MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 4, No. 6, pp. 553-562, 1991

Gene-for-Gene Interactions Between *Pseudomonas syringae* pv. *phaseolicola* and *Phaseolus*

Carol Jenner[1], Ed Hitchin[1], John Mansfield[1], Karen Walters[1], Peter Betteridge[2], Dawn Teverson[3], and John Taylor[3]

[1]Department of Biochemistry and Biological Sciences, Wye College, Ashford, Kent, TN25 5AH, U.K.; [2]Shell Research Ltd., Sittingbourne, Kent, ME9 8AC, U.K.; and [3]Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, U.K.
Received 25 January 1990. Revised 21 March 1991. Accepted 2 May 1991.

The gene for cultivar-specific avirulence to *Phaseolus vulgaris* cv. Tendergreen in races 3 and 4 of *Pseudomonas syringae* pv. *phaseolicola* was isolated and sequenced. Genomic clones from libraries of race 3 in pLAFR1 and race 4 in pLAFR3, which altered the phenotype of race 5 from virulent to avirulent in Tendergreen, were found to possess a common ~15-kb region of DNA that contained the determinant of avirulence. Subcloning and insertion mutagenesis with Tn*1000* located an avirulence gene within a 1.4-kb *Bgl*II/*Hin*dIII DNA fragment in races 3 and 4. Comparison of the nucleotide sequences of regions of DNA that confer avirulence confirmed that both races have an identical gene for avirulence (designated *avrPph3*) comprising 801 base pairs (bp) and predicted to encode a cytoplasmic protein of 28,703 Da. A sequence, TGCAACCGAAT, 91% homologous to the motif found in promoter regions of *avrB* and *avrD* from *P. s.* pv. *glycinea* was located 89-99 bp upstream of the start of the open-reading frame 1. Hybridization experiments showed that *avrPph3* was not plasmid-borne and was absent from isolates of *P. s.* pv. *phaseolicola* races 1, 2, 5, 6, 7, and 8, *P. cichorii*, *P. s.* pvs. *coronafaciens*, *glycinea*, *maculicola*, *pisi*, *syringae*, and *tabaci*. Cosegregation studies of crosses between cultivars resistant (Tendergreen) and susceptible (Canadian Wonder) to races 3 and 4 and transconjugants of race 5 confirmed that a gene-for-gene relationship controls specificity in the interaction between Tendergreen and races 3 and 4 of *P. s.* pv. *phaseolicola*.

Additional keywords: halo-blight disease, hypersensitive reaction, pathogenicity, race-specific reaction.

Analysis of the virulence of isolates of *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young *et al.* and the susceptibilities of cultivars and lines of *Phaseolus vulgaris* L. and related species of *Phaseolus* from Africa has provided new insights into specificity in halo-blight disease of bean. Taylor *et al.* (1987) described the identification of race 3 of *P. s.* pv. *phaseolicola*, which was recognized by the induction of a very rapid hypersensitive reaction (HR) in leaves and pods of cultivar Tendergreen (Harper *et al.* 1987; Hitchin *et al.* 1989). Subsequent studies have revealed the existence of at least eight races that may be differentiated based on the presence of five putative genes for avirulence in *P. s.* pv. *phaseolicola* and five matching genes for resistance as outlined in Table 1 (D. Teverson and J. Taylor, *in preparation*).

A determinant of cultivar-specific avirulence in Tendergreen was cloned from the race 3 isolate 1301A (Hitchin *et al.* 1989). A cosmid library of race 3 was prepared in pLAFR1 (Friedman *et al.* 1982), and individual clones were conjugated into *P. s.* pv. *phaseolicola* isolate NCPPB 52, which must now be described as race 5, which is a particularly efficient recipient for triparental matings. Transconjugants were tested for their virulence on cultivars Canadian Wonder, Red Mexican, and Tendergreen (Table 1). In this way, the presence of genes controlling avirulence or virulence could be examined. None of the clones tested altered the race 5 phenotype from avirulent to virulent on Red Mexican (i.e., no virulence factors were found), but two clones with a common ~19-kb *Eco*RI fragment conferred avirulence in Tendergreen (Hitchin *et al.* 1989). A second gene for avirulence, which regulates induction of the HR in Red Mexican, has been cloned from a race 1 isolate of *P. s.* pv. *phaseolicola* (Shintaku *et al.* 1989).

The isolation of genes that control avirulence in phytopathogenic bacteria has clarified the molecular control of race specificity (Staskawicz *et al.* 1987, 1988; Daniels *et al.* 1988; Keen and Staskawicz, 1988; Bonas *et al.*, 1989; Kobayashi *et al.*, 1989, 1990; Keen *et al.*, 1990). However, as pointed out by Crute (1986) there is a need for genetical analyses of both host and parasite in any studies of putative gene-for-gene relationships. Cosegregation of resistance to a race of a pathovar of *P. syringae* and to transconjugants that contain cloned avirulence genes has now been demonstrated in two host-parasite interactions, the infection of pea with *P. s.* pv. *pisi* (Vivian *et al.* 1989) and soybean with *P. s.* pv. *glycinea* (Keen and Buzzell 1991). As part of a program to use the bean-*P. s.* pv. *phaseolicola* interaction as a model system to study determinants of specificity at the classical genetic and molecular levels, we have characterized a gene for avirulence towards Tendergreen in races 3 and 4 and examined the inheritance of resistance in the host plant. The gene has been designated *avrPph3* as an avirulence gene from *P. s.* pv. *phaseolicola* complementary to gene R3 for resistance to halo-blight in *Phaseolus vulgaris*. Use of the conventional capital letter designation

Current address of Ed Hitchin: Institute of Animal Physiology and Genetics, Edinburgh Research Station, Roslin, Scotland, U.K.

Address correspondence to John Mansfield.

Nucleotide and/or amino acid sequence data is to be submitted to GenBank, EMBL, and DDBJ as accession number J03704.

for the gene, according to Demerec *et al.* (1966) (i.e., *avrA*), has been avoided to prevent possible confusion with *avrA* from *P. s.* pv. *glycinea.*

MATERIALS AND METHODS

Bacteria and plasmids. Principal bacterial isolates, cosmids, and plasmids used are noted in Table 2; additional strains and constructs are given in Hitchin *et al.* (1989). Isolates and transconjugants of *P. s.* pv. *phaseolicola* were grown on King's medium B agar at 25° C and *Escherichia coli* strains on Luria-Bertani (LB) agar or in LB broth at 37° C (King *et al.* 1954; Miller 1972). Antibiotics, obtained from Sigma (Poole, Dorset), were usually used at the following concentrations ($\mu g\ ml^{-1}$): nalidixic acid, Nal (50); streptomycin, Sm (25); tetracycline, Tc (15), and ampicillin, Ap (40).

Cloning procedures. A genomic library of race 4 was prepared with the basic methods described by Hitchin *et al.* (1989), except that total DNA from *P. s.* pv. *phaseolicola* was partially digested with *Sau*3AI and fragments of 20–30 kb were ligated into the *Bam*HI site of pLAFR3 (Staskawicz *et al.* 1987). The library was screened for determinants of avirulence by conjugation of individual clones into race 5 with the helper plasmid pRK2013 (Figurski and Helinski 1979). Transconjugants were tested for pathogenicity in pods of Canadian Wonder, Red Mexican, and Tendergreen (Hitchin *et al.* 1989).

DNA of pRK290, pLAFR3, and pIJ3200 was isolated from *E. coli* HB101 by the cleared lysate procedure (Clewell and Helinski 1969) and was purified by CsCl/EtBr gradient centrifugation (Maniatis *et al.* 1982). Restriction fragments from pPPY310 and pPPY420 were cloned into pRK290 (*Bgl*II digests only), pLAFR3, or pIJ3200 after recovery of DNA from agarose gels using Geneclean (Stratech Scientific, Luton). Ligations were performed with T4 DNA ligase (Boehringer Mannheim Corp., Lewes, Sussex) according to the manufacturer's instructions. Avirulence genes were also subcloned into pLAFR3 from pPPY423 by religation of a *Hin*dIII/*Bgl*II double digest. Small scale preparations of plasmids were done with the methods of Birnboim and Doly (1979) or Holmes and Quigley (1981). Basic procedures for handling DNA were as described in Maniatis *et al.* (1982) or Ausubel *et al.* (1987).

