Conservation of Plasmid DNA Sequences and Pathovar Identification of Strains of *Xanthomonas campestris*

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ABSTRACT

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One hundred and seventeen different strains of *Xanthomonas campestris*, representing 26 different pathovars, were examined for plasmid content and restriction fragment length polymorphism of the plasmid DNAs. All strains tested of 10 pathovars contained plasmids. All strains tested of 13 pathovars contained no detectable plasmids, and strains of three pathovars were variable in plasmid content. Restriction endonuclease digests of plasmid DNAs from strains within a given plasmid-containing pathovar gave surprisingly similar, but not always identical, digestion profiles on agarose gels. When strains were purified by repeated single-colony isolations, the plasmid DNAs were found to be stable. In most cases, strains of *X. campestris* that contained plasmids could be differentiated at the pathovar level on the basis of their characteristic plasmid profiles. In no instance was the same plasmid profile seen in more than one pathovar. Plasmids that appeared to be similar by restriction fragment length profiles were confirmed to be similar in DNA sequence by Southern hybridization analyses. All 60 strains tested of *X. c.* pv. *glycines*, *X. c.* pv. *malvacearum*, *X. c.* pv. *phaseoli*, and *X. c.* pv. *vignicola* could be accurately identified by pathovar from determination of the restriction fragment profile and/or by Southern hybridizations of that profile. The apparent stability of the plasmids provides a natural genetic marker that can be strain specific and perhaps useful in epidemiological investigations.

Additional key word: DNA probes.

More than 125 different pathovars of *Xanthomonas campestris* (Pammel 1895) Dowson 1939 are currently recognized (2,8), and the primary means for differentiating them is by inoculation of the plant host(s) of that pathovar. It would be a difficult task to inoculate every plant that could serve as a host to an *X. campestris* isolate, therefore the potential host range of a given isolate is unknown. Most often, the pathovar name assigned to a strain of *X. campestris* is determined by the host it was isolated from. Such designations may be artifactual because the primary host may be different from the one the strain was isolated from; some *X. campestris* pathovars are known to be pathogenic on more than one host. Epiphytes cannot be classified in this pathovar identification system. Possible taxonomic relationships among pathovars are also elusive. It would be helpful if alternative means to differentiate among *X. campestris* pathovars were available. Some suggested approaches for differentiating *X. campestris* pathovars have included serology (1,30), membrane protein profiles (24), phage-typing (13), and gas chromatography of fatty acids (21,28). These approaches suffer because they are often strain specific, dependent on constant environmental parameters, and/or so cumbersome that no extensive evaluative tests have been performed.

Plasmid DNA has been identified in several pathovars of *X. campestris* (4,7,10,14–18,27). It is relatively simple to extract large numbers of strains and visualize their plasmids with standardized alkaline lysis procedures (15,22). To characterize the plasmids, restriction endonucleases are used to digest the DNA into distinct fragments that can be separated by size, resulting in fragment patterns visualized by agarose gel electrophoresis. To date there have been no systematic attempts to examine the extent of plasmid variation among a large number of strains involving a large number of *X. campestris* pathovars. Our preliminary studies on selected pathovars of *X. campestris* suggested that there was a surprisingly high degree of plasmid sequence conservation within some pathovars (11,16,17). These studies further suggested that plasmids of purified strains were quite stable and hence useful in epidemiological studies (11), similar to those used to monitor the spread of selected human pathogens (9). The purpose of this study was to survey the extent of variation of plasmid DNAs within a large number of *X. campestris* pathovars, and to determine if the plasmid content of strains based on restriction fragment polymorphism and Southern hybridization could be used in differentiating the pathovars of *X. campestris*.

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MATERIALS AND METHODS

Bacterial strains. The *X. campestris* strains used in this study, their pathovar designations, geographic origin, and sources are listed in Table 1. Some of the stock cultures of *X. c.* pv. *malvacearum* were mixtures of different strains, maintained and described as 'races' for purposes of screening cotton host differentials and breeding lines for disease resistance. Described 'races' of *X. c.* pv. *malvacearum* may or may not be purified strains; as many as seven different strains have been derived from a single race 1 isolate (3). All strains used in this study were repeatedly purified from single colonies and confirmed to be pathogenic on their designated hosts. Broth cultures of bacteria were grown in a peptone-glycerol medium (10.0 ml of glycerol, 20.0 g of peptone, and 1.5 g of K_2HPO_4 per liter). Bacterial strains were commonly stored at −80 C in the same medium containing 15% glycerol.

