# Host-Specific Symptoms and Increased Release of *Xanthomonas citri* and *X. campestris* pv. *malvacearum* from Leaves Are Determined by the 102-bp Tandem Repeats of *pthA* and *avrb6*, Respectively

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**Six avirulence genes (*avrB4, avrb6, avrb7, avrBIn, avrB101,* and *avrB102*) found in *Xanthomonas campestris* pv. *malvacearum* strain XcmH1005 and a host-specific pathogenicity gene (*pthA*) found in *X. citri* belong to an *avr/pth* gene family and are characterized by tandemly arranged, 102-bp repeats in the central portions of the genes. Marker exchange mutagenesis and complementation experiments revealed that *avrb6* was required for XcmH1005 to cause severe water-soaking and subsequent necrosis in susceptible Acala-44 cotton lines. An average of 240 times more bacteria were released onto the cotton leaf surface from water-soaked spots caused by XcmH1005 than from those caused by an isogenic *avrb6*$^-$ strain, strongly indicating a role for *avrb6* in pathogen dispersal. However, *avrb6* did not affect *in planta* bacterial growth rate or yield. By constructing chimeric genes among *pthA, avrB4, avrb6, avrb7, avrBIn, avrB101,* and *avrB102,* the 102-bp tandem repeats of the genes were found to determine the gene-for-genes specificity of the avirulence reactions on cotton. In addition, the repeat regions of *avrb6* and *pthA* determined their specificity in enhancing water-soaking of cotton and causing cankers on citrus, respectively. When the native promoters of each gene were replaced by the *Escherichia coli lacZ* promoter, the hypersensitive response elicited in resistant host lines was stronger in all cases tested, while the pathogenic specificities of *avrb6* for cotton and *pthA* for citrus were unaltered. These results indicate that some members of this *avr/pth* gene family may help condition host range by increasing the release of *Xanthomonas* cells from the mesophyll to the leaf surface, leading to increased dispersal on specific hosts.**

*Additional keywords:* citrus canker, cotton blight, gene-for-gene specificity.

Microbial genes involved in plant-microbe interactions may be functionally classified into four broad categories: those involved in parasitism, pathogenicity, host range, and avirulence (Gabriel 1986). Genes involved in parasitism are absolutely required for growth *in planta* and are widely conserved at the family or genus level. Examples include most *hrp* (hypersensitive response and pathogenicity) genes of *Erwinia, Pseudomonas,* and *Xanthomonas* and the common *nod* (nodulation) genes of *Rhizobium* (Boucher *et al.* 1992; Denarie *et al.* 1992; Willis *et al.* 1991). Genes involved in pathogenicity are required for induction of symptoms. Examples include pectate lyase, polygalacturonase, and endoglucanase genes (Collmer and Keen 1986; Schell *et al.* 1988; Roberts *et al.* 1988), *dsp* (disease-specific) genes (Arlat and Boucher 1991), *wts* (water-soaking) genes (Coplin *et al.* 1992), phytohormone biosynthesis genes (Smidt and Kosuge 1978), and toxin biosynthesis genes (Mitchell 1984). Genes involved in conditioning host range are host-specific and required for growth on specific hosts. Examples include *hsv* (host-specific virulence), *pth* (pathogenicity), and *hsn* (host-specific nodulation) genes of *Pseudomonas, Xanthomonas,* and *Rhizobium* (Denarie *et al.* 1992; Kingsley *et al.* 1993; Ma *et al.* 1988; Salch and Shaw 1988; Swarup *et al.* 1991; Waney *et al.* 1991; Gabriel *et al.* 1993). Information on conservation of these genes within species, pathovars, and biovars is scarce, but they appear to determine biovar and pathovar status (Djordjevic *et al.* 1987; Gabriel *et al.* 1993). Genes in the fourth group are termed avirulence (*avr*) genes because they negatively affect virulence. The *avr* genes are superimposed on basic compatibility (Ellingboe 1976), are not highly conserved, and determine pathogenic races below the species, biovar, or pathovar level. These four broad categories are not mutually exclusive.

The interaction of microbes having *avr* genes and host plants having resistance (*R*) genes can result in plant defense responses, often observed visually as a hypersensitive reaction (HR) and characterized by the rapid necrosis of plant cells at the site of infection and the accumulation of phytoalexins. Most *avr* genes do not appear to confer selective advantage to the pathogen (Gabriel 1989; Keen and Staskawicz 1988). Pleiotropic functions have been identified for only three of the 30 *avr* genes cloned to date (Gabriel *et al.* 1993). Furthermore, the DNA sequences of the cloned *avr* genes have been remarkably uninformative in terms of function (Keen 1990). The presence of most *avr* genes in plant pathogens therefore remains enigmatic.

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Recently, an *avr* gene family has been discovered in many different xanthomonads; members include *avrBs3* (Bonas *et al.* 1989) and *avrBsP* (Canteros *et al.* 1991) of *X. campestris* pv. *vesicatoria; avrB4, avrb6, avrb7, avrBIn, avrB101,* and *avrB102* of *X. campestris* pv. *malvacearum* (De Feyter and Gabriel 1991a; De Feyter *et al.* 1993); and *avrxa5, avrXa7,* and *avrXa10* of *X. oryzae* (Hopkins *et al.* 1992). Interestingly, this gene family includes a gene, *pthA,* that is required for pathogenicity of *X. citri* on citrus. This gene is not known to function for avirulence in *X. citri* (Swarup *et al.* 1992) but is required for *X. citri* to induce cell divisions in the leaf mesophyll of citrus, leading to epidermal rupture and subsequent release of bacteria onto the leaf surface. The gene also confers on *X. campestris* strains from several different pathovars this ability to induce cell divisions in citrus (Swarup *et al.* 1991, 1992).

De Feyter and Gabriel (1991a) observed that *avrb6* and *avrb7* enhanced the water-soaking ability of several *X. campestris* pv. *malvacearum* strains on cotton, but the role of these genes in pathogenicity was not determined. The family therefore consists primarily of *avr* genes, but it includes at least one and perhaps more host-specific pathogenicity genes. The most conspicuous feature of this highly homologous gene family is the presence of nearly identical, tandemly arranged, 102-bp repeats in the central region of the genes, as shown in Figure 1. These repeats are known to determine the gene-for-gene specificity of *avrBs3* (Herbers *et al.* 1992). The purpose of this study was to characterize the water-soaking functions of *avrb6* and to investigate the role of the 102-bp repeats of *pthA, avrB4, avrb6, avrb7, avrBIn, avrB101,* and *avrB102* in pathogenicity and avirulence.

## RESULTS

### Pleiotropic pathogenicity functions of *avrb6*.

*X. campestris* pv. *malvacearum* strain XcmH carries *avrB4, avrb6, avrb7, avrBIn, avrB101,* and *avrB102* on a single plasmid, pXcmH, and elicits an HR in cotton lines carrying any one of many different resistance (*R*) genes (De Feyter *et al.* 1993). Strains XcmH and XcmH1005, a spontaneous rifamycin-resistant derivative of XcmH, are virulent on susceptible cotton line Acala-44 (Ac44), and both elicit severe water-soaking and necrosis associated with growth *in planta* (XcmH1005 on Ac44 is shown in Figs. 2 and 3). Mutations of *avrb6, avrb7,* and *avrBIn* were individually generated in XcmH1005 by marker exchange mutagenesis, and each mutant was confirmed to carry a single Tn*5-gusA* insertion in the appropriate DNA fragment by Southern blot hybridization (see Fig. 4; additional data not shown). As predicted by gene-for-gene theory, marker exchange mutants XcmH1407 (*avrb6*::Tn*5-gusA*), XcmH1427 (*avrb7*::Tn*5-gusA*), and XcmH1431 (*avrBIn*::Tn*5-gusA*) gained virulence on cotton lines with resistance genes *b6, b7,* and *BIn,* respectively (Table 1). Plasmids pUFR127 (*avrb6*$^+$), pUFR163 (*avrb7*$^+$), and pUFR156 (*avrBIn*$^+$) were able to fully complement the specific avirulence defects of XcmH1407, XcmH1427, and XcmH1431, respectively.