Insertion mutagenesis with Tn*1000*. The 8.5-kb *Bgl*II fragment of race 3 DNA cloned from pPPY310 into pRK290 to form pPPY311 was found to confer cultivar-specific avirulence. The avirulence gene was located on pPPY311 by Tn*1000* insertions (Auyer 1978). DNA of pPPY311 was transformed into the *E. coli* F^+ strain K12S and conjugated into the F^- strain AB1157; positive selection for transconjugants was with streptomycin (25 $\mu g\ ml^{-1}$) and tetracycline (10 $\mu g\ ml^{-1}$). Plasmid DNA derived from colonies of AB1157 (pPPY311) was isolated, and the Tn*1000* insertions were localized by restriction mapping with *Bam*HI and *Hin*dIII. Only about 50% of the plasmids recovered were found to contain Tn*1000*. Those with insertions in cloned DNA were mobilized into race 5 by triparental mating and transconjugants were tested for virulence.

Plants and pathogenicity tests. Leaves and pods of cultivars of French bean were inoculated as previously described (Harper *et al.* 1987; Hitchin *et al.* 1989). The compatible interaction in pods is expressed by the development of water-soaked lesions at sites of stab-inoculation and in leaves by the collapse of infiltrated tissue during the second day after inoculation and formation of chlorotic halos after 5 days. Incompatibility to races 3 and 4 is expressed by the HR observed as the formation of sunken brown lesions in pods and the collapse of infiltrated leaf tissue within 24 hr of inoculation (Harper *et al.* 1987).

The inheritance of resistance was examined in the F_1 and F_2 populations of crosses between Canadian Wonder (susceptible) and Tendergreen (resistant to races 3 and 4). Ten families were derived from crosses with both cultivars as the female parent. Crossing was performed as described by Bliss (1980). Crosses with Canadian Wonder as the female parent were more frequently successful. In the analysis of the segregating F_2 progeny, all strains and transconjugants examined were inoculated into at least three pods detached from each plant.

DNA hybridization experiments. DNA of genomic clones and plasmids containing subcloned fragments was recovered from *E. coli* by the cleared lysate method and purified by CsCl/EtBr gradient centrifugation. Plasmid DNA was isolated from *P. s.* pv. *phaseolicola* by the methods of Birnboim and Doly (1979) and Kado and Liu (1981). Total DNA was obtained from races of *P. s.* pv. *phaseolicola*, pathovars of *P. syringae*, and *P. cichorii* with the CTAB procedure described in Ausubel *et al.* (1987) and Walters *et al.* (1990).

For genomic Southerns the 3.4-kb *Bam*HI fragment containing *avrPph3* and the predominantly intragenic 558-bp *Ssp*I fragment were isolated from pPPY3001 and pPPY4001, respectively, and ^{32}P-labeled in low melting point agarose by the random priming method (Feinberg and Vogelstein 1983) by using a Pharmacia oligolabeling kit (Pharmacia Ltd., Milton Keyner, Bucks.). In other

Table 1. Proposed gene-for-gene relationships among bean cultivars and races of *Pseudomonas syringae* pv. *phaseolicola*

Cultivars/lines and resistance (*R*) genes	Races and avirulence (*A*) genes							
	1(*A1*)	2(*A2*)	3(*A3*)	4(*A2* + *A3*)	5(*A1* + *A2* + *A4*)	6(no *A* gene)	7(*A1* + *A2*)	8(*A5*)
Canadian Wonder (no *R* genes)	S[a]	S	S	S	S	S	S	S
Tendergreen (*R3*)	S	S	R	R	S	S	S	S
Red Mexican UI3 (*R1* + *R4*)	R	S	S	S	R	S	R	S
P. acutifolius 1072 (*R2* + *R4*)	S	R	S	R	R	S	R	S
A53 (*R3* + *R4*)	S	S	R	R	R	S	S	S
A43 (*R2* + *R3* + *R4* + *R5*)	S	R	R	R	R	S	R	R

[a] S or R, susceptible or resistant reactions in leaves characterized by the development of water-soaked lesions or limited, hypersensitive responses, respectively.

Table 2. Bacterial strains and plasmids used in this study

Strain		Relevant characteristics	Source or reference
Pseudomonas syringae pv. *phaseolicola*			
Principal isolates used			
52N	Race 5[a] Nal^r		Hitchin *et al.* 1989
1301AN	Race 3 Nal^r		Hitchin *et al.* 1989
1302AN	Race 4 Nal^r		J.D. Taylor[b]
Additional isolates used in hybridization experiments			
1281A	Race 1		D. Teverson[b]
882	Race 2		D. Teverson
1375A	Race 5		D. Teverson
1299A	Race 6		D. Teverson
1449B	Race 7		D. Teverson
2656A	Race 8		D. Teverson
P. cichorii			
2379		Lettuce pathogen	NCPPB[c]
P.s. pv. *coronafaciens*			
1354		Oat pathogen	Harper *et al.* 1987
P.s. pv. *glycinea*			
1416A		Soybean pathogen	J. D. Taylor
P.s. pv. *maculicola*			
1820		Brassica pathogen	NCPPB
P.s. pv. *syringae*			
281		Lilac pathogen	NCPPB
E. coli			
AB1157		Sm^r, F$^-$	Howard-Flanders *et al.* 1964
K12S		F$^+$	Bachmann 1972
HB101		Sm^r, *recA pro gal*	Boyer and Roulland-Dussoix 1969
Helpers and vectors			
pRK2013		Km^r, Tra$^+$, Mob$^+$, ColEI replicon, helper plasmid	Figurski and Helinski 1979
pRK290		Tc^r, Tra$^-$, Mob$^+$, RK2 replicon	Ditta *et al.* 1980
pLAFR1		Tc^r, Tra$^-$, Mob$^+$, RK2 replicon, cosmid	Friedman *et al.* 1982
pLAFR3		pLAFR1 containing *Hae*II fragment of pUC8	Staskawicz *et al.* 1987
pIJ3200		pLAFR3 containing pBluescript polylinker	Liu *et al.* 1990
pBluescriptII SK+		Ap^r, ColEI replicon, multiple cloning and priming sites	Stratagene (Cambridge, U.K.)
pBR322		Ap^r, Tc^r, ColEI replicon	Bolivar *et al.* 1977
Clones containing the avirulence gene			
pPPY310		pLAFR1-based clone of race 3, previously called p3A1000	Hitchin *et al.* 1989
pPPY311		8.5-kb *Bgl*II fragment from pPPY310 in pRK290	This study
pPPY312		3.4-kb *Bam*HI fragment from pPPY310 in pLAFR3	This study
pPPY313		1.9-kb *Pst*I/*Bgl*II fragment from pPPY312 in pIJ3200	This study
pPPY410 and pPPY420		pLAFR3-based clones of race 4	This study
pPPY422		3.4-kb *Bam*HI fragment from pPPY420 in pLAFR3	This study
pPPY423		2.1-kb *Pst*I fragment from pPPY422 in pLAFR3	This study
pPPY424		1.4-kb *Bgl*II/*Hin*dIII fragment from pPPY423 in pLAFR3	This study
pPPY3001		3.4-kb *Bam*HI fragment from pPPY312 in pBluescriptII SK+, internal *Bgl*II site near the vector *Xho*I site	This study
pPPY3002		As pPPY3001 but with *Bam*HI fragment in opposite orientation	This study
pPPY4001		2.1-kb *Pst*I fragment from pPPY423 in pBR322	This study
Clones without avirulence activity			
pPPY425		0.54-kb *Bgl*II/*Hin*dIII fragment of pPPY424	This study
pPPY426		0.86-kb *Hin*dIII fragment of pPPY424	This study

[a] This isolate was previously described as race 1 (Hitchin *et al.* 1989). All isolates of *P. s.* pv. *phaseolicola* were differentiated into races according to Table 1.
[b] Horticulture Research International, U.K.
[c] National Collection of Plant Pathogenic Bacteria, Harpenden, U.K.

experiments to confirm restriction maps, fragments from various clones were labeled in the same way.

