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Gene-For-Gene Relationships Specifying Disease Resistance in *Xanthomonas campestris* pv. *vesicatoria* - Pepper Interactions

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Three groups of *Xanthomonas campestris* pv. *vesicatoria* strains were distinguished, based on the response to infection of the tomato cultivar Walter and a set of near-isogenic lines of pepper. The near-isogenic pepper lines were derived from Early Calwonder (ECW), ECW-10R, ECW-20R, and ECW-30R, and contain the resistance genes *Bs1*, *Bs2*, and *Bs3*, respectively. The XcvT group was avirulent on all pepper lines and virulent on Walter; the XcvP group was avirulent on specific pepper lines and also avirulent on Walter; and the XcvPT group was avirulent on specific pepper lines but virulent on Walter. To test whether avirulence genes played a role in these responses, genomic and plasmid DNA libraries were constructed from two strains of *X. c.* pv. *vesicatoria*. Three avirulence genes were identified that converted normally virulent strains to avirulence on specific lines of pepper. The three avirulence genes were characterized by restriction enzyme analysis, Southern blot analysis, and analysis of the phenotype of the hypersensitive reaction (HR). The avirulence gene *avrBsT* controlled the ability of the XcvT group to induce an HR on pepper. The avirulence genes *avrBs2* and *avrBs3* allowed strains carrying these genes to induce an HR on pepper lines containing the corresponding disease resistance genes *Bs2* and *Bs3*. The *avrBsT* and *avrBs3* genes were localized to indigenous plasmids while *avrBs2* appears to be localized to the chromosome. To further characterize the HR resistance induced by *X. c.* pv. *vesicatoria* avirulence genes, four avirulence genes from *X. c.* pv. *vesicatoria* were conjugated into a spontaneous mutant of *X. c.* pv. *vesicatoria* that was virulent on all pepper lines. Comparisons of the electrolyte leakage patterns of resistant pepper lines inoculated with these transconjugants revealed that strains carrying the cloned avirulence genes induced patterns similar to those of the wild-type strains with that avirulence gene.

Additional keywords: *Capsicum annuum*, bacterial spot.

Xanthomonas campestris pv. *vesicatoria* (Doidge) Dye causes a foliage and fruit spot disease of tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.). Three groups of *X. c.* pv. *vesicatoria* have been distinguished on the basis of virulence for tomato and pepper: the tomato group (XcvT) is virulent on tomato only, the pepper group (XcvP) on pepper only, and the pepper-tomato group (XcvPT) on both pepper and tomato (Reifschneider *et al.* 1985). Within the XcvPT and XcvP groups, races of the pathogen can be distinguished by their ability to cause disease on various pepper lines.

Resistance in pepper to *X. c.* pv. *vesicatoria* is generally associated with a hypersensitive response (HR). An HR is observed as a confluent necrosis when leaves are infiltrated with bacterial suspensions of 10^8 colony forming units (cfu) per milliliter. Growth of bacterial populations within the intercellular spaces of leaves is arrested during the development of an HR and disease symptoms are not evident (Stall and Cook 1966; Hibberd *et al.* 1987a). HR resistance is thought to be controlled according to the gene-for-gene model of resistance, that is, it is controlled by an avirulence gene in the pathogen and a resistance gene in the host (Flor 1955; Ellingboe 1984).

Support for the gene-for-gene model of resistance operating in the interaction between pepper and members of the XcvPT and XcvP groups has been developed through breeding of pepper for resistance to *X. c.* pv. *vesicatoria*. Three sources of resistance to strains of *X. c.* pv. *vesicatoria* have been described in pepper (Cook and Stall 1963; Cook and Guevara 1984; Kim and Hartmann 1985), and each resistance gene is specific for particular strains of the pathogen (Hibberd *et al.* 1987b). Furthermore, each resistance gene is simply inherited, and each of the three genes segregates independently from each other (Hibberd *et al.* 1987b).

Support for the gene-for-gene model operating in the interaction of pepper with XcvT is difficult to obtain. The inheritance of resistance to XcvT in pepper cannot be studied because no line is susceptible (Cook 1973), and therefore, crosses between resistant and susceptible lines cannot be made. However, the HR resistance of pepper to XcvT is frequently overcome by pathogen change to virulence (Dahlbeck and Stall 1979), a characteristic that often indicates an interaction which is controlled in a simple genetic manner (Kearney *et al.* 1988).

Evidence that resistance in pepper is controlled in a gene-for-gene fashion is forthcoming from analysis of avirulence in *X. c.* pv. *vesicatoria*. The genetic locus for avirulence of XcvPT race 2 that corresponds to the *Bs1* gene for resistance in pepper has been located on a self-transmissible plasmid which also encodes copper resistance (Stall *et al.* 1986). The avirulence gene *avrBs1* was cloned from the plasmid (Swanson *et al.* 1988). Sequence analysis of *avrBs1* revealed two open-reading frames, with avirulence activity residing in the open-reading frame encoding a 49.8-kDa protein (Ronald and Staskawicz 1988). Thus it appears that a single gene in the host, *Bs1*, and a single gene in

the pathogen, *avrBs1*, specify the resistant reaction.