Neither XcmH1427 nor XcmH1431 showed any change in water-soaking ability, compared with XcmH1005. However,


**Fig. 1.** General structure of the *Xanthomonas avr/pth* gene family. The arrow indicates the open reading frame of a typical member, starting from the 5′ *Bam*HI site. The promoter is indicated by the dark box. The hatched region in the middle of the gene represents the 102-bp tandem repeats; 14 tandem repeats are shown, but the actual number varied from 14 to 23 repeat units, depending on the gene. Restriction enzyme cleavage sites relevant to this work and found in most members of the gene family are *Bam*HI (B), *Pst*I (P), *Stu*I (St), *Hin*cII (H), and *Sst*I (S).

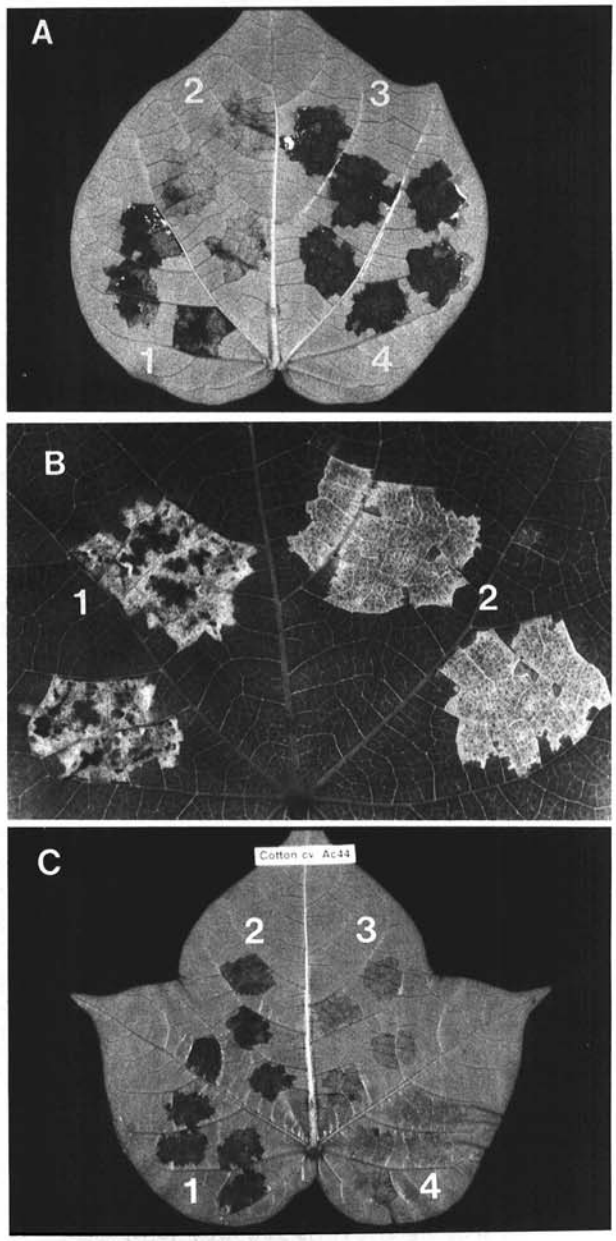


**Fig. 2.** Water-soaked lesions caused by *Xanthomonas campestris* pv. *malvacearum* strains on the susceptible cotton line Acala-44. **A,** Leaf inoculated 5 days previously with (1) XcmH1005, (2) XcmH1407, (3) XcmH1407/pUFR127, and (4) XcmH1431. **B,** Leaf inoculated 7 days previously with (1) XcmH1005 and (2) XcmH1407. **C,** Leaf inoculated 5 days previously with (1) XcmH1005, (2) Xcm1003/pUFR127, (3) Xcm1003/pUFR156, and (4) Xcm1003.

marker exchange mutant XcmH1407 not only lost avirulence on a cotton line with the *b6* resistance gene but also elicited significantly less water-soaking and necrosis in susceptible cotton line Ac44 (Table 1 and Fig. 2A and B). Plasmid pUFR127 was able to fully complement the pathogenicity defect(s) of XcmH1407 on susceptible cotton plants, in addition to complementing the specific avirulence defect. Despite its reduced ability to elicit pathogenic symptoms in susceptible cotton lines, mutant XcmH1407 exhibited the same growth rate and yield as that of the wild-type XcmH1005 and XcmH1431 on Ac44 (Fig. 3A).

*X. campestris* pv. *malvacearum* strain Xcm1003, which carries no known *avr* genes (De Feyter and Gabriel 1991a), caused less water-soaking of Ac44 than XcmH1005 and exhibited a lower growth rate on Ac44 than XcmH1005. Introduction of pUFR127 (*avrb6*$^+$) into Xcm1003 conferred increased water-soaking ability (Fig. 2C), but growth rate and yield *in planta* of the transconjugant containing pUFR127 were not increased in comparison with the growth and yield of Xcm1003 or the transconjugant Xcm1003/pUFR156 (*avrBIn*$^+$) (Fig. 3B). Therefore pUFR127 affected pathogenic symptoms in both XcmH1005 and Xcm1003, but not bacterial growth rate or yield *in planta* of either strain.

Strains containing *avrb6* (e.g., XcmH1005 and Xcm1003/pUFR127) elicited more severe water-soaking and necrosis and were associated with much more slime oozing from water-soaked areas than strains lacking *avrb6* (e.g., XcmH1407 and Xcm1003) (Fig. 2). The peak number of total colony-forming units (cfu) per square centimeter of water-soaked leaf was basically the same for leaves inoculated with strains XcmH1005 and XcmH1407 ($1.41 \pm 0.09 \times 10^9$ cfu/cm$^2$ vs. $1.48 \pm 0.17 \times 10^9$ cfu/cm$^2$). However, 14.1% of the XcmH1005 bacteria present in the lesion ($2.32 \pm 0.56 \times 10^8$ cfu/cm$^2$) were released onto the surface of the leaf, whereas only 0.06% of the XcmH1407 bacteria ($9.64 \pm 4.42 \times 10^5$ cfu/cm$^2$) were released onto the surface. Therefore, more than 240 times more bacteria were present on the external surface of water-soaked lesions caused by XcmH1005 than on those caused by XcmH1407. In experiments in which the leaves were moistened periodically after inoculation with a hand-held mist sprayer, *X. campestris* pv. *malvacearum* strains carrying *avrb6* always exhibited many secondary infections around the original inoculation site. In contrast, strains lacking *avrb6* rarely exhibited secondary infections.