DNA digested with the appropriate restriction endonucleases was electrophoresed in 0.8% agarose gels before Southern transfer onto Hybond nylon membrane (Amersham International, Aylesbury, Bucks.). Hybridizations were performed in 5× SSPE (0.9 M NaCl, 0.05 M sodium phosphate, 0.005 M ethylenediaminetetraacetic acid, pH 7.7), 5× Denhardt's solution (Maniatis *et al.* 1982), 0.5% sodium dodecyl sulfate (SDS), and 20 $\mu g\ ml^{-1}$ salmon sperm DNA with gentle shaking for 18 hr at 65° C. Blots were washed twice in 2× SSPE, 0.1% SDS at room temperature for 10 min and then in 1× SSPE, 0.1% SDS at 65° C for 15 min before preliminary autoradiography. A subsequent high stringency wash was done with 0.1× SSPE, 0.1% SDS at 65° C for 15 min before exposure to X-ray film. Previously hybridized blots were stripped of probes in 0.4 M NaOH (30 min at 45° C) and then neutralized in 0.1× SSC (15 mM NaCl, 1.5 mM trisodium citrate) 0.1% SDS, 0.2 M Tris-HCl, pH 7.5, for 15 min at 45° C.

DNA sequencing. The 3.4-kb *Bam*HI fragment from pPPY312 was cloned in both orientations into pBluescriptII SK+ to produce pPPY3001 and pPPY3002. These plasmids were linearized with *Kpn*I and *Xho*I, followed by exonuclease III digestion at the *Xho*I terminus for various periods of time to produce a series of nested deletions. Single-stranded DNA ends were removed by mung bean nuclease digestion prior to recircularization of the plasmids. Experimental details are as described in the Stratagene handbook.

The 2.1-kb *Pst*I fragment from pPPY423 was cloned into pBR322 at the *Pst*I site within the β-lactamase gene to produce the plasmid pPPY4001. Tn*1000* insertions in this plasmid were generated as described previously for pPPY311. The positions and orientations of insertions were deduced from *Bam*HI and *Pst*I digestions, and six different clones were chosen as useful priming sites for sequencing reactions.

The DNA sequence was determined by the dideoxynucleotide method (Sanger *et al.* 1977) using T7 DNA polymerase (Pharmacia) and modified T7 DNA polymerase (Sequenase version 2.0, Pharmacia) and deoxyadenosine 5′-([α-^{35}S] thio) triphosphate (1,200 Ci/mmol; Amersham), according to the manufacturer's protocol for CsCl gradient-purified plasmid DNA. Polyacrylamide gels contained 6% acrylamide and 7 M urea. Oligonucleotide primers were produced with an Applied Biosystems (Warrington, Lancs.) DNA synthesizer. The M13 and T7 priming sites on pBluescriptII SK+ were used on the nested deletion series derived from pPPY3001 and pPPY3002 to obtain the sequence of the gene from race 3. Primers corresponding to 53-37 bases from the γ end and 48-32 bases from the δ end of Tn*1000*, and to pBR322 bases 3556-3571 and 3638-3624 were used on the Tn*1000* insertion clones of pPPY4001 to obtain the sequence of the gene from race 4. Additional 17-mer primers were made as required for sections not obtainable from the series of deletions or Tn*1000* insertions. Occasionally it was necessary to substitute deoxyinosine triphosphate for deoxyguanosine triphosphate to obtain the sequence in areas of "compressions."

The sequences were analyzed with the PC-Gene version 6.26 (Genofit) computer package on an IBM PC-2 personal computer. The EMBL (24), GenBank (65), SwissProt (15), and Brookhaven databases were searched for homology to the resulting DNA and predicted protein sequences. The published sequences of other avirulence genes were also compared individually with *avrPph3*.

RESULTS

Isolation of a gene for avirulence from race 4. Two of the 960 clones examined (pPPY410 and pPPY420) from the pLAFR3-based genomic library of race 4 conferred avirulence towards Tendergreen on the race 5 *P. s.* pv. *phaseolicola* strain 52. Restriction mapping indicated that the clones contained about 20 kb of race 4 DNA in common. Transconjugants of race 5 containing pPPY410 or pPPY420 caused a rapid HR in leaves as well as pods of Tendergreen. During initial screening, another clone appeared to confer virulence to race 5 on Red Mexican, because the transconjugant caused water-soaked lesions to develop in pods of the three test cultivars. However, after repeated mating the clone was found to have no effect on pathogenicity. Analysis of the original recipient bacterium cured of the clone by growth in the absence of tetracycline selection showed that it was a spontaneous mutant of race 5 that had reduced ability to cause the HR on Red Mexican.

Location of the gene for avirulence in genomic clones from race 3 and race 4. The genomic clones containing determinants of avirulence from races 3 and 4 were analyzed by restriction mapping and hybridization experiments. The clones contained a region of about 15 kb of homologous DNA. Restriction fragment length polymorphism was indicated by the presence of an additional *Eco*RI site in race 4 DNA as noted in Figure 1.

Different subcloning strategies were adopted with DNA from race 3 in pPPY310 and from race 4 in pPPY420. Thus, *Bgl*II and *Bam*HI fragments were initially cloned from insert DNA in pPPY310 and pPPY420, respectively.

The 8.5-kb *Bgl*II fragment from pPPY310 subcloned into pRK290 (pPPY311) was found to be fully active. None of the other *Bgl*II fragments from pPPY310 affected the virulence of race 5 to cultivars of bean. Mutagenesis of pPPY311 with Tn*1000* located the determinant of avirulence to a 0.9-kb region spanning the *Hin*dIII site within a 3.4-kb *Bam*HI fragment (Fig. 2). Most insertions within the 0.9-kb region abolished HR-inducing activity in Tendergreen but one, at the left border, caused an intermediate phenotype, a mixture of water-soaking and tissue browning, to develop.

Initial subcloning from pPPY420 showed that only the 3.4-kb *Bam*HI fragment (cloned as pPPY422) conferred avirulence. Further subcloning (Fig. 2) from pPPY310 and pPPY420 with *Hin*dIII, *Pst*I, and *Bgl*II/*Hin*dIII digests to give pPPY313, 423, and 424 (Table 2) demonstrated that the 1.4-kb *Bgl*II/*Hin*dIII fragment containing the region of pPPY310 defined by Tn*1000* insertions alone conferred avirulence to Tendergreen.

