Gene-for-Genes Interactions Between Cotton *R* Genes and *Xanthomonas campestris* pv. *malvacearum avr* Genes

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Received 13 April 1992. Revised 20 November 1992. Accepted 25 November 1992.

Six plasmid-borne avirulence (*avr*) genes were previously cloned from strain XcmH of the cotton pathogen, *Xanthomonas campestris* pv. *malvacearum*. We have now localized all six *avr* genes on the cloned fragments by subcloning and Tn*5-gusA* insertional mutagenesis. None of these *avr* genes appeared to exhibit exclusively gene-for-gene patterns of interactions with cotton *R* genes, and *avrB4* was demonstrated to confer *avr* gene-for-*R* genes (plural) avirulence to *X. c.* pv. *malvacearum* on congenic cotton lines carrying either of two different resistance loci, *B1* or *B4*. Furthermore, the *B1* locus appeared to confer *R* gene-for-*avr* genes resistance to cotton against isogenic *X. c.* pv. *malvacearum* strains carrying any one of three *avr* genes: *avrB4, avrb6,* or *avrB102*. Restriction enzyme, Southern blot hybridization, and DNA sequence analyses showed that the XcmH *avr* genes are all highly similar to each other, to *avrBs3* and *avrBsP* from the pepper pathogen *X. c.* pv. *vesicatoria*, and to the host-specific virulence gene *pthA* from the citrus pathogen *X. citri*. The XcmH *avr* genes differed primarily in the multiplicity of a tandemly repeated 102-base pair motif within the central portions of the genes, repeated from 14 to 23 times in members of this gene family. The complete nucleotide sequence of *avrb6* revealed that it is 97% identical in DNA sequence to *avrB4, avrBs3, avrBsP,* and *pthA* and that 62-bp inverted terminal repeats mark the boundaries of homology between *avrb6* and all members of this *Xanthomonas* virulence/avirulence gene family sequenced to date. The terminal 38 bp of both inverted repeats are highly similar to the 38-bp consensus terminal sequence of the Tn*3* family of transposons. Up to 11 members of the *avr* gene family appear to be present in North American strains of *X. c.* pv. *malvacearum*, including XcmH. The high level of homology observed among these *avr* genes and their presence in multiple copies may explain the gene-for-genes interactions and also the observed high frequencies (10^{-3} to 10^{-4} per locus) of *X. c.* pv. *malvacearum* race change mutations. Five spontaneous race change mutants of XcmH suffered *avr* locus deletions, strongly indicating intergenic recombination as the primary mechanism for generating new races in *X. c.* pv. *malvacearum*.

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MPMI, Vol. 6, No. 2, pp. 225-237

Additional keywords: cotton blight, horizontial gene transfer, race specificity.

When a plant species is within the host range of a group of pathogenic microbes, variation is often observed in relative levels of susceptibility vs. resistance within the host species. Host resistance is almost always inherited as a single gene trait, and each resistance (*R*) gene confers a characteristic level of resistance, ranging from immunity to barely detectable (Ellingboe 1976). Since conventional plant breeders exploit host resistance for pest control purposes, the genetic data on host resistance are extensive. Host *R* genes are not effective against all strains of a given pathogenic microbe generally, but instead are only effective against strains of the pathogen carrying specific "target" genes. These strain-specific, microbial "target" genes are termed avirulence (*avr*) genes. Mutational inactivation of *avr* gene "targets" renders the host *R* genes ineffective. Perhaps surprisingly, each different host *R* gene appears to require a specific microbial *avr* gene "target" in order to confer resistance to that strain. Such interactions fit the gene-for-gene model, first discovered by Flor (1946). The spectrum of *avr* genes carried by a given strain determines its race. A large number of *avr* genes have been identified directly or have been inferred to exist (based on the gene-for-gene model) in fungal and bacterial plant pathogens (Sidhu 1987). The genetic data on microbial avirulence, however, are relatively limited. Although there are hints that some *avr* genes may react with different *R* genes and vice versa (reviewed by Gabriel and Rolfe 1990), there are no reports demonstrating such gene-for-genes interactions.

Given the negative effect of *avr* genes on virulence, their surprisingly ubiquitous presence in plant pathogens is enigmatic, and usually explained in terms of some hypothetical pleiotropic effect. Many *avr* genes have been cloned from plant pathogenic bacteria, including *Xanthomonas, Pseudomonas, Erwinia,* and *Rhizobium* and an *avr* gene has been cloned from the fungus *Cladosporium fulvum* (reviewed by Keen 1992). Nevertheless, and despite determined effort to find evidence for pleiotropic effects of *avr* genes, such effects have been found only rarely (for example, Kearney and Staskawicz 1990; Swarup *et al.* 1992). DNA sequencing of *avr* genes published to date has been uninformative in terms of revealing potential function or homology to other genes of known function in sequence data banks. Most *avr* genes appear to be mutable at high frequencies (based on race change mutants) and dispens-

able (based on marker-exchange mutagenesis) and therefore gratuitous in observed plant/microbe interactions (reviewed by Gabriel 1989).

The interaction between cotton and *Xanthomonas campestris* pv. *malvacearum*, the causal agent of bacterial blight of cotton, has been studied for many years. At least 16 genetically well-characterized resistance genes against *X. c.* pv. *malvacearum* have been identified in cotton, and race-change mutations in *X. c.* pv. *malvacearum* that "defeat" these genes are common (Brinkerhoff 1970). Many of these *R* genes have been introduced into a common (Acala-44) genetic background by repeated backcrossing (Hunter and Brinkerhoff 1961). Six avirulence genes were recently isolated from pXcmH, a 90.4-kb plasmid carried by XcmH, a North American strain of *X. c.* pv. *malvacearum* (De Feyter and Gabriel 1991a). Each was shown to govern a distinct set of incompatible interactions with a series of resistant cotton lines. Subcloning of these genes had localized them to regions of 5–10 kb on the cloned pXcmH DNA fragments. We now define the boundaries of these genes, compare their structure, and present the nucleotide sequence of one of them, *avrb6*. These analyses show: 1) that these genes are highly homologous to each other and to *avrBs3* and *avrBsP* from *X. c.* pv. *vesicatoria* and to *pthA* from *X. citri*; 2) that these *avr* genes are representative of a multigene family of *avr* genes, widespread in the genus *Xanthomonas*; 3) that *avrB4* confers gene-for-genes avirulence on cotton lines with either of two unlinked resistance gene loci *B1* and *B4*; 4) that cotton locus *B1* may confer gene-for-genes resistance to isogenic *X. c.* pv. *malvacearum* strains carrying *avrB4*, *avrb6*, or *avrB102;* and 5) that spontaneous race-change mutants of *X. c.* pv. *malvacearum* exhibit deletions of specific *avr* loci.

RESULTS

Localization and characterization of six avirulence genes from *X. c.* pv. *malvacearum*.

Six avirulence genes had previously been isolated from a 90.4-kb plasmid (pXcmH) found in XcmH, separately cloned, and localized to regions of 5–10 kb on the cloned DNA fragments (DeFeyter and Gabriel 1991a). Subcloning experiments were carried out to determine whether these phenotypes were conferred by single or multiple avirulence genes on the cloned fragments, and to further localize these genes. *Bam*HI and *Sst*I fragments were generated by either complete or partial digestion of plasmids pUFR101 (AvrB4$^+$ Avrb6$^+$), pUFR107 (AvrB101$^+$ AvrBIn$^+$), and pUFR114 (AvrB102$^+$ Avrb7$^+$), and inserted into the shuttle vectors pUFR042 (DeFeyter and Gabriel 1991a) or pUFR047 (Fig. 1). The resultant plasmids were introduced into Xcm1003 by conjugation, and the transconjugants were inoculated onto Acala-44 congenic lines carrying the appropriate single *R* genes and on cultivar 101-102B, which is thought to carry multiple different *R* genes. The results of the pathogenicity assays using specific subclones are shown schematically in Figure 2. All fragments with avirulence activity in Xcm1003 on AcB4, Acb6, Acb7, or AcBIn also exhibited avirulence activity on cultivar 101-102B. Cultivar 101-102B also was used to detect and define *avrB101* and *avrB102*. Each of the six *avr* genes contained one *Sst*I site and at least one *Bam*HI site, as shown by the requirement for adjacent *Sst*I fragments or *Bam*HI fragments for avirulence activity. The distance between these internal *Bam*HI and *Sst*I sites was similar for each gene. The avirulence activity encoded by some *Bam*HI fragments was dependent on the orientation relative to the vector *lac* promoter, indicating the direction of transcription of some of the *avr* genes, and the lack of an *X. c.* pv. *malvacearum*-derived promoter on these fragments (e.g., compare pUFR135 vs. pUFR136, pUFR150 vs. pUFR151, pUFR160 vs. pUFR161).