**The pathogenicity functions encoded by *pthA* and *avrb6* are host-specific and determined by 102-bp repeats.**

Single *Stu*I and *Hin*cII sites are found in *avrb6* at positions 1281 and 2860, respectively, which closely flank the 102-bp tandemly repeated region of the gene, as shown in Figure 1 (De Feyter *et al.* 1993). Unique *Stu*I and *Hin*cII sites are also found at the same relative positions in *pthA*, and the DNA sequences of *avrb6* and *pthA* are identical in the flanking regions from the 102-bp tandem repeats to these restriction sites (Swarup *et al.* 1992; Y. Yang and D. W. Gabriel, unpublished data). Chimeric genes were constructed by swapping the *Stu*I/*Hin*cII fragments containing the internal 102-bp repeated regions between *pthA* and *avrb6*. At least three individual clones of both chimeric genes were introduced into the mutant *X. citri* strain B21.2 (*pthA*::Tn*5-gusA*) and wild-type strains of the following pathogens: *X. phaseoli* strain G27 (host range on bean), *X. campestris* pv. *citrumelo* strain 3048 (host range on bean and citrus), *X. campestris* pv. *alfalfae* strain KX-1 (host range on alfalfa, bean, and citrus), and Xcm1003. The strains and transconjugants were used to inoculate citrus, bean, and cotton leaves. As shown in Figure 5, pUFY020, carrying a chimeric gene containing the 5′ and 3′ ends of *avrb6* and the internal repeats of *pthA*, complemented

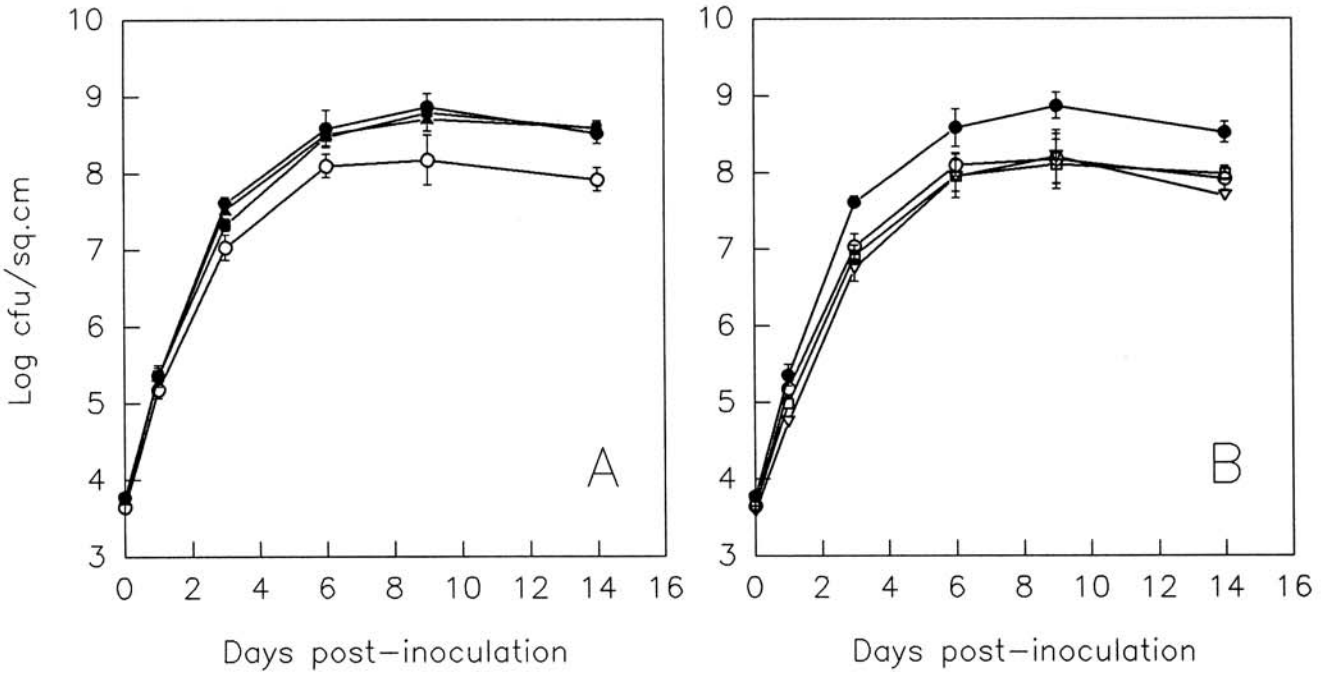


**Fig. 3.** Growth kinetics of *Xanthomonas campestris* pv. *malvacearum* strains on the susceptible cotton line Acala-44: **A,** XcmH1005 (solid circle), XcmH1407 (solid triangle), XcmH1431 (solid square), and Xcm1003 (open circle); **B,** XcmH1005 (solid circle), Xcm1003/pUFR127 (open triangle), Xcm1003/pUFR156 (open square), and Xcm1003 (open circle). The data are means and standard errors from three separate experiments

the mutant B21.2 to full virulence on citrus. The citrus canker symptoms caused by B21.2/pUFY020 were indistinguishable from the symptoms caused by B21.2/pZit45 (*pthA*$^+$). When pUFY020 was present in 3048 or KX-1, both of which cause water-soaked leaf spots on citrus, canker symptoms were formed by the transconjugants that were indistinguishable from those caused by the same strains carrying pZit45. Also, like pZit45 (Swarup *et al.* 1992), pUFY020 in 3048, KX-1, and G27 conferred avirulence on bean, and in Xcm1003 it conferred avirulence on cotton.

A chimeric gene containing the 5′ and 3′ ends of *pthA* and the internal repeats of *avrb6* on pUFY019 enhanced the ability of Xcm1003 and XcmH1407 to water-soak cotton and cause necrosis. The water-soaking symptoms caused by Xcm1003/pUFY019 were not distinguished from those caused by Xcm1003/pUFR127. This chimeric gene on pUFY019 behaved like *avrb6* on pUFR127 and conferred on B21.2, 3048, or G27 no ability to induce detectable symptoms in citrus or bean.

### Cultivar-specific avirulence is determined by 102-bp repeats.

Each of the seven *avr/pth* genes (*avrB4, avrb6, avrb7, avrBIn, avrB101, avrB102,* and *pthA*) exhibits unique avirulence specificity in Xcm1003 on cotton resistance lines differing by single *R* genes (De Feyter *et al.* 1993; Swarup *et al.* 1992). Each of these seven genes contains two *Bam*HI sites, one near the 5′ end and one near the 3′ end of each gene, and single *Stu*I and *Hin*cII sites flanking the 102-bp direct repeat region (De Feyter *et al.* 1993; Swarup *et al.* 1992). To localize the region that determines the specificity of the reactions, a series of chimeric genes was constructed by swapping *Bam*HI and *Stu*I/*Hin*cII internal fragments between these seven members of the gene family. One to three individual clones of each chimeric gene were introduced into Xcm1003, which was used to inoculate cotton cultivar Ac44 and its congenic resistance lines, each differing by a single *R* gene (*B1, B2, B4, b6, b7, BIn,* or *BIn3*). The avirulence specificities of the seven *avr/pth* genes were first localized within the internal *Bam*HI fragments (Table 2) and then were further localized within the internal *Stu*I/*Hin*cII fragments (Table 3). In all cases, the avirulence specificity of a given gene was determined inside the *Stu*I/*Hin*cII (tandem repeat) region.

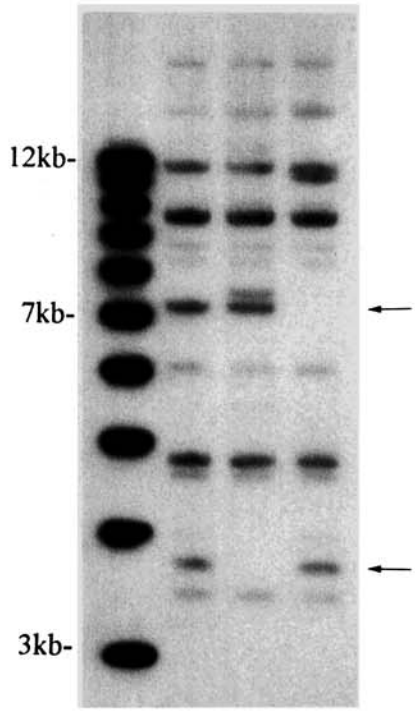


**Fig. 4.** Southern blot hybridization of total DNA from *Xanthomonas campestris* pv. *malvacearum* strain XcmH1005 and marker-exchanged mutants of XcmH1005 after *Eco*RI/*Sst*I digestion. The blot was probed with the internal 2.9-kb *Bam*HI fragment from *avrb6*. The expected positions of fragments carrying each plasmid-borne member of the *avr/pth* gene family were determined from the restriction map of pXcmH (De Feyter and Gabriel 1991a). The upper arrow indicates the expected position of the DNA fragment carrying *avrBIn*. The lower arrow indicates the position of *avrb6*. Lane 1, 1-kb DNA ladder; lane 2, XcmH1005; lane 3, XcmH1407; lane 4, XcmH1431.