Gene-for-gene interaction. Subcloning and transposon mutagenesis indicated that a single gene controlled the avirulence of races 3 and 4 to Tendergreen. To test the hypothesis that the gene for avirulence may have a matching gene for resistance in Tendergreen, the virulence of wild type races 3, 4, and 5, and transconjugants of race 5 containing the cloned avirulence gene was examined on segregating F_2 progeny. After the initial crosses between Canadian Wonder and Tendergreen, all F_1 progeny were susceptible to race 5 but resistant to races 3 and 4, and transconjugants expressing the avirulence gene. Results of a typical pathogenicity test of pods from F_2 progeny are illustrated in Figure 3. In the pods expressing resistance (A,B,D), the HR has developed at sites inoculated with

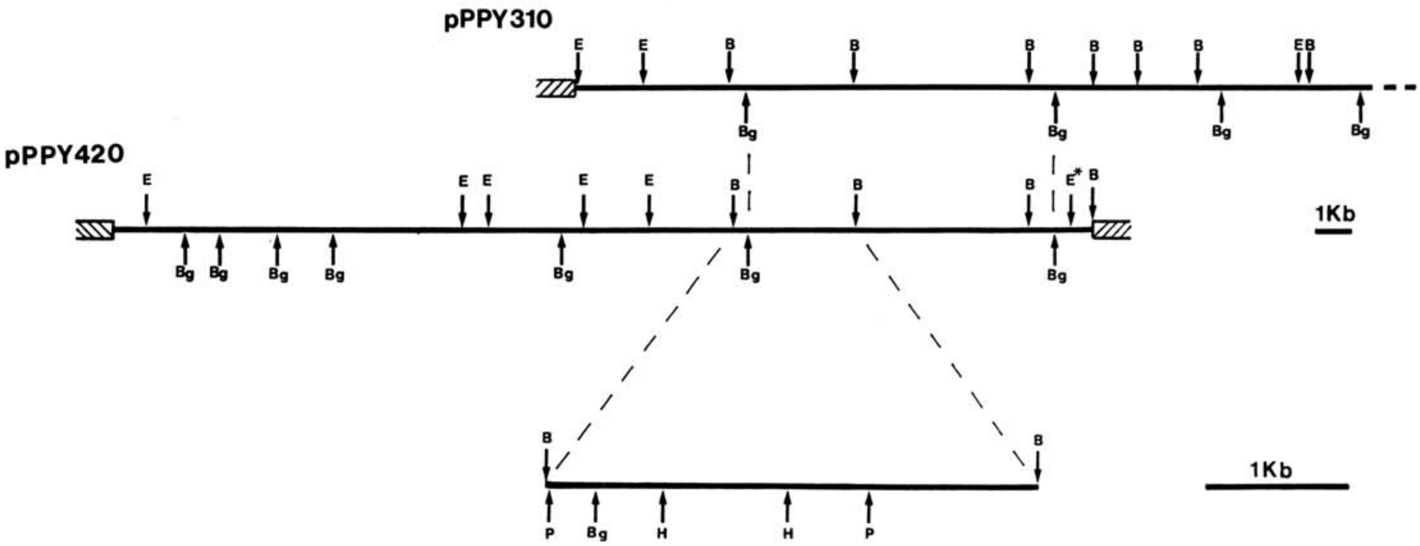

Fig. 1. Restriction maps of pPPY310 and pPPY420. Sites for *Bam*HI (B), *Bgl*II (Bg), and *Eco*RI (E) are given in the genomic clones and *Hin*dIII (H), and *Pst*I (P) in the common *Bam*HI fragment. The *Eco*RI site unique to pPPY420 is marked with an asterisk.

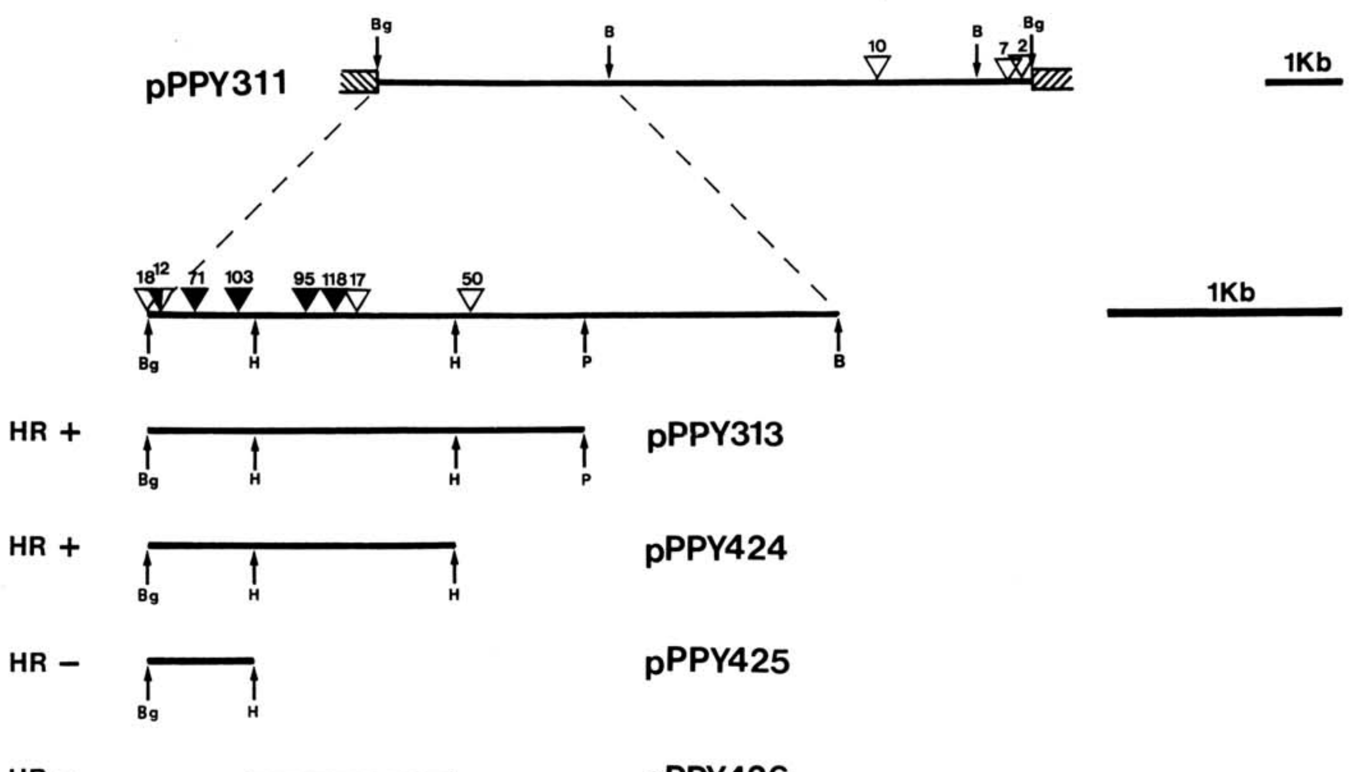

Fig. 2. Transposon mutagenesis of pPPY311 and subcloning of pPPY310 and pPPY420 to locate the avirulence gene. Sites of Tn*1000* insertions preventing or having no effect on ability to cause the hypersensitive reaction (HR) in cultivar Tendergreen are indicated by ▼ or ▽, respectively. Mixed symptoms were produced by transconjugants harbouring insertion 12, marked ▼.

races 3 and 4, and all of the transconjugants, but a water-soaked (susceptible) lesion has been formed at the site of inoculation with race 5. In the other pod (C), all inoculations have produced water-soaked lesions. Without exception, when progeny expressed the HR to race 3, they gave the same reaction to race 4 and all transconjugants. That is, cosegregation for resistance to races 3 and 4 and transconjugants was demonstrated.

Numbers of resistant and susceptible progeny in families derived from either Canadian Wonder or Tendergreen as the female parent are given in Table 3. Few F_2 seeds were recovered from each family but, overall, numbers of resistant and susceptible progeny were 95 and 26, respectively, giving a good fit to the 3:1 ratio ($\chi^2 = 0.796$; $P = 0.50$–0.30), which was expected if resistance to races 3 and 4 in Tendergreen is controlled by a single dominant gene.

During the analysis of symptom development on numerous pods, variations were observed in the form of water-soaked lesions produced by certain transconjugants compared with the race 5 recipient strain or isolates of race 3 or 4. Particularly on young pods, the lesions produced by transconjugants containing small, subcloned fragments were noticeably smaller and more sunken, although they remained water-soaked and did not develop any browning during the first four days after inoculation. The phenomenon was quantified after the inoculation of detached pods of Canadian Wonder, and the results from one experiment are given in Table 4.

