Genetic Analysis of a DNA Region Involved in Expression of Two Epitopes Associated with Lipopolysaccharide in *Xanthomonas campestris* pv. *vesicatoria*

J. B. Jones, G. V. Minsavage, R. E. Stall, R. O. Kelly, and H. Bouzar

First, fourth, and fifth authors: Gulf Coast Research & Education Center, University of Florida, 5007 60th St. East, Bradenton 34203. Second and third authors: Department of Plant Pathology, University of Florida, Gainesville 32611.
Florida Agricultural Research Station Journal Series R-02250.
Accepted for publication 18 January 1993.

ABSTRACT

Jones, J. B., Minsavage, G. V., Stall, R. E., Kelly, R. O., and Bouzar, H. 1993. Genetic analysis of a DNA region involved in expression of two epitopes associated with lipopolysaccharide in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 83:548-551.

We report on the cloning of DNA involved in expression of two epitopes associated with lipopolysaccharide (LPS) in *Xanthomonas campestris* pv. *vesicatoria* and on the use of part of this region for differentiating A (nonpectolytic/amylolytic) and B strains (pectolytic/amylolytic) of this pathogen. From a genomic library of A-strain 75-3 that expresses two epitopes, two recombinant cosmids conferred expression of two epitopes in 87-13, an A strain that does not express the two epitopes. One of the two overlapping clones, pEC795, contained a 27-kb insert and was used for further analysis. Immunoblots revealed that the epitopes are components of LPS in 75-3. Transposon mutagenesis of the insert identified a 4.5- to 5.5-kb region necessary for expression of the two epitopes in 87-13. A 0.65-kb internal fragment from this region reacted strongly in hybridization tests with A strains, weakly with B strains, and with only two of eight pathovars of *X. campestris*. PCR (polymerase chain reaction) amplification using primers from the 0.65-kb fragment resulted in the predicted DNA product in all A strains (12), in one of 12 B strains, and in one of 10 other pathovars (i.e., *X. c.* pv. *alfalfae*). Digestion of the PCR products by two enzymes resulted in identical restriction patterns for A strains and *X. c.* pv. *alfalfae* but resulted in a different pattern for the B strain.

Xanthomonas campestris pv. *vesicatoria*, causal agent of bacterial spot on tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) is pathogenically (22,33), serologically (4; H. Bouzar, J. B. Jones, A. M. Alvarez, and A. A. Benedict, *unpublished data*), and physiologically diverse (6). Strains on tomato are divided into two races, based on the differential reaction on tomato genotype Hawaii 7998 (31). One group of strains, A, contains the avirulence gene *avrRxv*, which confers avirulence of the bacterium in tomato genotype Hawaii 7998. The second race consists of strains of *X. c. vesicatoria* that do not induce a hypersensitive reaction in Hawaii 7998 and that are differentiated from the A strains by being pectolytic and/or starch hydrolytic. Characterization of these strains (hereafter, B strains) by DNA hybridization, carbon-utilization patterns, and