### Effects of promoter strength on avirulence and pathogenicity.

To study the effect of promoter strength on avirulence, a *lacZ* promoter was fused with the coding regions of *avrB4, avrb6, avrBIn, avrB101,* and *avrB102.* The resulting *lacZ*::*avr* fusions were introduced into Xcm1003, and transconjugants were used to inoculate cotton cultivar Ac44 and congenic lines containing resistance genes *B1, B2, B4, b6, b7, BIn,* or *BIn3.* When driven by their own promoters, all pXcmH *avr* genes conferred on Xcm1003 weak or no detectable avirulence on cotton lines AcB1 and AcB2 (De Feyter *et al.* 1993). Upon fusion with the *lacZ* promoter, however, all *avr* genes tested except *avrBIn* conferred on Xcm1003 strong avirulence on cotton lines with the *B1* or *B2* resistance genes,

**Table 1.** Phenotypes of marker exchange mutants of *Xanthomonas campestris* pv. *malvacearum* strain XcmH1005 on cotton cultivar Acala-44 (Ac44) and congenic lines[a]

| Strain | Ac44 | Acb6 | Acb7 | AcBIn |
|---|---|---|---|---|
| XcmH1005 | ++ | − | − | − |
| XcmH1407 (*avrb6*::Tn*5-gusA*) | + | + | − | − |
| XcmH1427 (*avrb7*::Tn*5-gusA*) | ++ | − | ++ | − |
| XcmH1431 (*avrBIn*::Tn*5-gusA*) | ++ | − | − | ++ |

[a]Acb6, Acb7, and AcBIn are congenic lines of Ac44 containing resistance genes *b6, b7,* and *BIn,* respectively. ++ = Strong water-soaking symptoms; + = weak water-soaking symptoms; − = hypersensitive response.

**Table 2.** Avirulence specificity of *Bam*HI fragment-swapped chimeric genes in *Xanthomonas campestris* pv. *malvacearum* strain Xcm1003 on cotton

| Plasmid | Chimeric gene: 5′ and 3′ ends | Chimeric gene: *Bam*HI fragment | Avirulence specificity[a] |
|---|---|---|---|
| pUFR190 | *avrb7* | *avrB4* | *avrB4* |
| pUFR191 | *avrb7* | *avrb6* | *abrb6* |
| pUFR192 | *avrb7* | *avrBIn* | *avrBIn* |
| pUFR193 | *avrb7* | *avrB101* | *avrB101* |
| pUFR194 | *avrb7* | *avrB102* | *avrB102* |
| pUFR196 | *avrBIn* | *avrB4* | *avrB4* |
| pUFR197 | *avrBIn* | *avrb6* | *avrb6* |
| pUFR198 | *avrBIn* | *avrb7* | *avrb7* |
| pUFR199 | *avrBIn* | *avrB101* | *avrB101* |
| pUFR200 | *avrBIn* | *avrB102* | *avrB102* |

[a]Chimeric genes were introduced into Xcm1003 and tested on cotton cultivar Acala-44 and its congenic resistance lines AcB1, AcB2, AcB4, Acb6, Acb7, AcBIn, and AcBIn3 for avirulence specificity.

in some cases converting an apparently compatible interaction into an obviously incompatible one. Figure 6 shows the reactions of six cloned *avr* genes with their native promoters in Xcm1003 (odd numbers 3–13 in Fig. 6) in comparison with the same *avr* genes transcribed from the *lacZ* promoter in Xcm1003 (even numbers 4–12 in Fig. 6).

No qualitative changes were evident in the reactions of the *lacZ*::*avr* fusions in Xcm1003 used to inoculate AcB4, Acb6, Acb7, AcBIn, or AcBIn3. However, in all incompatible interactions involving these lines, the HR elicited by the *lacZ* promoter fusions appeared quantitatively faster and stronger than the HR elicited by the same genes transcribed from their native promoters.

In contrast to its obvious strengthening effect on avirulence, the *lacZ* promoter did not affect the strength of the pathogenicity functions of *avrb6* or *pthA*. In terms of specificity, pUFR135 (*lacZ*::*avrb6*$^+$) conferred the same cotton-specific pathogenicity on Xcm1003 as pUFR127 (*avrb6*$^+$). Similarly, the *pthA* chimeric gene with the *lacZ* promoter on pUFY020 exhibited the same citrus-specific pathogenicity function as *pthA* on pZit45. Neither pUFR135 nor pUFR127 could complement *X. citri* B21.2 to virulence on citrus or enhance the water-soaking of citrus by *X. campestris* pv. *citrumelo* 3048. These two clones conferred no obvious phenotypic change on either strain on citrus. Neither pUFY020 nor pZit45 conferred on Xcm1003 the ability to elicit cankers on cotton. Instead, both clones conferred the ability to elicit an HR in cotton, with Xcm1003/pUFY020 eliciting the stronger HR.

**Table 3.** Avirulence specificity of *StuI*/*HincII* fragment-swapped chimeric genes in *Xanthomonas campestris* pv. *malvacearum* strain Xcm1003 on cotton

| Plasmid | Chimeric gene | | Avirulence specificity[a] |
|---|---|---|---|
| | 5′ and 3′ regions | *StuI*/*HincII* fragment | |
| pUFR205 | *avrBIn* | *avrB4* | *avrB4** |
| pUFR206 | *avrBIn* | *avrb6* | *avrb6** |
| pUFR207 | *avrBIn* | *avrb7* | *avrb7** |
| pUFR208 | *avrBIn* | *avrB101* | *avrB101* |
| pUFR209 | *avrBIn* | *avrB102* | *avrB102* |
| pUFR211 | *avrb7* | *avrB4* | *avrB4* |
| pUFR212 | *avrb7* | *avrb6* | *avrb6* |
| pUFR213 | *avrb7* | *avrBIn* | *avrBIn** |
| pUFR214 | *avrb7* | *avrB101* | *avrB101* |
| pUFR215 | *avrb7* | *avrB102* | ...[b] |
| pUFY019 | *pthA* | *avrb6* | *avrb6* |
| pUFY020 | *lacZ*::*avrb6* | *pthA* | *pthA*+ |

[a]Chimeric genes were introduced into Xcm1003 and tested on cotton cultivar Acala-44 and its congenic resistance lines AcB1, AcB2, AcB4, Acb6, Acb7, AcBIn, and AcBIn3 for avirulence specificity. Asterisk indicates weak avirulence; + = strong avirulence.
[b]No avirulence detected.