In this study, we describe the cloning and characterization of three avirulence genes from *X. c.* pv. *vesicatoria*. We present evidence that these avirulence genes restrict the host range of *X. c.* pv. *vesicatoria* strains.

MATERIALS AND METHODS

Plasmids, bacterial strains, and culture conditions. Plasmids used in this study are listed in Table 1. Bacterial strains and their relevant phenotypes in leaves of tomato and pepper are listed in Tables 2, 3, and 4. Strains of *X. c.* pv. *vesicatoria* were stored in sterile tap water after isolation from diseased tissue by R. E. Stall. *X. c.* pv. *vesicatoria* was subcultured on nutrient agar (NA) or nutrient yeast-glycerol agar (NYGA; Daniels *et al.* 1984). Rifampicin-resistant strains were isolated by plating 10^9 cells on NA containing rifampicin and selecting resistant colonies with wild-type pathogenicity characteristics. *Escherichia coli* HB101 and DH5α were maintained on Luria-Bertani agar medium (Maniatis *et al.* 1982). The following antibiotic concentrations were used: 50 to 100 μg/ml of rifampicin and 10 μg/ml of tetracycline.

Table 1. Plasmids used in this study

Designation	Relevant characteristics	Reference
pLAFR3	*tet*^{r}	Staskawicz *et al.* 1987
pEC103	pLAFR3 clone from XcvT race 1 75-3 with *avrBsT* activity	This study
pXV943	4.3-kbp *Pst*I subclone of pEC103 with *avrBsT* activity	This study
pXV2007	5.3-kbp *Bgl*II-*Pst*I pLAFR3 subclone with *avrBs1* activity	Swanson *et al.*1988
pEC815	pLAFR3 clone from XcvT race 1 75-3 with *avrBs2* activity	This study
p81538	2.4-kbp *Bam*HI subclone of pEC815 with *avrBs2* activity	This study
pEC83	pLAFR3 clone with *avrBs3* activity from library of a 41-kbp plasmid from XcvP race 1 71-21	This study
pBS3	4.5-kbp *Sau*3A subclone of pEC83 with *avrBs3* activity	This study

Table 2. Virulence of groups and races of *Xanthomonas campestris* pv. *vesicatoria* classified according to reactions of pepper and tomato[a]

		Pepper				Tomato
Group	Race	ECW[b]	ECW-10R[c] (*Bs1*)	ECW-20R (*Bs2*)	ECW-30R (*Bs3*)	Walter
Tomato (XcvT)	1	−	−	−	−	+
Pepper (XcvP)	1	+	+	−	−	−
Pepper-Tomato (XcvPT)	2	+	−	−	+	+
Pepper-Tomato (XcvPT)	3	+	+	−	+	+

[a]+, virulent; −, avirulent.
[b]ECW refers to the Early Calwonder cultivar.
[c]ECW-10R refers to a line derived from ECW containing the resistance gene, *Bs1*.

Growth of plants and plant inoculations. The pepper cultivar Early Calwonder (ECW) and the tomato cultivar Walter were used to distinguish three groups of *X. c.* pv. *vesicatoria*. Three lines of pepper, ECW-10R, ECW-20R, and ECW-30R carrying the *Bs1*, *Bs2*, and *Bs3* genes for resistance, respectively, were used to differentiate races within XcvPT and XcvP (Table 2). These lines are near-isogenic derivatives of ECW (R. E. Stall, unpublished data). In early work, the breeding line 3-25-2-7 was used instead of ECW-20R. This line originated from the progeny of a second backcrossed F_2 plant homozygous for the *Bs2*

Table 3. Virulence phenotypes of strains and transconjugants of *Xanthomonas campestris* pv. *vesicatoria*

	Pepper			
Designation	ECW[a]	ECW-10R	ECW-20R 3-25-2-7	ECW-30R
XcvT race 1 75-3	A[b]	B	C	A
XcvPT race 2 81-23	+[c]	B	C	+
XcvPT race 2 81-23 (pEC103)	A	B	C	A
XcvPT race 2 81-23 (pXV943)	A	B	C	A
XcvT race 1 75-3 *avrBsT*$^-$	+	B	C	+
XcvT race 1 75-3 *avrBsT*$^-$ (pXV943)	A	B	C	A
XcvT race 1 75-3 *avrBsT*$^-$, *1*$^-$	+	+	C	+
XcvT race 1 75-3 *avrBsT*$^-$, *1*$^-$ (pXV2007)	+	B	C	+
XcvP race 1 82-8	+	+	C	D
XcvP race 1 82-8 *avrBs2*$^-$	+	+	+	D
XcvP race 1 82-8 *avrBs2*$^-$ (pEC815)	+	+	C	D
XcvP race 1 82-8 *avrBs2*$^-$ (p81538)	+	+	C	D
XcvP race 1 71-21	+	+	C	D
XcvPT race 2 81-23 (pEC83)	+	B	C	D
XcvPT race 2 81-23 (pBS3)	+	B	C	D

[a]See footnotes to Table 2.
[b]Phenotypes of avirulent reactions. A, light brown, nonconfluent necrosis appearing within 24 to 36 hr, associated with *avrBsT*. B, dark, papery-brown, confluent necrosis appearing within 8 to 12 hr, associated with *avrBs1* and *Bs1*. C, medium brown, confluent necrosis appearing within 12 to 36 hr, associated with *avrBs2* and *Bs2*. D, medium brown, confluent necrosis appearing within 24 to 36 hr, associated with *avrBs3* and *Bs3*.
[c]Virulent reaction, water-soaked lesion.

gene. ECW was the recurring parent in the backcrossing program, and XVR-3-25 (Cook 1984) was the source of the *Bs2* gene.

