactions between *Xanthomonas campestris* pv. *vesicatoria* and pepper, including immunocytochemical localization of extracellular polysaccharides and the AvrBs3 protein. Mol. Plant-Microbe Interact. 6:376-386.

Casadaban, M. J. 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. 104:541-555.

Cornelis, G. R. 1994. *Yersinia* pathogenicity factors. Pages 243-263 in: Bacterial Pathogenesis in Plant and Animals: Molecular and Cellular Mechanisms. Vol. 192. J. L. Dangl, ed. Springer-Verlag, Berlin.

Culver, J. N., and Dawson, W. 1991. Tobacco mosaic virus elicitor coat protein genes produce a hypersensitive phenotype in transgenic *Nicotiana sylvestris* plants. Mol. Plant-Microbe Interact. 4:458-463.

Dangl, J. L. 1994. The enigmatic avirulence genes of phytopathogenic bacteria. Pages 99-118 in: Bacterial Pathogenesis of Plants and Animals. Vol. 192. J. L. Dangl, ed. Springer-Verlag, Berlin.

Dangl, J. L. 1995. Piece de Resistance: Novel classes of plant disease resistance genes. Cell 80:363-366.

Debener, T., Lehnackers, H., Arnold, M., and Dangl, J. 1991. Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. Plant J. 1:289-302.

Ellis, J. G., Lawrence, G. J., Finnegan, E. J., and Anderson, P. A. 1995. Contrasting of two rust resistance loci in flax. Proc. Natl. Acad. Sci. USA 92:4185.

Fenselau, S., Balbo, I., and Bonas, U. 1992. Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in secretion in bacterial pathogens of animals. Mol. Plant-Microbe Interact. 5:390-396.

Finlay, B. B. 1994. Molecular and cellular mechanisms of *Salmonella* pathogenesis. Pages 163-185 in: Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms. Vol. 192. J. L. Dangl, ed. Springer-Verlag, Berlin.

Flor, H. H. 1971. Current status of the gene-for-gene concept. Annu.

