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Cloned Avirulence Gene of *Xanthomonas campestris* pv. *vesicatoria* Complements Spontaneous Race-Change Mutants

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The copper resistance plasmid pXvCul, which specifies avirulence on pepper cultivars containing the Bs_1 locus, was isolated from *Xanthomonas campestris* T-55 transconjugants. Purified plasmid DNA was partially digested with the restriction enzyme *Sau*3A, ligated into the single *Bam*H1 site of the wide host range plasmid cloning vehicle pLAFR3 and transduced into *Escherichia coli* HB101. A single cosmid clone, pXv2000, was identified that specifically converted virulent race 1 isolates of *X.c.* pv. *vesicatoria* to avirulence when inoculated into the pepper cultivar ECW10R containing the dominant resistance gene Bs_1. The avirulence gene, $avrBs_1$, was characterized by restriction enzyme mapping, subcloning, and deletion analysis and has been localized to a 5.3-kb fragment of DNA. In addition, it was shown by Southern hybridization that a DNA fragment containing the $avrBs_1$ locus only hybridized to DNA from races of *X.c.* pv. *vesicatoria* that were avirulent on the pepper cultivar ECW10R. Spontaneous race-change mutants of *X.c.* pv. *vesicatoria* were isolated and were stably changed from avirulence to virulence on the pepper cultivar ECW10R. Conjugation of the wild type $avrBs_1$ subclone (pXv2007) into the spontaneous race-change mutants restored their avirulent phenotype.

Additional key words: copper resistance, cosmid cloning, durable disease resistance, gene-for-gene hypothesis

The ability of plants to resist pathogen attack depends on the genotype of both the plant and the pathogen. In a gene-for-gene interaction, a host will be resistant to a particular pathogen if the host carries a dominant resistance gene that interacts with a dominant avirulence gene in the pathogen (Ellingboe 1984; Flor 1955). This pattern of gene-for-gene relationship has been observed in many plant-pathogen interactions between fungi, bacteria, viruses, nematodes, and their respective hosts (Day 1974). In our laboratory, we are studying *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot of peppers and tomatoes, a serious pathogen that severely limits the production of these crops in growing areas of high humidity and frequent rain (Marco and Stall 1983). Our initial studies focused on the dominant disease resistance locus, Bs_1 (Cook and Stall 1963). The Bs_1 locus has been introduced into the susceptible pepper cultivar, Early Cal wonder (ECW), creating a near-isogenic line (ECW10R) that specifically confers resistance to *X.c.* pv. *vesicatoria* pepper race 2 strains containing a self-transmissible copper plasmid that specifies avirulence. This interaction fulfills the formal genetic requirements of the gene-for-gene hypothesis as the corresponding gene pairs in both the host and the pathogen have been identified (Stall *et al.* 1986).

Single gene resistance in the host is often short-lived under natural field conditions (Johnson 1983). This phenomenon has been attributed to the selection of either naturally occurring races of the pathogen that lack corresponding avirulence genes or spontaneous race-change mutants that are able to overcome host resistance. In this regard, Dahlbeck and Stall (1979) demonstrated that pepper race 2 strains of *X.c.* pv. *vesicatoria* spontaneously mutate from avirulence to virulence at a high frequency (5×10^{-4}) when inoculated into a pepper cultivar containing the Bs_1 locus.

Although spontaneous race-change mutants can be readily isolated, the mutated genetic locus in the pathogen has yet to be identified. In this paper we report the cloning and molecular characterization of an avirulence gene in race 2 strains of *X.c.* pv. *vesicatoria* and demonstrate that the cloned wild type avirulence gene ($avrBs_1$) can complement race 2 spontaneous race-change mutants.