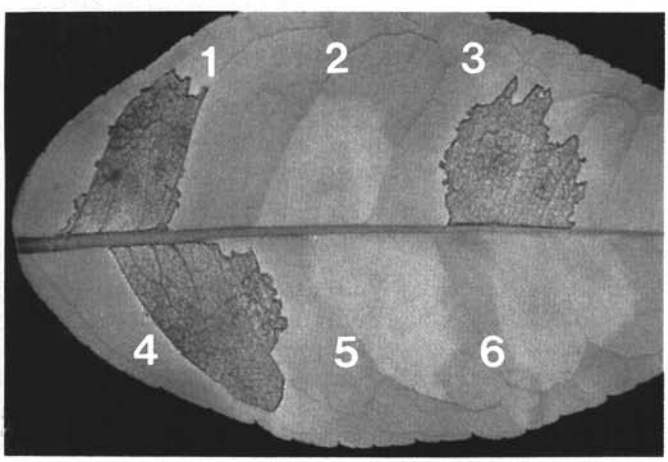


**Fig. 5.** Phenotypes of *Xanthomonas citri* wild-type and mutant strains on a grapefruit leaf (*Citrus paradisi* 'Duncan'): (1) wild-type strain 3213, (2) B21.2, a marker exchange mutant of strain 3213, (3) B21.2/pZit45, (4) B21.2/pUFY020, (5) B21.2/pUFR135, and (6) B21.2/pUFY019.

## DISCUSSION

Plant pathologists have long been puzzled by the presence of avirulence genes in pathogens. These genes act as negative factors to limit virulence and in most cases do not appear to provide selective advantage to the pathogens (Ellingboe 1976; Gabriel 1989; Keen and Staskawicz 1988). Rare exceptions have been reported. For example, *avrBs2* from *X. campestris* pv. *vesicatoria* is required for optimal growth *in planta* (Kearney and Staskawicz 1990). Both *avrb6* and *avrb7* from *X. campestris* pv. *malvacearum* strain XcmH are known to enhance the water-soaking ability of *X. campestris* pv. *malvacearum* strain Xcm1003 on cotton (De Feyter and Gabriel 1991a). In this study we demonstrated that the ability of strain XcmH1005 to cause strong water-soaking and necrosis in cotton requires the presence of *avrb6* but not *avrb7*. Although *avrb6* increased symptom elicitation by both Xcm1003 and XcmH1005, neither their growth rates nor their maximum bacterial counts per square centimeter of leaf were affected by the presence or absence of *avrb6*. Therefore *avrb6* functions as a pathogenicity gene and increases symptoms of cotton blight, but without eliciting an HR in cotton lines lacking *b6*.

The strong water-soaking ability conferred by *avrb6* was correlated with significantly (240 times) higher levels of bacterial cells released from inside the plant leaf to the surface. Since bacterial blight of cotton is usually spread by rain

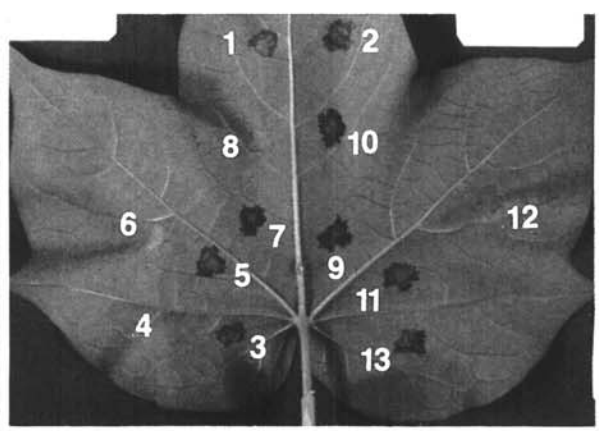


**Fig. 6.** Avirulence genes driven by their natural promoters or by the *lacZ* promoter in *Xanthomonas campestris* pv. *malvacearum* strain Xcm1003, inoculated on a leaf of cotton line AcB1: (1) *X. campestris* pv. *malvacearum* strain XcmH, (2) Xcm1003, (3) Xcm1003/pUFR115, (4) Xcm1003/pUFR131, (5) Xcm1003/pUFR127, (6) Xcm1003/pUFR135, (7) Xcm1003/pUFR142, (8) Xcm1003/pUFR144, (9) Xcm1003/pUFR156, (10) Xcm1003/pUFR150, (11) Xcm1003/pUFR157, (12) Xcm1003/pUFR160, and (13) Xcm1003/pUFR163. Cotton line AcB2, inoculated with the same strains (not shown), reacted similarly.

splash, the presence of large numbers of bacteria on the leaf surface undoubtedly contributes to the dissemination of the population. Strains carrying *avrb6* would thereby have a selective advantage on cotton plants lacking the *b6* gene. Similarly, *pthA* appears to aid in the dissemination of *X. citri* by rupturing leaf epidermis and releasing bacteria, although it does so by inducing tissue hyperplasia (Swarup *et al.* 1991). Therefore, *pthA* and *avrb6* not only contribute to the amount of damage to hosts of these xanthomonads but may also contribute to the ecological fitness of their respective bacterial populations as pathogenicity genes.

Both *pthA* and *avrb6* may help determine host range in a positive manner, and not as *avr* genes. When *pthA* was transferred to *X. campestris* pv. *malvacearum* Xcm1003 and *X. phaseoli* G27, it conferred avirulence on their respective hosts and did not induce tissue hyperplasia (Swarup *et al.* 1992). In the present study, when *avrb6* was transferred to *X. citri* B21.2, *X. campestris* pv. *citrumelo* 3048, and *X. phaseoli* G27, it conferred no detectable effect when these strains were used to inoculate their respective hosts. Therefore, the pathogenicity functions of *avrb6* and *pthA* are host-specific. If release of the pathogen to the leaf surface is host-specific, and if it contributes to the ecological fitness of the pathogen, as we propose, then *avrb6* and *pthA* function to determine host range. Furthermore, the avirulence conferred by *pthA* appears to be gratuitous in terms of restricting host range (Swarup *et al.* 1992), and in the present study *avrb6* failed to confer avirulence on three other pathogens. Therefore if *avrb6* and *pthA* help determine host range, it is not because of their function as *avr* genes. In a formal genetic sense, these pleiotropic *avr/pth* genes resemble some *Rhizobium* host-specific nodulation (*hsn*) genes, which are required for host range on some hosts, but which can also confer avirulence when transferred to other *Rhizobium* strains with a different host range (Debelle *et al.* 1988; Faucher *et al.* 1989; Lewis-Henderson and Djordjevic 1991).

By swapping the tandemly repeated regions, the avirulence specificities of *avrB4, avrb6, avrb7, avrBIn, avrB101, avrB102,* and *pthA* were shown to be determined by the 102-bp tandem repeats. These results are consistent with and extend the findings of Herbers *et al.* (1992), who showed that the avirulence specificity of *avrBs3* is determined by the 102-bp repeats of that gene. In addition, the swapping experiments clearly demonstrated that the pathogenicity functions of *pthA* and *avrb6* are distinct (cankers vs. water-soaking) and host-specific, and in both cases the pathogenic specificity was determined by their 102-bp tandemly repeated regions. Furthermore, the use of chimeric genes also ruled out the possibility that the host-specific pathogenicity on cotton and citrus are the result of additional, unidentified pathogenicity genes encoded on the plasmids used (pUFR127 and pZit45).

Extensive deletion analyses of both the 5′ and the 3′ ends of the pXcmH *avr* genes has shown that all sections of these genes are required for avirulence activity (De Feyter *et al.* 1993). In the present study, all sections of *pthA* and *avrb6* were required in order to confer pathogenicity functions. However, the 5′ and 3′ ends of the genes outside of the repeats appeared to be isofunctional among members of the gene family.

