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 electolyte 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* (Dodge) 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 and pepper, the pepper group (XcvP) on pepper only, and the pepper-tomato group (XcvPT) on both pepper and tomato (Reischneider 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 10^6 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-glucose agar (NYGA; Daniels *et al.* 1984). Rifampicin-resistant strains were isolated by plating 10⁶ 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**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Relevant characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLAFR3</td>
<td>tet'</td>
<td>Staskawicz <em>et al.</em> 1987</td>
</tr>
<tr>
<td>pEC103</td>
<td>pLAFR3 clone from XcvT race 1 75-3 with avrBsT activity</td>
<td>This study</td>
</tr>
<tr>
<td>pXV943</td>
<td>4.3-kbp PsI subclone of pEC103 with avrBsT activity</td>
<td>This study</td>
</tr>
<tr>
<td>pXV2007</td>
<td>5.3-kbp BglII-PstI plLAFR3 subclone with avrBsl activity</td>
<td>Swanson <em>et al.</em> 1988</td>
</tr>
<tr>
<td>pEC815</td>
<td>pLAFR3 clone from XcvT race 1 75-3 with avrBs2 activity</td>
<td>This study</td>
</tr>
<tr>
<td>p81538</td>
<td>2.4-kbp BamHI subclone of pEC815 with avrBs2 activity</td>
<td>This study</td>
</tr>
<tr>
<td>pEC83</td>
<td>pLAFR3 clone with avrBs3 activity from library of a 41-kbp plasmid from XcvP race 1 71-21</td>
<td>This study</td>
</tr>
<tr>
<td>pBS3</td>
<td>4.5-kbp Sau3A subclone of pEC83 with avrBs3 activity</td>
<td>This study</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Pepper</th>
<th>Tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ECW&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ECW-10R&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tomato (XcvT)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Pepper (XcvP)</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>Pepper-Plant (XcvP)</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>Pepper-Plant (XcvP)</td>
<td>3</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> +, virulent; -, avirulent.

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

<table>
<thead>
<tr>
<th>Designation</th>
<th>ECW&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ECW-10R</th>
<th>ECW-20R</th>
<th>ECW-30R</th>
</tr>
</thead>
<tbody>
<tr>
<td>XcvT race 1 75-3</td>
<td>A&lt;sup&gt;b&lt;/sup&gt;</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>XcvPT race 2 81-23</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>B</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>XcvPT race 2 81-23 (pEC103)</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>XcvPT race 2 81-23 (pXV943)</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>XcvT race 1 75-3 *avrBsT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>B</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>XcvT race 1 75-3 *avrBsT&lt;sup&gt;c&lt;/sup&gt;, 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>XcvT race 1 75-3 *avrBsT&lt;sup&gt;c&lt;/sup&gt;, 1&lt;sup&gt;c&lt;/sup&gt; (pXV2007)</td>
<td>+</td>
<td>B</td>
<td>C</td>
<td>+</td>
</tr>
<tr>
<td>XcvP race 1 82-8</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>XcvP race 1 82-8 *avrBs&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>D</td>
</tr>
<tr>
<td>XcvP race 1 82-8 *avrBs&lt;sup&gt;c&lt;/sup&gt; (pEC815)</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>XcvP race 1 82-8 *avrBs&lt;sup&gt;c&lt;/sup&gt; (p81538)</td>
<td>+</td>
<td>+</td>
<td>C</td>
<td>D</td>
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<td>XcvP race 1 71-21</td>
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<td>+</td>
<td>C</td>
<td>D</td>
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<tr>
<td>XcvPT race 2 81-23 (pEC83)</td>
<td>+</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>XcvPT race 2 81-23 (pBS3)</td>
<td>+</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

<sup>a</sup>See footnotes to Table 2.

<sup>b</sup>Phenotypes for 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*.