To localize the *avr* genes more precisely, plasmids pUFR180 (AvrB4$^+$ Avrb6$^+$), pUFR156 (AvrBIn$^+$), pUFR157 (AvrB102$^+$), and pUFR163 (Avrb7$^+$) were subjected to Tn*5-gusA* insertional mutagenesis. The sites of transposon insertion were mapped by restriction enzyme analysis, and the avirulence phenotypes conferred by the insertional derivatives were determined by introduction of the plasmids into Xcm1003 and inoculation of transconjugants into cotton. Results are presented for the genes *avrB4, avrb6, avrBIn, avrB102,* and *avrb7* in Figure 3. At least six insertions were identified in each gene. For each fragment, several insertions were also mapped that had not inactivated the avirulence genes. This analysis indicated the minimum extent of each gene. The five genes all occupied regions of greater than 2.9 kb. In each case, the inactivating insertions lay within a *Bam*HI fragment of 2.9–3.6 kb in size. Moreover, every insertion within one of these *Bam*HI fragments inactivated one particular gene. Loss of an avirulence phenotype on cotton AcB4, Acb6, Acb7, or AcBIn was always accompanied by loss of the avirulence phenotype on cultivar 101-102B, indicat-

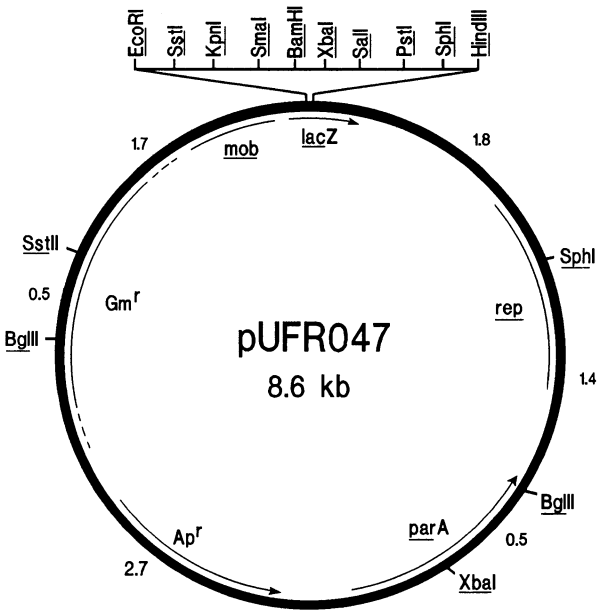

Fig. 1. Schematic representation of pUFR047, which was constructed as described in Materials and Methods. Arrows indicate the direction of transcription where known. Abbreviations: *rep,* replication origin from plasmid Sa (IncW replicon); *parA,* partition locus; *mob,* conferring plasmid mobilization ability (derived from plasmid RK2); Ap^R, resistance to ampicillin; Gm^R, resistance to gentamycin.

ing that single genes were responsible for the observed avirulence phenotypes.

To determine the direction of transcription of each *avr* gene, Xcm1003 transconjugants containing the insertional derivatives were assayed for Gus activity. For three of the genes, derivatives with Tn*5-gusA* inserted in one orientation within the gene expressed detectable β-glucuronidase activity, whereas the transposon in the opposite orientation did not express the *gusA* reporter gene (Fig. 3). For *avrB4* and *avrb6*, the proximity of the vector *lac* promoter did not allow a conclusion to be drawn as to the direction of transcription.

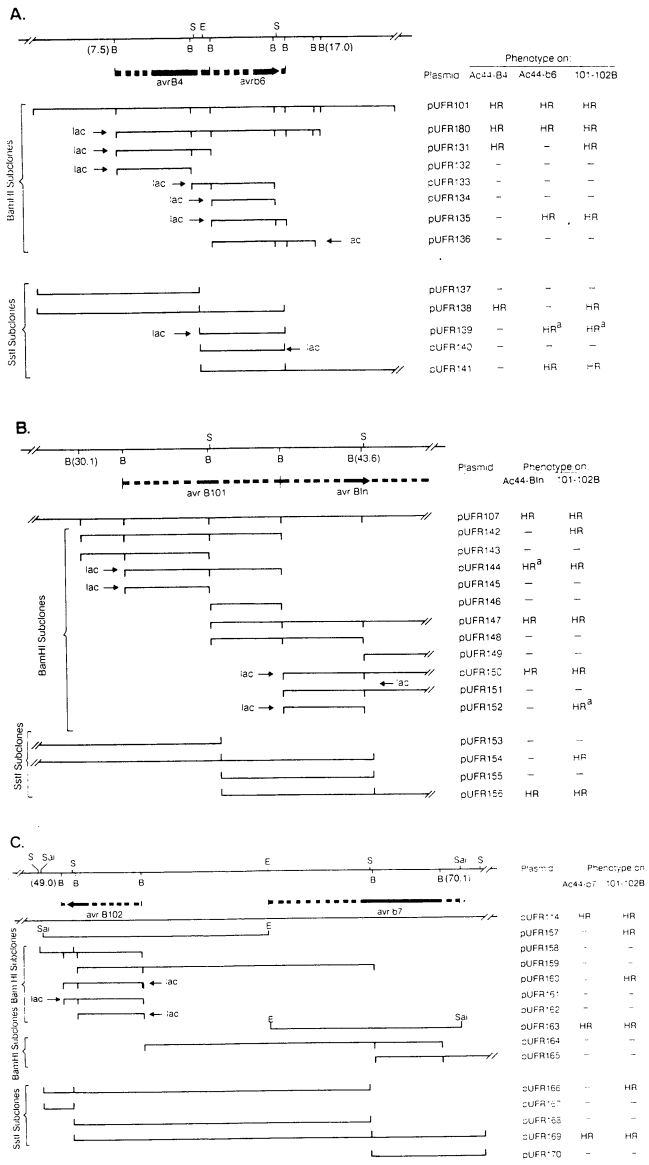

Fig. 2. Avirulence phenotypes conferred by *Bam*HI and *Sst*I subclones of **A,** pUFR101, **B,** pUFR107 and, **C,** pUFR114. Restriction site abbreviations: B, *Bam*HI; E, *Eco*RI; S, *Sst*I. *Bam*HI sites are shown as strokes below the horizontal lines, *Sst*I sites are strokes above the lines. Where known, the orientation of the vector *lacZ* promoter relative to cloned inserts is shown by small arrows. The locations of the avirulence genes deduced from the subcloning data are shown as thick lines, with the large arrows indicating direction on transcription. Figures in parentheses show the segment location of the pXcmH map (DeFeyter and Gabriel 1991a) in kilobases. Avirulence phenotypes of Xcm1003 transconjugants containing each plasmid are indicated as: HR, a strong hypersensitive response; HR[a], a much weaker hypersensitive response; –, fully virulent.

Gene-for-genes interactions.

Based on the results obtained from the subcloning and transposon localization experiments, the smallest cloned fragments carrying different single *avr* genes were selected. These were transferred to Xcm1003 by conjugation and tested on a full set of Ac44 congenic resistance lines, each thought to carry a different, single *R* gene effective against *X. c.* pv. *malvacearum*. The results of repeated pathogenicity tests are presented in Table 1. If only congenic AcB4, Acb6, Acb7, and AcBIn lines are considered, their interactions with isogenic *X. c.* pv. *malvacearum* strains with *avrB4, avrb6, avrb7,* and *avrBIn* are gene-for-gene. However, lines AcB1, AcB2, and AcBIn3 each responded hypersensitively with multiple different *avr* genes in an isogenic *X. c.* pv. *malvacearum* background. Cotton line AcB1, developed by recurrently selecting the *B1* resistance in six backcrosses with Ac44, conferred resistance to

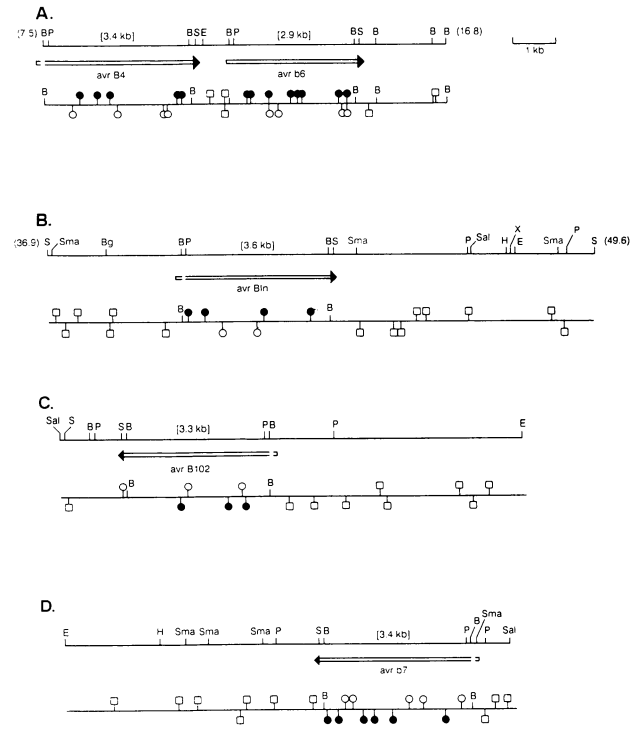

Fig. 3. Sites and orientations of Tn*5-gusA* insertion into avirulence gene clones are shown for derivatives of **A,** pUFR180; **B,** pUFR156; **C,** pUFR157; and **D,** pUFR163. Arrows indicate the deduced positions and orientations of the avirulence genes on the cloned inserts. Restriction enzyme site abbreviations: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sst*I; Sal, *Sal*I; Sma, *Sma*I; X, *Xba*I. Insertion sites shown above horizontal lines correspond to a rightward orientation of the *gusA* reporter gene; sites shown below the lines correspond to a leftward *gusA* orientation. Symbols: □, sites of insertion that did not affect the avirulence gene activity when in Xcm1003; ○, sites that inactivated the particular gene; ●, sites that inactivated the particular gene with resultant expression of gusA. Figures in parentheses show the segment location on the pXcmH map (DeFeyter and Gabriel 1991a) in kilobases.

Xcm1003 carrying any one of the following different *avr* genes: *avrB4*, *avrb6*, or *avrB102*. Similarly, line AcB2 responded to Xcm1003 with any of five *avr* genes tested, and line AcBIn3 responded to Xcm1003 carrying any of the six *avr* genes tested.