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MATERIALS AND METHODS

Bacterial strains and media. Defined races of *X.c.* pv. *vesicatoria* were provided by R. E. Stall, and their reactions on pepper are described in Table 1. *Escherichia coli* HB101 was used as a recipient in construction of the pXvCul cosmid library. *X.c.* pv. *vesicatoria* was routinely subcultured on nutrient yeast glycerol agar (NYGA; Daniels *et al.* 1984) or nutrient agar (Miller 1972) media. *E. coli* strains were routinely subcultured on Luria medium (Miller 1972). The concentration of antibiotics used were kanamycin 50 μg/ml, tetracycline (Tc) 10 μg/ml, ampicillin 50 μg/ml, and rifampicin (Rif) 100 μg/ml. Copper resistance in *X.c.* pv. *vesicatoria* was determined by growth on nutrient agar containing 200 μg/ml copper sulfate.

Plasmids. Cosmid pLAFR3 (Staskawicz *et al.* 1986) was used to construct the pXvCul library. The copper resistance plasmid pXvCul contained the avirulence gene activity described by Stall *et al.* (1986).

Plasmid pWB5A, containing the *Eco*R1 polylinker cloned from the plasmid πVX (Maniatis *et al.* 1982), was used as a vector for subcloning avirulence gene activity.

Bacterial conjugations. Transmission of the 200-kb Cu^R plasmid was accomplished by mixing equal volumes (100 μl) of Cu^R Rif^S *X.c.* pv. *vesicatoria* E3 donor cells with the Cu^S Rif^R *X.c.* pv. *vesicatoria* 82-4 recipient cells. The 200-μl mixture was placed on NYGA and incubated for at least 18 hr at 30° C. The cells were then resuspended in H_2O and

plated on NYGA containing 200 μg/ml $CuSO_4$ and 100 μg/ml Rif.

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).

Isolation of the Cu^R plasmid pXvCul. pXvCul DNA was isolated from *X. campestris* T-55 (pXvCul) according to the procedure described by Rosenberg *et al.* (1982).

Growth of plants and plant inoculations. Seed of ECW susceptible to *X.c.* pv. *vesicatoria* E3 and the near-isogenic derivative (ECW10R) resistant to E3 were provided by R. E. Stall. Plants were grown from seeds planted in 6-in. clay pots filled with standard potting soil. The race reactions were determined by inoculating the underside of a fully expanded pepper leaf. *X.c.* pv. *vesicatoria* was prepared for inoculation studies by growing fresh, overnight cultures in NYG broth. Cells were resuspended in sterile water to give a final concentration of 10^8 cells per milliliter. Small wounds were made by nicking the underside of fully expanded leaves with the corner of a razor blade. Approximately 15 μl of a bacterial suspension was infiltrated into the leaves with a disposable plastic Pasteur pipet. Inoculated plants were incubated in a growth chamber with a 16-hr photoperiod followed by 8 hr of darkness at 30° C. Resistant phenotypes (evidenced by the hypersensitive reaction) were scored beginning 16 hr after inoculation. Susceptible phenotypes (appearance of a water-soaked lesion) developed approximately 48–72 hr after inoculation.

Standard recombinant DNA techniques. Cosmid cloning, enzyme digestions, alkaline phosphatase treatments, nick-translations, Southern hybridization, cloning procedures, plasmid rapid lysate procedure, and agarose gel electrophoresis of DNA fragments were essentially as described by Maniatis *et al.* (1982).

Hybridizations were performed in 6× SSPE (0.9 M NaCl, 60 mM $NaH_2PO_4 \cdot H_2O$, 6 mM EDTA, pH 7.4), 0.01 M EDTA, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 100 μg/ml denatured salmon sperm DNA, and 10^6 cpm ^{32}P-labeled probe at 65° C for 16 hr with gentle rocking. Probed filters were washed at room temperature in 2× SSPE, 0.5% SDS for 5 min, in 2× SSPE, 0.1% SDS for 15 min at room temperature, and at 65° C in 0.1× SSPE, 0.5% for 2.5 hr (Maniatis *et al.* 1982). Filters were dried and autoradiography was carried out at −80° C using Kodak XAR-5 film and Dupont Cronex Lightning Plus intensifying screens.