TABLE 1. Bacterial strains and plasmids used in this study

Bacterium or plasmid	Relevant characteristics[a]	Reference
Xanthomonas campestris		
pv. *armoraciae* A		R. E. Stall
pv. *begoniae* XB9		J. W. L. van Vuurde[b]
pv. *begoniae* XB40		J. B. Jones
pv. *campestris* 33913		R. E. Stall
pv. *carotae* 13		R. E. Stall
pv. *citri* 9771		R. E. Stall
pv. *dieffenbachiae* X729		A. R. Chase[c]
pv. *malvacearum* RIATC		R. E. Stall
pv. *pelargonii* XCP58		J. B. Jones
pv. *phaseoli* G28		R. E. Stall
pv. *phaseoli* 85-6		R. E. Stall
pv. *pruni* FLA1		R. E. Stall
pv. *vignicola* 81-20		R. E. Stall
pv. *vesicatoria* 75-3	A-strain; strain used to prepare MAbs 2H10 and 5D12	R. E. Stall
87-13	A strain; does not react with MAbs 2H10 and 5D12	R. E. Stall
XT5	A strain	AVRDC[d]
89-10	A strain	R. E. Stall
86-46	A strain	R. E. Stall
XVP26	A strain	AVRDC
86-2	A strain	R. E. Stall
87-44	A strain	AVRDC
86-46	A strain	AVRDC
82-4	A strain	R. E. Stall
89-10	A strain	R. E. Stall
86-22	A strain	R. E. Stall
87-21	A strain	AVRDC
XV56	B strain; reacts with MAb 2H10	H. Nagai[e]
BV5-4a	B strain	B. Canteros[f]
71-4	B strain	R. E. Stall
0226	B strain	B. Canteros
0350	B strain	B. Canteros
BV#3	B strain	B. Canteros
BV20-3A	B strain	B. Canteros
BV5-3A	B strain	B. Canteros
BV5-4B	B strain	B. Canteros
XV56	B strain	R. E. Stall
81-6	B strain	R. E. Stall
Opportunistic xanthomonad INA 42		R. E. Stall
Opportunistic xanthomonad T55		R. E. Stall
Pseudomonas solanacearum K60		A. Kelman[g]
Escherichia coli		
HB101	F⁻ *recA* F⁻ *recA* 80d*lacZ*	BRL[h]
DH5α	M15	BRL
C2110	Nal^r *polA*	BRL
Plasmids		
pLAFR3	Tet^r Tra⁻ Mob⁺	Staskawicz et al (30)
pHoKmGus	Km^r, *tnpA*⁻; promoterless β-glucoronidase gene	Bonas et al (5)
pSShe	Cm^r, *tnpA*	Stachel et al (29)
pRK2073	Sp^r, Tra⁺, Mob⁺	Sawacyc et al (27)
pEC795	pLAFR3 clone from 75-3	This study
pBS-KS⁺, pBS-KS⁻	Amp^r, Bluescript	Stratagene

[a] MAb, monoclonal antibody; Nal, nalidixic acid; Tet, tetracycline; Km, kanamycin; Cm, chloramphenicol; Sp, spectinomycin; Amp, ampicillin; ^{r}, resistant. *X. c. vesicatoria* A strains contain the avirulence gene, *avrRxv*, and are positive for pectolytic and amylolytic activity; *X. c. vesicatoria* B strains do not contain the *avrRxv* and are positive for pectolytic and amylolytic activity.

[b] Institute of Plant Protection, Wageningen, Netherlands.

[c] University of Florida, Central Florida Research and Education Center, Apopka.

[d] Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan.

[e] Instituto Agronomico, Campinas, Brazil.

[f] EEA, INTA, Corrientes, Argentina.

[g] Department of Plant Pathology, University of Wisconsin, Madison.

[h] Bethesda Research Laboratories.

fatty acid profiles revealed they are distinct from A strains (R. E. Stall, *unpublished data*).

Monoclonal antibodies (MAbs) have been effective tools for demonstrating antigenic diversity in a number of *X. campestris* pathovars (1,2) and in some instances, are specific for an individual pathovar (4). MAbs developed against A strains were used to discriminate between A and B strains (H. Bouzar, J. B. Jones, A. M. Alvarez, and A. A. Benedict, *unpublished data*). In a survey of 306 strains from throughout the world, two MAbs (5D12 and 2H10) were useful for differentiating most of the A strains from the B strains; 91% of the A strains and 22% of the B strains reacted with both MAbs. Approximately 9% of the A strains did not react with either MAb, indicating the epitopes are not encoded by *avrRxv*. Nevertheless, genetic analysis of the region necessary for expression of antigenic determinants (epitopes) may reveal differences between the two groups of strains and may help explain differential responses to MAbs.

In this paper, we describe the cloning and characterization of a region required for expression of two epitopes in an A strain that does not react with MAbs 5D12 or 2H10. We also present information on the effect of this region in other pathovars of *X. campestris* and other phytopathogenic bacteria and its effects on expression of the two epitopes. Finally, we present information on a unique area of this DNA fragment that can be used for differentiating A strains from B strains.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. Strains of *X. c. vesicatoria* were stored in 15% glycerol at −80 C or in sterile tap water at room temperature. The bacteria were subcultured on nutrient agar (NA) or nutrient yeast-glycerol agar (NYGA). Rifampicin-resistant strains were selected on NA containing rifampicin at 75 μg/ml. *Escherichia coli* strains HB101 and DH5α (Bethesda Research Laboratories, Gaithersburg, MD) were maintained on Luria-Bertani (LB) medium (26). When necessary, antibiotics were added at the following concentrations: rifampicin, 75 μg/ml; kanamycin, 25 μg/ml; spectinomycin, 50 μg/ml; and tetracycline, 12.5 μg/ml.