To demonstrate that at least two of the *R* gene loci thought to be different were in fact different, congenic host lines AcB1 and AcB4 were chosen for segregation analysis. Isogenic strains carrying cloned *avrB4* (Xcm1003/pUFR138) and *avrb6* (Xcm1003/pUFR135) were used to distinguish the two resistance phenotypes (Table 2). The results of inoculating these strains onto 85 F_2 progeny of a cross between homozygous parents AcB1 and AcB4 are shown in Table 2. These results fit, at a 95% confidence level, the 9:3:3:1 ratio expected of two unlinked genes in such crosses, with a χ^2 value of 0.27. Avirulence gene *avrB4* conferred gene-for-genes avirulence to Xcm1003 in inoculations on cotton plants with either one of two different resistance genes, *B1* or *B4*. The genetics of race-cultivar specificity in *X. c.* pv. *malvacearum* on cotton therefore exhibited gene-for-genes, as well as gene-for-gene patterns of interaction, depending upon which *avr*/*R* gene combinations were examined.

Similarity to other *Xanthomonas* avirulence and virulence genes.

It is clear from the restriction enzyme mapping analysis (Fig. 3) that there were similarities among the pXcmH *avr* genes, namely in the arrangements of *Bam*HI and *Sst*I sites and in the sizes of the genes. To confirm these similarities, Southern blot hybridization experiments were carried out using the internal *Bam*HI fragment from *avrB4* (pUFR132) as a probe. This fragment hybridized strongly under stringent conditions to the internal *Bam*HI fragments from *avrBs3*, *pthA*, and the six pXcmH avirulence genes (data not shown). The cloned avirulence genes were analyzed with a series of restriction enzymes that were known to cut within *avrBs3*. Restriction sites for *Bam*HI, *Bcl*I, *Eag*I, *Nae*I, *Nar*I, *Nsi*I, *Pst*I, *Sst*I, and *Stu*I were present in identical positions in the 5′ and 3′ portions of the avirulence genes relative to *avrBs3*. No polymorphisms were found with any of these enzymes within the 5′ and 3′ portions of the pXcmH *avr* genes.

To determine the degree of homology between *avrBs3* and *avrBsP* of *X. c.* pv. *vesicatoria*, *pthA* of *X. citri*, and the pXcmH *avr* genes, the complete DNA sequence of *avrb6* and a portion of *avrB4* was determined. The nucleotide sequence of *avrb6* is presented in Figure 4, together with the predicted amino acid sequence of the protein. The central region of the gene is characterized by 13.5 nearly perfect, tandemly repeated, 102-bp repeats (Fig. 5). The ends of the gene are characterized by 62-bp nearly perfect, inverted repeats. DNA sequence comparisons revealed that these inverted repeats define the boundaries of DNA sequence homology between *avrb6*, *avrB4* (refer below), *avrBs3* (Bonas *et al.* 1989), and *pthA* (refer Swarup *et al.* 1992). From the beginning of the left inverted repeat (*avrb6L*; position 314) to the end of the right inverted repeat (*avrb6R*; position 3763), *avrb6* is 98% identical in DNA sequence along its entire length to the complete nucleotide sequence of *avrBs3*. Three 200-bp fragments of *avrB4* were also sequenced, comprising sequences at the

Table 1. Gene-for-genes interactions[a]

X. campestris pv. *malvacearum* strains	Cotton cv. Acala-44 congenic lines							
	AC44	AcB1	AcB2	AcB4	Acb6	Acb7	AcBln	AcBln3
Xcm1003/pUFR047 (vector)	+	+	+	±	+	+	+	+
Xcm1003/pUFR138 (*avrB4*)	+	±	±	−	+	+	+	−
Xcm1003/pUFR141 (*avrb6*)	+	−	±	+	−	+	+	−
Xcm1003/pUFR163 (*avrb7*)	+	+	±	+	+	−	+	−
Xcm1003/pUFR150 (*avrBln*)	+	+	+	±	+	+	−	−
Xcm1003/pUFR142 (*avrB101*)	+	+	±	+	+	+	+	−
Xcm1003/pUFR157 (*avrB102*)	+	±	±	+	+	+	+	−

[a] Acala-44 congenic lines carrying the indicated *R* genes were inoculated with the indicated Xcm1003 isogenic strains carrying the indicated *avr* genes. + means a compatible interaction, as indicated by a watersoaking lesion; − means as incompatible interaction, as indicated by a strong hypersensitive response (HR); + − indicates a weak hypersensitive response. The dashed box illustrates results expected of classical gene-for-gene interactions.

Table 2. Independent segregation of two cotton blight resistance loci, *B1* and *B4*[a]

Pathogen	AcB1	AcB4	F_1	F_2			
	B1B1 *b4b4*	*b1b1* *B4B4*	*B1b1* *B4b4*	*B1-* *B4-*	*B1-* *b4-*	*b1b1* *B4-*	*b1b1* *b4b4*
Xcm1003	+	+	+	+	+	+	+
Xcm1003/pUFR135 (*avrb6*)	−	+	−	−	−	+	+
Xcm1003/pUFR138 (*avrB4*)	±	−	−	−	±	−	+
Observed				54	19	17	5
Expected[b]				54	18	18	6

[a] Cultivar AcB4, an Acala-44 line homozygous for *B4*, was used as the male parent in a cross with cultivar AcB1, homozygous for *B1*. Pathogenic reactions of Xcm1003 and transconjugants of Xcm1003 carrying *avrB4* and *avrb6* on plasmids pUFR138 and pUFR135, respectively, on parental lines and F_2 plants are shown.

[b] $\chi^2 = 0.27 << \chi^2_{0.05} = 7.82$. Symbols as in Table 1.

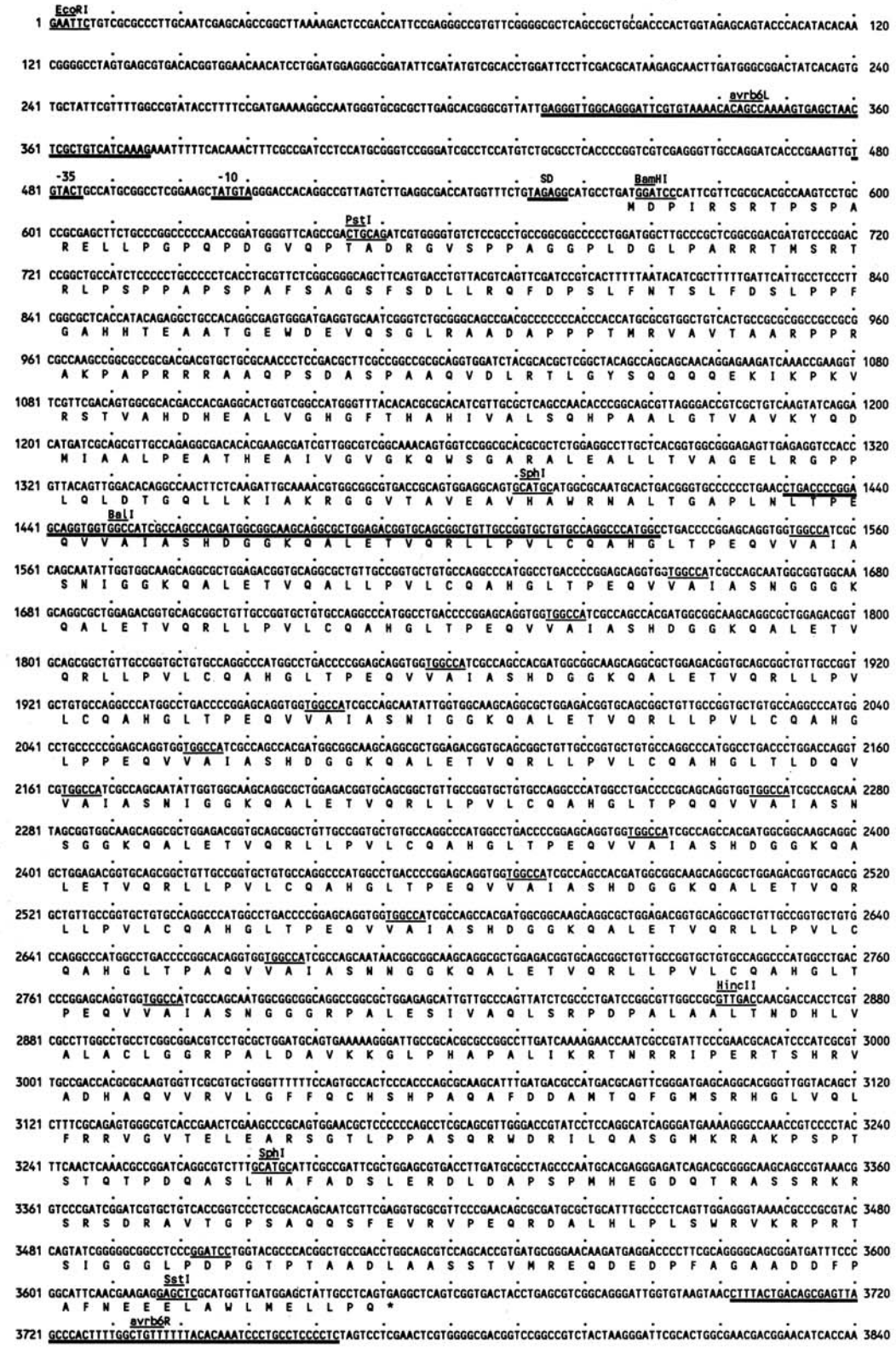

Fig. 4. Nucleotide and predicted amino acid sequence of a 3,840-bp fragment of pXcmH carrying *avrb6* activity. (Genbank Accession # L06634). Sequences homologous to *Escherichia coli* -35, -10 promoter and Shine-Dalgarno (SD) regions are double-underlined and labeled, as are the left and right terminal inverted repeats, *avrb6L* and *avrb6R*. The *Eco*RI, *Bam*HI, *Sph*I, *Bal*I, *Hin*cII, *Sst*I, and *Pst*I sites are underlined, and the first (or only) occurrence of each site is labeled.