Sequence analysis. Sequences of 3064 and 2081 bp were determined from within the 3.4-kb *Bam*HI and 2.1-kb *Pst*I fragments from races 3 and 4, respectively. The sequences differed only in the presence of cytosine at base 724 in race 4 instead of adenine as found in race 3. A single open-reading frame, ORF1, of 801 bp, was located within the sequence of the region defined by Tn*1000* mutagenesis and subcloning to control avirulence (Fig. 4). An incomplete, possible coding region (ORF2) was identified downstream, beginning at base 2348 (ATG). ORF1 was therefore identified as *avrPph3*.

There were no sequences similar to those of *E. coli*-like transcription promoters upstream of ORF1 (*viz.* −35

Table 3. Cosegregation of resistance in the F_2 generation of the cross of cultivars Canadian Wonder × Tendergreen to strains of race 3 and race 4 and to transconjugants[a] of race 5 that harbor the cloned avirulence gene

Original female parent	Numbers observed[b]		χ^2 analysis		χ^2 value
	Resistant	Susceptible	Source	df	(3:1)
Tendergreen (four families[c])	37	8	Deviations	1	1.25 ($P = 0.3$–0.2)
Canadian Wonder (six families)	58	18	Deviations	1	0.0675 ($P = 0.8$–0.7)
Combined	95	26	Deviations	1	0.796 ($P = 0.5$–0.3)
			Heterogeneity	1	0.579 ($P = 0.5$–0.3)

[a] For details of the transconjugants examined see Figure 3.
[b] Resistance was expressed by the formation of restricted sunken brown lesions and susceptibility by the development of spreading water-soaked lesions with no browning within four days of inoculation.
[c] Each family made up the progeny from a single F_1 plant.

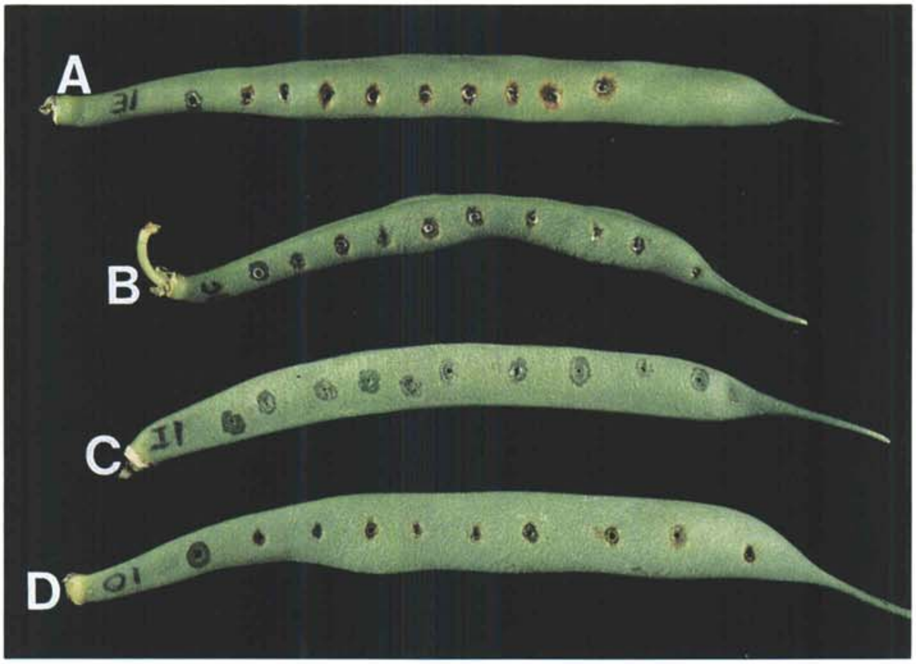

Fig. 3. Pathogenicity tests on pods from the F_2 progeny of a cross between cultivars Canadian Wonder and Tendergreen. The pods were from plants A, B, C, and D from family 1. Dark green water-soaked or sunken brown hypersensitive lesions developed 5 days after inoculation with (from left to right) wild types: races 5, 4, and 3; transconjugants: race 5 with genomic clone pPPY420 and subclones of *Pst*I, 2.1 kb (pPPY423), and *Bgl*II/*Hin*dIII, 1.4 kb (pPPY424), and race 5 with genomic clone pPPY310 and subclones of *Bgl*II, 8.5 kb (pPPY311); *Bam*HI, 3.4 kb (pPPY312), and *Pst*I/*Bgl*II, 1.9 kb (pPPY313).

TNTTGACA and −10 TATAAT; Rosenberg and Court 1979), nor for various *P. putida* consensus promoters (Inouye *et al.* 1984; Mermod *et al.* 1984; Minton and Clarke 1985). There were no sequences showing similarity to rho-independent transcription termination signals (Brosius *et al.* 1981). The sequence TGGAACCGAAT, located 89-99 bp upstream of the start of ORF1, was almost identical to TGGAACCTAAT and TGGAACCAAAT found within the promoter regions of *avrB* and *avrD*, respectively, from *P. s.* pv. *glycinea*. Each of these sequences shares homology with the conserved motif TGA/CAANC within the "harp box" upstream of genes regulated by *hrpS* (Fellay *et al.* 1991). A purine-rich sequence resembling a ribosome-binding site (Shine and Dalgarno 1974) was also situated just upstream of the ATG codon.