Construction of pLAFR3 cosmid library. A cosmid library of purified pXvCul DNA was constructed in the cosmid vector pLAFR3. Purified pXvCul DNA was partially digested with *Sau*3A and treated with alkaline phosphatase. The partially digested DNA was then separated by sucrose density gradient (5–20%) centrifugation in a Beckman SW27 rotor for 18 hr at 20,000 rpm. The vector pLAFR3 was cut with *Bam*H1 and ligated to the partially digested *Sau*3A DNA that was enriched for fragments 25–30 kb in size. The ligated DNA was packaged in *in vitro* phage heads and tails and transduced into HB101 selecting for tetracycline resistance according to published procedures (Staskawicz *et al.* 1984).

Isolation and detection of large plasmids in *X.c.* pv. *vesicatoria*. We isolated plasmids according to the procedure of Kado and Liu (1981) with the following modifications. *X.c.* pv. *vesicatoria* was grown overnight in nutrient broth at 30° C and 1 ml of this culture was pelleted. The pellet was resuspended in a small volume (25 μl) of the following buffer: 25 mM Tris at pH 8.0, 10 mM EDTA, and 50 mM glucose. Resuspended cells were lysed by adding 35 μl of 3% SDS, 5 mM EDTA, 50 mM Tris at pH 8.0, 30 mM NaCl, and 0.0625 N NaOH with gentle mixing. The supernatant from the centrifuged lysate was incubated at 65° C for 5 min, extracted with chloroform and isoamyl alcohol (24:1), and electrophoresed in a 0.5% agarose Tris-acetate gel. Plasmids were visualized by staining the gel with 0.5 μg/ml ethidium bromide.

Isolation of spontaneous race-change mutants. Spontaneous race-change mutants were isolated by infiltrating 5×10^6 colony forming units per milliliter of an avirulent race 2 isolate of *X.c.* pv. *vesicatoria* into leaves of the pepper cultivar ECW10R. Water-soaked lesions appeared in 5–6 wk and virulent colonies were isolated by streaking tissue containing a single lesion for single colonies on NYGA supplemented with 100 μg/ml of rifampicin. To verify a stable change to virulence, a single colony was resuspended in H_2O and inoculated into ECW10R.

Table 1. Phenotypes of near-isogenic cultivars of pepper inoculated with races of *Xanthomonas campestris* pv. *vesicatoria* and pepper race 1 transconjugants

X.c. pv. *vesicatoria*	Race designation	ECW[a]	ECW10R[a]
81-23	Pepper race 2	C	I-B
E3	Pepper race 2	C	I-B
71-21	Pepper race 1	C	C
82-4	Pepper race 1	C	C
82-8	Pepper race 1	C	C
68-1	Pepper race 3	C	C
75-3	Tomato race	I-A	I-B
T-55	Nonpathogenic epiphyte	NR	NR
	Pepper race 1 transconjugants		
82-4	(pXvCu1)	C	I-B
82-8	(pXv2000)	C	I-B
82-8	(pXv2001)	C	I-B
82-8	(pXv2004)	C	I-B
82-8	(pXv2003)	C	C
82-8	(pXv2007)	C	I-B

[a]ECW = Early Cal Wonder, ECW10R = Early Cal Wonder containing the Bs_1 locus, C = compatible interaction (water-soaked lesions), I = incompatible interaction (hypersensitive reaction), I-A = phenotypic appearance of this hypersensitive reaction appeared in 48 hr and was characterized by light brown necrosis, I-B = phenotypic appearance of the hypersensitive reaction appeared in 8-10 hr and was characterized by dark brown necrosis, NR = no observable phenotypic reaction.

RESULTS

Avirulence gene *avrBs*$_1$ cosegregates with copper resistance. Previous studies by Stall *et al.* (1986) showed that the race specificity of race 2 strains of *X.c.* pv. *vesicatoria* corresponding to the Bs_1 locus in pepper is linked to the self-transmissible copper resistance plasmid, pXvCul. We have corroborated this observation by performing conjugations between a copper resistant race 2 isolate (*X.c.* pv. *vesicatoria* E3) that is avirulent on ECW10R and a copper sensitive race 1 isolate (*X.c.* pv. *vesicatoria* 82-4) that is normally virulent on ECW10R (Fig. 1). The *X.c.* pv. *vesicatoria* 82-4 (pXvCul) transconjugants were inoculated on the resistant cultivar ECW10R that contains the Bs_1 locus and were changed from virulence to avirulence as evidenced by the induction of a hypersensitive reaction in pepper cultivar ECW10R (Table 1, Fig. 1). Figure 2 depicts the plasmid profiles of the parent strains (lanes A and B) and a copper resistant transconjugant (lane C). A large plasmid

corresponding to the same molecular weight of the pXvCul plasmid can be observed in the *X.c.* pv. *vesicatoria* 82-4 copper resistant transconjugant (lane C).