Plasmid extraction and visualization. Cultures were grown to mid- to late logarithmic growth phase and extracted by either of two small-scale alkaline lysis extraction procedures (15,22). Extracted DNA was resuspended in TE (10 mM tris(hydroxymethyl)aminomethane (Tris), 1 mM sodium ethylenediaminetetraacetate (Na_2EDTA), and 20 μg/ml of DNase-free pancreatic RNase; pH 7.6) and digested with either of two restriction

TABLE 1. Strains used in this investigation

Bacterium (no. of strains)	Strain designation (geographic origin)	Source[a]
Xanthomonas campestris pathovar		
alfalfae (2)	KS (Kansas);	D. L. Stuteville
	FL (Florida);	R. E. Stall
argemones (1)	084-1052 (Florida);	DPI
begoniae (1)	084-155 (Florida);	DPI
campestris (4)	XC1 (Oklahoma);	this study
	084-720,084-809,	
	084-1318 (Florida);	DPI
carotae (3)	G1 (Idaho),G5,G7	
	(California);	R. E. Stall
citri (5+)	X59 (Brazil), X62 (Japan),	
	X69 (Argentina),	
	X70 (Brazil);	E. L. Civerolo
	F11 (Florida);	DPI
cyamopsidis (5)	13D5;	C. I. Kado
	X002, X005, X0016,	
	X0017 (Arizona);	J. Mihail
dieffenbachiae (2)	084-729,084-1373 (Florida);	DPI
esculenti (1)	084-1093 (Florida);	DPI
glycines (3)	B-9-3 (Brazil),1717 (Africa),	
	17915;	W. F. Fett
hederae (3)	084-1789,084-3928,	
	251G (Florida);	DPI
holcicola (2)	Xh66 (Kansas);	L. Claflin
	XH1;	this study
maculifoliigardeniae (2)	084-6006,084-6166	
	(Florida);	DPI
malvacearum-cotton (32)	A,B,E,F,G,H (Oklahoma);	M. Essenberg
	HV25 (Upper Volta),	
	Ch1,Ch2 (Chad),	
	Su2,Su3 (Sudan);	L. S. Bird
	FL79 (Florida);	DPI
	D,M,N,O,U,V,W,X,Y,Z,	
	TX84 (Texas),	
	I,Q,R,S,T (Oklahoma)	
	C,J,K,L (Upper Volta);	this study
malvacearum-hibiscus (8)	X10,X27,X52,X102,	
	X103,X108 (Florida);	A. R. Chase
	083-4344,	DPI
	M84-11 (Florida;	
mangiferaeindicae (1)	084-166 (Florida);	DPI
pelargonii (1)	084-190 (Florida);	DPI
phaseoli (13)	EK11,Xph25,Xpf11	
	(Nebraska);	M. Schuster
	Xpa,Xp11 (Wisconsin);	A. W. Saettler
	82-1,82-2 (Florida);	R. E. Stall
	LB-2,SC-3B (Nebraska);	A. K. Vidaver
	XP2 (New York);	J. A. Laurence
	XP-JL (Kansas);	J. L. Leach
	XP-JF (Missouri),	this study
	XP-DPI;	
pisi (1)	XP1 (Japan);	M. Goto
poinsettiicola (1)	083-6248 (Florida);	DPI
pruni (3)	068-1008,084-1793	
	(Florida);	DPI
	82-1 (Florida);	R. E. Stall
translucens (2)	82-1 (Florida);	R. E. Stall
	XT1;	this study
vesicatoria (5)	E-3,69-13,71-21,	
	82-8,82-23 (Florida);	R. E. Stall
vignicola (7)	A81-331,C-1,CB5-1,	
	Xv19,SN2,432,82-38	
	(Georgia);	R. D. Gitaitis
vitians (3)	084-2057,084-2848,	
	084-4348 (Florida);	DPI
zinniae (1)	084-1944 (Florida);	DPI
unknown (1)	G65	this study
X. albilineans (1)	Xalb (Florida);	M. J. Davis
X. fragariae (1)	Xfra (Florida);	R. E. Stall