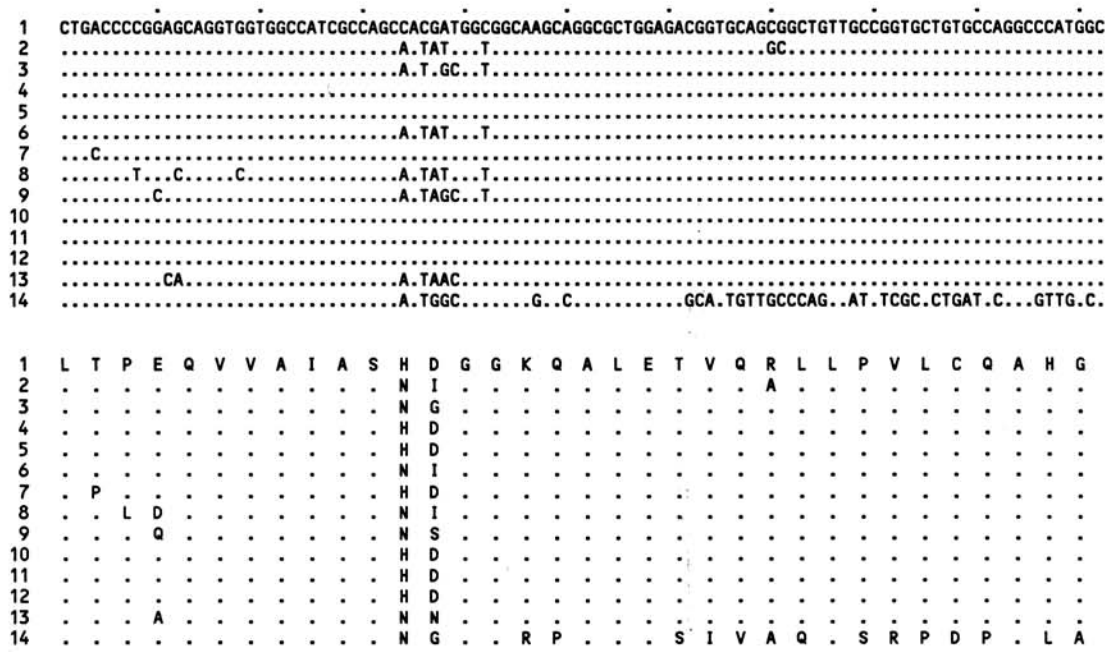

Fig. 5. Alignment of the 102-bp nucleotide direct repeat region and the 34-bp predicted amino acid sequenceof *avrb6*. Dots indicate identity with the sequence in repeat number 1, which is also the concensus.

5′ end of the gene downstream from the *Bam*HI site, the repeat region downstream from the *Sph*I site, and the 3′ end downstream from the *Sst*I site (including the right inverted repeat; sequence data not shown). These *avrB4* sequences were 97% identical to those of the corresponding regions from *avrb6*. The sequences of *avrb6, avrB4, avrBs3,* and *pthA* diverge immediately beyond the conserved inverted repeats. The terminal 38 bases of both *avrb6* inverted repeats are 87% similar to a consensus terminal inverted repeat sequence from 11 members of the transposon Tn*3* family (Heffron 1983): (5′-GGGGNNNNNNNNNN-ANNNGNANNANANNNNACGNTAAG-3′). That is, 5/38 (13%) of the bases of *avrb6L* and *avrb6R* are in conflict with this consensus, and 11/16 (69%) of the defined bases are identical to the consensus.

Each direct repeat of *avrb6* has conserved *Bal*I and *Nco*I restriction sites and therefore the other pXcmH *avr* genes were examined for multiple sites with these enzymes. The internal *Bam*HI fragments from the pXcmH avirulence genes were inserted into pGem11Zf(+) and the resultant plasmids were digested partially with *Bal*I and completely with *Hin*dIII. The digested DNA was electrophoresed on agarose gels, revealing a laddered pattern of fragments (Fig. 6). By counting the number of bands in each "ladder," the number of repeated units in each *avr* gene was determined. The number of repeat units of the six pXcmH *avr* genes ranged from a low of 14 for *avrb6* to 23 for *avrB101*. Only *avrB4* and *avrb7* contain the same number (19) of repeats. DNA sequencing of fragments from *avrB4* revealed that the first two *Bal*I fragments of the gene were 102 bp in size and were nearly identical with each other and with the 102-bp repeats found in *avrb6* (Fig. 5). The detailed restriction maps, partial *Bal*I digests, hybridization data, and DNA sequence analyses together showed that the six pXcmH avirulence genes were all members of a

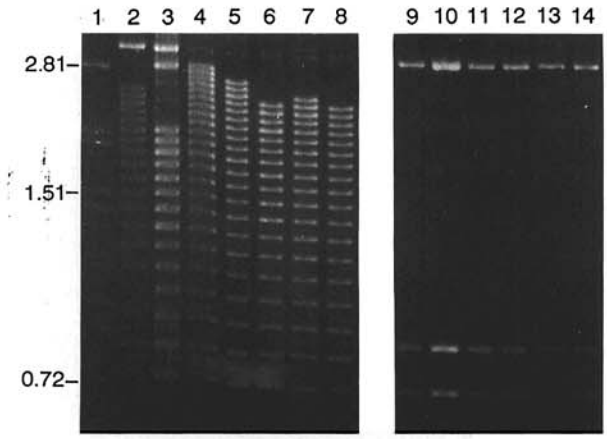

Fig. 6. Analysis of cloned avirulence genes by partial digestion with *Bal*I. Plasmids were digested partially (lanes 2–8) or completely (lanes 9–14) with *Bal*I, then digested to completion with *Hin*dIII, and electrophoresed through 0.8% agarose. Digested plasmids were loaded as follows: lanes 2, 9, pUFR171 (*avrB4*); lanes 3, 10, pUFR172 (*avrb6*); lanes 4, 11, pUFR173 (*avrB101*); lanes 5, 12, pUFR174 (*avrBIn*); lanes 6, 13, pUFR175, (*avrB102*); lanes 7, 14, pUFR176 (*avrb7*); lane 8, pUFR177 (*avrBs3*). Sizes of molecular weight markers (lane 1) are indicated in kilobases.

Xanthomonas virulence/avirulence gene family, and that they differed in the number of repeated units within the central portions of the genes.

Multiple members of the *avr* gene family are in many xanthomonads.

To determine how many genes in this *avr* gene family are present in *X. c.* pv. *malvacearum* strains, total genomic DNA from 25 *X. c.* pv. *malvacearum* strains was analyzed in Southern blot hybridization experiments. The strains were of different races and origins, including 19 isolated

from cotton and six from hibiscus. An internal gene probe from *avrB4* (pUFR132) hybridized not only to the six pXcmH (plasmid-borne) avirulence genes in XcmH, but also to at least five other fragments in the XcmH genome (Fig. 7, compare lanes 7 and 8). From band intensities, the plasmid-derived fragments were clearly present at higher copy number in total XcmH DNA than the other five fragments, suggesting that the latter group are chromosomally borne. DNA from other strains of *X. c.* pv. *malvacearum* isolated in the United States contained from eight to 11 hybridizing fragments (lanes 8–21). By comparison, only four or five hybridizing fragments were present in *X. c.* pv. *malvacearum* strains from West Africa (lanes 2–6), and none in six *X. c.* pv. *malvacearum* strains isolated from hibiscus (lanes 22–28).

To determine how widespread genes in this *avr* gene family are in *Xanthomonas*, total genomic DNA isolated from 25 strains representing 12 *Xanthomonas* pathovars or species was similarly analyzed. Besides *X. c.* pv. *malvacearum*, strains of *X. citri, X. phaseoli,* and *X. c.* pvs. *vignicola, glycines, alfalfae, cyamopsidis* and *translucens* contained multiple members of the avirulence gene family (data not shown; the blot is identical to Figure 1 of Swarup *et al.* 1992). Some strains of *X. c.* pvs. *translucens* and *vesicatoria* contained members of the gene family, while others did not.

Deletions in *avr* genes among race-change mutants.

Spontaneous race-change mutants Xcm1102 (Avrb6$^-$, AvrBIn$^-$), Xcm1113 (AvrBIn$^-$), and KM46 were derived from XcmH. Such mutants arose at a frequency in the range 10^{-3} to 10^{-4} (DeFeyter and Gabriel 1991a; McNally 1990). Strains Xcm1201 and Xcm1216 were similarly isolated as spontaneous, virulent mutants of XcmH on AcB4 plants at similar frequencies. All mutants were tested on all of the congenic lines in Table 1, except AcB1. Xcm1201 was virulent on Ac44 and AcB4 (AvrB4$^-$); Xcm1216 was virulent on Ac44, AcB4, and Acb6 (AvrB4$^-$, Avrb6$^-$); and KM46 was virulent on Ac44, AcB4, and AcBIn (AvrB4$^-$, AvrBIn$^-$). (The wild-type parent is avirulent on all lines except Ac44.) All five spontaneous race-change mutants appeared stable in phenotype in repeated tests.