<sup>c</sup>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 B2 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 10⁶ to 10⁹ cfu/ml. Leaves were inoculated using 1-ml syringes and 27-gauge needles. Each inoculated area, 1–2 cm², 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 gtr was added to the standardized inoculum and rubbed with a cotton swab onto leaves that were not fully expanded. An area of 4–5 cm² 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

<table>
<thead>
<tr>
<th>Designation</th>
<th>ECW⁺</th>
<th>ECW-10R</th>
<th>ECW-20R 3:24-2:7</th>
<th>ECW-30R</th>
</tr>
</thead>
</table>
| XcvP race 1 82-8
avrB2⁺, 3⁺ | +⁺⁺⁺ | +⁺⁺⁺ | +⁺⁺⁺ | +⁺⁺⁺ |
| XcvP race 1 82-8
avrB2⁺, 3⁻
(p XV943) | A⁺⁺⁺⁺ | A⁺⁺⁺⁺ | A⁺⁺⁺⁺ | A⁺⁺⁺⁺ |
| XcvPT race 2 E3 | +⁺⁺⁺ | B⁺⁺⁺⁺ | C⁺⁺⁺⁺ | A⁺⁺⁺⁺ |
| XcvP race 1 82-8
avrB2⁺, 3⁻
(p XV2007) | +⁺⁺⁺ | B⁺⁺⁺⁺ | +⁺⁺⁺ | +⁺⁺⁺ |
| XcvPT race 3 88-5 | +⁺⁺⁺ | +⁺⁺⁺ | C⁺⁺⁺⁺ | +⁺⁺⁺ |
| XcvP race 1 82-8
avrB2⁺, 3⁻
(p 81538) | +⁺⁺⁺ | +⁺⁺⁺ | C⁺⁺⁺⁺ | +⁺⁺⁺ |
| XcvP race 1 84-1 | +⁺⁺⁺ | +⁺⁺⁺ | C⁺⁺⁺⁺ | D⁺⁺⁺⁺ |
| XcvP race 1 82-8
avrB2⁺, 3⁻
(p BS3) | +⁺⁺⁺ | +⁺⁺⁺ | C⁺⁺⁺⁺ | D⁺⁺⁺⁺ |

*See footnotes in Table 2.
*Virulent reaction, water-soaked lesion.
*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 x 10⁴, 3 x 10⁵, and 3 x 10⁶ cfu/cm², 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 re inoculated 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 (Dita 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 3²P-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).

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 avr Bs2**. 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 PstI 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 avr Bs2. The avirulence gene avr Bs2 was localized to an indigenous plasmid of approximately 41 kbp in XcvT race 1 75-3 (data not shown). Hybrizing 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 (Dahbeck 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 avr Bs2 activity was isolated and designated XcvT race 1 75-3 avr Bs2- (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 avr Bs2 (Fig. 1A, lane 2). The subclone pXV943 complemented the loss of avr Bs2 activity on ECW and ECW-30R (Table 3). XcvT race 1 75-3 avr Bs2- retained avirulence activity on ECW-10R suggesting that XcvT race 1 75-3 avr Bs2- carried avr Bs1. To learn if a second race change mutation could be induced, XcvT race 1 75-3 avr Bs2- was infiltrated into leaves of ECW-10R. A virulent strain lacking avr Bs2 and avr Bs1 activity was isolated and designated XcvT race 1 75-3 avr Bs2-, Bs1-. 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 the insertion of IS476 into avr Bs1; total DNA hybridized to the probe containing avr Bs1 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 avr Bs1, pXV2007, converted the phenotype of XcvT race 1 75-3 avr Bs2-, Bs1- back to avirulence on ECW-10R (Table 3).

**Identification and cloning of avr Bs2 and avr Bs3**. 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 avr Bs2-, was isolated (Table 3). XcvP race 1 82-8 avr Bs2- was still avirulent on ECW-30R. To isolate a strain that was virulent on all three pepper lines, XcvP race 1 82-8 avr Bs2- was infiltrated into leaves of ECW-30R, and the virulent strain XcvP race 1 82-8 avr Bs2-, Bs3- was isolated (Table 4). This