Computer analysis of the amino acid sequence encoded by ORF1 could find no sequences indicative of N-terminus secretory signal peptides nor of sufficient hydrophobicity as to be integral or associated with membranes. There was no similarity to amino acid sequence motifs found in DNA-

```
       610        620        630        640        650        660
                                             BglII
TTTCCACTAGGACGTCGAAATTGCGGAAGGTTATCAACGGCGAGATCTTTCAGGGCGGCG

       670        680        690        700        710        720
GTGATCCCCTTCACAACCTCATAGCTCAGTCCTACCCAAATTTTTTGGAACCGAATGGGT

  *    730        740        750        760        770        780
  A
CAGCTGGACACTTAGATACCGATTCTAGGTACGTAGGCCAAATTTTTAGTCTAAGAACCA

       790        800        810        820        830        840
CATCGGAAATGTTTTGAGGTTTATATGAAAATAGGTACGCAGGCCACCTCGCTGGCCGTT
                          METLysIleGlyThrGlnAlaThrSerLeuAlaVal

       850        860        870        880        890        900
TTGCACAATCAGGAGTCGCATGCTCCACAGGCACCAATTGCTGTAAGACCTGAGCCTGCT
LeuHisAsnGlnGluSerHisAlaProGlnAlaProIleAlaValArgProGluProAla

       910        920        930        940        950        960
CACGCAATCCCAGAAATTCCTTTAGACCTCGCCATTAGGCCTAGAACACGTGGCATACAC
HisAlaIleProGluIleProLeuAspLeuAlaIleArgProArgThrArgGlyIleHis

       970        980        990       1000       1010       1020
                                                          XbaI
CCATTCCTGGCAATGACACTGGGCGATAAAGGGTGTGCATCCTCTTCAGGCGTAAGTCTA
ProPheLeuAlaMETThrLeuGlyAspLysGlyCysAlaSerSerSerGlyValSerLeu

      1030       1040       1050       1060       1070       1080
                     HindIII
GAAGATGATTCTCACACGCAAGTAAGCTTGAGTGATTTTTCTGTGGCTTCAAGAGATGTT
GluAspAspSerHisThrGlnValSerLeuSerAspPheSerValAlaSerArgAspVal

      1090       1100       1110       1120       1130       1140
       SspI
AATCATAACAATATTTGTGCCGGCCTCTCCACGGAGTGGCTTGTAATGAGTAGTGACGGA
AsnHisAsnAsnIleCysAlaGlyLeuSerThrGluTrpLeuValMETSerSerAspGly

      1150       1160       1170       1180       1190       1200
GATGCAGAATCACGCATGGACCATCTCGACTATAACGGAGAGGGACAAAGCAGGGGAAGC
AspAlaGluSerArgMETAspHisLeuAspTyrAsnGlyGluGlyGlnSerArgGlySer

      1210       1220       1230       1240       1250       1260
GAAAGGCATCAGGTTTATAATGATGCGTTACGTGCCGCACTATCAAATGATGATGAAGCA
GluArgHisGlnValTyrAsnAspAlaLeuArgAlaAlaLeuSerAsnAspAspGluAla

      1270       1280       1290       1300       1310       1320
CCTTTTTTCACAGCCAGTACCGCTGTTATCGAAGATGCTGGTTTCTCTCTACGCAGGGAA
ProPhePheThrAlaSerThrAlaValIleGluAspAlaGlyPheSerLeuArgArgGlu

      1330       1340       1350       1360       1370       1380
CCAAAAACTGTTCATGCGTCAGGTGGCAGTGCGCAATTGGGCCAGACAGTAGCCCATGAT
ProLysThrValHisAlaSerGlyGlySerAlaGlnLeuGlyGlnThrValAlaHisAsp

      1390       1400       1410       1420       1430       1440
GTAGCTCAATCTGGTAGAAAACATTTGCTGAGCTTACGTTTTGCAAACGTTCAAGGACAC
ValAlaGlnSerGlyArgLysHisLeuLeuSerLeuArgPheAlaAsnValGlnGlyHis

      1450       1460       1470       1480       1490       1500
GCAATCGCGTGCTCCTGTGAGGGAAGTCAGTTCAAGCTGTTTGACCCTAACCTCGGTGAG
AlaIleAlaCysSerCysGluGlySerGlnPheLysLeuPheAspProAsnLeuGlyGlu

      1510       1520       1530       1540       1550       1560
TTTCAATCATCCCGCAGCGCAGCACCGCAGCTCATTAAGGGCTTAATAGATCACTATAAT
PheGlnSerSerArgSerAlaAlaProGlnLeuIleLysGlyLeuIleAspHisTyrAsn

      1570       1580       1590       1600       1610       1620
AGCTTAAACTACGACGTAGCTTGCGTAAACGAGTTTAGAGTTTCGTAATTTAATCTTTTA
SerLeuAsnTyrAspValAlaCysValAsnGluPheArgValSer---

      1630       1640       1650       1660       1670       1680
                           SspI
AGAATCTAAATAAACTGTAACGCCGGCAATATTTACCACTCACATGCGAAAAACTTACGC

      1690       1700       1710       1720       1730       1740
AACATCGCCGCATTCCTAAAACAAGTCTGTCTCGAATCGACTCCATTGTTAGTGGAAGCA

      1750       1760       1770       1780       1790       1800
AAAAACCCAGCCTGAAAGGGCTGGGCCTGATACCAACTCATCCTCAAAGAAAACAATCCA

      1810       1820       1830       1840       1850       1860
CACACACATAAGAACTTGGTGCCAGAAACAAAGACAATCTTAGTTTAACTCGTGAAAAAG

      1870       1880       1890       1900       1910       1920
                                                 HindIII
GCATATATCAATTGGCGGAAAATCATGAAACCGCTTAGAATCTTTTAAAGCTTTCCTATG
```

Fig. 4. Sequence of the *avrPph3* gene (ORF1) and its deduced amino acid sequence derived from the 3.4-kb *Bam*HI fragment cloned from *Pseudomonas syringae* pv. *phaseolicola* race 3 isolate 1301A. The single base difference in race 4 isolate 1302A at position 724 is marked as are selected restriction endonuclease sites, a possible Shine-Dalgarno sequence (underlined) and the sequence with close homology to the motif found in the promoter regions of *avrB* and *avrD* (boxed).

binding proteins. A search of DNA and protein databases revealed no sequences with significant homology to ORF1 or its deduced protein.

Hybridization to pathovars and races. Autoradiographs from Southern blot analyses of total DNA are illustrated in Figure 5. Sequences hybridizing to the 3.4-kb *Bam*HI fragment were found in races 3 and 4 of *P. s.* pv. *phaseolicola* as expected and also to race 8 under conditions of high stringency. Hybridizing bands were also found in digests of DNA from pathovars *syringae* and *tabaci*. When the 558-bp *Ssp*I fragment was used as a probe, hybridization was only observed to DNA from races 3 and 4 even after washing at low stringency (1× SSPE). Polymorphism expected from analysis of genomic clones was clearly observed in *Eco*RI digests of total DNA. Hybridization to the *Ssp*I fragment was also observed with other isolates of races 3 and 4 from diverse origins (data not shown). Neither probe hybridized to plasmid DNA from races 3 or 4 isolated by the methods of Birnboim and Doly (1979) or Kado and Liu (1981).

Table 4. Formation of sunken water-soaked lesions in detached pods of cultivar Canadian Wonder four days after inoculation[a]

Isolate or transconjugant	Mean lesion diameter ± SEM[b] (mm)	Total sunken score[c] (range)
Race 5	4.7 ± 1.1	3 (0–1)
Race 5 (pLAFR3)	4.6 ± 0.7	3 (0–1)
Race 5 (pPPY400)[d]	4.1 ± 0.8	2 (0–1)
Race 5 (pPPY420)	3.8 ± 0.4	4 (0–2)
Race 5 (pPPY423)	3.3 ± 0.8	10 (1–2)
Race 5 (pPPY424)	2.9 ± 0.5	7 (0–3)

[a] Inoculations were made into six pods each with unexpanded seeds; inocula were distributed so that, overall, no variation was introduced by their position on the pod.
[b] Lesion diameter was measured at its widest point.
[c] The degree of sunkenness was scored on a 0–3 scale: 0, no sunken areas; 1, some sunken tissue; 2, more than 50%; and 3, 100% of the water-soaked lesion sunken below the pod surface. The total score recorded for each isolate or transconjugant and the range observed at individual inoculation sites are given.
[d] pPPY400 is a genomic clone from the race 4 library that does not affect the virulence of race 5. Details of other plasmids are given in Table 3.

DISCUSSION

The discovery of isolates of *P. s.* pv. *phaseolicola* that give the same HR phenotype on bean cultivar Tendergreen but are differentiated by their abilities to colonize *Phaseolus acutifolius* line 1072 and the *P. vulgaris* line A43 has allowed a critical appraisal of the genetic basis underlying cultivar-specific avirulence in halo-blight disease. Analysis of the *Sau*3AI genomic library of race 4 prepared in pLAFR3 failed to yield clones altering the phenotype of race 5 from avirulent to virulent on cultivar Red Mexican. Similar results were obtained with race 3 using an *Eco*RI library prepared in pLAFR1 and, therefore, potentially less representative (Hitchin *et al.* 1989). Thus, there is no evidence for the presence of genes in races 3 and 4 that confer cultivar-specific virulence towards Red Mexican. The search for the putative *avrPph2* in race 4 (Table 1) has been hampered by the absence of a *P. s.* pv. *phaseolicola* isolate virulent on A43 (e.g., races 3 or 6), which functions as an efficient recipient in triparental matings.