Total DNA from these five independently isolated, spontaneous race-change mutants of XcmH were included in the Southern blot analysis of *X. c.* pv. *malvacearum* DNA (Fig. 7, lanes 17–21). All five race-change mutants exhibited losses of hybridizing DNA fragments consistent with their mutant phenotypes. Strain Xcm1201 (AvrB4$^-$) appears to be missing one of the two 15.1-kb fragments; *avrB4* is located on one of these fragments. Strain Xcm1216 (AvrB4$^-$, Avrb6$^-$) is missing the 4.0-kb *avrb6* fragment; the 15.1-kb *avrB4* fragment is not obviously affected. Strain

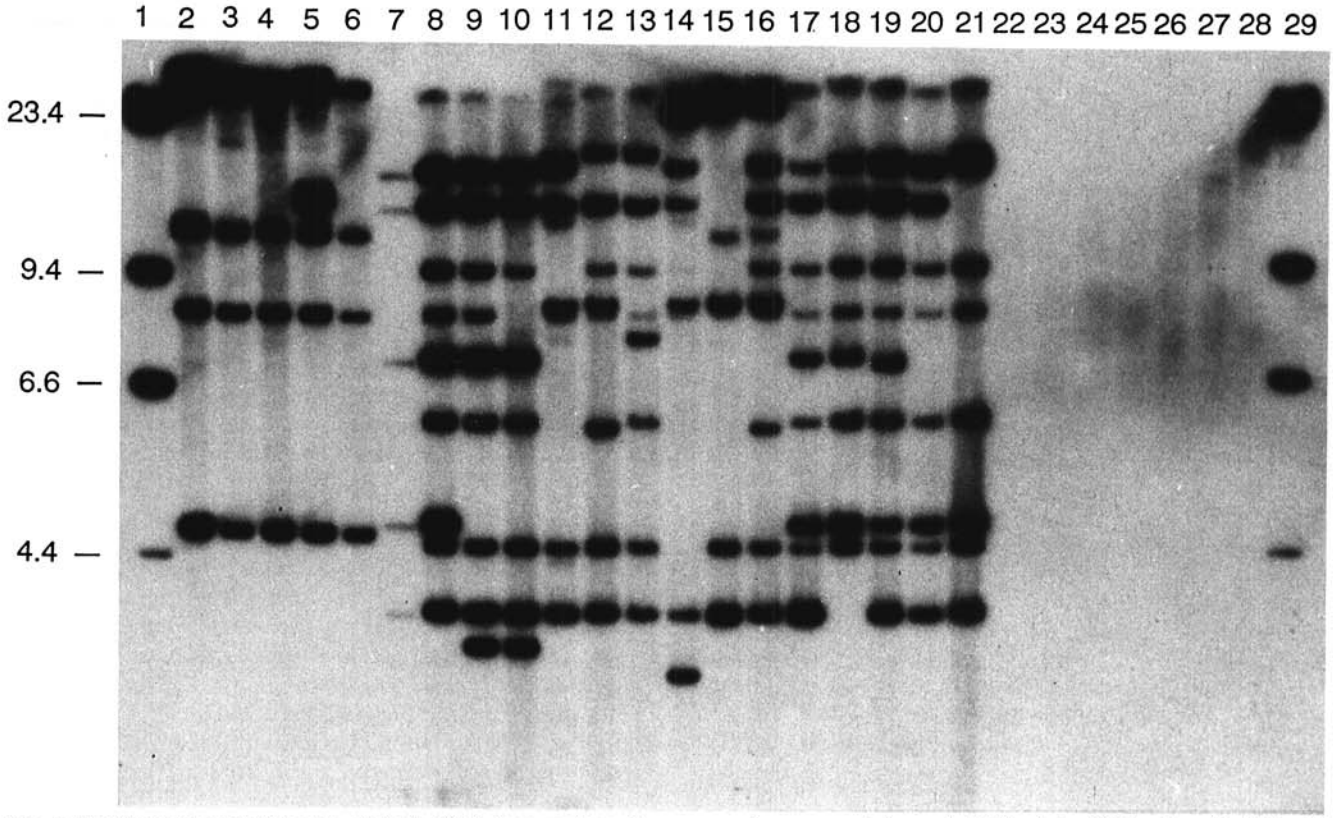

Fig. 7. Southern blot hybridization of total DNA from various *X. c.* pv. *malvacearum* strains and DNA of the 90-kb plasmid of XcmH (pXcmH) after *Sst*I digestion. The blot was probed with the internal 3.4-kb *Bam*HI fragment from *avrB4* (pUFR132). DNA was loaded in the following order. Lanes 2–6: strains XcmJ, XcmL, XcmC, XcmN, and XcmNSp (all African origin). Lane 7: plasmid pXcmH. Lanes 8–16: strains XcmH, XcmA, XcmF, XcmI, XcmD, XcmM, XcmQ, XcmX, XcmY (all U.S. origin). Lanes 17–21: Xcm1201, Xcm1216, Xcm1102, Xcm1113, KM46 (race-change mutants of XcmH). Lanes 22–28, strains X10, X27, X52, X102, X103, X108, and 083–4344 (hibiscus strains). Sizes of molecular weight markers (Lanes 1 and 29) are shown in kilobases.

Xcm1102 (Avrb6$^-$, AvrBIn$^-$) exhibited a 6.8-kb fragment that is slightly reduced in size from the 6.9-kb fragment carrying *avrBIn*; the 4.0-kb *avrb6* fragment is not obviously affected. Strain Xcm1113 (AvrBIn$^-$) is missing the 6.9-kb *avrBIn* fragment. Strain KM46 (AvrB4$^-$, AvrBIn$^-$) is missing the 6.9-kb *avrBIn* fragment as well as the 12.7-kb *avrB101* fragment; the 15.1-kb *avrB4* fragment is not obviously affected.

DISCUSSION

Six avirulence genes had previously been isolated from pXcmH, a 90.4-kb plasmid found in XcmH, separately cloned, and localized to regions of 5–10 kb on the cloned DNA fragments (DeFeyter and Gabriel 1991a). Four of the genes were designated *avrB4, avrb6, avrb7,* and *avrBIn* based on the HR, elicited by *X. c.* pv. *malvacearum* transconjugants carrying the *avr* genes, on cotton cultivars AcB4, Acb6, Acb7, and AcBIn, respectively. These interactions were cultivar-specific, that is, "gene-for-gene" interactions. Each of the six *avr* genes, however, conferred an avirulent phenotype to the widely virulent strain Xcm1003 when transconjugants were inoculated onto cotton cultivars that were thought to have *R* genes other than *B4, b6, b7,* or *BIn*. Attempts were made by subcloning and transposon insertion analyses to separate these activities, without success (Figs. 2 and 3). For example, we have shown here that all insertions of Tn*5-gusA* into the 3.4-kb *Bam*HI fragment of pUFR163 resulted in the loss of all *avr* activity. Insertions only 200 bp on either side had no effect, ruling out the possibility of polar effects on any putative, adjacent *avr* gene(s). Analogous results were obtained with *avrB4, avrb6,* and *avrBIn*. We conclude that single avirulence genes were responsible for conferring multiple avirulence activities on different cotton cultivars.

The question then addressed was, do these cultivars contain different *R* genes? We have shown here, by formal segregation analyses of a cross between AcB1 and AcB4 that these two cultivars carried unlinked resistance loci, i.e., *B1* and *B4* are genetically distinct. Both loci conferred resistance to strain Xcm1003 carrying *avrB4*. AcB1 reacts with other *avr* genes in addition to *avrB4* in Xcm1003, while AcB4 reacts only with *avrB4*. Since the *B4* locus reacts with Xcm1003/*avrB4* to give a stronger HR than that given by *B1*, and since *avr*/*R* gene interactions are dominant and epistatic, it cannot be argued that *B1* is a compound locus containing *B4* also. Avirulence gene *avrB4* therefore exhibited gene-for-genes activity on congenic cotton lines with *B1* or *B4*. From Table 1 it is clear that there are *R* genes (*B4, b6, b7,* and *BIn*) that react with one and only one *avr* gene tested, but there are no cases of an *avr* gene reacting with only one *R* gene. We conclude that individual members of this *avr* gene family from *X. c.* pv. *malvacearum* interact with multiple cotton *R* genes as a rule, and not according to the gene-for-gene hypothesis.

Do single cotton *R* genes interact with multiple *X. c.* pv. *malvacearum avr* genes? Previous studies have shown that in most cases cotton *R* genes against *X. c.* pv. *malvacearum* are unlinked (Brinkerhoff 1970). In this study, AcB1 was selectively backcrossed six times to Ac44 (99.22% Ac44), without evidence of other *R* gene loci independently segregating. AcB1 was also crossed with AcB4, again without evidence of more than a single locus present in either AcB1 or AcB4. Because of the epistatic effects of avirulence over virulence, AcB1 could not carry *B2, B4, b7, BIn,* or *BIn3* (refer Table 1). The B1 locus exhibited resistant interactions with isogenic *X. c.* pv. *malvacearum* strains with *avrB4, avrb6,* or *avrB102*. It is possible that the *B1* locus might be compound, carrying homologues of *B4, b6,* and *B102*. By similar logic, the *B2* locus would have to carry homologues of *B4, b6, b7, B101,* and *B102*. AcB2 cannot carry the *B1* locus because AcB2 reacts more weakly than AcB1 to Xcm1003/*avrb6*. Therefore, for the compound locus interpretation to be correct, at least two (*B1* and *B2*) and probably three (*BIn3*), independent, compound loci must be invoked with homologues for *B4, b6,* and *B102*. Different compound *R* gene loci with homologues conferring identical gene-for-gene specificity have never before been described. We favor a simpler explanation: that some interactions are not gene-for-gene, and some individual cotton *R* genes (such as *B1, B2,* and *BIn3*) react with multiple *avr* genes, while others (such as *B4, b6, b7,* and *BIn*) do not. However, without molecular characterization of a locus, it is virtually impossible to prove that the locus is not compound.