Although the specificity of all of these genes was determined by the internal repeats, the strength of the promoters had an effect on avirulence in some cases. The *avr* genes driven by the *lacZ* promoter in Xcm1003 elicited a stronger HR than did the *avr* genes driven by their native promoters on all resistant cotton lines. This was most obvious in lines with resistance gene *B1* or *B2,* which responded with a strong HR to Xcm1003 carrying any of five *lacZ::avr* fusions but reacted only weakly or not at all when the *avr* genes were expressed from their native promoters. Superficially, the *lacZ* promoter altered the avirulence specificities of these *avr* genes. However, the fusion of the *lacZ* promoter to the coding regions of these *avr* genes always resulted in gains of avirulence activity, and never losses of avirulence. The apparent loss of specificity due to the *lacZ* promoter may therefore be due to enhanced *avr* gene expression and the nature of the genes-for-genes interactions between cotton and *X. campestris* pv. *malvacearum* (De Feyter *et al.* 1993). Since these *avr* genes are highly homologous and recognized by multiple *R* genes, the *lacZ* promoter appears to have converted very weak avirulence (already present due to *avrB4, avrb6, avrB101,* or *avrB102*) on cotton lines with resistance gene *B1* or *B2* into strong avirulence. Therefore, the strength of the promoter more likely affected the intensity of the avirulence reactions rather than specificity *per se.*

Besides the members of this *Xanthomonas avr/pth* gene family, internal tandem repeats are found in some pathogenicity genes of animal pathogens. Examples include the outer membrane protein A (*ompA*) gene of some *Rickettsia* species (Anderson *et al.* 1990; Gilmore 1993), the internalin (*inl*) gene of *Listeria monocytogenes* (Gaillard *et al.* 1991), the toxin A gene of *Clostridium difficile* (Dove *et al.* 1990), and the M protein genes (*emm*) of *Streptococcus* (Hollingshead *et al.* 1987). Tandem repeats have also been found in many genes from protozoan and metazoan parasites, such as *Plasmodium* (McConkey *et al.* 1990), *Trypanosoma* (Hoft *et al.* 1989), *Leishmania* (Wallis and McMaster 1987), and *Meloidogyne* (Okimoto *et al.* 1991). Genes from *T. cruzi* (the protozoan agent of American trypanosomiasis) and mitochondria of *M. javanica* (plant root-knot nematode) contain 102-bp tandem repeats. Most of these genes encode surface proteins, and the distinctive arrangement of the tandem repeats in these genes are thought to encode a protective, strain-specific conformational epitope for evasion of host immunity (Gilmore 1993; Hoft *et al.* 1989; McConkey *et al.* 1990). By contrast, AvrBs3 is mainly located in cytosol (Knoop *et al.* 1991; Brown *et al.* 1993), and it is not clear how it may interact with the plant cell.

Southern hybridization has shown that potential members of the *avr/pth* gene family exist, often in multiple copies, in nine of 12 *Xanthomonas* species or pathovars examined (Bonas *et al.* 1989; Swarup *et al.* 1992; De Feyter *et al.* 1993). In all *X. citri* and *X. campestris* pv. *malvacearum* cotton strains tested to date, multiple DNA fragments hybridizing to *pthA* and *avrb6* have been found. Based on these data and knowledge of the functions of *avrb6* and *pthA,* we assume that all strains of *X. campestris* pv. *malvacearum* capable of strongly water-soaking cotton carry an *avrb6* gene or homologue that functions for pathogenicity. Similarly, we assume that all strains of *X. citri* capable of causing cankers on citrus carry a *pthA* gene. A number of pathogenic strains and pathovars in the genus *Xanthomonas* do not carry members of the gene family, and therefore these genes are not required for

*Xanthomonas* virulence generally. In pathovars in which members of the *avr/pth* gene family are found in some but not all strains tested (such as *X. campestris* pv. *vesicatoria*), there may be no pleiotropic pathogenicity function; for example, there is no evidence of a pathogenicity function of *avrBs3* and *avrBsP* (Bonas *et al.* 1989; Canteros *et al.* 1991).

The high degree of homology among members of this *avr/pth* family and their presence in phylogenetically distinct xanthomonads indicates that these genes might have transferred horizontally. The presence of Tn*3*-like terminal inverted repeat sequences suggests that the genes may transpose (De Feyter *et al.* 1993). Many of these genes are present on plasmids, although demonstration of horizontal transfer has not yet been reported. The pleiotropic pathogenicity functions of *pthA* and *avrb6* and their potential fitness value may explain why these genes are maintained in plant pathogens. The fitness value might be satisfied by one member of the family, leaving other copies free to mutate. Of the six pXcmH genes examined, only *avrb6* is required for the strong water-soaking function. It is possible that the other genes confer the ability to cause water-soaking in some other host, perhaps a different cotton species. However, the indication of a high frequency of intergenic recombination among members of this gene family in XcmH (De Feyter *et al.* 1993) leads us to favor the idea that most members of the gene family are mutant copies of a few genes with pathogenicity function but are nonfunctional in pathogenicity.

The mechanism or mechanisms by which leaf-spotting pathogens elicit water-soaking and necrosis are unknown, but the process appears to involve both damage to leaf cell membranes (without eliciting an HR) and the production of extracellular polysaccharide (EPS). The EPS does not appear to be involved in suppressing a potential HR, since production levels of EPS by *X. campestris* pv. *malvacearum* are similar in both susceptible and resistant cotton lines (Pierce *et al.* 1993). Instead, mutational analyses of *Xanthomonas* EPS biosynthesis genes and inoculations with purified EPS have shown that EPS contributes to water-soaking by trapping water and nutrients in intercellular spaces after they are released (reviewed by Leigh and Coplin [1992]). Coplin *et al.* (1992) proposed that water-soaking caused by and pathogenicity of *Erwinia stewartii* involve EPS plus a cell leakage

**Table 4.** Bacterial strains and plasmids used in this study

| | Relevant characteristics | Reference or source |
|---|---|---|
| *Escherichia coli* | | |
| DH5α | F⁻, *endA1*, *hsdR17* ($r_k^-m_k^+$), *supE44*, *thi-1*, *recA1*, *gyrA*, *relA1*, *ϕ80dlacZ* ΔM15, Δ(*lacZYA-argF*)U169 | Gibco-BRL, Gaithersburg, MD |
| HB101 | *supE44*, *hsdS20*($r_k^-m_k^+$), *recA13*, *ara-14*, *proA2*, *lacY1*, *galK2*, *rpsL20*, *xyl-5*, *mtl-1* | Boyer and Roulland-Dussoix 1969 |
| ED8767 | *supE44*, *supF58*, *hsdS3*($r_k^-m_k^+$), *recA56*, *galK2*, *galT22*, *metB1* | Murray *et al.* 1977 |
| *Xanthomonas citri* | | |
| 3213 | ATCC 49118, citrus canker type strain | Gabriel *et al.* 1989 |
| B21.2 | *pthA*::Tn*5*-*gusA*, marker exchange mutant of 3213 | Swarup *et al.* 1991 |
| *X. phaseoli* | | |
| G27 | ATCC 49119, bean blight type strain | Gabriel *et al.* 1989 |
| G27Sp | Spc$^r$ derivative of G27 | Swarup *et al.* 1991 |
| *X. campestris* pv. *citrumelo* | | |
| 3048 | ATCC 49120, citrus leaf spot pathotype strain | Gabriel *et al.* 1989 |
| 3048Sp | Spc$^r$ derivative of 3048 | Swarup *et al.* 1991 |
| *X. campestris* pv. *alfalfae* | | |
| KX-1Sp | Spc$^r$ derivative of KX-1, isolated from alfalfa, causing citrus leaf spot | Swarup *et al.* 1991 |
| *X. campestris* pv. *malvacearum* | | |
| XcmH | Natural isolate from cotton from Oklahoma, carrying six *avr* genes used in this study on pXcmH, plus additional *avr* genes | De Feyter and Gabriel 1991a |
| XcmH1005 | Spontaneous Rif$^r$ derivative of XcmH | This study |
| XcmH1407 | *avrb6*::Tn*5*-*gusA*, marker exchange mutant of XcmH1005 | This study |
| XcmH1427 | *avrb7*::Tn*5*-*gusA*, marker exchange mutant of XcmH1005 | This study |
| XcmH1431 | *avrBIn*::Tn*5*-*gusA*, marker exchange mutant of XcmH1005 | This study |
| XcmN | Natural isolate from cotton from Upper Volta (Burkina Faso) | Gabriel *et al.* 1986 |
| Xcm1003 | Spc$^r$, Rif$^r$ derivative of XcmN | De Feyter and Gabriel 1991a |
| Plasmids | | |
| pRK2013 | ColE1, Km$^r$, Tra$^+$, helper plasmid | Figurski and Helinski 1979 |
| pRK2073 | pRK2013 derivative, npt::Tn*7*, Km$^s$, Sp$^r$, Tra$^+$, helper plasmid | Leong *et al.* 1982 |
| pUFR042 | IncW, Km$^r$, Gm$^r$, Mob$^+$, *lacZα*$^+$, Par$^+$ | De Feyter and Gabriel 1991a |
| pUFR047 | IncW, Gm$^r$, Ap$^r$, Mob$^+$, *lacZα*$^+$, Par$^+$ | De Feyter *et al.* 1993 |
| pUFR049 | RSF1010 replicon, Cm$^r$, Sm$^r$, IncW$^+$, Mob$^+$ displacement vector | Swarup *et al.* 1991 |
| pUFR054 | IncP, Tc$^r$, Mob$^+$, containing methylases *Xma*I and *Xma*III | De Feyter and Gabriel 1991b |
| pUFR115 | 7.5-kb fragment containing *avrB4* in pUFR042 | De Feyter and Gabriel 1991a |
| pUFR127 | 5-kb fragment containing *avrb6* in pUFR042 | De Feyter and Gabriel 1991a |
| pUFR131 | *lacZ*::*avrB4* fusion in pUFR042 | De Feyter *et al.* 1993 |
| pUFR135 | *lacZ*::*avrb6* fusion in pUFR042 | De Feyter *et al.* 1993 |
| pUFR142 | 9-kb fragment containing *avrB101* in pUFR047 | De Feyter *et al.* 1993 |
| pUFR144 | *lacZ*::*avrB101* fusion in pUFR047 | De Feyter *et al.* 1993 |
| pUFR150 | *lacZ*::*avrBIn* fusion in pUFR047 | De Feyter *et al.* 1993 |

