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The Avirulence Gene *avrBs*$_1$ from *Xanthomonas campestris* pv. *vesicatoria* Encodes a 50-kD Protein

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A gene cloned from *Xanthomonas campestris* pv. *vesicatoria* race 2, *avrBs*$_1$, specified avirulence on pepper cultivars containing the resistance gene *Bs*$_1$. A series of exonuclease III deletions were made on a 3.2-kbp DNA fragment that determined full avirulence activity, observed as hypersensitive response (HR) induction. The deletion products were subcloned into the broad host range cloning vector pLAFR3, conjugated into a virulent *X. c.* pv. *vesicatoria* race 1 strain, 82-8, and scored for their ability to induce a HR on a pepper cultivar (ECW10R) containing the resistance gene *Bs*$_1$. A span of approximately 1.8 kbp of DNA was necessary for full induction of the HR. The nucleotide sequence revealed two open reading frames (ORFs) capable of encoding proteins of 12.3 and 49.8 kD, designated ORF1 and ORF2, respectively. Deletions into ORF1 altered the HR-inducing activity to give an intermediate phenotype. Deletions into ORF2 completely destroyed activity. When the ORF2 coding region was driven by the *lacZ* promoter on plasmid pLAFR3 (placD), full avirulence activity was restored, indicating that ORF2 alone can induce the HR. Antisera raised to a β-galactosidase-ORF2 fusion protein reacted with a 50-kD protein in *X. c.* pv. *vesicatoria* race 1 (placD) transconjugants. The deduced amino acid sequence of ORF2 had ≈47% overall homology to the carboxyl terminus of the avirulence gene, *avrA*, isolated from *Pseudomonas syringae* pv. *glycinea* race 6, and 86% homology over a region of 49 amino acids. *P. s.* pv. *glycinea*, however, did not induce an HR on ECW10R plants. Coding sequences for *avrBs*$_1$ were also present in *X. campestris* pathovars *vitians* and *campestris*; both of these pathovars induced a HR on ECW10R plants.

Additional keywords: disease resistance, gene-for-gene, incompatibility, race specificity.

The factors involved in specifying disease resistance in plants are poorly understood. In most cases, expression of the resistant phenotype requires the interaction of a host resistance gene and a pathogen avirulence gene, as was first shown by the classic genetic studies of flax and flax rust (Flor 1956). This gene-for-gene pattern has also been shown to function in several bacterial-plant systems (Staskawicz *et al.* 1984; Gabriel *et al.* 1986; Staskawicz *et al.* 1987; Swanson *et al.* 1988). Considerable progress has been made in isolating the bacterial genes involved in the interaction. However, the gene products encoded by these loci have not yet been characterized.

The phytopathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot disease on peppers and tomatoes, provides a model system for addressing this problem. The host's resistance genes, *Bs*$_1$, *Bs*$_2$, and *Bs*$_3$ (Cook and Stall 1963; Cook and Guevara 1984; Kim and Hartmann 1985), each corresponding to a specific avirulence gene in the bacterium (*X. c.* pv. *vesicatoria*), have been genetically defined. The resistance genes are inherited independently (Hibberd *et al.* 1987), and the phenotype of the hypersensitive response (HR) associated with each resistance interaction can be distinguished by both the intensity and the timing of the response. The gene *Bs*$_1$ controls a HR to *X. c.* pv. *vesicatoria* race 2 strains of the pathogen (Cook and Stall 1963) containing the avirulence gene, *avrBs*$_1$, whereas the *Bs*$_2$ and *Bs*$_3$ loci correspond to the *avrBs*$_2$ and *avrBs*$_3$ genes in *X. c.* pv. *vesicatoria* race 1 strains (Minsavage *et al.*, unpublished).

Nucleotide and/or amino acid sequence data is to be submitted to GenBank as accession number J03672.

A 200-kbp self-transmissible plasmid present in *X. c.* pv. *vesicatoria* race 2 was found to confer both copper resistance and avirulence to bacteria inoculated into pepper plants (ECW10R) containing the *Bs*$_1$ gene (Stall *et al.* 1986). A 5.3-kbp clone was isolated from the megaplasmid carrying copper resistance that, when conjugated into *X. c.* pv. *vesicatoria* race 1 and inoculated into the pepper cultivar ECW10R, specifically converted the bacterial phenotype from virulence to avirulence. The avirulence gene, designated *avrBs*$_1$, complemented 13 race 2 mutants that had spontaneously become virulent on ECW10R plants (Swanson *et al.* 1988). To further characterize the gene product(s) encoded by *avrBs*$_1$ and to begin to understand the biochemical basis of recognitional specificity, we have determined the nucleotide sequence of *avrBs*$_1$. This locus encodes two ORFs. Only the ORF2-encoded protein is required to induce an HR. Antisera raised to ORF2 react specifically with a 50-kD protein in protein extracts of *Escherichia coli* and *X. c.* pv. *vesicatoria* expressing *avrBs*$_1$.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacterial conjugations. Races of *X. c.* pv. *vesicatoria* used (race 1 strain 82-8rif^r and race 2 81-23rif^r) and their reactions on pepper are described by Swanson *et al.* (1988). Strains 81-23m13 and 81-23m1 are spontaneous mutants of *X. c.* pv. *vesicatoria* race 2 containing the insertion element IS476 in the 12.5-kbp and 824-bp *Eco*RV fragments, respectively, of *avrBs*$_1$ (Kearney *et al.* 1988). Both of these mutants cause watersoaking when inoculated into ECW10R plants containing the resistance gene *Bs*$_1$. *X. campestris* pathovars were obtained from R. E. Stall. *Pseudomonas syringae* pv. *glycinea* race 0 and race 6 were supplied by Noel Keen. A rifampicin-resistant (rif^r)

isolate of *X. c.* pv. *vitians* was obtained as described previously (Staskawicz *et al.* 1984).

All *E. coli* strains and vectors used are described in Table 1 and plasmid constructions described in Table 2. Clones of *X. c.* pv. *vesicatoria* DNA maintained in *E. coli* were mobilized to the appropriate *X. c.* pv. *vesicatoria* race by employing the helper plasmid pRK2013 in triparental matings (Ditta *et al.* 1980). The identity of plasmids from all *X. c.* pv. *vesicatoria* transconjugants was verified by recovering plasmid DNA, transforming it into *E. coli*, reisolating, and digesting with the appropriate restriction endonucleases.

Growth of plants, plant inoculations, and media. The maintenance and inoculation of pepper cultivars ECW and the near-isogenic ECW10R containing the resistance gene Bs_1 are described by Swanson *et al.* (1988). *X. c.* pv. *vesicatoria* was routinely subcultured on nutrient yeast agar (NYGA; Daniels *et al.* 1984). *E. coli* strains were cultured on Luria-Bertani media (LB; Miller 1972). The following concentrations of antibiotics were used: tetracycline, 10 μg/ml; ampicillin, 50 μg/ml; and rifampicin, 100 μg/ml.

Recombinant DNA techniques. Cloning procedures, restriction enzyme digestions, agarose gel electrophoresis, Southern hybridization, and DNA small-scale preparations were essentially as described by Maniatis *et al.* (1982). Genomic DNA was isolated from *Xanthomonas* and *Pseudomonas* as described previously (Staskawicz *et al.* 1984). DNA restriction endonuclease fragments were ^{32}P-labeled using random primers (Feinberg and Vogelstein 1983).