Most *avr*/*R* gene interactions reported with both fungi and bacteria appear to be gene-for-gene, and there is a good possibility that the gene-for-genes interactions reported here are anomalous and due to the fact that all of the *avr* genes reported here are members of the same gene family. In fact, given the surprising degree of homology among members of this gene family, a harder and more obvious question to answer is how nearly identical genes can specifically "interact" with different plant *R* genes. The DNA sequence of *avrb6* (Fig. 4) showed ≥97% identity with avrB4 of *X. c.* pv. *malvacearum, pthA* of *X. citri* (Swarup *et al.* 1992), and *avrBs3* (Bonas *et al.* 1989) and *avrBsP* (Canteros *et al.* 1991) of *X. c.* pv. *vesicatoria*. The *X. c.* pv. *malvacearum* genes differ most obviously in the multiplicity of the 102-bp motif (Fig. 5) in the central portions of the genes. As shown by partial *Bal*I digests of the cloned pXcmH genes (Fig. 6), the 102-bp motif is repeated from 14 (in *avrb6*) to 23 times (in *avrB101*). The only genes with the same number of repeats are *avrB4* and *avrb7*, both having 19. We conclude that the avirulence specificity of *X. c.* pv. *malvacearum avr* genes is not determined solely by the number of repeated units. Deletion of some of the repeats of *avrBs3* (Herbers *et al.* 1992) and *pthA* (Swarup and Gabriel, unpublished) can result in the generation of altered avirulence specificities, indicating that particular repeats or the order of particular repeats may be important. The repeated units of *avrb6, avrB4, pthA, avrBsP,* and *avrBs3* are highly similar (>92% among the repeats), and the consensus repeat sequences of *avrb6* and *avrBs3* are identical. The most obvious differences in both of these genes appear be in positions 12 and 13 of the 34 amino acid repeated motif (Fig. 5), involving histidine-aspartate vs. asparagine-isoleucine, -glycine, or -asparagine.

To date, the biochemical function(s) of any member of this gene family remains unknown. Comparisons of the

avrb6 nucleotide coding sequence and inferred amino acid sequence with sequence data banks did not reveal significant homology with known genes or proteins outside of this gene family. The 102-bp direct repeats within these genes are remarkable and are required for function. Outside of this gene family, highly repetitive elements of similar size and periodicity are found in structural proteins that interact with cytoskeletal elements such as the ankyrin family of proteins (Lux *et al.* 1990) and in a number of genes involved in cell cycle control in yeast (Sikorski *et al.* 1990) and nuclear migration in yeast (Kormanec *et al.* 1991). Most of these proteins are associated with membranes or structural components of the cell. The putative protein product of *avrb6* apparently lacks any amino-terminal signal sequence that would indicate a transmembrane subcellular localization. Expression of *avrBs3* (Knoop *et al.* 1991) and *pthA* (Swarup *et al.* 1992) is constitutive, and *avr::gusA* fusions to three of the *X. c.* pv. *malvacearum* genes were expressed from their native promoters when bacteria containing these genes were grown in rich media. Constitutive expression might indicate a function for these genes other than that involving plant interactions, since a certain amount of metabolic energy must be expended in the constitutive expression of the genes. By contrast, the *Xanthomonas hrp* genes reported to date are essential for virulence and are well regulated (Willis *et al.* 1991).

DNA fragments that hybridize to internal probes derived from members of this gene family were found in nine of 12 *Xanthomonas* species or pathovars examined, including *X. citri, X. phaseoli,* and *X. campestris* pvs. *alfalfae, aurantifolii, cyamopsidis, glycines, malvacearum, translucens,* and *vignicola,* and almost always with multiple members per strain (Bonas *et al.* 1989; Swarup *et al.* 1992). Since not all *Xanthomonas* strains carry DNA fragments that hybridize to members of this gene family, these genes do not appear to be needed in the genus generally. By contrast, all strains tested of *X. citri, X. phaseoli,* and *X. c.* pv. *malvacearum* carried members of this gene family (Fig. 7 and Swarup *et al.* 1992), suggesting that these genes may be needed in some strains. At least one member of this family, *pthA*, is a host-specific virulence gene and confers the ability to elicit cankers on citrus (and only on citrus) to several other xanthomonads (Swarup *et al.* 1991). (Gene *pthA* also confers gratuitous avirulence to *X. citri* on nonhosts [Swarup *et al.* 1992]). Similarly, *avrb6* confers water-soaking ability to *X. c.* pv. *malvacearum* strains, and the phenotype is also host-specific (for cotton) (De Feyter and Gabriel 1991a; Yang and Gabriel 1992). *X. c.* pv. *malvacearum* strains originating in North America, such as XcmH, had at least eight to 11 potential members of the gene family, including four to six hybridizing fragments on large plasmids. Besides the six plasmid-borne *avr* genes cloned from XcmH, the previously cloned, chromosomally encoded *avrBn* (Gabriel *et al.* 1986) was recently determined to be the seventh member of this gene family expressing *R* gene-specific avirulence (Yang and Gabriel, unpublished). The function(s) of the other one to four hybridizing fragments is unknown. Strains originating in West Africa (such as XcmN) had four to five potential members of the family, including at least one on a plasmid. Yet strain XcmN exhibits no known avirulence activity, and most of the other African strains are virulent on a wide range of cotton cultivars. The presence of potential members of the gene family in all *X. c.* pv. *malvacearum* strains—some with no known avirulence activity—was therefore unexpected and indicates that at least one or more members (perhaps including *avrb6*) may be needed for virulence on cotton. None of the *X. c.* pv. *malvacearum* strains isolated from Hibiscus contained genes with homology to this avirulence gene family (Fig. 7), and cotton is not a host for *X. c.* pv. *malvacearum* strains isolated from Hibiscus. This gene family may be considered a *Xanthomonas* host-specific virulence/avirulence gene family.

The presence of inverted repeat sequences flanking all members of this gene family sequenced to date was unexpected, as was the fact that *avrb6* of XcmH is 98% identical in DNA sequence with *avrBs3* of *X. c.* pv. *vesicatoria.* Since strains of *X. c.* pv. *malvacearum* and *X. c.* pv. *vesicatoria* are only 34–42% similar by DNA-DNA hybridizations (Kingsley and Gabriel, unpublished), these genes have obviously moved horizontally among genetically dissimilar strains. The wide distribution of multiple copies of hybridizing fragments among natural strains of the genus *Xanthomonas* may indicate that the horizontal transfer of these genes is not rare. The presence of terminal inverted repeats, the evidence for horizontal gene transfer, and the presence of multiple hybridizing bands in nearly all strains examined suggested transposition as a possible mechanism of genetic exchange. In fact, the terminal 38 bases of both *avrb6R* and *avrb6L* are 87% similar to consensus sequence, 5′-GGGGNNNN-NNNNNNANNNGNANNANANNNNACGNTAAG-3′, shared by 11 members of the Tn*3* transposon family (Heffron 1983). A characteristic of the Tn*3* family is that the transposase function can be supplied in *trans*; only the terminal inverted repeats are required in *cis* for transposition of the intervening DNA (Heffron 1983). Although this evidence is not conclusive, we suggest that the similarity of the terminal repeats found in *avrb6, avrB4, pthA, avrBs3,* and probably other members of this gene family to the Tn*3* family of repeats indicates that these genes may be capable of transposition. This idea is currently under investigation.

The generation of race-change mutations at high frequencies has been well documented in fungi (for example, Statler [1985]) and in bacterial pathogens, including *X. c.* pv. *malvacearum* (for example, Brinkerhoff 1970). These race-change mutations, presumably involving *avr* genes, occur independently of host cultivar (or *R* gene) selection against avirulence (Alexander *et al.* 1985). Therefore many, if not most, *avr* genes may be selectively neutral (Gabriel 1989). If a multi-gene family is found within a given strain, any essential function might be satisfied by one of the members of the family, leaving the others free to mutate. For example, four members of the *avr* gene family reported here are found in *X. citri*, but only one member, *pthA*, is required for virulence (Swarup *et al.* 1992). Spontaneous race-change mutations in *X. c.* pv. *malvacearum* were readily obtained at unusually high frequencies of from 10^{-3} to 10^{-4} (DeFeyter and Gabriel 1991a; McNally 1990). In

all five race-change mutants examined (Fig. 7), deletions appeared in DNA fragments corresponding to members of this *avr* gene family, usually including the fragment carrying the predicted *avr* gene. It is apparent that multiple members of this avirulence gene family may have arisen in *Xanthomonas* strains by duplication of an existing member, and divergence of the copies by intragenic or intergenic recombination. The highly conserved and reiterated structure of the members of this *avr* gene family provides opportunities for the evolution of new avirulence/virulence phenotypes via homologous recombination. All seven cotton *R* genes examined so far interact with *X. c.* pv. *malvacearum* through members of this *avr* gene family. On the basis of the high frequency of race change mutations observed in *X. c.* pv. *malvacearum* and the observed deletions in fragments known to carry the specific *avr* genes involved, we conclude that homologous recombination among duplicated members of this *avr* gene family is the most likely mechanism for race change in *X. c.* pv. *malvacearum*.

MATERIALS AND METHODS

Bacterial strains and plasmids.

The bacterial strains and plasmids used in this study are listed in Table 3. The virulent race-change mutants Xcm1201 and Xcm1216 were isolated from cotton line AcB4 (refer below) as described previously (DeFeyter and Gabriel 1991a).

Media.

Escherichia coli strains HB101 and DH5α were grown in LB medium (Sambrook *et al.* 1989) at 37° C and *X. c.* pv. *malvacearum* strains at 30° C in PYGM (peptone-yeast extract-glycerol-MOPS) medium (DeFeyter *et al.* 1990). When appropriate, antibiotics were added at the following final concentrations (in mg/L): ampicillin (Ap), 25; gentamycin (Gm), 2; kanamycin sulphate (Km), 20; and rifampicin (Rif), 75.

Recombinant DNA methods.

Plasmids were isolated by alkaline lysis methods (Birnboim and Doly 1979) and digested with restriction enzymes as recommended by the manufacturers. All other recombinant DNA methods were according to Sambrook *et al.* (1989).