*(continued on next page)*

factor encoded by *wts* (water-soaking) genes. This hypothesis may well apply to *X. campestris* pv. *malvacearum*, with *avrb6* encoding a cell leakage factor. Like some *wts* genes of *E. stewartii, avrb6* was not required for bacterial growth *in planta*, but strongly affected water-soaking of its host.

The ion channel defense model of the gene-for-gene hypothesis invokes a cell leakage factor as the product of an *avr* gene (Gabriel *et al.* 1988; Gabriel and Rolfe 1990). In this model, *avr* genes produce a protein or compound that opens an ion channel in the plant cell membrane, which rapidly depolarizes the membrane, causing electrolyte leakage and host cell death. This gene-for-gene model is not inconsistent with the idea that the product of an *avr* gene might induce slower cell leakage in susceptible hosts. The only difference might be the allelic form of the host *R* gene. If the leakage were slow enough to avoid cascade amplification of a wound-response signal, changes in the osmotic gradient could cause a net loss of water from the cell and redistribution to the apoplast, thereby increasing the fluidity of the EPS (M. Essenberg and M. Pierce, personal communication). Increased fluidity or amounts of EPS may increase the number of bacteria exuding onto the leaf surface through stomata (Thiers and Blank 1951). Another possible function is that the increased levels of necrosis induced by *avrb6* may serve to collapse the palisade layer and physically squeeze more bacteria onto the leaf surface. We are currently investigating these possibilities.

## MATERIALS AND METHODS

### Bacterial strains, plasmids, and culture media.

The bacterial strains and plasmids used in this study are listed in Table 4. Strains of *Escherichia coli* were grown in Luria-Bertani medium (Sambrook *et al.* 1989) at 37° C. Strains of *Xanthomonas* were grown in peptone–yeast extract–glycerol–MOPS medium at 30° C (De Feyter *et al.* 1990). For culture on solid medium, agar was added at 15 g/L. Antibiotics were used at the following final concentrations: ampicillin, 25 mg/L; kanamycin, 20 mg/L; gentamicin, 3 mg/L; spectinomycin, 50 mg/L; tetracycline, 15 mg/L; and rifamycin, 75 mg/L.

**Table 4.** (*continued from preceding page*)

| | Relevant characteristics | Reference or source |
|---|---|---|
| pUFR156 | 12.9-kb fragment containing *avrBIn* in pUFR042 | De Feyter *et al.* 1993 |
| pUFR157 | 11-kb fragment containing *avrB102* in pUFR042 | De Feyter *et al.* 1993 |
| pUFR160 | *lacZ*::*avrB102* fusion in pUFR042 | De Feyter *et al.* 1993 |
| pUFR163 | 10-kb fragment containing *avrb7* in pUFR042 | De Feyter *et al.* 1993 |
| pUFR163ΔBam | pUFR163 deleted for 3.4-kb *Bam*HI fragment | This study |
| pUFR171 | Internal *Bam*HI fragment of *avrB4*, in pGem11Zf(+) | De Feyter *et al.* 1993 |
| pUFR172 | Internal *Bam*HI fragment of *avrb6*, in pGem11Zf(+) | De Feyter *et al.* 1993 |
| pUFR173 | Internal *Bam*HI fragment of *avrB101*, in pGem11Zf(+) | De Feyter *et al.* 1993 |
| pUFR174 | Internal *Bam*HI fragment of *avrBIn*, in pGem11Zf(+) | De Feyter *et al.* 1993 |
| pUFR175 | Internal *Bam*HI fragment of *avrB102*, in pGem11Zf(+) | De Feyter *et al.* 1993 |
| pUFR176 | Internal *Bam*HI fragment of *avrb7*, in pGem11Zf(+) | De Feyter *et al.* 1993 |
| pUFR178 | Internal *Bam*HI fragment of *avrBIn*, in pGem11Zf(+), *Sal*I site filled | This study |
| pUFR179 | Internal *Bam*HI fragment of *avrb7*, in pGem11Zf(+), *Sal*I site filled | This study |
| pUFR180 | 9.5-kb fragment containing *avrb6* in pUFR042 | De Feyter *et al.* 1993 |
| pUFR186 | 10.3-kb *Bgl*II-*Eco*RI fragment containing *avrBIn* from pUFR156 in pUFR047 | This study |
| pUFR186ΔBam | pUFR186 deleted for 3.7-kb *Bam*HI fragment | This study |
| pUFR190–194 | *Bam*HI-swapped chimeric genes with *avrb7* 5′ and 3′ ends plus *Bam*HI fragment of *avrB4*, *avrb6*, *avrBIn*, *avrB101*, or *avrB102* in pUFR047 | This study |
| pUFR196–200 | *Bam*HI-swapped chimeric genes with *avrBIn* 5′ and 3′ ends plus *Bam*HI fragment of *avrB4*, *avrb6*, *avrb7*, *avrB101*, or *avrB102* in pUFR047 | This study |
| pUFR205–209 | *Stu*I/*Hin*cII-swapped chimeric genes with *avrBIn* 5′ and 3′ regions plus *Stu*I/*Hin*cII fragment of *avrB101*, *avrB4*, *avrb6*, *avrB102*, or *avrb7* in pUFR047 | This study |
| pUFR211–215 | *Stu*I/*Hin*cII-swapped chimeric genes with *avrb7* 5′ and 3′ regions plus *Stu*I/*Hin*cII fragment of *avrB101*, *avrB4*, *avrb6*, *avrBIn*, or *avrB102* in pUFR047 | This study |
| pUFR217 | pUFR156 derivative, *avrBIn*::Tn*5-gusA* | This study |
| pUFR220 | pUFR163 derivative, *avrb7*::Tn*5-gusA* | This study |
| pUFR227 | pUFR180 derivative, *avrb6*::Tn*5-gusA* | This study |
| pUFY019 | 3.7-kb *Stu*I/*Hin*cII-swapped fragment with *pthA* 5′ and 3′ regions of pZit45 plus *avrb6* internal repeat region of pUFR135 in pUFR047 | This study |
| pUFY020 | 3.8-kb *Stu*I/*Hin*cII-swapped fragment with *avrb6* 5′ and 3′ regions of pUFR135 plus *pthA* internal repeat region of pZit45 in pUFR047 | This study |
| pZit45 | 4.5-kb fragment containing *pthA* in pUFR047 | Swarup *et al.* 1992 |
| pGEM7Zf(+) | ColE1, $Ap^r$, $lacZ\alpha^+$ | Promega, Madison, WI |
| pGEM11Zf(+) | ColE1, $Ap^r$, $lacZ\alpha^+$ | Promega, Madison, WI |