Exonuclease III deletion analysis. The 5′ termini of a 3.2-kbp *Bgl*II-*Pvu*II DNA fragment from pXV2007 were filled in with T4 DNA polymerase and cloned in both orientations into the *Sma*I site of pUC18, creating the plasmids pXV2108 and pXV2109. DNA of each plasmid was digested with *Bam*HI and *Pst*I followed by exonuclease III and S1 nuclease treatment as described by Henikoff (1984). DNA was then treated with T4 polymerase to ensure formation of blunt ends. After ethanol precipitation, the samples were resuspended in 10 μl of a solution containing 0.05 M Tris, pH 7.6, 0.01 M MgCl2, 0.01 M DTT, 0.1 mg/ml of bovine serum albumin, 1 mM ATP, 0.2 mM dNtps, and 1 unit of T4 DNA polymerase and incubated 30 min at room temperature. The samples were ligated to 0.25 μg of unphosphorylated *Bam*HI linkers at 12° C overnight, diluted to 100 μl in TE (Maniatis *et al.* 1982), ethanol precipitated, and resuspended in TE containing 0.1 M NaCl. Cohesive ends were allowed to anneal by heating to 65° C for 5 min and then cooling slowly to 4° C. Thirty μl of this mix was used in transformation of *E. coli* DH5α.

Defining endpoints of *avrBs*$_1$ activity. Deletion products in pUC18 or pUC118 were digested with *Bam*HI and *Eco*RI, cloned into the *Bam*HI and *Eco*RI sites of pLAFR3, transformed into *E. coli*, and conjugated into *X. c.* pv. *vesicatoria* race 1 strain 82-8. All DNA fragments were oriented such that the *lacZ* promoter in pLAFR3 did not provide transcriptional readthrough into the ORFs as later determined by sequence analysis. The transconjugants were inoculated into ECW and ECW10R and checked for HR-inducing activity.

Nucleotide sequencing. Two deletions that still retained full *avrBs*$_1$ activity, pXV2108e8 and pXV2109e8, initially made in pUC18, were subcloned into the *Bam*HI and *Eco*RI sites of pUC118, and a series of exonuclease III deletions were created. Single-stranded templates were prepared as described by Viera and Messing (1987) and sequenced by using the dideoxy chain-termination sequencing method

Table 1. Bacterial strains and vectors

Strain or vector	Relevant characteristics	Source
E. coli		
DH5α	F$^-$ *recA thil lacZ*ΔM15	Bethesda Research Laboratories
AR68	λ lysogen, TS *cI*, htpR$^-$	Shatzman and Rosenberg 1986
MM294	λ lysogen *cI*$^+$	Shatzman and Rosenberg 1986
JM107	F′, *recA*	Stephen Elledge, unpublished
MV1193	Δ (*lacpro*AB) Tc^r *thi*, [F′ (*lacI*^{q}ZΔM15)]	Messing, unpublished
Vectors		
pUR278, 288, 289	*lacZ* fusion vectors	Rüther and Müller-Hill 1983
pOTS	bacteriophage λ P_L promoter vector under thermo-inducible control of a thermolabile CI represser	Shatzman and Rosenberg 1986
pLAFR3	pLAFR1 containing *Hae*II of pUC8 Tc^r Tra$^-$ Mob$^+$, RK2 replicon	Staskawicz *et al.* 1987
pUC18	ColE1 replicon	Norrander *et al.* 1983
pUC118	IG region of M13 in pUC18	Viera and Messing 1987
pRK2013	Km^r Tra$^+$ Mob$^+$, ColE1 replicon	Figurski and Helinski 1979

Table 2. Plasmid constructions

Plasmid	Vector	Insert
pXV2007	pLAFR3	5.3-kbp *Pst-Sst*I fragment cloned from *Xanthomonas campestris* pv. *vesicatoria* race 2 containing the *avrBs*$_1$ gene (Swanson *et al.* 1988)
pXV2108	pUC18	3.2-kbp *Bgl*II-*Puv*II fragment of pXV2007 cloned into *Sma Sma*I site of vector
pSV2109	pUC18	As above, cloned in opposite orientation
pXV2110	pLAFR3	3.2-kbp *Bam*HI-*Eco*RI fragment from pXV2108
pSV2111	pUC18	2.3-kbp *Sph*I fragment from pXV2109
pXV2112	pLAFR3	2.3-kbp *Pst-Bam*HI fragment from pXV2111
placA	pLAFR3	Transcriptional fusion of the *Bam*HI-*Eco*RI subclone from pXV2108e8 to the *lacZ* promoter
placD	pLAFR3	Transcriptional fusion of the *Bam*HI-*Eco*RI subclone from pXV2108e28 to the *lacZ* promoter
placE	pLAFR3	Transcriptional fusion of the *Bam*HI-*Eco*RI subclone from pXV2108e1 to the *lacZ* promoter
pOTS A	pOTS	*Bam*HI-*Eco*RI subclone of pXV2108e8 cloned in opposite orientation to the P_L promoter
pOTS B	pOTS	Transcriptional fusion of the *Bam*HI-*Eco*RI subclone from pXV2108e8 to the bacteriophage P_L promoter
pUR 6	pUR289	*avrBs*$_1$-ORF2-*lacZ* fusion protein; *Bam*HI subclone of placE
pXV2m105a	pLAFR3	*Sph*I subclone of an *avrBs*$_1$ mutant (81-23ml, Kearney *et al.* 1988) containing the transposon IS476 clone in the direction of vector *lacZ* promoter

(Sanger *et al.* 1977). Both strands of a 2,061-bp fragment of *X. c.* pv. *vesicatoria* race 2 containing full avirulence activity were sequenced.

Construction of transcriptional fusions to *avrBs*$_1$. Transcriptional fusions of three deletions, pXV108e8, pXV2108e28, and pXV2108e1, were constructed to the β-galactosidase promoter in pLAFR3. The plasmids were digested with *Eco*RI, the 5′ termini filled in with T4 polymerase and ligated to *Bam*HI linkers. The resulting plasmids were then digested with *Bam*HI, and the DNA fragments were cloned into the *Bam*HI site of pLAFR3 and named placA, placD, and placE (Fig. 1C and Table 2).

β-galactosidase fusions and preparation of antisera. The *E. coli* β-galactosidase-*avrBs*$_1$ ORF2 fusion plasmid was constructed by using the pUR series of vectors containing polylinker sequences at the carboxyl terminus of the β-galactosidase gene (Rüther and Müller-Hill 1983). Plasmid pXV2108e1 (Fig. 1B) was cleaved with *Eco*RI, the 5′ extended termini were filled in with T4 DNA polymerase, ligated to unphosphorylated *Bam*HI linkers, cleaved with *Bam*HI, and the DNA fragment ligated to *Bam*HI cleaved pUR289. The plasmid orientation producing an in-frame fusion protein approximately 50 kD greater than β-galactosidase was called pUR6 (Table 2). The construct pUR6 contained all but the first eight amino acids of the coding sequence of ORF2 fused to the 3′ end of the *lacZ* gene. The preparative purification of the bacterial fusion protein aggregates and immunization of rabbits was accomplished as described by Rio *et al.* (1986). The crude antisera were affinity purified according to the method of Smith and Fisher (1984), and the β-galactosidase specific antibodies were removed by absorption of the affinity purified antisera to an *E. coli* extract containing β-galactosidase.

β-galactosidase fusions were also constructed to a *Bam*HI-*Eco*RI DNA fragment from pXV2108e8 containing both ORFs in all three pUR vectors. These constructs gave the expected size fusion proteins as predicted by the DNA sequence for translational stops in all three frames (data not shown).