Rev. Phytopathol. 9:275-296.
Gabriel, D. W., and Rolfe, B. G. 1990. Working models of specific recognition in plant-microbe interactions. Annu. Rev. Plant Physiol. 28:365-391.
Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattlier, A., Innes, R. W., and Dangl, J. L. 1995. Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. Science 269:1543.
He, S. Y., Huang, H. C., and Collmer, A. 1993. *Pseudomonas syringae* pv. syringae Harpin$_{Pss}$: A protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. Cell 73:1255-1266.
Heu, S., and Hutcheson, S. W. 1993. Nucleotide sequence and properties of the *hrm*A locus associated with the *P. syringae* pv. *syringae* 61 *hrp* gene cluster. Mol. Plant-Microbe Interact. 6:553-564.
Huang, H. C., He, S. Y., Bauer, D. W., and Collmer, A. 1992. The *Pseudomonas syringae* pv. *syringae* 61 *hrpH* product: An envelope protein required for elicitation of the hypersensitive response in plants. J. Bacteriol. 174:6878-6885.
Huang, H. C., Hutcheson, S. W., and Collmer, A. 1991. Characterization of the *hrp* cluster from *Pseudomonas syringae* pv. *syringae* 61 and Tn*phoA* tagging of exported or membrane-spanning Hrp proteins. Mol. Plant-Microbe Interact. 4:469-476.
Huang, H. C., Lin, R. H., Chang, C. J., Collmer, A., and Deng, W. L. 1995. The complete *hrp* gene cluster of *Pseudomonas syringae* pv. *syringae* 61 includes two blocks of genes required for harpin$_{Pss}$ secretion that are arranged colinearly with *Yersinia ysc* homologs. Mol. Plant-Microbe Interact. 8: 733-746.
Huang, H. C., Schuurink, R., Denny, T. P., Atkinson, M. M., Baker, C. J., Yucel, I., Hutcheson, S. W., and Collmer, A. 1988. Molecular cloning of a *Pseudomonas syringae* pv. *syringae* gene cluster that enables *Pseudomonas fluorescens* to elicit the hypersensitive response in tobacco. J. Bacteriol. 170:4748-4756.
Huang, H. C., Xiao, Y., Lin, R.-H., Lu, Y., Hutcheson, S. W., and Collmer, A. 1993. Characterization of the *Pseudomonas syringae* pv. *syringae* 61 *hrp*J and *hrp*I genes: Homology of HrpI to a superfamily of proteins associated with protein translocation. Mol. Plant-Microbe Interact. 6:515-520.
Hutcheson, S. W., Heu, S., Jin, S., Lidell, M. C., Pirhonen, M. U., and Rowley, D. L. 1996. Function and regulation of *Pseudomonas syringae hrp* genes. In: Pseudomonas: Molecular Biology and Biotechnology. American Society for Microbiology, Washington, DC. In press.
Huynh, T., Dahlbeck, D., and Staskawicz, B. J. 1989. Bacterial blight of soybean: Regulation of a pathogen gene determining host cultivar specificity. Science 245:1374-1377.
Innes, R. W., Bent, A. F., Kunkel, B. N., Bisgrove, S. R., and Staskawicz, B. J. 1993. Molecular analysis of avirulence gene *avr*Rpt2 and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. J. Bacteriol. 175: 859-4869.
Jarvis, K. G., Giron, J. A., Jerse, A. E., McDaniel, T. K., Donnenberg, M. S., and Kaper, J. B. 1995. Enteropathogenic *Escherichia coli* contains a putative type III secretion system necessary for the export of proteins involved in attaching and effacing lesion formation. Proc. Natl. Acad. Sci. USA 92:7996-8000.
Jenner, C., Hitchin, E., Mansfield, J., Walters, K., Betteridge, P., Teverson, D., and Taylor, J. 1991. Gene-for-gene interactions between *Pseudomonas syringae* pv. *phaseolicola* and Phaseolus. Mol. Plant-Microbe Interact. 4:553-562.
Jones, D. A., Thomas, C. A., Hammond-Kosack, K. E., Balint-Kurti, P. J., and Jones, J. D. G. 1994. Isolation of the tomato Cf-9 gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789.
Joosten, M. H. A. J., Cozijnsen, T. J., and DeWit, P. G. M. 1994. Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. Nature (London) 367:384-386.
Keen, N. 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. 24:447-463.
Keen, N. T. 1992. The molecular biology of disease resistance. Plant Mol. Biol. 19:109-122.
Keen, N. T., and Buzzell, R. I. 1991. New 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.
Keen, N. T., Tamaki, S., Kobayshi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D., and Staskawicz, B. 1990. Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. Mol. Plant-Microbe Interact. 3:112-121.
Klement, Z. 1977. Cell contact recognition versus toxin action in induction of bacerial hypersensitive reaction. Acta Phytopathol. Sci. Hung. 12:257-261.
Knoop, V., Staskawicz, B. J., and Bonas, U. 1991. The expression of the avirulence gene *avrBs*3 from *Xanthomonas campestris* pv. *vesicatoria* is not under the control of *hrp* genes and is independent of plant factors. J. Bacteriol. 173:7142-7150.
Lidell, M., and Hutcheson, S. W. 1994. Characterization of the *hrpJ* and *U* operons of *Pseudomonas syringae* pv. *syringae* Pss61: Similarity with components of enteric bacteria involved in flagellar biogenesis and demonstration of their role in harpin$_{Pss}$ translocation. Mol. Plant-Microbe Interact. 7:488-497.
Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. Gene cluster of *Pseudomonas syringae* pv. *phaseolicola* controls pathogenicity on bean plants and hypersensitivity on nonhost plants. J. Bacteriol. 168:512-522.
Martin, G. B., Frary, A., Wu, T., Brommonschenkel, S., Chunwongse, J., Earle, E. D., and Tanksley, S. D. 1994. A member of the tomato *Pto* gene family confers sensitivity to fenthion resulting in rapid cell death. Plant Cell 6:1543.
Midlands, S. L., Keen, N. T., Sims, J. J., Midland, M. M., Stayton, M. M., Burton, V., Smith, M. J., Mazzola, E. P., Graham, K. J., and Clardy, J. 1993. The structure of syringolides 1 and 2, novel C-glycosidic elicitors from *Pseudomonas syringae* pv. *tomato*. J. Org. Chem. 58: 2940-2945.
Mindrinos, M., Katagiri, F., Yu, G.-L., and Ausubel, F. M. 1994. The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78:1089-1099.
Murillo, J., Shen, H., Gerhold, D., Sharma, A., Cooksey, D. A., and Keen, N. T. 1994. Characterization of pPT23B, the plasmid involved in syringolide production by *Pseudomonas syringae* pv. tomato PT23. Plasmid 31:275-287.
Napoli, C., and Staskawicz, B. J. 1987. Molecular characterization and nucleic acid sequence of an avirulence gene from race 6 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 169:572-578.
Parsot, C. 1994. *Shigella flexneri*: Genetics of entry and intercellular dissemination in epithelial cells. Pages 217-241 in: Bacterial Pathogenesis of Plants and Animals. Vol. 192. J. L. Dangls, eds. Springer-Verlag, Berlin.
Parsot, C., Menard, R., Gounon, P., and Sansonetti, P. J. 1995. Enhanced secretion through the *Shigella flexneri* Mxi-Spa translocon leads to assembly of extracellular proteins into macromolecular structures. Mol. Microbiol. 16:291-300.
Preston, G., Huang, H. C., He, S. Y., and Collmer, A. 1995. The HrpZ proteins of *Pseudomonas syringae* pvs. *syringae, glycinea*, and *tomato* are encoded by an operon containing *Yersinia ysc* homologs and elicit the hypersensitive response in tomato but not soybean. Mol. Plant-Microbe Interact. 8:717-732.
Ronald, P. C., Salmeron, J., Carland, F. M., and Staskawicz, B. J. 1992. Cloned avirulence gene *avr*Pto induces disease resistance in tomato cultivars containing the Pto resistance gene. J. Bacteriol. 174:1604-1611.
Rosqvist, R., Magnusson, K. E., and Wolf-Watz, H. 1994. Target cell contact triggers expression and polarized transfer of *Yersinia* YopE cytotoxin into mammalian cells. EMBO J. 13:964-972.
Salmeron, J. M., and Staskawicz, B. J. 1993. Molecular characterization and *hrp*-dependence of the avirulence gene *avr*Pto from *Pseudomonas syringae* pv. *tomato*. Mol. Gen. Genet. 239:6-10.
Salmond, G. P. C. 1994. Secretion of extracellular virulence factors by plant pathogenic bacteria. Annu. Rev. Phytopathol. 32:181-200.
Shen, H., and Keen, N. T. 1993. Characterization of the promoter of avirulence gene D from *Pseudomonas syringae* pv. tomato. J. Bacteriol. 175:5916-5924.
Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G., and Jones, J. D. G. 1995. Molecular genetics of plant disease resistance. Science 268:661-667.
Staskawicz, B. J., Dahlbeck, D., and Keen, N. T. 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.
Tamaki, S., Dahlbeck, D., Staskawicz, B. J., and Keen, N. T. 1988.