Seedlings were cultured in a peat-vermiculite mix in 10-cm-diameter plastic pots in a greenhouse that varied in temperature from 20 to 35° C. A soluble 20-20-20 fertilizer (0.4 g per pot) was added to the plants on a biweekly schedule. Fully expanded leaves were inoculated near the time when the main stem branched. For analysis of electrolyte leakage, plants were transplanted into 15-cm-diameter pots after 6 wk of growth, and fully expanded leaves were inoculated when secondary stems branched. After inoculation, plants were transferred to growth chambers (28 to 30° C, 12-hr light period).

For screening libraries of DNA from *X. c.* pv. *vesicatoria*, transconjugants were grown on NA and then suspended in 2 ml of sterile tap water. The concentration of cells was visually adjusted to 10^8 to 10^9 cfu/ml. Leaves were inoculated using 1-ml syringes and 27-gauge needles. Each inoculated area, 1–2 cm^2, 10 per leaf, received an individual transconjugant carrying a different clone. To distinguish races of XcvPT and XcvP, fewer areas per leaf were infiltrated, and the inoculum concentration was standardized to an absorbance of 0.3 at a wavelength of 600 nm. For identification of *X. c.* pv. *vesicatoria* strains pathogenic to pepper and not to tomato, that is, XcvP, Carborundum of 600 grit was added to the standardized inoculum and rubbed with a cotton swab onto leaves that were not fully expanded. An area of 4–5 cm^2 was infiltrated for analysis of electrolyte leakage.

Table 4. Virulence phenotypes of strains and transconjugants of *Xanthomonas campestris* pv. *vesicatoria* used in electrolyte leakage analysis

	Pepper			
Designation	**ECW[a]**	**ECW-10R**	**ECW-20R 3-24-2-7**	**ECW-30R**
XcvP race 1 82-8 *avrBs2*$^-$, *3*$^-$	+[b]	+	+	+
XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pXV943)	A[c]	A	A	A
XcvPT race 2 E3	+	B	C	+
XcvP race 1 82-8 *avrBs2*$^-$, *3*$^-$ (pXV2007)	+	B	+	+
XcvPT race 3 88-5	+	+	C	+
XcvP race 1 82-8 *avrBs2*$^-$, *3*$^-$ (p81538)	+	+	C	+
XcvP race 1 84-1	+	+	C	D
XcvP race 1 82-8 *avrBs2*$^-$, *3*$^-$ (pBS3)	+	+	+	D

[a]See footnotes in Table 2.
[b]Virulent reaction, water-soaked lesion.
[c]Phenotypes of avirulent reactions are described in the footnotes of Table 3.

To select for strains that lost specific avirulence gene activity, bacterial cells were infiltrated into entire leaves with syringes. In normally resistant plants, strains losing avirulence activity were able to multiply and form lesions, whereas those carrying an active copy of the specific avirulence gene could not. Three concentrations were used, 3×10^1, 3×10^2, and 3×10^3 cfu/cm^2, and three leaves were inoculated with each concentration. After inoculation, leaves were examined weekly for up to 6 wk for small blackish-brown lesions. Bacteria were isolated from such lesions, single-colony purified, and then reinoculated into leaves. Those strains giving a virulent reaction upon reinoculation were saved for further analysis.

Conjugations. To mobilize clones of DNA maintained in *E. coli* to *X. c.* pv. *vesicatoria*, the helper plasmid pRK2013 was used in triparental matings (Ditta *et al.* 1980). For screening libraries, 50 matings were plated on NYGA. After incubation at 30° C for 24 hr, bacteria were suspended in 4 ml of sterile water, and portions of this suspension were spread on NA containing rifampicin and tetracycline to select for transconjugants. All *X. c.* pv. *vesicatoria* recipients were resistant to rifampicin.

Recombinant DNA techniques. Techniques used for cosmid cloning, enzyme digestions, alkaline phosphatase treatments, nick translation, Southern hybridizations, the plasmid rapid lysate procedure, and agarose gel electrophoresis are described in Maniatis *et al.* (1982). The cosmid libraries of total DNA from XcvT race 1 75-3 and plasmid DNA from XcvP race 1 71-21 were constructed in pLAFR3 (Staskawicz *et al.* 1987). To ascertain whether particular DNA fragments were localized to indigenous plasmids or to chromosomal DNA of *X. c.* pv. *vesicatoria*, indigenous plasmids were separated (Swanson *et al.* 1988; Kado and Liu 1981), Southern blotted, and probed with ^{32}P-labeled DNA fragments. Molecular weights of *X. c.* pv. *vesicatoria* plasmids were approximated using the plasmids of *Erwinia stewartii* as molecular weight standards (Coplin *et al.* 1981). Genomic DNA from *X. c.* pv. *vesicatoria* was isolated as described by Staskawicz *et al.* (1984).