Protein preparations. *E. coli* carrying plasmids with the β-galactosidase fusion proteins were grown in LB and 100 μg/ml of ampicillin. One-ml cultures were induced with 1 mM isopropylthiogalactoside (IPTG) at $A_{590} \approx 0.2$–0.4 and grown 2 hr at 37° C following induction. The bacteria were harvested, resuspended in 100 μl of sodium dodecyl sulfate (SDS) gel sample buffer (Laemmli 1970), and boiled for 90 sec. After a 10-min centrifugation, 10 μl was run on a 10%

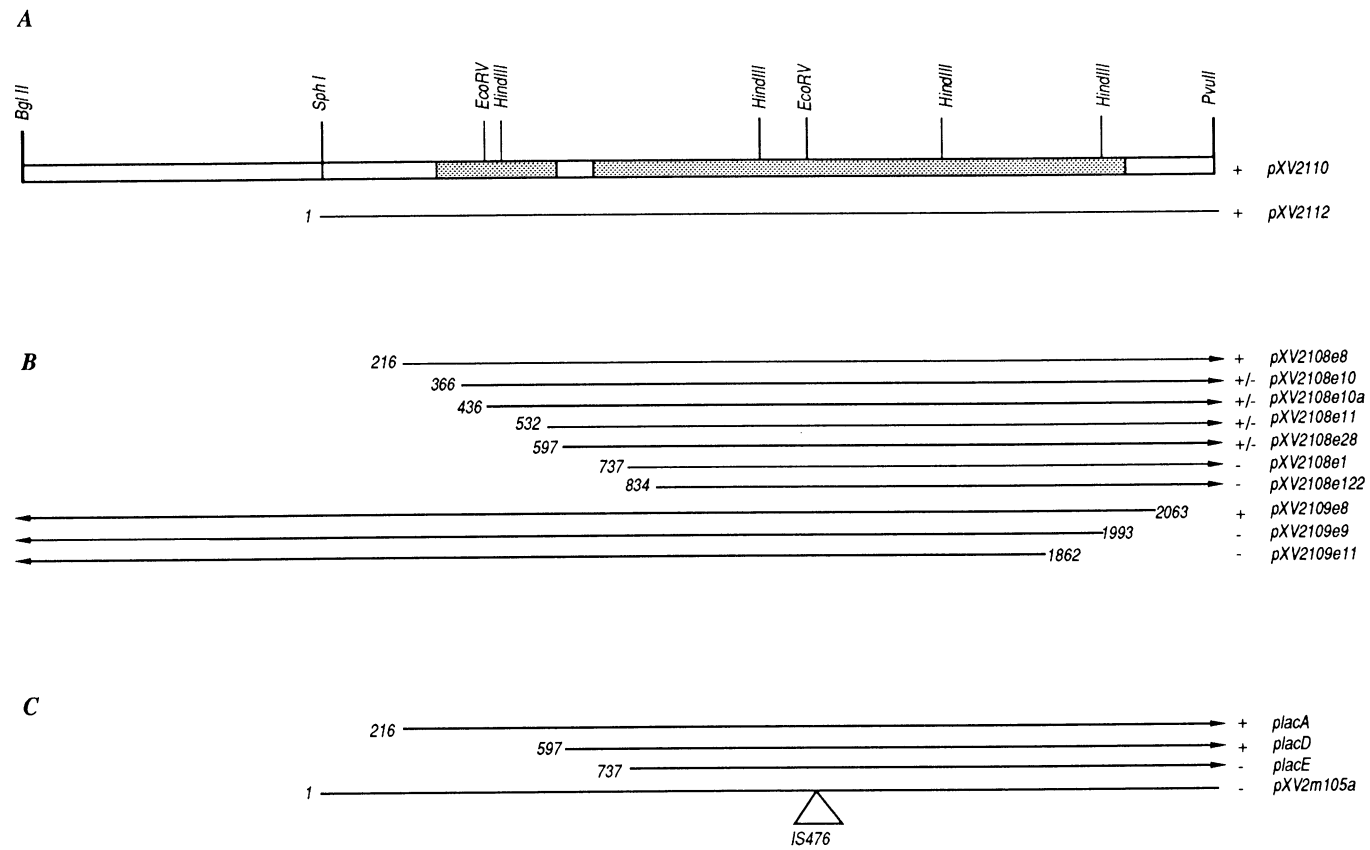

Fig. 1. A, Restriction map and position of open reading frames (ORFs) of *avrBs*$_1$ on the 3.2-kbp subclone pXV2110. The first shaded region designates ORF1 (bp 308–619), and the second designates ORF2 (bp 713–2,047). Both ORFs are in the same reading frame directed from left to right. The 2.3-kbp *Sph*I-*Pvu*II subclone pXV2112 is also shown. **B,** Deletion analysis of *avrBs*$_1$. Exonuclease III deletions made from the 5′ end of the 3.2-kbp subclone are indicated by arrows directed right (→). Deletions made from the 3′ end are indicated by (←). The deletions are oriented in pLAFR3 such that the *lacZ* promoter drives transcription in the opposite direction to the presumed transcription of *avrBs*$_1$. **C,** Transcriptional fusions of *avrBs*$_1$ to the *lacZ* promoter. The *lacZ* promoter transcribes from the left into *avrBs*$_1$ coding sequences. The triangle indicates the insertion site of IS476. Numbering starts with position 1 at the *Sph*I site and corresponds to the sequence in Figure 2. All plasmids indicated on the right were conjugated into *X. c.* pv. *vesicatoria* race 1 and inoculated into ECW10R plants. The ability of the transconjugants to induce an HR are shown as +, full activity; +/−, intermediate activity; and −, watersoaking.

denaturing discontinuous polyacrylamide gel and stained with Coomassie blue R-250 (Laemmli 1969). *X. c.* pv. *vesicatoria* cells carrying plasmids were grown to saturation in NYG broth containing 100 μg/ml of rifampicin and 10 μg/ml of tetracycline. One-ml cultures were pelleted and resuspended in 200-μl sample buffer.

pOTS vector constructs. To overexpress the cloned gene *avrBs*$_1$ in *E. coli*, a derivative of the vector system pAS, pOTS (Shatzman and Rosenberg 1986), was used. This system provides the strong transcriptional promoter P_L from the bacteriophage λ genome, and the plasmids constructed are under thermoinducible control of the *cI* thermolabile repressor. The plasmid pXV2108e8 was digested with *Eco*RI, the 5′ termini filled in with T4 DNA polymerase and ligated to *Bam*HI linkers. The plasmid was then digested with *Bam*HI, and the 2.1-kbp *Bam*HI DNA fragment containing ORF1 and ORF2 was cloned in both orientations into the *Bam*HI site of the pOTS vector. The plasmid pOTSB is a transcriptional fusion such that translation of ORF2 must initiate from the *avrBs*$_1$ sequence. Translation initiating from the ribosome binding site present in the vector will terminate upstream from ORF2 due to translational stops in all three frames. All cloning experiments were carried out in a cI^+ lysogen (MM294cI$^+$) to maximize stability of the vector. To express the cloned gene, pOTSA and pOTSB were transformed into a λ lysogen (AR68) carrying a temperature-sensitive mutation in its repressor gene (*cI*). Host cells were protease-deficient to ensure stability of the translated product (Baker *et al.* 1984). The transformed cells were grown in LB containing ampicillin at 30° C until mid-log phase. At this time the cells were moved to 42° C and incubated for an additional hour. The cells were then harvested and the proteins prepared for gel electrophoresis as described above.

Western blotting. SDS-PAGE and electrophoretic transfer to nitrocellulose were performed as described by Towbin *et al.* (1979). The blots were reacted overnight with a 1:500 dilution of the affinity purified, β-galactosidase depleted antibody. After washing, the blot was reacted with ^{125}I-labeled protein A for 2 hr before autoradiography.