Characterization and expression of two avirulence genes cloned from *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 170:4846-4854.

Tamaki, S. J., Kobayashi, D. Y., and Keen, N. T. 1991. Sequence domains required for the activity of avirulence genes *avrB* and *avrC* from *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 173:301-307.

Van der Ackerveken, G. F. J. M., Van Kan, J. A. L., and DeWit, P. J. G. M. 1992. Molecular analysis of the avirulence gene avr9 of *Cladosporium fulvum* fully supports the gene-for-gene hypothesis. Plant J. 2:359-366.

VanGijsegem, F., Genin, S., and Boucher, C. 1994. Conservation of secretion pathways for pathogenicity determinants of plant and animal bacteria. Trends Microbiol. 1:175-180.

VanGijsegem, F., Gough, C., Zischek, C., Niqueux, E., Ariat, M., Genin, S., Barberis, P., German, S., Castello, P., and Boucher, C. 1995. The *hrp* gene locus of *Pseudomonas solanacearum,* which controls the production of a type III secretion system, encodes eight proteins related to components of the bacterial flagellar biogenesis complex. Mol. Microbiol. 15:1095-1114.

Wei, Z.-M., Laby, R. J., Zumoff, C. H., Bauer, D. W., He, S. H., Collmer, A., and Beer, S. V. 1992. Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. Science 257:85-88.

Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C., and Baker, B. 1994. The product of the tobacco mosaic virus resistance gene N: Similarity to Toll and the interleukin-1 receptor. Cell 78:1101-1115.

Willis, D. K., Rich, J. J., and Hrabak, E. M. 1991. The *hrp* genes of phytopathogenic bacteria. Mol. Plant-Microbe Interact. 4:132-138.

Xiao, Y., Heu, S., Yi, J., Lu, Y., and Hutcheson, S. W. 1994. Identification of a putative alternate sigma factor and characterization of a multicomponent regulatory cascade controlling the expression of *Pseudomonas syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. J. Bacteriol. 176:1025-1036.

Xiao, Y., and Hutcheson, S. W. 1994. A single promoter sequence recognized by a newly identified alternate sigma factor directs expression of pathogenicity and host range determinants in *Pseudomonas syringae*. J. Bacteriol. 176:3089-3091.

Yang, Y., and Gabriel, D. W. 1995. *Xanthomonas* avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. Mol. Plant-Microbe Interact. 8:627-631.