Plasmid constructions.

Plasmid pUFR044 was constructed from pUFR042 (DeFeyter and Gabriel 1991a) by deletion of the 1.4-kb *Pst*I fragment specifying Km^R. pUFR044 retained the Gm^R and $lacZ^+$ markers. The *Hae*II fragment encoding resistance to ampicillin from pUC19 was inserted into a *Pst*I site of pUFR044, after both digested DNAs had been treated briefly with *Bal*31. This treatment removed approximately 0.4 kb of DNA in total from the fragment ends. The resultant plasmid, pUFR046, has two *Sma*I sites. One *Sma*I site was deleted by partial digestion with *Sma*I, brief *Bal*31 treatment, and ligation. The product, pUFR047 (Fig. 1) is 8.6 kb in size, has unique restriction sites for *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Sal*I, *Sma*I, and *Sst*I, and is stably maintained at low copy number in both *E. coli* and *Xanthomonas*.

Transposon mutagenesis.

Insertion of Tn*5-gusA*, a transcriptional operon fusion transposon (Sharma and Signer 1990) into cloned *X. c.* pv. *malvacearum* DNA fragments was achieved essentially as described previously (Swarup *et al.* 1991). In this method, plasmids to be mutagenized are transferred by conjugation through the *E. coli* strain C600-387, which has the transposon inserted into the chromosome. The site and orientation of insertion into the target plasmids were determined by restriction enzyme analyses. Assays for β-glucuronidase (Gus) activity were performed after *X. c.* pv. *malvacearum* transconjugants were grown in PYGM broth. Ten microliters of fresh overnight culture was added to 0.1 ml of 0.1 M phosphate buffer, pH 7.0, containing 40 μl/ml X-glucuronic acid (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid) (Molecular Probes, Inc., Eugene, OR). Gus activity was indicated by a blue color developing within 24 hr.

Bacterial conjugation.

Triparental conjugations were carried out as described elsewhere (DeFeyter and Gabriel 1991a) to transfer plasmids from one *E. coli* strain to another, or from *E. coli* to *Xanthomonas*. Transfer of plasmids into Xcm1003 used the modifier plasmid pUFR054 to enhance the transfer frequency (DeFeyter and Gabriel 1991b). Selection for *X. c.* pv. *malvacearum* transconjugants was achieved using PYGM plates containing Rif, Km and Gm, or Rif and Gm.

Cotton lines and plant inoculations.

The Acala-44 (Ac44) cotton lines used in this study were originally created by crossing cotton lines carrying the indicated cotton blight (*B*) resistance genes to a common parent, Ac44 (Hunter and Brinkerhoff 1961; M. Essenberg, unpublished data). For example, AcB1 carries the *B1* resistance gene. The *B* gene was then identified in the F_2 population using pathogenicity tests, and was then backcrossed repeatedly to the same Ac44 parent line. The resulting lines are congenic with Ac44, and each is known to have been backcrossed to Ac44 at least the following number of times: AcB1, 6×; AcB2, 2×; AcB4, 3×; Acb6, 2×; Acb7, 6×; AcBIn, 5×; and AcBIn3, 3×. Following the last backcross, all lines except AcB2 were self-pollinated, and the F_3 plants exhibiting homozygosity were retained for pathogenicity tests. All lines used except AcB2 were homozygous for the indicated blight gene. Cotton line 101-102B confers immunity to all North American strains of *X. c.* pv. *malvacearum* and has multiple blight resistance genes (including *B2, B3, b6, b7,* and *BSm*) (Brinkerhoff *et al.* 1984; DeFeyter and Gabriel 1991a).

To determine if the resistance genes carried in lines AcB1 and AcB4 were linked, AcB1 (*B1B1*) and AcB4 (*B4B4*) were crossed. The AcB1 line used in this study carried *B1B1* in a 99.22% Ac44 background (six backcrosses), and the AcB4 line used in this study carried *B4B4* in a 93.75% Ac44 background (three backcrosses). The F_2 progeny were inoculated with isogenic *X. c.* pv. *malvacearum* strains differing only by single, cloned *avr* genes. Inoculation and

Table 3. Bacterial strains, phage, and plasmids used in this study

Bacterial strain	Relevant characteristics	Reference or Source
E. coli		
C600-387	*hsdR17* ($r_k^-m_k^+$), *supE44, thr-1, thi-1, leuB6, lacY1, tonA21, hflA150*[chr::Tn*t-gusA* (Km^r,Tc^r)]	Swarup *et al.* 1991
HB101	*supE44, hsdS20*($r_k^-m_k^+$), *recA13, ara-14, proA2, lacY1, galK2, rpsL20, xyl-5, mtl-1*	Boyer and Roulland-Dussoix 1969
DH5∝	F^-, *endA1, hsdR17* ($r_k^-m_k^+$), *supE44, thi-1, recA1, gyrA, relA1, ϕ80dlacZ* ΔM15, Δ(*lacZYA-argF*)U169	Gibco-BRL, Gaithersburg, MD
X. campestris pv. *malvacearum*		
XcmC, XcmJ, XcmL and XcmN	Natural isolates from cotton from Upper Volta, Africa	Lazo and Gabriel 1987
XcmNSp	Spc^R derivative of XcmN	De Feyter *et al.* 1990
XcmH	Natural isolate from cotton from Oklahoma; *abrB4*$^+$, *avrb6*$^+$, *avrBn*$^+$, *avrBIn*$^+$, *avrB101*$^+$ and *avrB102*$^+$	De Feyter and Gabriel 1991a
XcmHSp	Spc^R derivative of XcmH	
XcmA, XcmD, XcmF, XcmI, XcmM, XcmQ, XcmX, and XcmY	Natural isolates from cotton from Oklahoma or Texas	Lazo and Gabriel 1987
Xcm1003	Spc^R Rif^R derivative of XcmN	DeFeyter and Gabriel 1991a
Xcm1102	Spc^R, *avrb6, avrBIn* derivative of XcmH	DeFeyter and Gabriel 1991a
Xcm1113	Spc^R, Δ*avrBIn* derivative of XcmH	DeFeyter and Gabriel 1991a
Xcm1201	Spc^R, *avrB4* derivatives of XcmH	This study
Xcm1216	Spc^R, *avrB4*, Δ*avrb6* derivative of XcmH	This study
KM46	Δ(*avrB101* - *avrBIn*) derivative of XcmH	McNally 1990
X10, X27, X52, X102, X103, X108, and 083-4344	Natural isolates from hibiscus; not virulent on cotton	Lazo and Gabriel 1987
Plasmid		
pXcmH	Natural plasmid from XcmH carrying six *avr* used in this study	DeFeyter and Gabriel 1991a
pUFR042	IncW, Km^R, Gm^R, Mob^+, *mob*(P), *lacZ*α$^+$, Par^+	DeFeyter and Gabriel 1991a
pUFR044	IncW, Gm^R, Mob^+, *mob*(P), *lacZ*α$^+$, Par^+	This study
PUFR046	IncW, Gm^R, Ap^R, Mob^+, *mob*(P), *lacZ*α$^+$, Par^+	This study
pUFR047	IncW, Gm^R, Ap^R, Mob^+, *mob*(P), *lacZ*α$^+$, Par^+	This study
pUFR054	IncP, Tc^R, Mob^+, *mob*(P), containing M.*Xma*I and M.*Xma*III	DeFeyter and Gabriel 1991b
pUFR101	Cosmid clone, AvrB4$^+$, Avrb6$^+$	DeFeyter and Gabriel 1991a
pUFR107	Cosmid clone, AvrB101$^+$, AvrBIn$^+$	DeFeyter and Gabriel 1991a
pUFR114	Cosmid clone, AvrB102$^+$, Avrb7$^+$	DeFeyter and Gabriel 1991a
pUFR131-6	*Bam*HI subclones of pUFR101, in pUFR042	This study, see Fig. 2
pUFR137-38	*Sst*I deletion derivatives of pUFR101	This study, see Fig. 2
pUFR139-41	*Sst*I subclones of pUFR101, in pUFR042	This study, see Fig. 2
pUFR142-52	*Bam*HI subclones of pUFR107, in pUFR047	This study, see Fig. 2
pUFR153-56	*Sst*I subclones of pUFR107, in pUFR042	This study, see Fig. 2
pUFR157	10.7-kb *Eco*RI-*Sal*I fragment of pUFR114, in pUFR042	This study, see Fig. 2
pUFR158	*Bam*HI deletion derivative of pUFR166	This study, see Fig. 2
pUFR159-62	*Bam*HI subclones of pUFR114, in pUFR042	This study, see Fig. 2
pUFR163	10.4-kb *Eco*RI-*Sal*I fragment of pUFR114, in pUFR042	This study, see Fig. 2
pUFR164	*Bam*HI subclone of pUFR114, in pUFR042	This study, see Fig. 2
pUFR165	*Bam*HI deletion derivative of pUFR114	This study, see Fig. 2
pUFR166-70	*Sst*I subclones of pUFR114, in pUFR042	This study, see Fig. 2
pUFR171	Internal *Bam*HI fragment of *avrB4*, in pGem11Zf(+)	This study
pUFR172	Internal *Bam*HI fragment of *avrb6*, in pGem11Zf(+)	This study
pUFR173	Internal *Bam*HI fragment of *avrB101*, in pGem11Zf(+)	This study
pUFR174	Internal *Bam*HI fragment of *avrBIn*, inpGem11Zf(+)	This study
pUFR175	Internal *Bam*HI fragment of *avrB102*, in pGem11Zf(+)	This study
pUFR176	Internal *Bam*HI fragment of *avrb7*, in pGem11Zf(+)	This study
pUFR177	Internal *Bam*HI fragment of *avrBs3*, in pGem11Zf(+)	This study
pUFR180	*Bam*HI subclone of pUFR101, in pUFR042	This study, see Fig. 2
pUFR185	*Hin*dIII-*Kpn*I reclone of insert from pUFR180, in pUC119	This study
pUC119	ColE1, Ap^R, *lacZ*α$^+$	Vieira and Messing 1987
pGEM11Zf(+)	ColE1, Ap^R, *lacZ*α	Promega Co., Madison, WI

assay methods are described elsewhere (DeFeyter and Gabriel 1991a).