Electrolyte leakage. Time courses of electrolyte leakage were obtained from measurements of the electrical conductivity of baths containing tissue from inoculated leaves. The procedure used for determining electrical conductivity was that reported by Hibberd *et al.* (1987a). For each assay, six leaf disks of a 0.5 cm^2 area were placed into 3 ml of deionized water in 1.6 × 10 cm test tubes. Three leaves per plant were inoculated with each strain. Tests on each combination were repeated three times.

RESULTS

Grouping of *X. c.* pv. *vesicatoria* strains. Three groups of *X. c.* pv. *vesicatoria* strains were distinguished by their phenotypes on pepper and tomato (Table 2). Members of the tomato group, XcvT, were avirulent on all pepper plants and virulent on the tomato cultivar Walter. Members of the pepper group, XcvP, were virulent on some pepper lines and avirulent on the tomato cultivar Walter. Members of the pepper-tomato group, XcvPT, were virulent on

tomato and on most lines of pepper. XcvP could only be distinguished from XcvT or XcvPT on the basis of HR induction in tomato in response to inoculation by swabbing Carborundum-amended suspensions of bacteria onto leaves. Races within XcvPT and XcvP were differentiated by inoculation of the three pepper lines with high concentrations of bacteria (Table 2). All XcvPT and XcvP races were avirulent on ECW-20R containing *Bs2*; race 1 strains were avirulent on ECW-30R containing *Bs3*; and race 2 strains were avirulent on ECW-10R containing *Bs1* (Table 2).

Identification and cloning of *avrBsT*. To determine if an avirulence gene in XcvT race 1 75-3 prevented it from causing disease on ECW, a library of DNA from XcvT race 1 75-3 was mobilized into normally virulent XcvPT race 2 81-23 and transconjugants were inoculated into leaves of ECW. Five cosmid clones were found that converted XcvPT race 2 81-23 from virulence to avirulence on ECW. One of the cosmid clones, pEC103, was subcloned by *Pst*I digestion (Table 3). An active subclone of 4.3 kilobase pairs (kbp), pXV943, was isolated that had full avirulence activity on ECW (Table 3). XcvPT race 2 81-23 (pXV943) is avirulent on all pepper lines and virulent on tomato (Table 3). The avirulence locus carried by pXV943 has been designated *avrBsT*. The avirulence gene *avrBsT* was localized to an indigenous plasmid of approximately 41 kbp in XcvT race 1 75-3 (data not shown). Hybridizing fragments of DNA were found in genomic DNA of XcvT race 1 75-3 and XcvPT race 2 E3, but not in XcvP race 1 82-8 or 84-1, XcvPT race 2 81-23, or XcvPT race 3 88-5 (Fig. 1A).

Isolation and characterization of XcvT race change mutants. In laboratory studies, XcvT undergoes spontaneous and sequential race changes (Dahlbeck and Stall 1979). To study the mechanism of race change in laboratory studies, the normally avirulent XcvT race 1 75-3 was infiltrated into leaves of ECW. A spontaneous virulent strain without *avrBsT* activity was isolated and designated XcvT race 1 75-3 *avrBsT*$^-$ (Table 3). The virulence was associated with the loss of the approximately 41-kbp indigenous plasmid (data not shown) and with the loss of a fragment that hybridized to a probe containing *avrBsT* (Fig. 1A, lane 2). The subclone pXV943 complemented the loss of *avrBsT* activity on ECW and ECW-30R (Table 3). XcvT race 1 75-3 *avrBsT*$^-$ retained avirulence activity on ECW-10R suggesting that XcvT race 1 75-3 *avrBsT*$^-$ carried *avrBs1*. To learn if a second race change mutation could be induced, XcvT race 1 75-3 *avrBsT*$^-$ was infiltrated into leaves of ECW-10R. A virulent strain lacking *avrBsT* and *avrBs1* activity was isolated and designated XcvT race 1 75-3 *avrBsT*$^-$,*1*$^-$. This strain was virulent on ECW, ECW-10R, and ECW-30R, but was still avirulent on ECW-20R (Table 3). Virulence on ECW-10R was associated with an insertion of IS476 into *avrBs1*; total DNA hybridized to the probe containing *avrBs1* with a decrease in mobility consistent with the insertion of a 1.2-kbp element (Kearney and Staskawicz 1987; Kearney *et al.* 1988). A subclone of *avrBs1*, pXV2007, converted the phenotype of XcvT race 1 75-3 *avrBsT*$^-$,*1*$^-$ back to avirulence on ECW-10R (Table 3).