[a] DPI = Florida Department of Agricultural and Consumer Services, Division of Plant Industry, Gainesville.

endonucleases, *Eco*RI or *Bam*HI, using manufacturer (Bethesda Research Laboratories, Gaithersburg, MD) specifications. Plasmid DNA fragments were separated by size using agarose gel electrophoresis (0.6% agarose (Sigma Type I:low EEO), 2-5 V/cm) in Tris-acetate buffer (40 mM Tris, 1 mM Na_2EDTA, adjusted to pH 7.6 with glacial acetic acid). Fragments were visualized by ultraviolet irradiation (302 nm) after staining agarose gels in ethidium bromide (0.5 μg/ml). Photographs were taken with Polaroid Type 55 (or Type 57) film using a yellow filter (Tiffen No. 12). All restriction fragment size estimates were based on the relative mobilities of linear DNA fragments using lambda phage DNA digested with *Hind* III as a molecular size standard. Plasmid sizes were estimated by addition of the sized restriction digested plasmid DNA fragments. All plasmid experiments were repeated at least twice for each strain examined.

Cloning of plasmid restriction endonuclease fragments. Plasmid DNA isolations from *X. campestris* were by a modification of either of two alkaline lysis extraction procedures (15,23). The extracted plasmids were purified on CsCl-ethidium bromide gradients by centrifugation at 55,000 rpm in a Beckman VTi65.2 rotor for 17 hr at 20 C. The purified plasmids were digested with the restriction enzyme *Eco*RI. The cloning vector, pUCD5 (5), was digested with *Eco*RI, treated with calf intestinal alkaline phosphatase (Boehringer-Mannheim, Indianapolis, IN), and ligated to the *Eco*RI digested *X. campestris* plasmid fragments with T4 DNA ligase (Bethesda Research Laboratories). The ligation products were transformed into *E. coli* strain ED8767, selecting transformed colonies on Luria-Bertani medium containing ampicillin (50 μg/ml) or kanamycin (30 μg/ml). Selected colonies were analyzed for the vector containing desired cloned DNA fragments. These general cloning procedures are outlined in Maniatis et al (22).

DNA/DNA hybridization. Plasmid DNAs were transferred from agarose gels to nitrocellulose membranes by the method of Southern as described by Maniatis et al (22) and hybridized against radioactively labeled DNA probes. The DNA probes derived from plasmid DNA of *X. campestris* pathovars were either cloned restriction digested DNA fragments of plasmid DNA in the cosmid vector pUCD5, or of the complete *X. campestris* plasmid. DNA probes were labeled in vitro with use of a nick-translation kit (Bethesda Research Laboratories) using ^{32}P-deoxycytidine triphosphate and hybridized against DNA bound to nitrocellulose membranes. The membranes were prehybridized and hybridized in plastic bags at 68 C. After hybridization, membranes were washed once in 2× standard saline citrate (SSC), 0.5% sodium dodecyl sulfate (SDS) and washed once in 2× SSC, 0.1% SDS at ambient temperature, and washed two times in 0.1× SSC, 0.5% SDS at 68 C as described by Maniatis et al (22) for stringent conditions. The membranes were then air-dried and exposed at −80 C to X-ray film (Kodak X-Omat AR) using intensifier screens. Similar methods were used to probe DNA transferred to nitrocellulose using a dot-blot manifold (Schleicher and Schuell Inc., Keene, NH). All hybridization experiments were repeated at least once.

RESULTS

Detection of plasmid DNA. Indigenous, cryptic plasmids were detected in all strains of the following *X. campestris* pathovars: *cyamopsidis*, *dieffenbachiae*, *glycines*, *malvacearum* (cotton), *pelargonii*, *phaseoli*, *pruni*, *vesicatoria*, *vignicola*, and *vitians* (Table 2). Plasmids were not detected in any strains of *X. campestris* pathovars *alfalfae*, *argemones*, *begoniae*, *carotae*, *esculenti*, *holcicola*, *maculifoliigardeniae*, *malvacearum* (hibiscus), *mangiferaeindicae*, *pisi*, *poinsettiicola*, *translucens*, and *zinniae*. Plasmids were found in some, but not all strains of *X. campestris* pathovars *campestris* and *hederae*. Similarly, plasmids were found in all type strains of *X. c.* pv. *citri* (A, B, and C types), but not in all strains of *X. campestris* isolated from leaf spots of citrus in Florida. Not only were four highly polymorphic plasmid variants found in 17 out of 44 Florida strains tested, but Southern hybridization revealed no homology between some of the plasmids (11). Furthermore, there were no similarities in plasmid digestion

patterns between *X. c.* pv. *citri* type A, type B, and any of the Florida citrus leaf spot strains that carried plasmids. These Florida isolates are presumed to be *X. c.* pv. *citri* because they were found on citrus, but they grow well and also cause disease symptoms on kidney bean and alfalfa, thus making their pathovar status questionable (12).