Computer analysis. The DNA sequence and deduced protein sequence of *avrBs*$_1$ (Fig. 2) were compared to the National Biomedical Research Foundation and NIH Genbank libraries by using the FASTP program available

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        10         20         30         40         50         60         70
CCATTGTCGG CGGTTATCCG GGTACTTGGC GTACACCAAA CAACTGGGGC AATGCTGGCA AATCACGTGA

        80         90        100        110        120        130        140
CGAAGCCTTG GCAGACGAGC AACAGAGGAT TCAAGCGCTT AAATCGCAAG AGACGGTACA TATCTTCCAT

       150        160        170        180        190        200        210
CGCAAAGATG TCAAGAGCGA ACCCGCAACC CACGCGGGGC GACGTTAAGT AAGCCACTGA TTTTTAGCGA

       220        230        240        250        260        270        280
AGAAGAGCTT GTGAGAGCTG CGGGCGCCAA ATATGTACGT TTGACAGTGA CAGATCATCT TTCACCACGG

       290        300                   322                   337
GCGGACGATA TTGATGCGTT TATTGCA ATG GAG CGG GAG ATG GCC CAT GAT GAG AGA CTG CAT
                              MET Glu Arg Glu MET Ala His Asp Glu Arg Leu His

       352                   367                   382                   397
GTA CAT TGT GGT ATG GGC CTA GGC CGT ACG ACA ATA TTT ATT GTC ATG CAT GAC ATA
Val His Cys Gly MET Gly Leu Gly Arg Thr Thr Ile Phe Ile Val MET His Asp Ile

           412                   427                   442                   457
CTA AGA AAT GCT GCA ATG TTA TCG TTT GAT GAT ATC ATC GAA CGG CAA CGT AAA TTT
Leu Arg Asn Ala Ala MET Leu Ser Phe Asp Asp Ile Ile Glu Arg Gln Arg Lys Phe

                472                   487                   502
AAT CCA GGG CGA AGC TTG GAT AAT AAT AAA GAC GTT TCT GAC AAG GGG CGC TCA GAA
Asn Pro Gly Arg Ser Leu Asp Asn Asn Lys Asp Val Ser Asp Lys Gly Arg Ser Glu

517                   532                   547                   562
TTT CGT AAT GAA CGG TCA GAG TTC CTT CCT CTA TTC TAC GAG TAC GCC AAG CAA AAT
Phe Arg Asn Glu Arg Ser Glu Phe Leu Pro Leu Phe Tyr Glu Tyr Ala Lys Gln Asn

    577                   592                   607                   622        632
CCA AAG GGC CAG CCA TTG TTA TGG TCC GAA TGG CTC GAC CAC AAT GCA TAA ATCGCAAGTA
Pro Lys Gly Gln Pro Leu Leu Trp Ser Glu Trp Leu Asp His Asn Ala

       642        652        662        672        682        692        702
CATTTTCGGC TATGACGGAC TTGTGCTCGA TGCGCTGGCG GCTTTCTCGA TAAATATCAA TTAATATAAA

       712                   727                   742                   757
TATCGAACTA ATG TCC GAC ATG AAA GTT AAT TTC TCT TCA AAA ATA ATA GAT TCA ACA CCC
           MET Ser Asp MET Lys Val Asn Phe Ser Ser Lys Ile Ile Asp Ser Thr Pro

       772                   787                   802                   817
AGT GAA GAG GAG GTC GCC ACT CAG CAA GAT AGT TAT ACG AAA TCT GGA CTG GTG GCG
Ser Glu Glu Glu Val Ala Thr Gln Gln Asp Ser Tyr Thr Lys Ser Gly Leu Val Ala

           832                   847                   862                   877
CCA TCG CTC GAT TCA CAA GCC TTG AAA AAA GCA CCT AGA AAA AGA GTA ATA AAA GAA
Pro Ser Leu Asp Ser Gln Ala Leu Lys Lys Ala Pro Arg Lys Arg Val Ile Lys Glu

                892                   907                   922
AAT ATA GCT GCT TTG CAC ACC TCA TCG TTA GAG CGA GTT CAT CAA AAG AAG GTA TTA
Asn Ile Ala Ala Leu His Thr Ser Ser Leu Glu Arg Val His Gln Lys Lys Val Leu

937                   952                   967                   982
GTT CAG AAT TTA GCG CAG TTG CAG AGA GGG TTG GCT AAG ATA AAT GGT AGA GTC GAA
Val Gln Asn Leu Ala Gln Leu Gln Arg Gly Leu Ala Lys Ile Asn Gly Arg Val Glu

    997                  1012                  1027                  1042
CTC GAA GAG CTA ATT GAT GGA TTT TCA GTC AAG GAA TTG CTA ATA AAA AGA AAT CCA
Leu Glu Glu Leu Ile Asp Gly Phe Ser Val Lys Glu Leu Leu Ile Lys Arg Asn Pro

       1057                  1072                  1087                  1102
AAG ATT GCT GAA GAG TAT GGA GAA GGA AAT CCT TTA ATG ATT CGA TCT CTA AGA TTT
Lys Ile Ala Glu Glu Tyr Gly Glu Gly Asn Pro Leu MET Ile Arg Ser Leu Arg Phe

              1117                  1132                  1147                  1162
TCA AAC CCC CAA GAG GTG ACT AGT AAG CTT GGG GCG GAA GGA AAA ACG CCA GCC AAA
Ser Asn Pro Gln Glu Val Thr Ser Lys Leu Gly Ala Glu Gly Lys Thr Pro Ala Lys

                  1177                  1192                  1207
AGA GAG GTT GAT ACG ATT TGC AAT AAA TCC ACG CTG CAT GAC ATT GTC ATG ACG CCC
Arg Glu Val Asp Thr Ile Cys Asn Lys Ser Thr Leu His Asp Ile Val MET Thr Pro

1222                  1237                  1252                  1267
GCC TCC CTT GTA AAA AAG GAA GTG CGG ATG AAC CTG ATA TCT GAA GTC CCA AGG GCG
Ala Ser Leu Val Lys Lys Glu Val Arg MET Asn Leu Ile Ser Glu Val Pro Arg Ala

    1282                  1297                  1312                  1327
AAG GAT AAA CAA AAA TAC AGA GGT CTT CCT TCA GTC GTA TAT GGC CAA AGC AGC CGC
Lys Asp Lys Gln Lys Tyr Arg Gly Leu Pro Ser Val Val Tyr Gly Gln Ser Ser Arg

        1342                  1357                  1372                  1387
CGT AGT GAA TCA GAC TAT CTA ACG TCT CGA AAT GGT TTC GGC GAC GTG CAC TCT TTG
Arg Ser Glu Ser Asp Tyr Leu Thr Ser Arg Asn Gly Phe Gly Asp Val His Ser Leu

              1402                  1417                  1432                  1447
AAA TCC AAT AAC GCA TTT AAT TCC GAC TAC GAA AAA ATA TGT GGG TCG CTT AGC CAT
Lys Ser Asn Asn Ala Phe Asn Ser Asp Tyr Glu Lys Ile Cys Gly Ser Leu Ser His

                  1462                  1477                  1492
GCC GAA AAG TTG GGG TTA ATT GAA AGG AAT CTT ACT CCC TTT ATA AGG CAT GAT CCA
Ala Glu Lys Leu Gly Leu Ile Glu Arg Asn Leu Thr Pro Phe Ile Arg His Asp Pro

1507                  1522                  1537                  1552
GAT AGA ATC TCC ACC GAC TTT GTT CAC TCT ATT GAA GAA TTG GCT GAA CAC CAG ATG
Asp Arg Ile Ser Thr Asp Phe Val His Ser Ile Glu Glu Leu Ala Glu His Gln MET

    1567                  1582                  1597                  1612
CTA TTG CAA TCA AGA AAA CCT GCC AGT GCT TTG CGG CAT AAT GAA TAT TGC ACC AAG
Leu Leu Gln Ser Arg Lys Pro Ala Ser Ala Leu Arg His Asn Glu Tyr Cys Thr Lys