Identification and cloning of *avrBs2* and *avrBs3*. At the time of this study, no strain isolated from diseased tissue from nature was virulent on pepper lines carrying the *Bs2* gene. To isolate a virulent strain, suspensions of wild-type XcvP race 1 82-8 were infiltrated into leaves of ECW-20R, and the virulent strain, XcvP race 1 82-8 *avrBs2*$^-$, was isolated (Table 3). XcvP race 1 82-8 *avrBs2*$^-$ was still avirulent on ECW-30R. To isolate a strain that was virulent on all three pepper lines, XcvP race 1 82-8 *avrBs2*$^-$ was infiltrated into leaves of ECW-30R, and the virulent strain XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ was isolated (Table 4). This

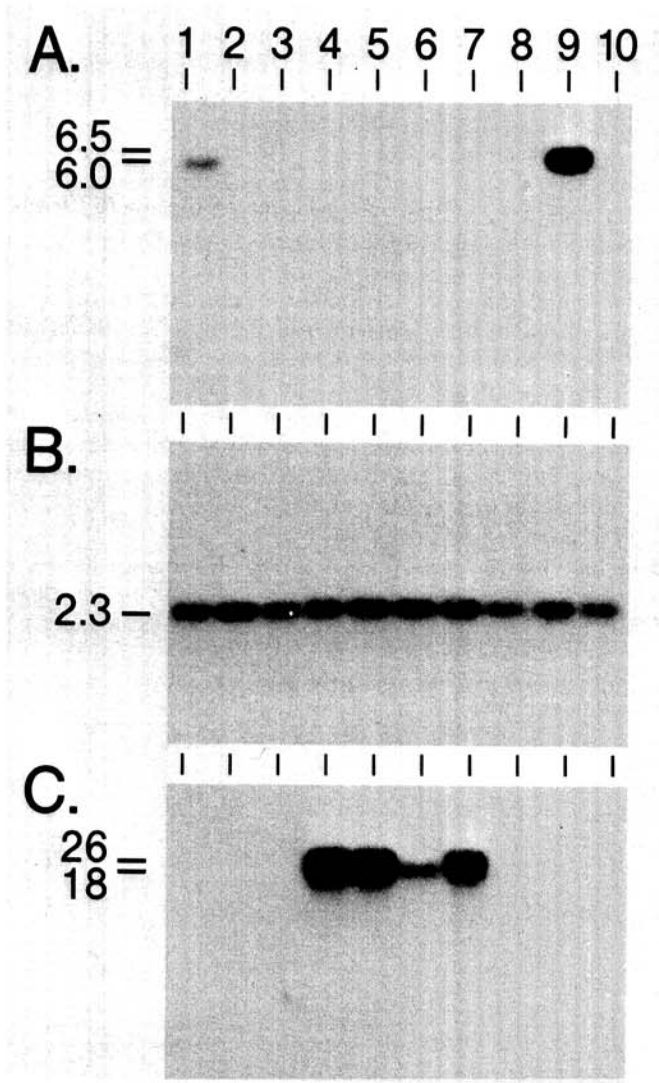

Fig. 1. Hybridization of avirulence gene-specific probes to total genomic DNA of *Xanthomonas campestris* pv. *vesicatoria* strains. Lane 1, XcvT race 1 75-3; lane 2, XcvT race 1 75-3 *avrBsT*$^-$; lane 3, XcvT race 1 75-3 *avrBsT*$^-$,*1*$^-$; lane 4, XcvP race 1 82-8; lane 5, XcvP race 1 82-8 *avrBs2*$^-$; lane 6, XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$; lane 7, XcvP race 1 84-1; lane 8, XcvPT race 2 81-23; lane 9, XcvPT race 2 E3; and lane 10, XcvPT race 3 88-5. Panel **A**, *avrBsT*, a 3.2-kilobase pair (kbp) *Eco*RI-*Pst*I fragment internal to the active 4.5-kbp subclone pXV943. *X. c.* pv. *vesicatoria* genomic DNA digested with *Eco*RV. Panel **B**, *avrBs2*, a 2.3-kbp *Sph*I fragment from p81538. *X. c.* pv. *vesicatoria* genomic DNA digested with *Sph*I. Panel **C**, *avrBs3*, a 3.2-kbp *Bam*HI fragment internal to the active 4.5-kbp subclone pBS3. *X. c.* pv. *vesicatoria* genomic DNA digested with *Eco*RI.

strain was used as the recipient for tests of avirulence gene specificity and electrolyte leakage activity (Table 4, Fig. 2).

To isolate an avirulence locus from *X. c.* pv. *vesicatoria* corresponding to the *Bs2* gene in ECW-20R, a library of DNA from XcvT race 1 75-3 was screened in XcvP race 1 82-8 *avrBs2*$^-$. Two cosmid clones were isolated that converted the normally virulent recipient to avirulence on the pepper line 3-25-2-7 carrying the *Bs2* gene. One cosmid clone, pEC815, was subcloned, and a 2.4-kbp *Bam*HI fragment of DNA in clone p81538 retained its activity (Table 3). This avirulence gene has been designated *avrBs2*. The *avrBs2* locus appears to be inseparable from chromosomal DNA (data not shown), and hybridizing fragments were present in DNA from all groups of *X. c.* pv. *vesicatoria* that were tested (Fig. 1B). A fragment hybridizing to a probe containing *avrBs2* was also present in the mutant strain lacking *avrBs2* activity, XcvP race 1 82-8 *avrBs2*$^-$ (Fig. 1B, lane 5).