Production of monoclonal antibodies. *X. c. vesicatoria* A-strain 75-3 was grown on nutrient agar for 24 h at 28 C. Cells were gently removed and suspended in 10 mM phosphate buffered saline (PBS). The cells were washed three times, resuspended in Tris buffer (50 mM Tris, 10 mM NaCl, and 25% [w/v] sucrose), and frozen at −70 C. BALB/c mice were immunized twice, 12 days apart, by intraperitoneal injection of approximately 10^8 cells mixed 1:1 with Ribi adjuvant (MPL + TDM adjuvant, RIBI Immunochem Research, Inc., Hamilton, MT). The mice were hyperimmunized 3 days before the fusion. The mouse spleen was removed, and the spleenocytes were mixed 7:1 with washed Sp2/0 myeloma cells, centrifuged, and fused with 50% polyethylene glycol for 1 min (17). During the next 5 min, 1 ml of HAT medium (DMEM plus 20% fetal calf serum, and hypoxanthine-aminopterin-thymidine selective agents) was added every 10 s. The mixture was centrifuged, resuspended in HAT medium, and dispensed into 96-well culture plates. Twelve days after the fusion the hybridoma supernates were screened against the homologous A strain (75-3) and a B strain (XV56) by ELISA (enzyme-linked immunosorbent assay) using plates coated with cell suspensions adjusted to the appropriate optical density as outlined below. Hybridomas 5D12 and 2H10 were selected for ascite production. A MAb, 4F1G9H6, produced against *X. c. pelargonii* strain XCP49 and determined to be pathovar specific (16), was also used in this study.

DNA manipulations. A cosmid library of total DNA from *X. c. vesicatoria* strain 75-3 was constructed according to the procedure of Minsavage et al (22) in which DNA was partially digested with *Sau*3AI, and 15- to 30-kb fragments were purified and ligated into the *Bam*HI site of pLAFR3. Ligated DNA was packaged in lambda phage using a packaging kit (Boehringer Mannheim, Indianapolis, IN) and was transduced into *E. coli*

HB101. The clones were mobilized into *X. c. vesicatoria* strain 87-13 by triparental mating (11) including helper plasmid pRK2073 (27). Cosmids containing the putative epitope-expressing genes were identified by selecting transconjugants that reacted with 5D12 or 2H10 in ELISA. Subsequent restriction enzyme analysis and cloning manipulations were done using standard techniques (26). Procedures for transferring DNA onto nitrocellulose membranes, labeling probes, and performing hybridizations using the Genius kit (Boehringer Mannheim) were previously described (21).

Screening clones for expression of epitopes. The clones were screened for expression of epitopes with an ELISA carried out according to standard procedures (10). For initial serological screening, transconjugant cells were removed from plates with a sterile loop or toothpick and were added directly to individual wells of Immulon 2 microtiter plates (Dynatech Laboratories, Chantilly, VA) containing 100 μl of 10 mM carbonate buffer (10). For more precise tests, bacterial suspensions were obtained by suspending cells from 24-h-old cultures grown on NA alone or amended with the appropriate antibiotics in carbonate buffer and adjusting the bacterial concentration to $A_{600} = 0.6$; each well received 100 μl of suspension. Coated plates were incubated overnight at 4 C. The substrate reaction was quantified by reading absorbance at 405 nm with an EAR400 AT plate reader (SLT-Labinstruments, Grödig, Austria).

LPS extraction, electrophoretic separation, and immunoblotting. Cells were grown in nutrient broth or in nutrient broth supplemented with rifampicin and tetracycline overnight at 28 C with vigorous shaking on a rotary shaker (200 rpm) and were harvested in the late log phase. LPS (lipopolysaccharide) was extracted from the cells using the hot phenol-water procedure (32). The aqueous phase was dialyzed in deionized water and freeze-dried. LPS samples (1%) were treated with 100 ng of Proteinase K (Boehringer Mannheim) per microliter of LPS sample, were incubated at 48 C for 50 min, and were subjected to SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) (18). The LPS bands were transferred to nitrocellulose membrane and immunoblotted with MAb 2H10 or 5D12, as described (7,8).