**Recombinant DNA techniques.**

Total DNA isolation from *Xanthomonas* was performed as described by Gabriel and De Feyter (1992). Plasmids were isolated from *E. coli* by alkaline lysis methods (Sambrook *et al.* 1989). Restriction enzyme digestion, alkaline phosphatase treatment, DNA ligation, and random priming reactions were performed as recommended by the manufacturers. Southern hybridization was performed by using nylon membranes as described by Lazo and Gabriel (1987). Otherwise, standard recombinant DNA procedures were used (Sambrook *et al.* 1989).

**Construction of chimeric genes.**

To construct *Bam*HI fragment–swapped chimeric genes from *avrB4, avrb6, avrb7, avrBIn, avrB101,* and *avrB102,* the *Bam*HI fragments from these genes were cloned into an *avrb7* shell (containing 5′ and 3′ ends of *avrb7* but deleted for its *Bam*HI fragment, pUFR163ΔBam) and an *avrBIn* shell (containing 5′ and 3′ ends of *avrBIn* but deleted for its *Bam*HI fragment, pUFR186ΔBam) on the pUFR047 vector. The resulting *Bam*HI fragment–swapped chimeric genes formed pUFR190–200. The *avr* genes from pXcmH and *pthA* from *X. citri* all have unique *Stu*I and *Hin*cII sites delimiting the 102-bp repeated regions, which allow the swapping of the internal repeated regions between genes. To construct *Stu*I/*Hin*cII fragment–swapped chimeras using the pXcmH *avr* genes, pUFR174 and pUFR176 were cut with *Sal*I, blunt-ended by means of the Klenow fragment, and religated to destroy the *Hin*cII sites on the pGem11Zf(+) portions of the plasmids, forming pUFR178 and pUFR179, respectively. These were then cut with *Stu*I and *Hin*cII to delete the internal fragments and used as recipients for the *Stu*I/*Hin*cII internal fragments from all pXcmH *avr* genes on pUFR171–176. The *Bam*HI fragments from pUFR178 and its *Stu*I/*Hin*cII chimeras were recloned into pUFR186ΔBam, forming pUFR205–209. The *Bam*HI fragments from pUFR179 and its *Stu*I/*Hin*cII chimeras were recloned into pUFR163ΔBam, forming pUFR211–215. To facilitate construction of chimeric genes between *pthA* and *avrb6,* the 4.1-kb *Sal*I fragment containing *pthA* from pZit45 and the 3.4-kb *Eco*RI/*Sal*I fragment containing *avrb6* from pUFR135 were inserted into pGEM7Zf(+). The *Stu*I/*Hin*cII internal fragments were swapped between the two pGem derivatives to create chimeric genes. The chimeric genes were then recloned as single *Eco*RI/*Hin*dIII fragments into pUFR047, forming pUFY019 and pUFY020.

**Bacterial conjugation.**

Triparental matings were carried out to transfer broad-host-range plasmids from *E. coli* DH5α to various *Xanthomonas* strains by the use of pRK2013 or pRK2073 as helper plasmids as described by De Feyter and Gabriel (1991a). To transfer plasmids into *X. campestris* pv. *malvacearum,* the modifier plasmid pUFR054, carrying the *Xcm*I and *Xcm*III methylase genes, was used to increase the transfer frequency (De Feyter and Gabriel 1991b).

**Marker exchange mutagenesis.**

Marker exchange mutagenesis of strain XcmH1005 was accomplished by introducing the displacement vector pUFR049 into XcmH1005 transconjugants harboring pUFR227 (*avrb6*::Tn*5-gusA*), pUFR220 (*avrb7*::Tn*5-gusA*), or pUFR217 (*avrBIn*::Tn*5-gusA*) derivatives. The procedure was the same as that described by Swarup *et al.* (1991), except that the modifier plasmid pUFR054 was used to facilitate plasmid transfer into XcmH1005.

**Plant inoculations.**

Cotton (*Gossypium hirsutum* L.) lines used were Acala-44 (Ac44) and its congenic resistance lines AcB1, AcB2, AcB4, Acb6, Acb7, AcBIn, and AcBIn3 as described by Swarup *et al.* (1992) and De Feyter *et al.* (1993). Cotton plants were grown in the greenhouse, transferred to growth chambers before inoculation, and maintained as described by De Feyter and Gabriel (1991a). Bacterial suspensions of *X. campestris* pv. *malvacearum* ($10^8$ cfu/ml) in sterile tap water were gently pressure-infiltrated into leaves of 4- to 5-week-old cotton plants. Pathogenic symptoms were observed periodically 2–7 days after inoculation.

All citrus (*Citrus paradisi* 'Duncan,' grapefruit) and bean (*Phaseolus vulgaris* 'California Light Red') plants were grown under greenhouse conditions. Plant inoculations involving *X. citri* or *pthA* or derivatives of *pthA* were carried out in BL-3P level containment (refer to Federal Register, vol. 52, no. 154, 1987) at the Division of Plant Industry, Florida Department of Agriculture, Gainesville. Bacterial suspensions were standardized in sterile tap water to $10^8$ cfu/ml and pressure-infiltrated into the abaxial leaf surface of the plants. All inoculations of cotton, citrus, and bean were repeated at least three times.

**Bacterial growth *in planta*.**

To determine the growth of *X. campestris* pv. *malvacearum* in the susceptible cotton line Ac44, bacterial suspensions were adjusted to $10^6$ cfu/ml, and cotton leaves were inoculated by pressure infiltration. Leaf disks (1 $cm^2$) were taken with a sterilized cork borer and then macerated in 1 ml of sterile tap water. At least three samples were taken at each time point for each strain inoculated. Viable counts were determined by serial dilutions on plates containing appropriate antibiotics. The data shown in Figure 3 are means and standard errors from three separate experiments.

To quantify the amount of bacteria present on the surface of water-soaked leaf spots, leaves were inoculated with bacterial suspensions containing $10^7$ cfu/ml from overnight cultures. Five days later, 100 μl of sterile tap water was dispensed onto the water-soaked leaf surface, spread to an area of approximately 1 $cm^2$, and mixed with the bacteria and slime on the leaf surface. This bacterial suspension was then collected with a pipette. Each 1-$cm^2$ water-soaked leaf area was washed 10 times, and a total of 1 ml of bacterial suspension was collected. To quantify the bacteria remaining inside the leaf, leaf disks (1 $cm^2$) were taken with a sterilized cork borer and then macerated in 1 ml of sterile tap water. Viable counts were determined by serial dilutions on plates containing appropriate antibiotics. The data reported in Results (external and total counts) are means and standard errors of six replicates from two separate experiments.

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