Subcloning and insertion mutagenesis with Tn*1000* located the *avrPph3* gene within a 1.4-kb *Bgl*II/*Hin*dIII fragment in races 3 and 4. Comparison of the nucleotide sequences of the regions of DNA that confer avirulence confirmed that they both have an identical gene for avirulence, ORF1 comprising 801 bp and predicted to encode a protein of 28,703 Da. The hydrophilic nature of the encoded protein suggests that it is cytoplasmic and therefore

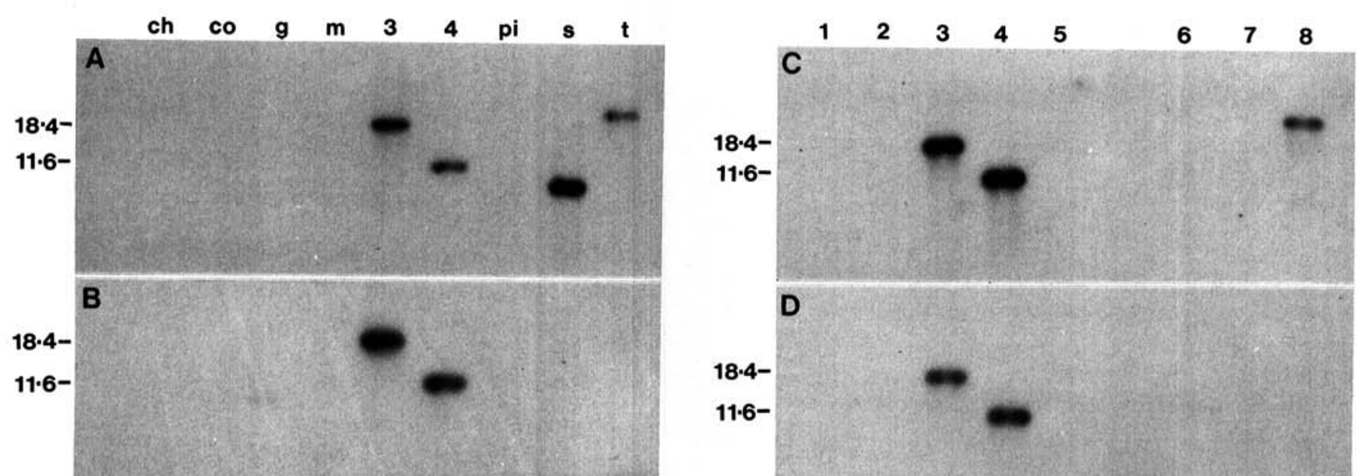

Fig. 5. Detection of hybridizing sequences in races of *Pseudomonas syringae* pv. *phaseolicola* and other pseudomonads. Southern blot analyses of total DNA from isolates of: **A** and **B,** *P. cichorii* (ch), *P. s.* pvs. *coronafaciens* (co), *glycinea* (g), *maculicola* (m), *phaseolicola* race 3 (3) and race 4 (4), *pisi* (pi), *syringae* (s), and *tabaci* (t); **C** and **D,** *P. s.* pv. *phaseolicola* races 1–8. Isolates are listed in Table 2. A and C show hybridization to the 3.4-kb *Bam*HI fragment containing *avrPph3*; B and D show hybridization to the 558 bp *Ssp*I fragment from the 3′ end of the avirulence gene (Fig. 4). Total DNA (about 5 μg) was digested with *Eco*RI and electrophoresed in 0.8% agarose, probes were labeled with [^{32}P]. No hybridizing bands were seen on regions of the blots not shown. Sizes of the hybridizing bands in digests of races 3 and 4 are marked in kilobases. A *Hin*dIII digest of lambda DNA was run between tracks of digests of races 5 and 6 DNA.

probably not directly recognized by any receptor in the plant. Preliminary analysis of the sequencing data for the gene cloned from race 4 reported briefly by Jenner *et al.* (1989) was found to be incorrect.

With the possible exception of *avrBs3* (Bonas *et al.* 1989) all the avirulence genes sequenced to date also appear to encode cytoplasmic proteins and it has been shown that for *avrC* and *avrD* (Tamaki *et al.* 1988; Keen *et al.* 1990), their protein products do not elicit the HR when injected into leaves of the appropriate cultivar of soybean. A cultivar-specific elicitor has been isolated from *E. coli* overexpressing *avrD* (Keen *et al.* 1990), but the links between *avrD*, the elicitor, and the corresponding resistance gene (*Rpg4* in soybean) remain unclear. We have been unable to express the *avrPph3* protein with the vectors pUC128 and pUC129 (*unpublished results*).

The function of avirulence genes in bacteria is unknown. A link with pathogenicity has been tentatively assigned to *avrBs2* (Kearney and Staskawicz 1990). Homology between different genes is rare, each sequence tending to be unique. The nucleic acid and amino acid sequences of ORF1 were compared directly with those of *avrA* (Napoli and Staskawicz 1987), *avrB* (Tamaki *et al.* 1988), *avrC* (Tamaki *et al.* 1988), *avrD* (Kobayashi *et al.* 1990), *avrBs1* (Ronald and Staskawicz 1988), and *avrBs3* (Bonas *et al.* 1989), but no regions of homology were found.

The discovery of a potential −60 promoter sequence similar to those thought to be regulated by *hrpS* (Fellay *et al.* 1991) provides further evidence for a possible link between genes for pathogenicity and avirulence. *hrpR* and *hrpS* form a two-component sensor system that has been shown to influence the expression of *avrB* (Huynh *et al.* 1989; Fellay *et al.* 1991). Although *avrPph3* appears to be located on the chromosome, restriction maps of the DNA region in which the gene was located bear no similarity to that of the *P. s.* pv. *phaseolicola hrp* region (Lindgren *et al.* 1986; Rahme *et al.* 1991).

The absence of sequences hybridizing to the 558-bp *Ssp*I probe clearly demonstrates that *P. cichorii* and the other *P. syringae* pathovars or races examined do not contain functional *avrPph3* genes. The significance of the hybridization observed when the 3.4-kb *Bam*HI probe was used remains unknown. A similar absence of sequences hybridizing to *avrA* and *avrB* was found in races of *P. s.* pv. *glycinea*, which lack these *avr* genes (Staskawicz *et al.* 1987).

In the analysis of F_2 progeny, the failure to obtain segregation between reactions to races 3 and 4, and to transconjugants of race 5 that harbor clones conferring avirulence demonstrates that a single gene controls the avirulence of isolates of races 3 and 4 to Tendergreen. The inheritance of resistance in *P. vulgaris* is consistent with the presence of a single dominant gene for resistance to both races. Thus, results confirm the operation of a gene-for-gene relationship in bean halo-blight disease. The biochemical regulation of induction of the HR remains to be determined.

Quantitative examination of symptoms produced by transconjugants of race 5, which harbor subclones that contain avirulence genes, revealed small but consistent alterations to the compatible phenotype in young pods. Water-soaked lesions developed more slowly and were more sunken than those produced by race 5 alone or those that harbored original genomic clones. Regions of DNA flanking the avirulence genes may therefore regulate their activity and allow the expression of full pathogenicity of races 3 and 4 on cultivars that lack the gene for resistance in Tendergreen.

ACKNOWLEDGMENTS

Ed Hitchin and Carol Jenner are joint first authors of this paper. We are indebted to Ruth Hockenhull for the blot of the eight races of *Pseudomonas syringae* pv. *phaseolicola*. We also wish to acknowledge support from the Science and Engineering Research Council and Shell Research Ltd (C. Jenner), the Agricultural and Food Research Council (E. Hitchin), and the Wolfson Foundation (K. Walters). This work was completed under the U.K. Ministry of Agriculture Food and Fisheries license numbers PHF 64/114(78) and 63/155(30).

LITERATURE CITED

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Siedman, J. G., Smith, J. A., and Struhl, K. 1987. Current Protocols in Molecular Biology. John Wiley & Sons, New York.

Bachmann, B. J. 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. 36:525-557.

Birnboim, H. C., and Doly, J. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7:1513-1523.

Bliss, F. A. 1980. Common bean. Pages 273-284 in: Hybridization of Crop Plants. W. R. Fehr and H. H. Hadley, eds. American Society of Agronomy/Crop Science Society of America, Madison, WI.

Bolivar, F., Rodriguez, R. L., Green, P. J., Beltach, M. C., Meyneker, M. L., Boyer, M. W., Crosa, J. H., and Falkow, S. 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene 2:95-113.

Bonas, U., Stall, R. E. and Staskawicz, B. 1989. Genetic and structural characterisation of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Gen. Genet. 218:127-136.

Boyer, H. W., and Roulland-Dussoix, D. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. 41:459-472.