Molecular cloning and physical characterization of *avrBs*$_1$. The previously described genetic experiments allowed us to devise a simple strategy to clone the avirulence gene from the copper resistant plasmid pXvCul. Because *X.c.* pv. *vesicatoria* E3 contains several plasmids (Fig. 2), pXvCul was transferred into a plasmid-free isolate of *X. campestris* T-55 that is naturally nonpathogenic on peppers. Plasmid DNA was isolated from T-55 carrying pXvCul and a cosmid library of pXvCul was constructed in the wide host range cosmid cloning vector pLAFR3. Forty random *E. coli* HB101 transductants were selected on tetracycline and individually conjugated into the race 1 isolate 82-8. The transconjugants were inoculated into the *Bs*$_1$ containing cultivar ECW10R and scored for avirulence. One cosmid clone, pXv2000, converted the race 1 isolate 82-8 from virulence to avirulence on this cultivar (Table 1, Fig. 1). Plasmid DNA from the *X.c.* pv. *vesicatoria* 82-8 (pXv2000) transconjugant was isolated by a rapid mini-lysate procedure and used to transform *E. coli* HB101 to tetracycline resistance. Isolation of plasmid DNA from this transformant and analysis by various restriction enzymes demonstrated this clone to be identical to the original cosmid clone that was conjugated from *E. coli* HB101 (data not shown).

To ascertain the location of the avirulence gene on the large DNA insert, we analyzed this clone by restriction enzyme mapping, subcloning, and deletion mapping (Fig. 3). The original cosmid clone, pXv2000, contains approximately 27 kb of insert DNA. Using information from the restriction enzyme digestion of the 27-kb insert, we constructed two large deletions. The plasmid pXv2001 was constructed by digestion of pXv2000 with the restriction enzyme *Bam*H1, religation of the plasmid, and transformation of *E. coli* HB101 to tetracycline resistance. To verify that a deletion of 9.0 kb had occurred, plasmid DNA from a Tc^R transformant was analyzed by digesting this plasmid with *Bam*H1. The resultant plasmid pXv2001 was mobilized into *X.c.* pv. *vesicatoria* 82-8 and inoculated onto ECW1OR; this plasmid still retained full avirulence activity (Table 1). The plasmid pXv2001 was then cut with *Cla*I and religated to produce pXv2004. This plasmid also retained full avirulence activity. Finally, two subclones were constructed from pXv2004 to produce pXv2003 and pXv2007. The 5.5-kb *Hin*dIII subclone pXv2003 was unable to confer avirulence gene activity when conjugated into *X.c.* pv. *vesicatoria* 82-8, whereas the 5.3-kb *Bgl*II-*Pst*I subclone pXv2007 retained full avirulence gene activity phenotypically identical to the wild type race 2 *X.c.* pv. *vesicatoria* E3 strain and the original cosmid clone pXv2000.

The pXv2007 subclone was then used as a ^{32}P-labeled probe to demonstrate definitively the location of this gene on the copper resistant plasmid pXvCul. The clone only hybridized to the copper plasmid pXvCul in the wild type *X.c.* pv. *vesicatoria* E3 and the *X.c.* pv. *vesicatoria* 82-4