        1627                  1642                  1657                  1672
CTT GAA CTG TGG GAT GCT AAA GCT ATA GCA GTT GGT GAA TCT CGT GCC TTG GCG GTC
Leu Glu Leu Trp Asp Ala Lys Ala Ile Ala Val Gly Glu Ser Arg Ala Leu Ala Val

              1687                  1702                  1717                  1732
GCT ACC CTG ATT GAA TTT AAT TTG GAG ATG TTG TCG ATA GCA CAA GAG ATA GAT GAT
Ala Thr Leu Ile Glu Phe Asn Leu Glu MET Leu Ser Ile Ala Gln Glu Ile Asp Asp

                  1747                  1762                  1777
GAT GGG CAC AAG AGT AAA ATG GTC GCC GAT TTT ATC GAG CGC CAA CTA TCA TGG CTT
Asp Gly His Lys Ser Lys MET Val Ala Asp Phe Ile Glu Arg Gln Leu Ser Trp Leu

1792                  1807                  1822                  1837
GGC CCA CAA ACC GCA CTT GAC AGC AAG TCA ACG CTT GAA AGG GTT TCA GCG GTG ACC
Gly Pro Gln Thr Ala Leu Asp Ser Lys Ser Thr Leu Glu Arg Val Ser Ala Val Thr

    1852                  1867                  1882                  1897
ATA CAA GAA AGG GAA TTT ATC GCT AAT GAG ATT AGC CGA TCG TTG CGT CAA GGT GTT
Ile Gln Glu Arg Glu Phe Ile Ala Asn Glu Ile Ser Arg Ser Leu Arg Gln Gly Val

        1912                  1927                  1942                  1957
TCA CTT TGC ACT TAC GAT AAA GAT GAA GCA GGA AGT CAT ATC CGT GAA ATG AGT TTG
Ser Leu Cys Thr Tyr Asp Lys Asp Glu Ala Gly Ser His Ile Arg Glu MET Ser Leu

              1972                  1987                  2002                  2017
TTG GAT TTT AGG GTT GAA GAA ATC ATA GAG GGG ATA AGT ATT TTT ATT TCC TCC AAG
Leu Asp Phe Arg Val Glu Glu Ile Ile Glu Gly Ile Ser Ile Phe Ile Ser Ser Lys

                  2032                  2047          2060        2070        2080
CTT TTA CAT GTT ACA AAT GCA GGA GAA GCG TAA GAGAAGAAGT ATCCGCCACA ATCGTGCGAC
Leu Leu His Val Thr Asn Ala Gly Glu Ala

      2090       2100
GGACCGACGT CCTAACGCCC
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Fig. 2. Nucleotide sequence of a 2,061-bp segment of DNA and amino acid translation of the two ORFs. The sequence shown begins at the *Sph*I site and extends to deletion pXV2109e8. The predicted molecular weight of products of the first ORF is 12.3 kD; that of the second is 49.8 kD.

from the NIH-sponsored Bionet National Computer Resource for Molecular Biology (Smith *et al.* 1986; Lipman and Pearson 1985). The algorithm of Hopp and Woods (1981) was used to produce the hydropathy patterns from the deduced proteins of *avrBs*$_1$ and *avrA*. The alignment of *avrA* and *avrBs*$_1$ in Figure 3 was accomplished by using the Bionet program FASTA (Lipman and Pearson 1985).

RESULTS

Defining endpoints of *avrBs*$_1$ activity. Previous work by Swanson *et al.* (1988) showed that a 5.3-kbp DNA fragment contained *avrBs*$_1$ activity. We further subcloned this DNA fragment to define better the segment of DNA necessary for activity. *X. c.* pv. *vesicatoria* race 1 (82-8) transconjugants containing plasmids pXV2110 or pXV2112 (Fig. 1A and Table 2) gave a HR when inoculated into ECW10R, indicating that the 2.3-kbp *Sph*I-*Pvu*II DNA fragment contained the gene *avrBs*$_1$. Exonuclease III deletions made on the 5′ and 3′ ends of the 3.2-kb *Bgl*II-*Pvu*II subclone were cloned into pLAFR3, conjugated into *X. c.* pv. *vesicatoria* race 1, and inoculated into ECW10R. The phenotypes of the reactions are shown in Figure 1 as full avirulence activity (+), intermediate avirulence activity (+/−), and watersoaking (−). The intermediate phenotype is characterized by partial watersoaking and partial necrosis. *X. c.* pv. *vesicatoria* race 1 transconjugants containing plasmid pXV2108e8, a 2.1-kbp subclone beginning at bp 216 and extending to the *Pvu*II site, gave full *avrBs*$_1$ activity (Fig. 1B). Transconjugants containing plasmid pXV2108e10, which contains a deletion extending 8 bp into ORF1, gave the intermediate phenotype. *X. c.* pv. *vesicatoria* race 1 transconjugants containing deletions further downstream into ORF1 (pXV2108e10a, pXV2108e11, pXV2108e28) also gave an intermediate reaction. Deletion products pXV2109e9 and pXV2109e11 extend into the 3′ side of ORF2 and abolish the ability of pXV2109 to express avirulence activity. Deletion product pXV2109e8 maintained activity. Sequence analysis (see below) revealed that this deletion maintained the ORF2 stop codon. Thus, deletion analysis delineated a span of approximately 1.8 kbp of DNA that was necessary for avirulence activity.

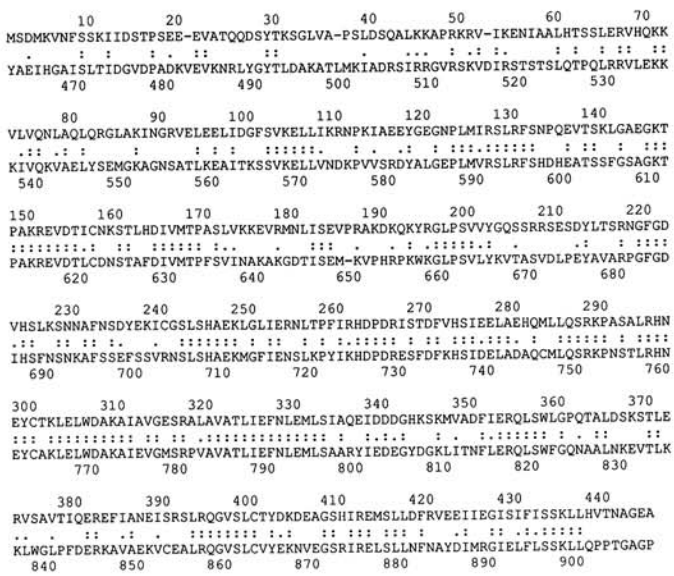

Fig. 3. Alignment of amino acids deduced from the nucleotide sequences of *avrBs*$_1$ from *X. campestris* pv. *vesicatoria* race 2 (top) and *avrA* from *Pseudomonas syringae* pv. *glycinea* race 6 (bottom). The optimized alignment is denoted by a colon for an identity and a dot for a conservative replacement. Insertions made during optimization are marked with a dash.

Sequence analysis. The nucleotide sequence of a 2,061-bp fragment of DNA containing full avirulence activity as defined by deletion analysis was determined (Fig. 2). The ORF1 began 308 bp downstream from the *Sph*I site and terminated at bp 622 (Fig. 1A). There were four more methionine codons downstream from the first ATG that could also serve as translation initiation sites. The largest peptide encoded in ORF1 was 12.3 kD. ORF2 began at bp 713 and extended to bp 2,047. A second ATG at position 722 and a third at 1,247 could also serve as translational starts. The largest protein encoded by ORF2 was 445 amino acids with a calculated molecular weight of 49.8 kD. DNA sequences upstream from ORF1 and ORF2 showed no homology to the *E. coli* consensus sequence for a ribosome binding site (Shine and Dalgarno 1974). An AG-rich Shine-Dalgarno consensus sequence was present preceding the ATG at position 1,247; however, translation initiating at this nucleotide produced a protein of only 30 kD. Sequences resembling the *E. coli* −10 and −35 promoter elements were found upstream of ORF1 at bp 246 and 224, respectively. A −10-like sequence at bp 702 preceded ORF2, but no −35 sequence was present.