PCR amplification. Polymerase chain reaction (PCR) amplification was performed using a DNA thermal cycler (M. J. Research, Watertown, MA) and *Taq* DNA polymerase (Boehringer Mannheim) according to standard protocols, with some modifications (15,24). The PCR-reaction solution contained PCR-amplification buffer (supplied by the manufacturer with the enzyme), 100 μM of each of the dNTP's, 50 μM of each primer, 50 ng of genomic template DNA, and 1.25 U of *Taq* DNA polymerase. Template DNA was initially denatured at 94 C for 1 min. A total of 30 PCR cycles were run under the following conditions: denaturation at 94 C for 1 min, primer annealing at 62 C for 0.5 min, and DNA extension at 72 C for 0.75 min.

A 560-bp region of the 75-3 genome was amplified using 22-mer primers RST13 (5′-TGGTTCCAGCCGTCCAGCAGGG) and RST14 (5′-CCCTAAAGGCACTGGGCGTCGG) chosen from sequences obtained from the 650-bp fragment G (Fig. 1). To find appropriate primers, fragment G was cloned into the *Eco*RV site of pBluescript II KS$^{+/-}$ (Stratagene, La Jolla, CA) and sequenced by the dideoxy method using T7-DNA polymerase (5). Oligonucleotide primers were synthesized using the Applied Biosystems 394 DNA synthesizer (Foster City, CA). PCR-amplified DNA was detected using agarose gel electrophoresis according to standard procedures (26).

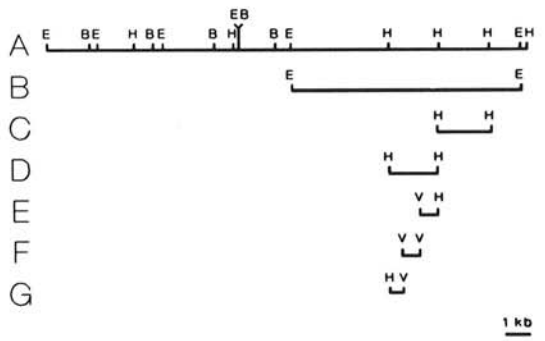

Fig. 1. Map showing relative positions and restriction-enzyme termini of DNA fragments selectively subcloned and labeled for hybridization experiments. E = *Eco*RI, B = *Bam*HI, H = *Hin*dIII, and V = *Eco*RV. Fragments B–G are fragments generated by digesting A, the insert in pEC795.

Insertion mutagenesis. Insertion mutagenesis of the cosmid pEC795, essential for expression of the two epitopes, was performed as described (5). *E. coli* HB101 (pHoKmGus, pSShe) was transformed with pEC795, and transformants were mated with Nal^r*polA E. coli* C2110 using pRK2073 as a helper plasmid (27). Transconjugants carrying pEC795 with transposon insertions were selected by plating on LB containing nalidixic acid, kanamycin, and tetracycline. The transconjugants were mated with 87-13 and plated on media containing rifampicin, kanamycin, and tetracycline. The transconjugants were screened by ELISA using MAbs 2H10 or 5D12.

Marker exchange. The 87-13 transconjugants containing mutant plasmids were cycled on NA plates containing rifampicin and kanamycin, at least 10 times, and subsequently, were plated onto NA containing rifampicin, kanamycin, and NaH_2PO_4 (10 g/L) to enrich for tetracycline-sensitive colonies.

Pathogenicity tests. Analyses of growth of plants and pathogenicity on pepper cv. Early Calwonder and tomato cv. Bonny Best were performed as reported previously (3,21). For inoculations, strains were grown for 24 h at 28 C on NA, the bacterial cells were suspended in sterile tap water to approximately 10^8 cfu/ml, and the suspension was infiltrated into the mesophyll of fully expanded leaves. Infiltrated areas were checked for disease development or incompatible reactions daily for 3 days.