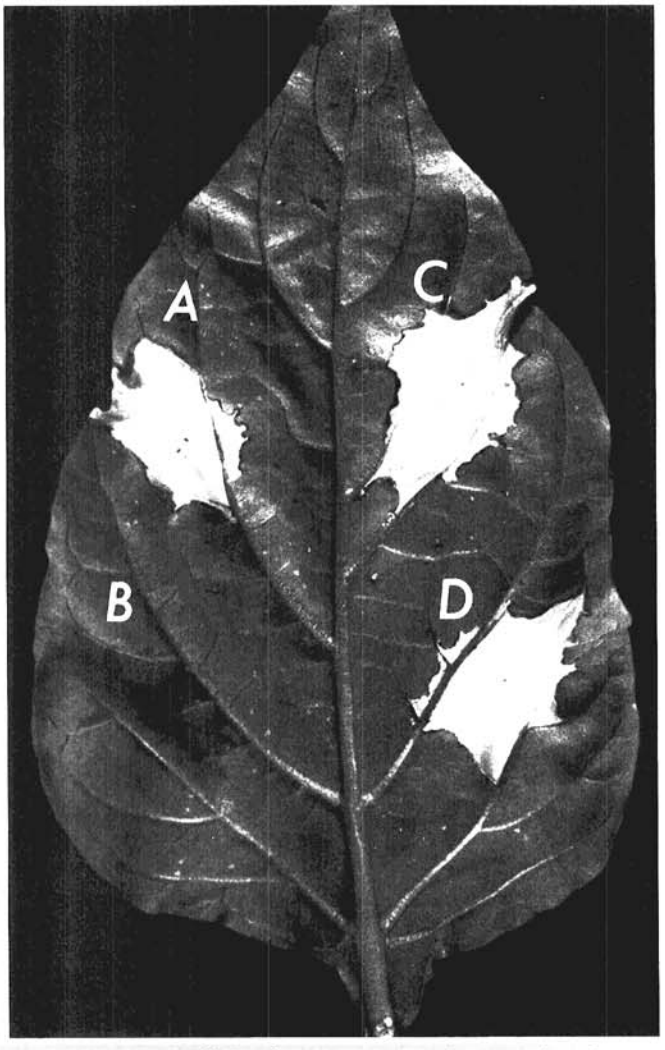

Fig. 1. Phenotypes of *Xanthomonas campestris* pv. *vesicatoria* race reactions on the pepper cultivar ECW10R after inoculation of 10^8 cells per milliliter. **A,** Wild type race 2 E3 produced a hypersensitive reaction. **B,** Wild type race 1 82-4 resulted in a compatible interaction indicated by a water-soaked lesion. **C,** 82-4 (pXvCul) transconjugant resulted in a hypersensitive reaction. **D,** 82-4 (pXv2000) transconjugant resulted in a hypersensitive reaction.

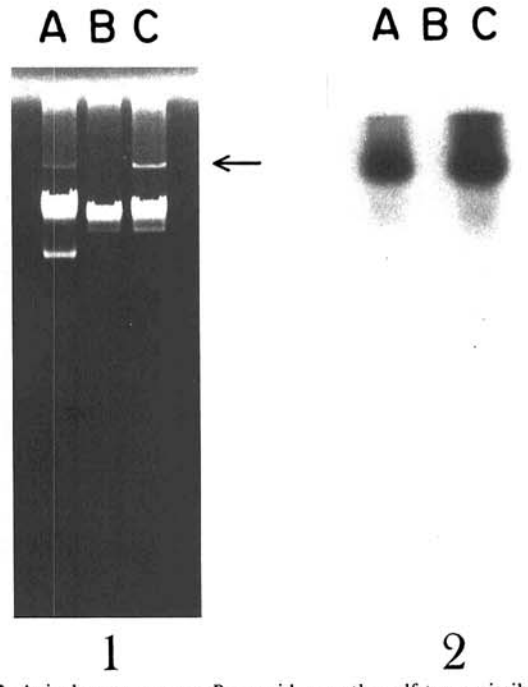

Fig. 2. Avirulence gene, *avrBs*$_1$, resides on the self-transmissible copper plasmid, pXvCul. **1,** Plasmid profiles of wild type *X.c.* pv. *vesicatoria* E3 (lane A), 82-4 (lane B), and the transconjugant 82-4, pXvCul (lane C). A large plasmid is marked by the arrow. **2,** Southern blot analysis of the plasmid DNA from panel 1 probed with the 5.3-kb *Bgl*II-*Pst*I insert of pXv2007. The ^{32}P-labeled probe only hybridized to the large plasmids in lanes A and C and did not hybridize to lane B (*X.c.* pv. *vesicatoria* 82-4).