To determine if the first ORF was necessary for the function of *avrBs*$_1$ or simply supplied transcriptional signals necessary for ORF2 transcription, we constructed plasmids that used a β-galactosidase promoter to drive ORF2. When the deletion fragment from plasmid pXV2108e28 was subcloned into pLAFR3 such that the *lacZ* promoter could provide transcriptional readthrough (placD, Fig. 1C), full avirulence activity was conferred to *X. c.* pv. *vesicatoria* race 1 (placD) transconjugants. In contrast, no avirulence activity was present in transcriptional fusions to constructs with deletions in ORF2 (placE, Fig. 1C) or insertions in ORF2 (pXV2m105a, Fig. 1C). Transconjugants containing a larger DNA fragment in plasmid pLAFR3 containing both ORFs gave full avirulence activity, in the presence or absence of the *lacZ* promoter (placA, Fig. 1C; and pXV2108e8, Fig. 1B).

Homology of *avrBs*$_1$ with *avrA*. Considerable homology was observed between the deduced amino acid sequences of avrBs$_1$ and *avrA*, an avirulence gene from race 6 of *P. s.* pv. *glycinea* (Napoli and Staskawicz 1987). Comparison of the 908 amino acids of *avrA* with *avrBs*$_1$ revealed extensive homology at the *avrA* carboxyl terminus with the *avrBs*$_1$ deduced protein sequence (Fig. 3). Forty-seven percent identity was observed between the amino acids of the carboxyl terminus of *avrA* and the *avrBs*$_1$ ORF2 coding regions. More significant homology (57%) was shown by allowing for conservative amino acid changes rather than perfect matches. Two large regions of the peptides were highly homologous. Forty-nine amino acids (amino acids 748–796 in *avrA*; and amino acids 285–333 in *avrBs*$_1$) shared 86% homology. An additional region spanning 28 amino acids (*avrA* 606–633, *avrBs*$_1$ 142–169) shared 75% homology. The DNA sequence homology stopped completely after the *avrA* stop codons (data not shown). Hydropathy analysis of *avrBs*$_1$ ORF1 and ORF2 indicated

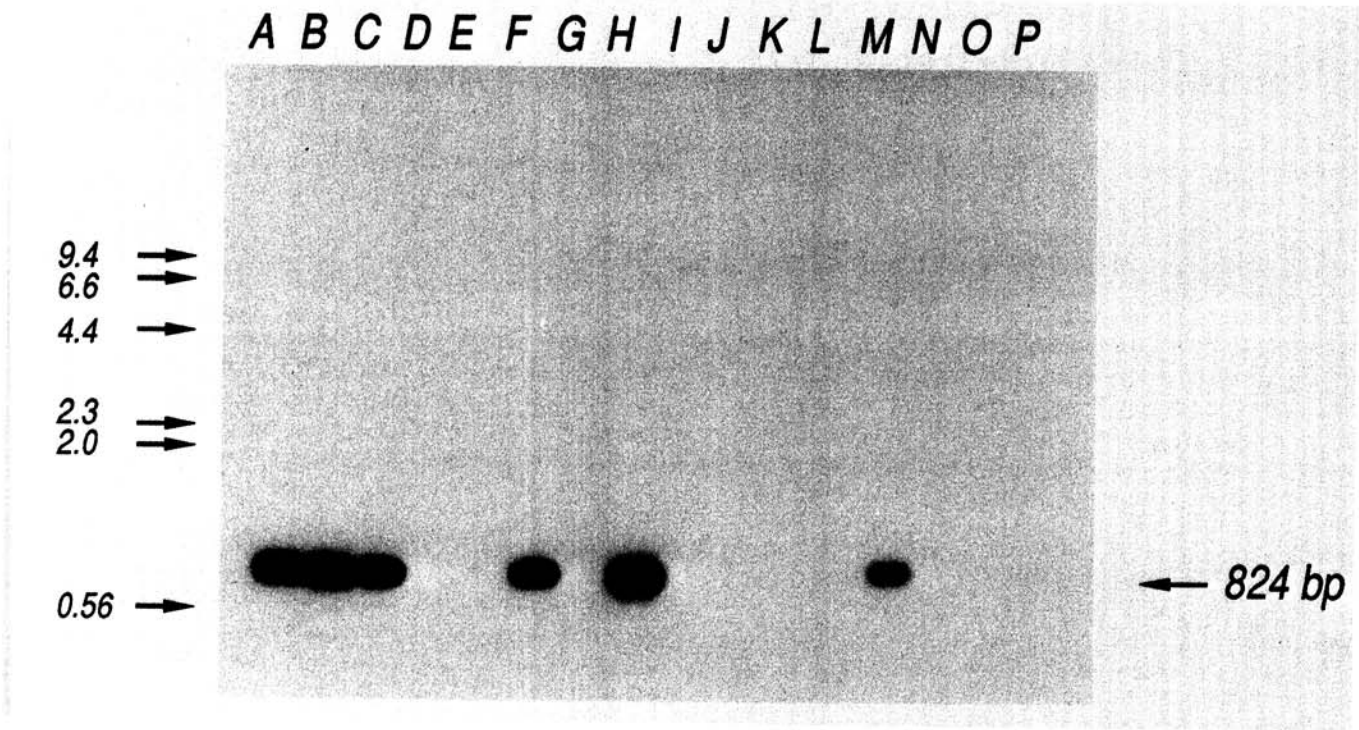

Fig. 4. Southern blot analysis of various xanthomonads and pseudomonads probed with an internal fragment to *avrBs*$_1$. Total genomic DNA (including plasmid DNA) was isolated and digested with *Eco*RV. Each lane was loaded with approximately 2 μg of DNA and electrophoresed in 0.7% agarose. The DNA was transferred to Nytran and probed with a ^{32}P-labeled 824-bp *Eco*RV fragment from pXV2-108. Lane A, *X. c.* pv. *vesicatoria* race 2 81-23; B, *X. c.* pv. *vesicatoria* race 2 85-10; C, *X. c.* pv. *vesicatoria* race 2 81-23m13; D, *X. c.* pv. *vesicatoria* race 3 68-1; E, *X. c.* pv. *vesicatoria* race 1 82-8; F, tomato race *X. c.* pv. *vesicatoria* 75-3; G, *X. campestris* T55; H, *X. c.* pv. *vitians* 164; I, *X. c.* pv. *malvacearum*; J, *X. c.* pv. *holcicola*; K, *X. c.* pv. *vignicola*; L, *X. c.* pv. *glycines*; M, *X. c.* pv. *campestris*; N, *X. c.* pv. *phaseoli*; O, *P. s.* pv. *glycinea* race 0; P, *P. s.* pv. *glycinea* race 6. Lambda DNA, digested with *Hind*III, was used as molecular weight markers.