RESULTS

Cloning of a region for expression of two epitopes in 75-3. When the library of 75-3 was conjugated into 87-13 and screened for expression of two epitopes, only two of 600 transconjugants reacted with both MAbs in ELISA. DNA from the two cosmid clones, EC795 and EC425, were digested using *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I and had overlapping restriction patterns (data not shown). The transconjugant containing pEC795 reacted more strongly in ELISA and was chosen for further characterization. A restriction map was made of the insert (Fig. 2A). To pinpoint the region necessary for expression of the two epitopes, insertion mutagenesis of pEC795 was followed by reintroduction of the

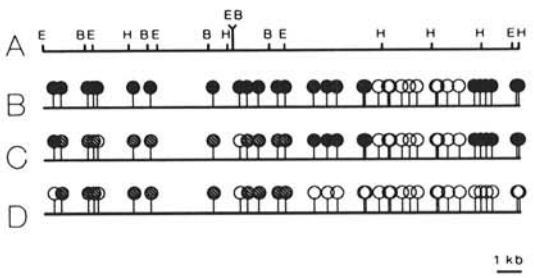

Fig. 2. A, Restriction map of pEC795: A 27-kb fragment of DNA that confers expression of two epitopes in A strain 87-13. **B,** Map of 87-13(pEC795) indicating transposon insertions and their effects on ELISA activity with MAbs 2H10 and 5D12. **C,** Map of pEC795 indicating transposon insertions and their effects on ELISA activity with MAb 5D12 of *Xanthomonas campestris* pv. *pelargonii* strain XCP58(pEC795). **D,** Map of *X. c. pelargonii* strain XCP58(pEC795) indicating transposon insertions and their effects on ELISA activity in XCP58 with a MAb to the pathovar-specific epitope of *X. c. pelargonii*. Open circles, cross-hatched circles, and solid-black circles represent ELISA activity in which transconjugants are inactivated completely or incompletely or are unaffected, respectively. E = *Eco*RI, B = *Bam*HI, and H = *Hin*dIII.

mutagenized plasmid into 87-13. The transconjugants were screened by ELISA to determine whether activity with 2H10 or 5D12 was reduced or eliminated. A 4.4- to 5.5-kb region was necessary for expression of both epitopes in 87-13 (Fig. 2B). Of the transconjugants altered by insertion mutagenesis, none were identified in which only one antibody reacted with the transconjugant. When 20 insertion mutants, spanning the 27-kb region, were marker exchanged back into 75-3, expression of both epitopes was eliminated. The wild-type strain and the marker-exchange mutants produced virulent reactions in Bonny Best tomato leaves and avirulent reactions in Early Calwonder pepper leaves.

Expression of the 75-3 epitope region in other bacteria. When pEC795 was conjugated into a diverse group of xanthomonads and *Pseudomonas solanacearum*, expression of one or both epitopes of *X. c. vesicatoria* was observed in all bacteria, with the exception of *X. c. begoniae*, one strain of an opportunistic xanthomonad (13), and *P. solanacearum* (Table 2). When pEC795 was mobilized into *X. c. pelargonii* strain XCP58, only MAb 5D12 reacted (Table 2). After insertion mutagenesis of pEC795 followed by reintroduction into XCP58, the same 4.5- to 5.5-kb region was essential for expression of the epitope that reacts with MAb 5D12 (Fig. 2C); however, there was reduced activity with MAb 5D12 because of insertions outside the 4.5- to 5.5-kb region of the fragment. The pathovar-specific pathogen of *X. c. pelargonii* (4F1G9H6) reacted with XCP58 but did not react with XCP58(pEC795). Insertion mutagenesis covering a large region of pEC795 resulted in partial expression of the *X. c. pelargonii* pathovar-specific epitope; mutagenesis in the region essential for expression of the two 75-3 epitopes still inhibited expression of that pathovar-specific epitope (Fig. 2D).

Immunoblotting with MAb 2H10 revealed that the epitope associated with LPS in 75-3 was also present in 87-13 containing pEC795, but not in 87-13 containing pLAFR3 without the insert (Fig. 3A). MAb 2H10 did not react with LPS from XCP58(pEC795) (Fig. 3A), and expression of the pathovar-specific epitope in that strain was inhibited (Fig. 3B). Using MAb 5D12, similar results were obtained, with the exception that the epitope reacting with this MAb was expressed in LPS of XCP58(pEC795) but not in the wild-type XCP58, confirming ELISA data (data not shown).