Brosius, J., Dull, T. J., Sleeter, D. D., and Noller, H. F. 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol. 148:107-127.

Clewell, D. B., and Helinski, D. R. 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*: Purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. USA 62:1159-1166.

Crute, I. R. 1986. Investigations of gene-for-gene relationships: The need for genetic analyses of both host and parasite. Plant Pathol. 35:15-17.

Daniels, K. J., Dow, J. M., and Osbourn, A. E. 1988. Molecular genetics of pathogenicity in phytopathogenic bacteria. Annu. Rev. Phytopathol. 26:285-312.

Demerec, M., Adelberg, E. A., Clark, A. J., and Hartman, P. E. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics 54:661-76.

Ditta, G., Stanfield, S., Corbin, D., and Helinski, D. R. 1980. Broad host range cloning system for gram-negative bacteria: Construction of a gene bank of *Rhizobium meliloti*. Proc. Natl. Acad. Sci. USA 77:7347-7351.

Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.

Fellay, R., Rahme, L. G., Mindrinos, M. N., Frederick, R. D., Pisi, A., and Panopoulos, N. J. 1991. Genes and signals controlling the *Pseudomomas syringae* pv. *phaseolicola*-plant interaction. Pages 45-52 in: Advances in Molecular Genetics of Plant-Microbe Interactions. Vol. 1. H. Heynecke and D. P. S. Verma, eds. Kluwer Academic Publishers, Dordrecht, Netherlands.

Figurski, D. H., and Helinski, D. R. 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.

Friedman, A. M., Long, S. R., Brown, S. E., Buikema, W. J., and Ausubel, F. M. 1982. Construction of a broad host range cosmid cloning vector and its use in the genetic analysis of *Rhizobium* mutants. Gene 18:289-

296.
Guyer, M. S. 1978. The $\gamma\delta$ sequence of F is an insertion sequence. J. Mol. Biol. 126:347-365.
Harper, S., Zewdie, N., Brown, I. R., and Mansfield, J. W. 1987. Histological, physiological and genetical studies of the responses of leaves and pods of *Phaseolus vulgaris* to three races of *Pseudomonas syringae* pv. *phaseolicola* and to *Pseudomonas syringae* pv. *coronafaciens*. Physiol. Mol. Plant Pathol. 31:153-172.
Hitchin, F. E., Jenner, C. E., Harper, S., Mansfield, J. W., Barber, C. E., and Daniels, M. J. 1989. Determinant of cultivar specific avirulence cloned from *Pseudomonas syringae* pv. *phaseolicola* race 3. Physiol. Mol. Plant Pathol. 34:309-322.
Holmes, D. S., and Quigley, M. 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. 114:193-197.
Howard-Flanders, P., Simson, E., and Theriot, L. 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K12. Genetics 49:237-246.
Huynh, T. V. D., Dahlbeck, D., and Staskawicz, B. J. 1989. Bacterial blight of soybean: Regulation of a pathogen gene determining host cultivar specificity. Science 245:1374-1377.
Inouye, S., Ebina, Y., Nakazawa, A., and Nakazawa, T. 1984. Nucleotide sequence surrounding transcription initiation site of *xylABC* operon on Tol plasmid of *Pseudomonas putida*. Proc. Natl. Acad. Sci. USA 81:1688-1691.
Jenner, C., Hitchin, E., Brown, I., Walters, K., Mansfield, J., and Betteridge, P. 1989. A gene for avirulence from *Pseudomonas syringae* pv. *phaseolicola*. Pages 175-181 in: Signal Molecules in Plants and Plant-Microbe Interactions. B. J. J. Lugtenberg, ed. NATO ASI Series H36. Springer-Verlag, Berlin.
Kado, C. I., and Liu, S. T. 1981. Rapid procedure for the detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-1373.
Kearney, B., and Staskawicz, B. 1990. Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. Nature (London) 346:385-386.
Keen, N. T., and Buzzell, R. I. 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.
Keen, N. T., and Staskawicz, B. J. 1988. Host range determinants in plant pathogens and symbionts. Annu. Rev. Microbiol. 42:421-440.
Keen, N. T., Tamaki, S., Kobayashi, 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 Inter. 3:112-121.
King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
Kobayashi, D. Y., Tamaki, S. J., and Keen, N. T. 1989. Cloned avirulence genes from the tomato pathogen *Pseudomonas syringae* pv. *tomato* confer cultivar specificity on soybean. Proc. Natl. Acad. Sci. USA 86:157-161.
Kobayashi, D. Y., Tamaki, S. J., and Keen, N. T. 1990. Molecular characterization of avirulence gene D from *Pseudomonas syringae* pv. *tomato*. Mol. Plant-Microbe Interact. 3:94-102.
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.
Liu, Y-N., Tang, J-L., Clarke, B. R., Dow, J. M., and Daniels, M. J. 1990. A multipurpose broad host range cloning vector and its use to characterise an extracellular protease gene of *Xanthomonas campestris* pathovar *campestris*. Mol. Gen. Genet. 220:433-440.
Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Mermod, N., Lehrbach, P. R., Reineke, W., and Timmis, K. N. 1984. Transcription of the TOL plasmid toluate catabolic pathway operon of *Pseudomonas putida* is determined by a pair of co-ordinately and positively regulated overlapping promoters. EMBO J. 3:2461-2466.
Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Minton, N. P., and Clarke, L. E. 1985. Identification of the promoter of the *Pseudomonas* gene coding for carboxypeptidase G2. J. Mol. Appl. Genet. 3:26-35.
Napoli, C., and Staskawicz, B. 1987. Molecular characterization and nucleic acid sequence of an avirulence gene from race 6 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 169:572-578.
Rahme, L. G., Mindrinos, M. N., and Panopoulos, N. J. 1991. The genetic and transcriptional organization of the *hrp* gene cluster of *Pseudomonas syringae* pv. *phaseolicola*. J. Bacteriol. 173:575-586.
Ronald, P. C., and Staskawicz, B. J. 1988. The avirulence gene *avrBs1* from *Xanthomonas campestris* pv. *vesicatoria* encodes a 50-kD protein. Mol. Plant-Microbe Interact. 1:191-198.
Rosenberg, M., and Court, D. 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. 13:319-353.
Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.
Shine, J., and Dalgarno, L. 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: Complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA 71:1342-1346.
Shintaku, M. H., Kluepfel, D. A., Yacoub, A., and Patil, S. S. 1989. Cloning and partial characterization of an avirulence gene from race 1 of *Pseudomonas syringae* pv. *phaseolicola*. Physiol. Mol. Plant Pathol. 35:313-322.
Staskawicz, B., Dahlbeck, D., Keen, N., and Napoli, C. 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 169:5789-5794.
Staskawicz, B. J., Bonas, U., Dahlbeck, D., Huynh, T., Kearney, B., Ronald, P., and Whalen, M. 1988. Molecular determinants of specificity in plant-bacterial interactions. Pages 124-130 in: Physiology and Biochemistry of Plant-Microbial Interactions. N. T. Keen, T. Kosuge, and S. Walling, eds. American Society of Plant Physiologists, Rockville, Maryland.
Tamaki, S., Dahlbeck, D., Staskawicz, B., and Keen, N. T. 1988. Characterization and expression of two avirulence genes cloned from *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 170:4846-4854.
Taylor, J. D., Teverson, D. M., and Davies, J. H. C. 1987. Halo-blight of *Phaseolus* bean. Pages 63-63 in: National Vegetable Research Station Annual Report, 1986. NVRS, Wellesbourne, Warwick.
Vivian, A., Atherton, G. T., Bevan, J. R., Crute, I. R., Mur, L. A. J., and Taylor, J. D. 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.
Walters, K., Maroofi, A., Hitchin, E., and Mansfield, J. W. 1990. A gene for pathogenicity and ability to cause the hypersensitive reaction cloned from *Erwinia amylovora*. Physiol. Mol. Plant Pathol. 36:509-521.