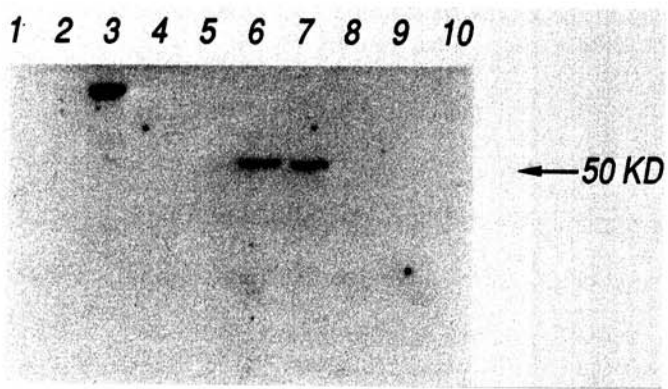

Fig. 5. Autoradiogram of western blot analysis of *avrBs*$_1$ ORF2. Protein samples were electrophoresed on a 10% sodium dodecyl sulfate polyacrylamide gel and the proteins electrophoretically transferred to nitrocellulose. The blot was first reacted with affinity purified, β-galactosidase depleted anti-ORF2 antisera (1:500 dilution) and then with ^{125}I-labeled protein A. Lanes are as follows: 1, Sigma molecular weight markers; 2, *E. coli* JM107recA (pUR289) IPTG treated; 3, *E. coli* JM107recA (pUR6) IPTG treated; 4, *E. coli* AR68 (pOTS) 42° C; 5, *E. coli* AR68 (pOTSA) 42° C; 6, *E. coli* AR68 (pOTSB) 42° C; 7, *X. c.* pv. *vesicatoria* 82-8 (placD); 8, *X. c.* pv. *vesicatoria* 82-8; 9, *X. c.* pv. *vesicatoria* 81-23; 10, *X. c.* pv. *vesicatoria* 81-23m13.

that the putative proteins were mostly hydrophilic (data not shown). As expected, the hydropathy profiles of the last 400 amino acids of the *avrA* protein and *avrBs*$_1$ ORF2 were similar. No homology to other DNA sequences or protein sequences in the NBRF or Genbank data bases was found.

Homology of *avrBs*$_1$ to other *X. campestris* pathovars. A Southern blot of total genomic DNA from various races and pathovars of *X. campestris* and *P. s.* pv. *glycinea* was probed with a ^{32}P-labeled 824-bp *Eco*RV fragment containing 188 bp of ORF1 and 545 bp of ORF2 (Fig. 1A). The 824-bp fragment hybridized to DNA of *X. c.* pv. *vesicatoria* race 2 and tomato race (Fig. 4, lanes A, B, and F) and to *X. c.* pv. *vesicatoria* race 2 81-23m13 (lane C) that contained an intact 824-bp *Eco*RV fragment. As shown previously (Swanson *et al.* 1988), there was no hybridization to *X. c.* pv. *vesicatoria* race 1 (lane E), race 3 (lane D), or to a nonpathogenic epiphyte, *X. campestris* strain T55 (lane G). Interestingly, two pathovars of *X. campestris*, *vitians* and *campestris*, also contained an 824-bp *Eco*RV DNA fragment that hybridized to the probe (lanes H and M). These two pathovars also gave a HR when inoculated into ECW10R (Bs$_1$) plants. *P. s.* pv. *glycinea* races 0 and 6 showed no homology to the probe.

Western blot analysis of *avrBs*$_1$. No reaction of the *avrBs*$_1$ ORF2-specific antisera was observed to the β-galactosidase marker (lane 1, Fig. 5) or to *E. coli* extracts containing the pUR289 vector (lane 2, Fig. 5). A 165-kD protein in extracts containing the *lacZ*-ORF2 fusion protein (lane 3) reacted with the antibody. The antibody did not react with *E. coli* extracts containing the pOTS vector alone (lane 4) nor with the pOTS vector containing an *avrBs*$_1$ ORF2 insert in the opposite orientation to the bacteriophage λ P_L promoter (lane 5). *E. coli* extracts containing pOTSB abundantly expressed a 50-kD protein, after temperature induction at 42° C, that reacted with the antisera (lane 6) and that comigrated with a protein from race 1 (placD) extracts (lane

7). No reaction with the antibody was observed with *X. c.* pv. *vesicatoria* race 1 (lane 8), 81-23m13 (lane 10), *X. c.* pv. *vitians*, *X. c.* pv. *campestris*, or *P. s.* pv. *glycinea* race 6 (data not shown). Unexpectedly, wild-type *X. c.* pv. *vesicatoria* race 2 strain 81-23 containing *avrBs*$_1$ (lane 9) did not react with the antibody.

DISCUSSION

Recent work on the *avrBs*$_1$ locus of *X. c.* pv. *vesicatoria* has shown that a 5.3-kbp fragment of DNA is necessary to specify the ability to induce an HR on pepper plants carrying the resistance gene *Bs*$_1$ (Swanson *et al.* 1988). In this paper, we further characterize the *avrBs*$_1$ locus and show that a 1.8-kbp fragment of DNA is necessary for full avirulence activity.

Exonuclease III deletion analysis in conjunction with sequence analysis defined two ORFs in *avrBs*$_1$. An intermediate phenotype was observed when portions of the first ORF were deleted. However, when the first ORF was replaced with the *lacZ* promoter (Fig. 1C, placD), full avirulence activity was restored, indicating that only the second ORF is necessary for full activity. Deletion analysis of the 3′ end of *avrBs*$_1$ ORF2 showed that the carboxyl terminus of the protein was necessary for avirulence activity. A deletion 13 bp downstream from the TAA stop codon had no effect on avirulence activity, whereas a deletion of the last 18 amino acids of the ORF2 protein prevented the *X. c.* pv. *vesicatoria* transconjugants from inducing a HR. These data corroborate a previous study that shows that insertions at the *avrBs*$_1$ locus disrupt the HR-inducing phenotype (Kearney *et al.* 1988).

Two mutants of *avrBs*$_1$ that had completely overcome resistance encoded by *Bs*$_1$ contained the insertion element IS476 located in ORF2 (Kearney *et al.* 1988). These mutants caused watersoaked lesions on *Bs*$_1$ plants identical to those observed for deletions into the second ORF. A mutant containing IS476 integrated into the central portion of *avrBs*$_1$ ORF1 gave an intermediate reaction when inoculated into ECW10R (Kearney *et al.* 1988). The same reaction was observed for *X. c.* pv. *vesicatoria* race 1 transconjugants containing plasmids with deletions into ORF1.

Although the first ORF had the coding capacity for a 12.3 kD protein, it is not known if this region is transcribed and translated. ORF1 was preceded by sequences resembling *E. coli* consensus promoter sequences; however, the function of these sequences in *Xanthomonas* is unknown. It is possible that deletions that gave the intermediate phenotype altered the level of *avrBs*$_1$ transcription and thereby reduced the active ORF2 protein product. Information on transcription initiation is necessary before one can conclude if the ORF1 sequences actually function as transcriptional start signals.

The similarity of an avirulence gene from *X. c.* pv. *vesicatoria*, a pathogen of pepper, with an avirulence gene from *P. s.* pv. *glycinea*, a pathogen of soybean, suggests that the proteins encoded by these two genes may function in an analogous manner to specify disease resistance in their respective hosts. Both *avrA* and *avrBs*$_1$ encode proteins that are hydrophilic, have similar hydropathy profiles, and have long stretches of conserved amino acids. In addition, neither protein contains recognizable transit signal sequences (Oliver 1985). Although other avirulence genes have been shown to be conserved among races (Tamaki *et al.* 1988) and between pathovars (Kobayashi and Keen 1986), this is the first report of conservation of avirulence genes between genera. *X. c.* pv. *vesicatoria* race 1 transconjugants containing *avrA* do not elicit an HR when inoculated into ECW10R plants (data not shown). Similarly, *P. s.* pv. *glycinea* race 4 transconjugants containing *avrBs*$_1$ clones (pXV2112 or placD; Table 2) do not induce an HR when inoculated into a soybean cultivar that recognizes *avrA*. It is unknown if the avirulence genes are expressed in the transconjugants. Although the avirulence gene proteins are highly conserved, the gene products still maintain specificity for their respective hosts in a gene-for-gene manner. No other proteins in the Bionet database have significant homology to *avrBs*$_1$.