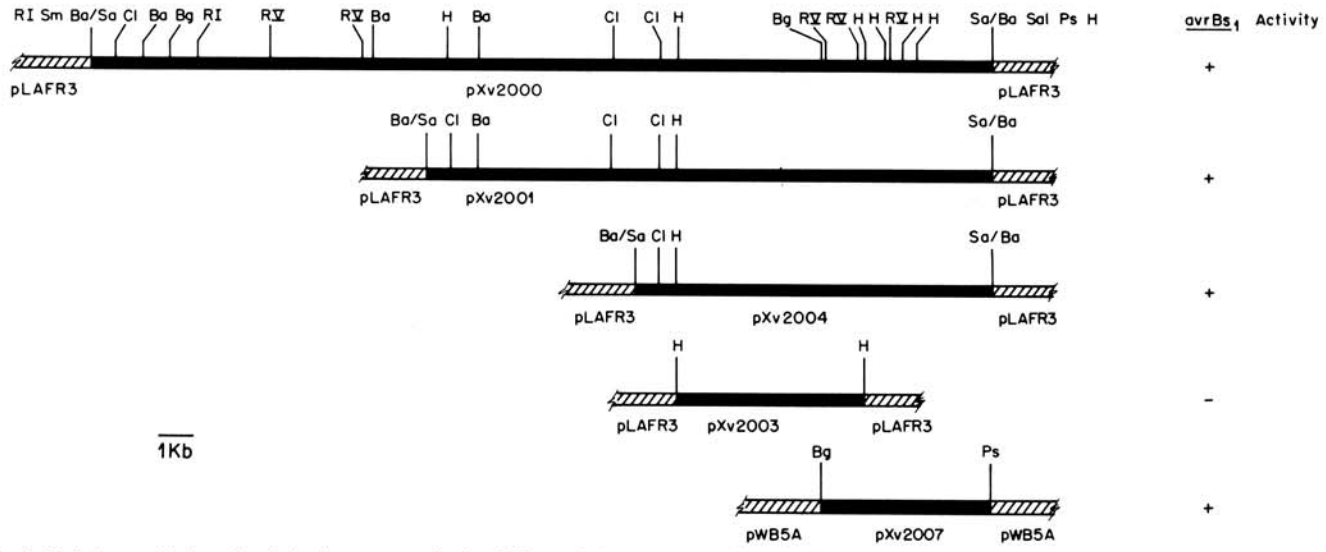

Fig. 3. Deletion analysis and subcloning strategy for localizing avirulence gene activity within pXv2000. The right column shows whether the clones are active (+) or inactive (−). The entire plasmid, including the vector and insert, are numbered in the center of each construction. Additional restriction enzyme sites not shown in plasmids pXv2001, 2004, 2003, and 2007 can be determined by direct comparison with pXv2000. RI, Sa, Cl, Bg, RV, H, and Ps designate the sites for the restriction endonucleases, *Eco*RI, *Sau*3A, *Cla*I, *Bgl*II, *Eco*Rv, *Hin*dIII, and *Pst*I, respectively. Note: Ba/Sa and Sa/Ba are the junction fragments between the insert and vector. The *Pst*I site in pXv2007 is a vector site in pWB5A.

(pXvCul) transconjugant (Fig. 2, lanes A and C).