XcvP race 1 71-21 was the source of the *avrBs3* locus. In previous experiments (G. V. Minsavage and R. E. Stall, unpublished data), the spontaneous loss of an approximately 41-kbp indigenous plasmid was correlated with the loss of avirulence on ECW-30R carrying *Bs3*. A library of DNA from the plasmid was conjugated into XcvPT race 2 81-23, and transconjugants were screened for *avrBs3* activity on ECW-30R. One cosmid clone, pEC83, converted the virulent recipient to avirulence on ECW-30R (Table 3). The insert was subcloned to a 4.5-kbp fragment of DNA, pBS3, by *Sau*3A digestion (Table 3). The plasmid-borne locus, designated *avrBs3*, hybridized to total genomic DNA from wild-type XcvP race 1 strains and mutants derived from XcvP race 1 strains (Fig. 1C, lanes 4–7). Genomic DNA from XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ hybridized to the probe containing *avrBs3*, although the strain lacked *avrBs3* activity (Fig. 1C, lane 6; Table 3) and the 41-kbp plasmid carrying the active copy of *avrBs3* (data not shown). The DNA that is not active which hybridized to the probe containing *avrBs3* has noticeably less homology than the active copy and is also carried on an indigenous plasmid (Bonas *et al.* 1989).

None of the avirulence genes described in this study hybridized to each other or to the previously described avirulence genes from *X. c.* pv. *vesicatoria*, *avrBs1*, or *avrRxv* (data not shown; Swanson *et al.* 1988; Whalen *et al.* 1988).

Phenotype of HR reactions. The phenotype of the HR observed upon inoculation varied according to the particular pair of interacting avirulence and resistance genes (Table 3). HRs varied in the timing of their appearance, the intensity of browning, and the degree of confluence. The same HR phenotype was associated with the presence of either the native copy of the particular avirulence gene in *X. c.* pv. *vesicatoria* or a copy carried by *X. c.* pv. *vesicatoria* transconjugants (Table 3). The phenotype of the HR of pepper lines ECW and ECW-30R associated with *avrBsT* was a light brown, nonconfluent necrosis that appeared within 24 to 36 hr. When *X. c.* pv. *vesicatoria* strains carrying *avrBs1* were inoculated on ECW-10R containing *Bs1*, a dark, papery-brown, confluent necrosis appeared within 8 to 12 hr. On ECW-20R containing *Bs2*, *X. c.* pv. *vesicatoria* strains carrying *avrBs2* induced a medium brown necrosis that slowly appeared within 12 to 36 hr. The phenotype of the HR associated with interactions between XcvP race 1 strains carrying *avrBs3* and ECW-30R containing the *Bs3* gene was a medium brown, confluent necrosis that appeared within 24 to 36 hr. On tomato, XcvP race 1 strains induced a medium brown, confluent necrosis that appeared within 24 to 36 hr.

Analysis of electrolyte leakage. Electrolyte leakage was analyzed using XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ as the recipient

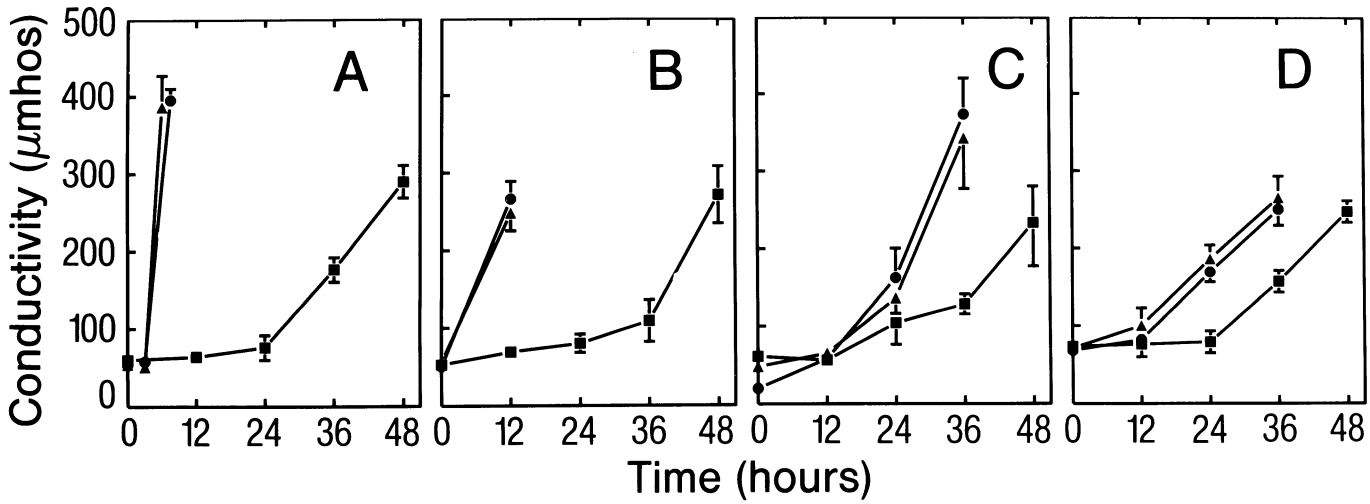

Fig. 2. Electrolyte leakage from leaves of pepper plants inoculated with strains of *Xanthomonas campestris* pv. *vesicatoria* at a concentration of 3×10^8 colony forming units per milliliter. Phenotypes of the reactions on leaves are given in Tables 3 and 4. Each point represents the mean from three experiments, each with three replicate measurements. Vertical lines represent standard error of the mean. **A**, Early Calwonder (ECW)-10R inoculated with XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (■); XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pXV2007) (●); and XcvPT race 2 E3 (▲). **B**, ECW-20R inoculated with XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (■); XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (p81538) (●); and XcvPT race 3 88-5 (▲). **C**, ECW-30R inoculated with XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (■); XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pBS3) (●); and XcvP race 1 84-1 (▲). **D**, ECW inoculated with XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (■); XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pXV943) (●), and XcvT race 1 75-3 (▲).