We have also shown that the *avrBs*$_1$ 824-bp DNA fragment is homologous to a fragment of the same size in *X. campestris* pathovars *vitians* and *campestris*. Both these pathovars cause an HR characteristic of the *avrBs*$_1$-*Bs*$_1$ interaction when inoculated into ECW10R plants. *X. c.* pv. *vitians* causes watersoaked lesions on ECW plants. *X. c.* pv. *campestris* gives a light HR on ECW that can be clearly distinguished from the HR induced on ECW10R plants. We are constructing *avrBs*$_1$ marker exchange mutants in *X. campestris* pathovars *vitians* and *campestris* to verify that *avrBs*$_1$ functions to specify avirulence when these pathovars are inoculated into *Bs*$_1$ plants.

The anti-ORF2 antisera showed no reaction with proteins of *X. c.* pv. *vesicatoria* race 2 grown in broth, indicating that the *avrBs*$_1$ ORF2 protein is not abundant under the conditions tested. The regulation of expression of this avirulence gene appears to be different from that of other avirulence genes so far studied. Preliminary evidence suggests that the *avrBs*$_1$ protein is not induced when *X. c.* pv. *vesicatoria* is grown *in planta*, as has been shown for the avirulence genes *avrB* and *avrC* from *P. s.* pv. *glycinea* (T. Huyunh, D. Dahlbeck, and B. J. Staskawicz, unpublished; S. Tamaki, personal communication). We are currently investigating more sensitive methods for detecting the protein in wild-type race 2 cells as well as other growth conditions in which the protein may be more highly expressed.

Western blots of *E. coli* and *X. c.* pv. *vesicatoria* cells expressing transcriptional fusions to *avrBs*$_1$ show that a 50-kD protein is produced and recognized by the antisera specific to *avrBs*$_1$ ORF2. The protein is stable in an *E. coli* protease deficient strain and in *X. c.* pv. *vesicatoria* race 1. It appears that *avrBs*$_1$ ORF2 is efficiently translated both in *E. coli* and *X. c.* pv. *vesicatoria* despite the lack of homology to the *E. coli* consensus sequence for a ribosome binding site. The 50-kD protein detected by the antisera correlates well with the 49.8 kD size protein predicted by the DNA sequence of *avrBs*$_1$ ORF2. In addition, *X. c.* pv. *vesicatoria* race 1 transconjugants that express the 50-kD protein also induce a HR on the host ECW10R. *X. c.* pv. *vesicatoria* race 1, and *X. c.* pv. *vesicatoria* race 1 containing transcriptional fusions to the insertionally mutated subclone in pLAFR3 (pXV2m105a), do not express a 50-kD protein and do not induce a HR when inoculated in ECW10R (data not shown). These data are consistent with the hypothesis that *avrBs*$_1$ ORF2 encodes a 50-kD protein necessary for eliciting the HR; however, it is currently unknown if this

protein acts directly or in conjunction with other molecules to induce the resistant plant response in the gene-for-gene interaction.

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LITERATURE CITED

Baker, T. A., Grossman, A. D., and Gross, C. A. 1984. A gene regulating the heat shock response in *Escherichia coli* also affects proteolysis. Proc. Natl. Acad. Sci. USA 81:6779-6783.

Cook, A. A., and Guevara, Y. G. 1984. Hypersensitivity in *Capsicum chacoense* to race 1 of the bacterial spot pathogen of pepper. Plant Dis. 68:329-330.

Cook, A. A., and Stall, R. E. 1963. Inheritance of resistance in pepper to bacterial spot. Phytopathology 53:1060-1062.

Daniels, M. J., Barber, C. E., Turner, D. C., Cleary, S. G., and Sawzyc, M. K. 1984. Isolation of mutants of *Xanthomonas campestris* pv. *campestris* showing altered pathogenicity. J. Gen. Microbiol. 130:2447-2455.

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

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

Figurski, D., 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.

Flor, H. H. 1956. The complementary genetic systems in flax and flax rust. Adv. Genet. 8:29-54.

Gabriel, D. W., Burges, A., and Lazo, G. R. 1986. Gene-for-gene interactions of five cloned avirulence genes from *Xanthomonas campestris* pv. *malvacearum* with specific resistance genes in cotton. Proc. Natl. Acad. Sci. USA 83:6415-6419.

Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28:351-359.

Hibberd, A. M., Basset, M. J., and Stall, R. E. 1987. Allelism tests of three dominant genes for hypersensitive resistance to bacterial spot of pepper. Phytopathology 77:1304-1307.

Hopp, T. P., and Woods, K. R. 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA 78:3824-3828.

Kearney, B., Ronald, P. C., Dahlbeck, D., and Staskawicz, B. J. 1988. Molecular basis for evasion of plant host defense in bacterial spot disease of pepper. Nature 332:541-543.

Kim, B.-S., and Hartmann, R. W. 1985. Inheritance of a gene (Bs_3) conferring hypersensitive resistance to *Xanthomonas campestris* pv. *vesicatoria* in pepper (*Capsicum annuum*). Plant Dis. 69:233-235.

Kobayashi, D. Y., and Keen, N. T. 1986. Analysis of an avirulence gene from *Pseudomonas syringae* pv. *tomato* which elicits hypersensistive response on soybean. (Abstr.) Phytopathology 76:1099.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Lipman, D. J., and Pearson, W. R. 1985. Rapid and sensitive protein similarity searches. Science 227:1435-1441.

Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Napoli, C., and Staskawicz, B. J. 1987. Molecular characterization of an avirulence gene from Race 6 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. 169:572-578.

Norrander, J., Kempe, T., and Messing, J. 1983. Construction of improved M13 vectors using oligodeoxynucleotide directed mutagenesis. Gene 26:101-106.

Oliver, D. 1985. Protein secretion in *Escherichia coli*. Annu. Rev. Microbiol. 39:615-648.

Rio, D. C., Laski, F. A., and Rubin, G. M. 1986. Identification and immunochemical analysis of biologically active *Drosophila* P element transposase. Cell 44:21-32.

Rüther, V., and Müller-Hill, B. 1983. Easy identification of cDNA clones. EMBO J. 2:1791-1794.

Sanger, F. Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.

Shatzman, A. R., and Rosenberg, M. 1986. Efficient expression of heterologous genes in *E. coli*: The pAS vector system and its applications. Annals of the New York Academy of Science. Allan Goodridge, ed., New York Academy of Science.

Shine, J., and Dalgarno, L. 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: Complementary to nonsense triplets and ribosomal binding sites. Proc. Natl. Acad. Sci. USA 71:1342-1346.

Smith, D. E., and Fisher, P. A. 1984. Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in *Drosophila* embryos: Application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. J. Cell Biol. 99:20-28.

Smith, D. H., Brutlag, D., Freidland, P., and Kedes, K. L. 1986. BIONET: National computer resource for molecular biology. Nucleic Acids Res. 14:17-20.

Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 76:240-243.

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.

Staskawicz, B. J., 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.

Swanson, J., Kearney, B., Dahlbeck, D., and Staskawicz, B. 1988. Cloned avirulence gene of *Xanthomonas campestris* pv. *vesicatoria* complements spontaneous race-change mutants. Mol. Plant Microbe Interact. 1:5-9.

Tamaki, S., Kobayashi, D., Staskawicz, B., and Keen, N. T. 1988. Construction of recombinant genes from *avr*B and *avr*C in order to localize regions of plant recognition specificity. 11th Annu. Symp. Plant Pathol., University of California, Riverside.

Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA 76:4350-4354.

Viera, J., and Messing, J. 1987. Production of single-stranded plasmid DNA. Methods Enzymol. 153:3-11.