Hybridization of pEC795 subclones to DNA of other bacteria. The 27-kb insert fragment (labeled A in Fig. 1) hybridized with DNA from A and B strains of *X. c. vesicatoria*, other pathovars of *X. campestris*, and opportunistic xanthomonads (Fig. 4A).

TABLE 2. ELISA reaction of wild-type strains and transconjugants carrying pEC795 with monoclonal antibodies 2H10 and 5D12

Organism	Strain	2H10	5D12
Xanthomonas campestris			
pv. *armoraciae*	A	++[a]	−
	A(pEC795)	++	+
pv. *dieffenbachiae*	X729	−	−
	X729(pEC795)	−	+
pv. *campestris*	33913	−	−
	33913(pEC795)	++	+
pv. *begoniae*	XCB40	−	−
	XCB40(pEC795)	−	−
pv. *pelargonii*	XCP58	−	−
	XCP58(pEC795)	−	+
pv. *vesicatoria*	87-13	−	−
	87-13(pEC795)	+	+
pv. *vesicatoria*	XV56	+	−
	XV56(pEC795)	++	+
pv. *vesicatoria*	75-3	++	+
Opportunistic xanthomonad	Ina42	−	−
	Ina42(pEC795)	−	−
Opportunistic xanthomonad	T55	−	−
	T55(pEC795)	−	+
Pseudomonas solanacearum	K60	−	−
	K60(pEC795)	−	−

[a] Relative reaction in ELISA.

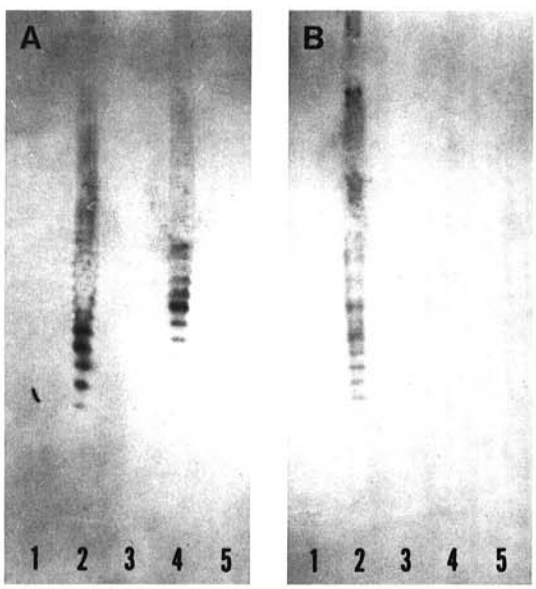

Fig. 3. Immunoblots of lipopolysaccharides from wild-type strains and transconjugants treated with **A,** MAb 2H10 prepared against *Xanthomonas campestris* pv. *vesicatoria* and **B,** MAb 4F1G9H6 prepared against *X. c. pelargonii*. **A:** lane 1, XCP58(pEC795); lane 2, 75-3; lane 3, *X. c. pelargonii* strain XCP58; lane 4, 87-13(pEC795); and lane 5, 87-13(pLAFR3). **B,** lane 1, 75-3; lane 2, *X. c. pelargonii* strain XCP58; lane 3, 87-13(pEC795); lane 4, 87-13(pLAFR3); and lane 5, XCP58(pEC795).