for avirulence genes because it was virulent on all pepper lines (Table 4). The time course of electrolyte leakage from pepper lines carrying the corresponding resistance gene infiltrated with XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pLAFR3), XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ carrying individual avirulence genes, and the wild-type strain containing the avirulence gene was compared. The timing and rate of electrolyte leakage induced by wild-type strains and XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ carrying each avirulence gene were indistinguishable (Fig. 2).

In all cases, electrolyte leakage from the virulent XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ occurred later and in all but one case, at a slower rate than the avirulent strains and transconjugants. Each combination showed a different overall pattern of electrolyte leakage (Fig. 2). Leaves of ECW-10R responded to XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pXV2007) and XcvPT race 2 E3 within 6 hr after infiltration with a high rate of electrolyte leakage (Fig. 2A). ECW-20R responded to infiltration with XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (p81538) and XcvPT race 3 88-5 with maximum electrolyte leakage within 12 hr after infiltration (Fig. 2B). ECW-30R responded to XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pBS3) and XcvP race 1 84-1 after 12 hr, with the rate of electrolyte leakage greatest between 24 and 36 hr (Fig. 2C). The electrolyte leakage of ECW in response to XcvT race 1 75-3 and XcvP race 1 82-8 *avrBs2*$^-$,*3*$^-$ (pXV943) slowly increased after 12 hr (Fig. 2D).

DISCUSSION

We provide evidence that the gene-for-gene model of resistance (Flor 1955; Ellingboe 1984) operates in the *X. c.* pv. *vesicatoria*-pepper interaction. We have identified and cloned three different *X. c.* pv. *vesicatoria* avirulence genes, *avrBs2, avrBs3*, and *avrBs1*, that specify disease resistance on lines of pepper carrying the corresponding resistance genes, *Bs2, Bs3*, and *Bs1*, respectively (Table 2). Avirulence genes restrict the host range of *X. c.* pv. *vesicatoria* within the pepper species. Moreover, we have cloned and characterized the avirulence gene *avrBsT* from the tomato pathogen XcvT that induces a resistant response in all pepper lines (Table 3).

These results show that avirulence genes are capable of not only restricting host range within a species but also host range between genera. These results are consistent with those from Whalen *et al.* (1988) and Kobayashi *et al.* (1989) showing that nonhost resistance in bean and soybean against two tomato pathogens can be partially explained by the recognition of avirulence genes. These reports did not address the possibility of additional avirulence genes specifying the resistant responses and of the lack of positive gene functions required for inducing disease on nonhosts. The spontaneous loss of *avrBsT* from the tomato pathogen XcvT race 1 75-3 allows the strain to cause disease on two normally resistant pepper lines showing that in this case, a single avirulence gene does indeed prevent the strain from causing disease on a nonhost (Table 3).

Five avirulence genes have now been cloned from strains of *X. c.* pv. *vesicatoria*, three of which correspond to genetically characterized resistance genes in pepper (Table 3; Hibberd *et al.* 1987b; Swanson *et al.* 1988; Whalen *et al.* 1988; Bonas *et al.* 1989). Interestingly, each host-pathogen gene pair gives rise to distinct HR phenotypes on pepper, distinguished by the timing of appearance, intensity of browning, and degree of confluence (Hibberd *et al.* 1987a). Gabriel *et al.* (1986) have also reported that the phenotypes of HR on cotton differed along with races of *X. c.* pv. *malvacearum* and the complementary resistance genes in the host. In their study, they show that the HR phenotype associated with cloned avirulence genes differed from that associated with the strain from which the genes were cloned.

Our results suggest that in *X. c.* pv. *vesicatoria*, background genotypes were unimportant in determining not only the HR phenotypes but also the pattern of electrolyte leakage from resistant plant lines (Table 4, Fig. 2). The timing of the onset of electrolyte leakage induced by a particular strain was correlated with the timing of the onset of a visible HR. This concurs with previous observations showing that electrolyte leakage is a measure of membrane disruption in the plant undergoing an HR-type resistant response (Klement 1982). Strains that induced a comparatively early onset of electrolyte leakage also induced HRs that could be observed early (for example, XcvPT race 2 E3). The HR phenotype observed when more than one avirulence gene was present in a strain was that associated with the faster-occurring HR. For example, XcvT race 1 75-3 has both *avrBsT*, which usually induces cell collapse within 24 to 36 hr, and *avrBs1*, which usually induces HR within 6 to 12 hr. On ECW-10R, the HR phenotype characteristic of the *avrBs1*:*Bs1* combination is epistatic to that associated with *avrBsT* (Table 3). Future research addressing the molecular and biochemical function of avirulence genes and, eventually, the pathway of HR induction will allow us to learn whether the HR phenotypes associated with each avirulence gene:resistance gene pair are derived independently or through a common pathway.