DNA sequencing and analysis.

The DNA insert from plasmid pUFR180 (*avrB4* and *avrb6*) was recloned into pUC119 using a *Hin*dIII and *Kpn*I double digest, which cut the fragment on each side of the polylinker, forming pUFR185. Sets of overlapping, deletion subclones were generated in pUFR185 from each end of the insert using DNase I as described (Sambrook *et al.* 1989). The DNA sequence of the relevant fragments was determined in both directions by the dideoxy chain termination method and the Amersham (Arlington Heights, IL) system RPN1590 as described by the manufacturer with the universal forward primer, the #1201 Reverse Sequencing Primer (New England Biolabs, Beverly, MA), or six custom synthesized (ICBR DNA Synthesis Core, University of Florida, Gainesville) 19- to 21-bp oligonucleotide primers. Some sequencing was performed by the ICBR DNA Sequencing Core, University of Florida, Gainesville. Overlapping DNA fragments comprising *avrb6* and *avrB4* were assembled the GCG Version 7 Sequence Analysis software package by Genetics Computer, Inc., Madison, WI. Computation was performed at the ICBR Biological Computing Facility, University of Florida, Gainesville. The Swiss-Prot 23.0, August 1992; PIR 34.0 (complete), September 30 1992; CDS translation from Genbank(R) Release 73.1, October 1 1992, databases were searched using the predicted amino acid sequence of *avrb6* and the BLAST program (Altschul *et al.* 1990), run at the National Center for Biotechnology Information (NCBI) network service in Bethesda, MD.

Nucleotide sequence accession number.

The nucleotide sequence of the *avrb6* gene has been submitted to GenBank and assigned accession number L06634.

NOTE ADDED IN PROOF

The DNA sequence of *avrXa10*, another member of the *avr* gene family described here, was recently published (C. M. Hopkins, F. F. White, S. H. Choi, A. Guo, and J. E. Leach. 1992. Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. Mol. Plant-Microbe Interact. 5:451-459). Gene *avrXa10* is less than 97% homologous to other published members of the *avr* gene family, and only half of the inverted terminal repeat concensus sequence identified here as marking the boundaries of homology between members of the gene family is preent in *avrXa10* (from position 3473 to 3501) at the 3′ end. Homology of *avrXa10* to other members of the gene family therefore ends within the concensus terminal inverted repeat. The 5′ boundary of homology of *avrXa10* to other members of the *avr* gene family is not indicated in the publication.

ACKNOWLEDGMENTS

We are indebted to Margaret Essenberg, Oklahoma State University, Stillwater, for her great help in preserving and providing cotton lines with resistance genes originally developed by Lloyd Brinkerhoff, for performing two of the backcrosses in the AcB1 line used here, and for helpful comments on the manuscript. We are grateful to Kenneth McNally for generously providing strain KM46 prior to publication. We also thank John Watson for helpful comments on the manuscript. Florida Agricultural Experiment Station Journal Series R-02326. This research was supported by USDA-CRGO 86-CRCR-1-2234 and USDA-OICD 58-319R-9-017.

LITERATURE CITED

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

Alexander, H. M., Groth, J. V., and Roelfs, A. P. 1985. Virulence changes in *Uromyces appendiculatus* after five asexual generations on a partially resistant cultivar of *Phaseolus vulgaris*. Phytopathology 75:449-453.

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

Brinkerhoff, L. A. 1970. Variation in *Xanthomonas malvacearum* and its relation to control. Annu. Rev. Phytopathol. 8:85-110.

Brinkerhoff, L. A., Verhalen, L. M., Johnson, W. M., Essenberg, M., and Richardson, P. E. 1984. Development of immunity to bacterial blight of cotton and its implications for other diseases. Plant Dis. 68:168-173.

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

Boyer, H. W., and Roulland-Dussoix, D. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. 41:459-465.

Canteros, B., Minsavage, G., Bonas, U., Pring, D., and Stall, R. 1991. A gene from *Xanthomonas campestris* pv. *vesicatoria* that determines avirulence in tomato is related to *avrBs3*. Mol. Plant-Microbe Interact. 4:628-632.

DeFeyter, R., and Gabriel, D. W. 1991a. At least six avirulence genes are clustered on a 90-kilobase plasmid in *Xanthomonas campestris* pv. *malvacearum*. Mol. Plant-Microbe Interact. 4:423-432.

DeFeyter, R., and Gabriel, D. W. 1991b. Use of cloned DNA methylase genes to increase the transfer frequency of foreign genes into *Xanthomonas campestris* pv. *malvacearum*. J. Bacteriol. 173:6421-6427.

DeFeyter, R., Kado, C. I., and Gabriel, D. W. 1990. Small, stable shuttle vectors for use in *Xanthomonas*. Gene 88:65-72.

Ellingboe, A. H. 1976. Genetics of host-parasite interactions. Pages 761-778 in: Encyclopedia of Plant Pathology, New Series, Vol 4: Physiological Plant Pathology. R. Heitefuss and P. H. Williams, eds. Springer-Verlag, Heidelberg.

Flor, H. H. 1946. Genetics of pathogenicity in *Melampsora lini*. J. Agric. Res. 73:335-357.

Gabriel, D. W. 1989. The genetics of plant pathogen population structure and host-parasite specificity. Pages 343-379 in: Plant-Microbe Interactions: Molecular and Genetic Perspectives, Vol 3. T. Kosuge and E. W. Nester, eds. Macmillan Publishing Co., New York.

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

Gabriel, D. W., and Rolfe, B. G. 1990. Working models of specific recognition in plant-microbe interactions. Annu. Rev. Phytopathol. 28:365-391.

Heffron, F. 1983. Tn*3* and its relatives. Pages 223-260 in: Mobile Genetic Elements. J. A. Shapiro, ed., Academic Press, New York.

Herbers, K., Conrads-Strauch, J., and Bonas, U. 1992. Race-specificity of plant resistance to bacterial spot disease determined by repetitive motifs in a bacterial avirulence protein. Nature 356:172-174.

Hunter, R. E., and Brinkerhoff, L. A. 1961. Report of the Bacterial Blight Committee. Proc. Cotton Disease Council 21:7-8.

Kearney, B., and Staskawicz, B. J. 1990. Widespread distribution

and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. Nature 346:385-386.
Keen, N. T. 1992. The molecular biology of disease resistance. Plant Mol. Biol. 19:109-122.
Knoop, V., Staskawicz, B., and Bonas, U. 1991. Expression of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria* is not under the control of *hrp* genes and is independent of plant factors. J. Bacteriol. 173:7142-7150.
Kormanec, J., Schaaff-Gerstenschlager, I., Zimmerman, F. K., Perecko, D., and Kuntzel, H. 1991. Nuclear migration in *Saccharomyces cerevisiae* is controlled by the highly repetitive 313 kDa NUM1 protein. Mol. Gen. Genet. 230:277-287.
Lazo, G. R., and Gabriel, D. W. 1987. Conservation of plasmid DNA sequences and pathovar identification of strains of *Xanthomonas campestris*. Phytopathology 77:448-453.
Lux, S. E., John, K. M., and Bennett, V. 1990. Analysis of cDNA from human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. Nature 344:36-42.
McNally, K. L. 1990. Useful minimal medium for growth of *Xanthomonas campestris* pv. *malvacearum*, molecular biology analysis of avirulence in Xcm, and identification of multiple resistance genes in the segregating plant line Acala B5. Ph.D. thesis. Oklahoma State University. 136 pp.
Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sharma, S. B., and Signer, E. R. 1990. Temporal and spatial regulation of the symbiotic genes of *Rhizobium meliloti in planta* revealed by transposon Tn*5-gusA*. Genes Dev. 4:344-356.
Sidhu, G. S. 1987. Host-parasite genetics. Pages 393-432 in: Plant Breeding Reviews. J. Janick, eds. Van Nostrand Reinhold Co., New York.
Sikorski, R. S., Boguski, M. S., Goebl, M., and Hieter, P. 1990. A repeating amino acid motif in CDC23 defines a family of aproteins and a new relationship among genes required for mitosis and RNA synthesis. Cell 60:307-317.
Statler, G. D. 1985. Mutations to virulence and avirulence in *Melampsora lini*. Phytopathology 75:771-773.
Swarup, S., DeFeyter, R., Brlansky, R. H., and Gabriel, D. W. 1991. A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *X. campestris* to elicit cankerlike lesions on citrus. Phytopathology 81:802-809.
Swarup, S., Yang, Y., Kingsley, M. T., and Gabriel, D. W. 1992. A *Xanthomonas citri* pathogenicity gene, *pthA*, pleiotropically encodes gratuitous avirulence on non-hosts. Mol. Plant-Microbe Interact. 5:204-213.
Vieira, J., and Messing, J. 1987. Production of single-stranded plasmid DNA. Methods Enzymol. 153:3-11.
Willis, D. K., Rich, J. J., and Hrabak, E. M. 1991. *hrp* genes of phytopathogenic bacteria. Mol. Plant-Microbe Interact. 4:132-138.
Yang, Y., and Gabriel, D. W. 1992. Functional analysis of avirulence genes in *Xanthomonas campestris* pv. *malvacearum*. (Abstr.) Phytopathology 82:1167.