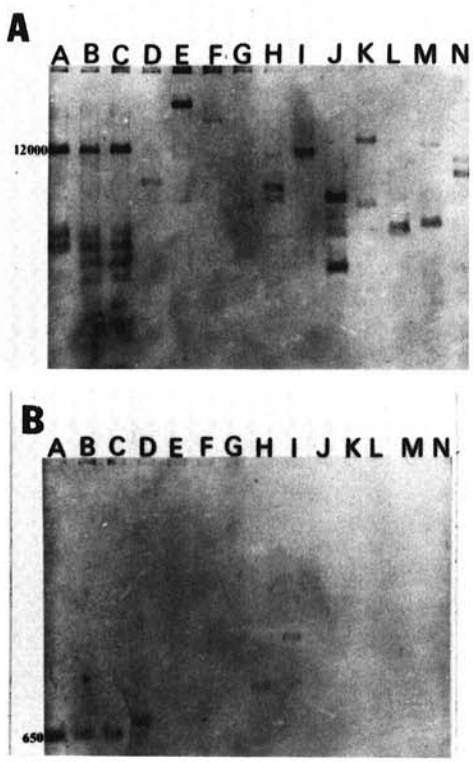

Fig. 4. Southern blot of **A,** *Eco*RI- and **B,** *Eco*RV-restricted genomic DNA from strains of *Xanthomonas campestris* pv. *vesicatoria*, other pathovars of *X. campestris*, an opportunistic xanthomonad probed with pEC795, and a 0.65-kb insert (labeled G in Fig. 1), respectively. Lane A, *X. c. vesicatoria* A-strain 75-3; B, *X. c. vesicatoria* A-strain 87-13; C, *X. c. vesicatoria* A-strain XVP26; D, *X. c. vesicatoria* B-strain XV56; E, *X. c. vesicatoria* B-strain BV5-4a; F, *X. c. pelargonii* strain XCP58; G, *X. c. begoniae* strain XB9; H, *X. c. campestris* strain 33913; I, *X. c. armoraciae* strain A; J, *X. c. phaseoli* strain G28; K, *X. c. vignicola* strain 81-20; L, *X. c. dieffenbachiae* strain X729; M, *X. c. pruni* strain FLA (according to table); and N, opportunistic-xanthomonad strain INA42.

When the 11-kb *Eco*RI fragment (labeled B in Fig. 1) was used as the probe, it hybridized strongly with the A strains of *X. c. vesicatoria* but much more weakly with the B strains of *X. c. vesicatoria* and the other xanthomonads (data not shown). Transconjugants containing this insert B clone expressed the two epitopes (data not shown). Two *Hin*dIII fragments from B, C, and D (Fig. 1), reacted strongly with the A strains in hybridization studies (data not shown). Fragment D reacted weakly with *X. c. campestris* and *X. c. armoraciae* (data not shown). When fragment D was digested further with *Eco*RV, three fragments, E, F, and G, were obtained that reacted the same as fragment D with all strains tested in hybridization studies; data for G are presented (Fig. 4B).

PCR amplification. Twenty-two base-pair primers were selected from sequences 560 bases apart in fragment G. The predicted 560-bp PCR product was detected in strains of *X. c. vesicatoria*, one strain of *X. c. alfalfae* (data not shown), but not in other xanthomonads tested (Fig. 5). The *X. c. alfalfae* strain also reacted strongly with both *X. c. vesicatoria* MAbs in ELISA. Within *X. c. vesicatoria*, the 560-bp product was detected in 12 A strains tested and in one (BV#3) of 11 B strains. When the products of four A strains, the B strain, and the *X. c. alfalfae* strain were digested with *Hae*III and *Sau*IIIA, all products from the A strains and the *X. c. alfalfae* strain had the same restriction pattern; the B strain had a different pattern (data not shown).

DISCUSSION

Altered LPS of various microorganisms has been shown to affect pathogenicity (9,28). In our study, LPS expression was modified in 87-13(pEC795), but pathogenicity did not appear to be altered. In a study with *P. solanacearum*, LPS-defective mutants could not be definitively associated with the ability or inability to induce a hypersensitive reaction or alter pathogenicity (14). Thus, minor and major changes in LPS do not necessarily result in altered pathogenicity or ability to induce a hypersensitive reaction.

A 4.5- to 5.5-kb region from 75-3 was required for expression of two LPS-associated epitopes in *X. c. vesicatoria* strain 87-13. The association with the LPS was evidenced by the pattern observed in immunoblots. By marker exchange of the transposon mutagenized insert of pEC795 back into 75-3, it was determined that the entire 27-kb insert was essential for expression of both epitopes in 75-3. The entire region is apparently associated with