The availability of the cloned *avrBs*$_1$ gene allowed us to search for hybridizing sequences in other races of *X.c.* pv. *vesicatoria*. Total genomic DNA from the various races was digested with the enzyme *Eco*RV, electrophoresed in agarose, and transferred to nitrocellulose. The Southern blot was probed with the ^{32}P-labeled 5.3-kb *Bgl*II-*Pst*I fragment electroeluted from pXv2007. As predicted from the restriction enzyme map (Fig. 3), three hybridizing bands corresponding to 0.85, 0.95, and 12.5 kb were observed (Fig. 4). The *avrBs*$_1$ gene only hybridized to strains of *X.c.* pv. *vesicatoria* previously classified as the tomato race (lane G) or the pepper race 2 (lanes D, E) that are avirulent on pepper plants containing the *Bs*$_1$ locus. The absence of hybridization to *X.c.* pv. *vesicatoria* pepper races 1 (lanes A, B, C) and 3 (lane F) suggests that there is no recessive allele for *avrBs*$_1$ in these strains and that these strains do not contain this locus.

Isolation and phenotypic characterization of spontaneous race-change mutants. Dahlbeck and Stall (1979) showed that spontaneous race-change mutations of *X.c.* pv. *vesicatoria* race 2 from avirulence to virulence on the pepper cultivar ECW10R containing the *Bs*$_1$ locus occurred at a frequency of 5×10^{-4}. Spontaneous race-change mutants of *X.c.* pv. *vesicatoria* pepper race 2 strain 81-23 Rif^R were isolated by infiltrating these bacteria into the resistant pepper cultivar ECW10R at a dilution of 5×10^6 colony forming units per milliliter. This dilution allows for sufficient spatial separation of the bacteria in the leaf mesophyll for the selection of virulent race-change mutants. The vast majority of the infiltrated bacteria remain avirulent and induce microscopic hypersensitive reactions that do not mask the development of water-soaked lesions caused by virulent mutants.

Approximately 4–5 wk after inoculation, isolated virulent lesions appeared. A sterile toothpick was touched to an isolated lesion and streaked on NYGA medium containing 100 μg/ml Rif and 50 μg/ml cycloheximide. A single colony was taken from each purification and inoculated into pepper cultivars ECW and ECW10R. A total of 13 independent spontaneous race-change mutants were obtained by this procedure. All the mutants were virulent on both ECW and ECW10R. The mutants were also scored for copper resistance and for the presence of a large plasmid. In all cases, the mutants remained copper resistant and contained a large plasmid. These data suggested that either there was a mutation in another locus that affected the expression of avirulence or that a mutation occurred at the *avrBs*$_1$ locus that did not detectably alter the mobility of the large plasmid on agarose gels. To distinguish these possibilities, we tested the ability of the wild type *avrBs*$_1$ gene to complement these mutants for avirulence.

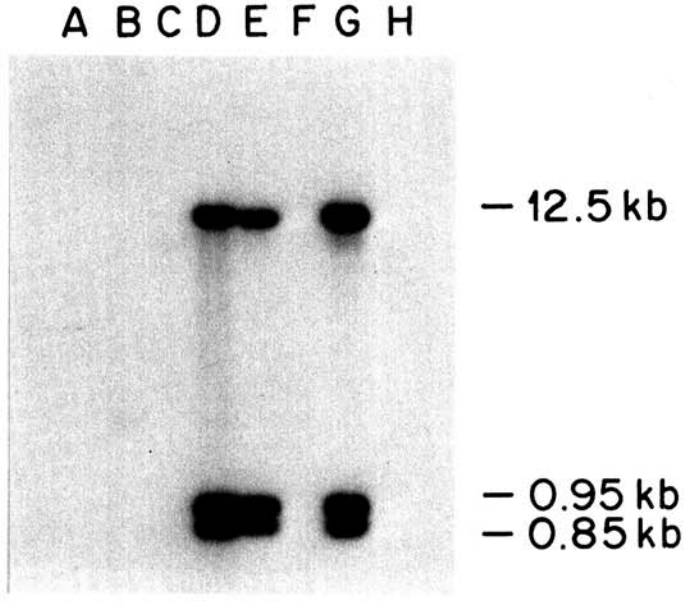

Fig. 4. Southern blot analysis of various *X.c.* pv. *vesicatoria* races probed with the *Bgl*II-*Pst*I insert of pXv2007. Total genomic DNA (including plasmid DNA) was isolated from eight races of *X.c.* pv. *vesicatoria* and digested with the restriction endonuclease *Eco*RV. Each lane was loaded with 2 μg of DNA and electrophoresed in 0.7% agarose. The DNA was transferred to nitrocellulose and probed with the ^{32}P 5.3-kb *Bgl*II-*Pst*I probe from pXv2007. Lane A, *X.c.* pv. *vesicatoria* 82-8; lane B, 82-4; lane C, 71-21; lane D, 81-23; lane E, E3; lane F, 68-1; lane G, 75-3; lane H, Xc T-55.