Previous work by Stall *et al.* (1986) and Swanson *et al.* (1988) showed that *avrBs1* was localized to a 200-kbp, self-transmissible copper resistance plasmid. We show that *avrBsT* and *avrBs3* are also located on unique indigenous plasmids, whereas *avrBs2* is probably on the chromosome. It may be significant that avirulence loci carried by plasmids occur sporadically in natural populations of *X. c.* pv. *vesicatoria*, whereas those chromosomally borne appear to be uniformly distributed (Fig. 1). Plasmid-borne avirulence loci may possibly be either lost from or acquired in particular strains, thereby altering their ability to cause disease on certain hosts.

Finally, this laboratory work furnishes a basis for the rational development of field resistance in pepper to bacterial spot disease. Each avirulence gene in the pathogen is subject to loss of activity with a concomitant change to virulence on pepper lines that previously were resistant. Several mechanisms may give rise to the loss of activity in laboratory experiments including: insertional inactivation by the transposable element IS476 (for example, *avrBs1*; Kearney *et al.* 1988), loss of a plasmid that carries an avirulence gene (for example, *avrBs3*), and

simple base pair mutations (for example, *avrBs2*; B. Kearney, unpublished data).

In the future, results from the use of laboratory isolates must be compared to those from the use of field isolates. The fact that many strains of the pathogen have more than one avirulence gene points out that multiple resistance genes should be used in the host to nullify the virulence associated with the loss of activity of any single avirulence gene. In addition, in the case of *avrBs2* shown to be present in all strains of *X. c.* pv. *vesicatoria*, one would expect resistance in pepper plants that contain the *Bs2* gene to be more durable than resistance in pepper plants encoded by *Bs1* or *Bs3* under natural field conditions.

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LITERATURE CITED

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

Cook, A. A. 1984. Florida XVR 3-25 bell pepper. HortScience 19:735.

Cook, A. A. 1973. Characterization of hypersensitivity in *Capsicum annuum* induced by the tomato strain of *Xanthomonas vesicatoria*. Phytopathology 63:915-918.

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.

Coplin, D. L., Rowan, R. G., Chisholm, D. A., and Whitmoyer, R. E. 1981. Characterization of plasmids in *Erwinia stewartii*. Appl. Environ. Microbiol. 42:599-604.

Dahlbeck, D., and Stall, R. E. 1979. Mutations for change of race in cultures of *Xanthomonas vesicatoria*. Phytopathology 69:634-636.

Daniels, M. J., Barber, C. E., Turner, D. C., Cleary, W. G., and Sawzyc, M. 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. 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.

Ellingboe, A. H. 1984. Genetics of host-parasite relations: An essay. Pages 131-151 in: Advances in Plant Pathology, Vol. 2. D. S. Ingram and P. H. Williams, eds. Academic Press, New York.

Flor, H. H. 1955. Host-parasite interactions in flax rust – Its genetics and other implications. Phytopathology 45:680-685.

Gabriel, D. W., Burges, A., and Lazlo, G. 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.

Hibberd, A. M., Stall, R. E., and Bassett, M. J. 1987a. Different phenotypes associated with incompatible races and resistance genes in the bacterial spot disease of pepper. Plant Dis. 71:1075-1078.

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

Kado, C. I., and Liu, S. T. 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145:1365-1373.

Kearney, B., and Staskawicz, B. J. 1987. Molecular analysis of spontaneous race change in *Xanthomonas campestris* pv. *vesicatoria*. Pages 155-161 in: Tomato Biotechnology. D. J. Nevins and R. A. Jones, eds. Alan R. Liss, Inc., New York.

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

Kim, B.-S., and Hartmann, R. W. 1985. Inheritance of a gene (*Bs3*) conferring hypersensitive resistance to *Xanthomonas campestris* pv. *vesicatoria* in pepper (*Capsicum annuum*). Plant Dis. 69:233-235.

Klement, Z. 1982. Hypersensitivity. Pages 150-175 in: Phytopathogenic prokaryotes, Vol. 2. M. S. Mount and G. S. Lacy, eds. Academic Press, New York.

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.

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

Reifschneider, G. J. B., Bongiolo, N. A., and Takatsu, A. 1985. Reappraisal of *Xanthomonas campestris* pv. *vesicatoria* strains – Their terminology and distribution. Fitopatol. Bras. 10:201-204.

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.

Stall, R. E., and Cook, A. A. 1966. Multiplication of *Xanthomonas vesicatoria* and lesion development in resistant and susceptible pepper. Phytopathology 56:1152-1154.

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. T., 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.

Whalen, M., Stall, R. E., and Staskawicz, B. J. 1988. Characterization of a gene from a tomato pathogen determining hypersensitive resistance in non-host species and genetic analysis of this resistance in bean. Proc. Natl. Acad. Sci. USA 85:6743-6747.