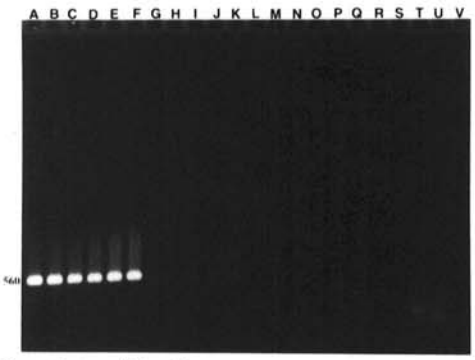

Fig. 5. PCR products of *Xanthomonas campestris* pv. *vesicatoria* strains, other pathovars of *X. campestris*, and an opportunistic xanthomonad, using PCR primers within the 650-bp fragment G (Fig. 1). Lane A, *X. c. vesicatoria* A-strain 75-3; B, *X. c. vesicatoria* A-strain 87-13; C, *X. c. vesicatoria* A-strain XT5; D, *X. c. vesicatoria* A-strain 89-10; E, *X. c. vesicatoria* A-strain 86-46; F, *X. c. vesicatoria* B-strain BV#3; G, *X. c. vesicatoria* B-strain XV56; H, *X. c. vesicatoria* B-strain BV#20; I, *X. c. vesicatoria* B-strain BV5-4a; J, *X. c. vesicatoria* B-strain 0226; K, *X. c. vesicatoria* B-strain 0350; L, *X. c. armoraciae* strain A; M, *X. c. campestris* strain 33913; N, *X. c. begoniae* strain XB9; O, *X. c. pelargonii* strain XCP58; P, *X. c. dieffenbachiae* strain X729; Q, *X. c. phaseoli* strain 85-6; R, *X. c. citri* strain 9771; S, *X. c. carotae* strain 13; T, *X. c. pruni* FLA1; U, *X. c. malvacearum* strain RIATC; and V, opportunistic-xanthomonad strain INA42.

expression of the two epitopes and is typical of other systems in which clustering of genes is associated with LPS biosynthesis (19,20). A similar situation was observed in *Rhizobium leguminosarum*, in which clustering of genes in a 20-kb region was associated with production of a slow-migrating LPS band (25). Although the genes located within fragment B were important for expression of the epitopes in 87-13, the region did not appear to be the only one responsible for expression of the two epitopes, because two B strains that reacted with both MAbs did not react with the PCR primers or did not have a high degree of homology with the fragments associated with the region. The reaction of 87-13 with the primers and the high degree of homology of its DNA with all fragments suggests that the 4.5- to 5.5-kb region in 87-13 is not expressed rather than not present. A similar situation was observed with regard to the pectate-lyase gene derived from a pectolytic strain of *X. c. vesicatoria* hybridizing with DNA of nonpectolytic *X. c. vesicatoria* strains (3).

Expression of the two epitopes in transconjugants containing pEC795 was not limited to *X. c. vesicatoria*. In certain strains of closely related bacteria, expression of epitopes depends on the sugars associated with the LPS core (23). Morona et al (23) observed that the *Vibrio cholerae* O-antigen is expressed in certain strains of *E. coli* that contain a diglucose associated with the core. Variation may exist in the core of xanthomonads, which could help explain the selective antigen expression observed in transconjugants of some *X. campestris* pathovars containing pEC795.

Based on DNA hybridization with fragments within the 27-kb region, there was a high degree of homology with A strains and less similarity with B strains and strains of other *X. campestris* pathovars. Specificity for A strains was increased using probes from the region critical for expression of the two epitopes in 87-13. Other than *X. c. vesicatoria* strains, *X. c. campestris* and *X. c. armoraciae* were the only other pathovars tested in which both antigenic determinants were expressed when pEC795 was present in transconjugants. These data indicate that there may be closer antigenic relatedness to *X. c. vesicatoria* of those two pathovars than of the other pathovars tested in the experiment. Elrod and Braun (12) noted that these two pathovars are closely related antigenically to *X. c. vesicatoria*.

PCR amplification was a more selective tool than either DNA hybridization or ELISA when using MAbs to discriminate between the A and B strains. With PCR, only one strain of another pathovar (*X. c. alfalfae*) reacted positively with the primers, whereas the DNA probes (depending on size) hybridized with several pathovars. Both MAbs used in this study reacted with one strain of *X. c. alfalfae*, and one MAb reacted with *X. c. armoraciae*; the strain of the former pathovar was pathogenic on alfalfa but not on pepper or tomato (data not shown). Another *X. c. alfalfae* strain neither reacted with the two MAbs nor with the primers in hybridization. Further genetic characterization of the region related to LPS production should offer better understanding of antigenic relationships within the *X. campestris* group.

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