***avrBs*$_1$ complements spontaneous race-change mutants.** The wild type *avrBs*$_1$ subclone (pXv2007) was introduced into each spontaneous race-change mutant and scored for avirulence on the cultivar ECW10R. All the spontaneous race-change mutant transconjugants containing pXv2007 were avirulent when inoculated on the near-isogenic cultivar ECW10R and remained virulent on the susceptible cultivar ECW. The introduced plasmid pXv2007 containing the *avrBs*$_1$ gene was recovered from these transconjugants by reintroducing the plasmid into *E. coli* HB101 and selecting for tetracycline resistance. Restriction enzyme analysis of the plasmids carried by these transformants demonstrated that the plasmids were identical to the one used in the original conjugation (data not shown). These results demonstrate that spontaneous race-change mutants can arise by mutation at the avirulence gene locus.

DISCUSSION

Although many plant-pathogen interactions suggest a gene-for-gene interaction, genetic proof in both the host and the pathogen must be provided to demonstrate the existence of complementary gene pairs that control the expression and phenotype of disease resistance. This has been accomplished in several fungal-plant interactions by taking advantage of natural genetic systems where the inheritance of avirulence genes in the pathogen and resistance genes in the host can be studied by following segregating populations in both the host and the pathogen (Day 1974).

In this paper we have described the identification and cloning of *avrBs*$_1$ and provided evidence that the interaction between *X.c.* pv. *vesicatoria* and pepper also fulfills the requirements of a bona fide gene-for-gene system. Thus, as previously shown for many fungal-plant interactions, gene-for-gene systems seem to be common in bacterial-plant interactions and provide a genetically simpler system with which to elucidate the molecular events involved in recognitional specificity and expression of disease resistance.

The *avrBs*$_1$ gene is only present in races of *X.c.* pv. *vesicatoria* that induce a hypersensitive reaction on the cultivar ECW10R containing the *Bs*$_1$ locus. These data differ from the results of Gabriel *et al.* (1986) who suggest that recessive alleles of avirulence genes occur in *X. c.* pv. *malvacearum*. The fact that *avrBs*$_1$ resides on a self-transmissible plasmid containing a gene conferring copper resistance may explain the lack of hybridizing sequences in other races of *X.c.* pv. *vesicatoria*. These data also suggest that avirulence genes may also provide a yet unrecognized selective advantage during some portion of the bacterium's life cycle and may explain why these plasmids are maintained in the bacterial population. Of course, this could be simply due to its linkage with copper resistance, but other data have shown that other avirulence genes in *X.c.* pv. *vesicatoria* are also borne on plasmids not carrying copper resistance (unpublished data).

More importantly, we have demonstrated that the wild type avirulence gene can complement spontaneous race-change mutants, which suggests that they arise by mutation at the *avrBs*$_1$ locus. This is significant in that it implies that mutants with an increased host range can arise by mutations at the avirulence locus and that the inactivation of this locus is not a lethal event for *X.c.* pv. *vesicatoria*. However, it is currently unknown whether the loss of this gene is deleterious to the long-term survival of this organism or makes it less able to survive under natural field conditions.

The cloning and characterization of the avirulence gene *avrBs*$_1$ in *X.c.* pv. *vesicatoria* will allow us to elucidate the molecular and biochemical basis of recognitional specificity and the subsequent expression of disease resistance in a genetically well-defined gene-for-gene interaction. Experiments are now in progress to examine the expression of the *avrBs*$_1$ gene, identify its protein product, and determine the subcellular location of the gene product. We will soon be in a position to test whether the avirulence gene product acts directly or indirectly with the plant to specify the expression of disease resistance.

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