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**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 avr Bs2-; lane 3, XcvT race 1 75-3 avr Bs2-, Bs1-; lane 4, XcvP race 1 82-8; lane 5, XcvP race 1 82-8 avr Bs2-; lane 6, XcvP race 1 82-8 avr Bs2-, Bs3-; 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, avr Bs2, a 2.3-kilobase pair (kbp) EcoRI-PstI fragment internal to the active 4.5-kbp subclone pXV943. X. c. pv. vesicatoria genomic DNA digested with EcoRV. Panel B, avr Bs2, a 2.3-kilobase pair (kbp) SphI fragment from pXV943. X. c. pv. vesicatoria genomic DNA digested with SphI. Panel C, avr Bs3, a 3.2-kilobase pair (kbp) BamHI fragment internal to the active 4.5-kbp subclone pBS3. X. c. pv. vesicatoria genomic DNA digested with EcoRI.
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 *Bs*2 gene in ECW-20R, a library of DNA from XcvT race 1 75-3 was screened in XcvP race 1 82-8 *avrBs2*². Two cosmid clones were isolated that converted the normally virulent recipient to avirulence on the pepper line 3-25-2-7 carrying the *Bs*2 gene. One cosmid clone, pEC815, was subcloned, and a 2.4-kbp BamHI 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. Stoll, unpublished data), the spontaneous loss of an approximately 41-kbp indigenous plasmid was correlated with the loss of avirulence on ECW-30R carrying *Bs*3. 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 Sau3A digestion (Table 3). The plasmid-borne locus, designated *avrBs3*, hybridizes 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*²,3⁻ 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 *avrRxx* (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 *Bs*1, a dark, papery-brown, confluent necrosis appeared within 8 to 12 hr. On ECW-20R containing *Bs*2, *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 *Bs*3 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*²,3⁻ as the recipient

![Graph](image-url)
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 \textit{avrBs2}, \textit{3}\textsuperscript{−} (pLAFR3), XcvP race 1 82-8 \textit{avrBs2}, \textit{3}\textsuperscript{−} 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 \textit{avrBs2}, \textit{3}\textsuperscript{−} carrying each avirulence gene were indistinguishable (Fig. 2).

In all cases, electrolyte leakage from the virulent XcvP race 1 82-8 \textit{avrBs2}, \textit{3}\textsuperscript{−} 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 \textit{avrBs2}, \textit{3}\textsuperscript{−} (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 \textit{avrBs2}, \textit{3}\textsuperscript{−} (pXV1538) 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 \textit{avrBs2}, \textit{3}\textsuperscript{−} (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 \textit{avrBs2}, \textit{3}\textsuperscript{−} (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 \textit{X. c. pv. vesicatoria}-pepper interaction. We have identified and cloned three different \textit{X. c. pv. vesicatoria} avirulence genes, \textit{avrBs2}, \textit{avrBs3}, and \textit{avrBs1}, that specify disease resistance on lines of pepper carrying the corresponding resistance genes, \textit{Bs2}, \textit{Bs3}, and \textit{Bs1}, respectively (Table 2). Avirulence genes restrict the host range of \textit{X. c. pv. vesicatoria} within the pepper species. Moreover, we have cloned and characterized the avirulence gene \textit{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 \textit{et al.} (1988) and Kobayashi \textit{et al.} (1989) showing that nonhost resistance in bean and soybean against two tomato pathogen 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 \textit{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 \textit{X. c. pv. vesicatoria}, three of which correspond to genetically characterized resistance genes in pepper (Table 3; Hibberd \textit{et al.} 1987b; Swanson \textit{et al.} 1988; Whalen \textit{et al.} 1988; Bonas \textit{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 \textit{et al.} 1987a). Gabriel \textit{et al.} (1986) have also reported that the phenotypes of HR on cotton differed along with race of \textit{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 \textit{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 \textit{avrBsT}, which usually induces cell collapse within 24 to 36 hr, and \textit{avrBs1}, which usually induces HR within 6 to 12 hr. On ECW-10R, the HR phenotype characteristic of the \textit{avrBs1}:\textit{Bs1} combination is epistatic to that associated with \textit{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 \textit{et al.} (1986) and Swanson \textit{et al.} (1988) showed that \textit{avrBs1} was localized to a 200-kbp, self-transmissible copper resistance plasmid. We show that \textit{avrBsT} and \textit{avrBs3} are also located on unique indigenous plasmids, whereas \textit{avrBs2} is probably on the chromosome. It may be significant that avirulence loci carried by plasmids occur sporadically in natural populations of \textit{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, \textit{avrBs1}; Kearney \textit{et al.} 1988), loss of a plasmid that carries an avirulence gene (for example, \textit{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. vescicatoria, one would expect resistance in pepper plants that the Bs2 gene to be more durable than resistance in pepper plants encoded by Bsl or Bs3 under natural field conditions.

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LITERATURE CITED