It appeared that plasmid-containing strains of *X. campestris* carried from one to three plasmids based on electrophoresis of extracted plasmid DNA. For example, a majority of the plasmid-containing *X. c.* pv. *malvacearum* strains contained only one plasmid, but some carried two or more. When plasmid-containing strains of *X. campestris* were purified by repeated single-colony isolations, the plasmid DNA content appeared to be stable. However, variation was present in bacterial stocks that were known to have been serially tranferred in agar medium over a period of years.

Restriction endonuclease profiles. Plasmid profiles for *X. campestris* were variable in over 60 different strains tested. Plasmids were placed into classes based on restriction endonuclease (*Eco*RI) digestion profiles on agarose gels. When a different restriction endonuclease (*Bam*HI) was used, the plasmid profiles were placed into the same classes. In all cases, strains that belonged to the same plasmid class also belonged to the same pathovar. There was obvious variability within classes, but there also appeared to be conservation of some DNA fragments of identical sizes (Fig. 1). Undigested plasmids were not reliable for strain classification because several strains had plasmids of apparently identical size, but they were clearly different after digesting the plasmid DNAs with restriction enzymes. By adding up the DNA fragment sizes yielded by restriction digests, plasmids in *X. campestris* were estimated as ranging from about 3 to 200 kb (kilobase pairs) in size. Estimation of some of these sizes was difficult for some strains because of the presence of more than one plasmid.

Plasmid profiles of strains of *X. c.* pv. *cyamopsidis*, *X. c.* pv. *glycines*, *X. c.* pv. *malvacearum*, *X. c.* pv. *phaseoli*, and *X. c.* pv. *vignicola* were compared. Restriction fragment length polymorphism was evident within each of these pathovars. Although more than one plasmid was present in some of these strains, a subset of restriction fragments of similar length and overall pattern appeared to be consistent for strains within a given pathovar (Fig. 2).

TABLE 2. Detection of plasmid DNA in strains of *Xanthomonas*

Bacterium	No. of strains containing plasmids/ No. of strains tested	Pathogenicity[a]
Xanthomonas campestris pathovar		
alfalfae	0/2	P
argemones	0/1	I
begoniae	0/1	I
campestris	2/4	R,I
carotae	0/3	R
citri	17/44	P,R,I
cyamopsidis	5/5	R
dieffenbachiae	2/2	I
esculenti	0/1	I
glycines	3/3	P
hederae	2/3	I
holcicola	0/2	R
maculifoliigardeniae	0/2	I
malvacearum-cotton	32/32	P
malvacearum-hibiscus	0/8	P
mangiferaeindicae	0/1	I
pelargonii	1/1	R
phaseoli	13/13	P
pisi	0/1	R
poinsettiicola	0/1	I
pruni	3/3	I
translucens	0/2	R
vesicatoria	5/5	P
vignicola	7/7	P
vitians	3/3	I
zinniae	0/1	I
X. albilineans	0/1	R
X. fragariae	0/1	I

[a] P = Pathogenicity of strains confirmed on appropriate host, conforms to current available information for particular pathovar. R = Received as named pathogen, appropriate host specificity, and pathovar designation of strain assumed. I = Isolated as a pathogen on host appropriate for designated pathovar; characterization of strain(s) incomplete.

DNA/DNA hybridization. Initial plasmid comparisons were done on strains of *X. c.* pv. *malvacearum*. Whole purified plasmid DNA from *X. c.* pv. *malvacearum* strain X, which contains only one plasmid, was hybridized against *Eco*RI digested plasmid DNAs of other strains of the same pathovar (not shown). This initial comparison demonstrated that the plasmids, although differing slightly in digestion patterns, were quite homologous as the radiolabeled plasmid hybridized to almost all *Eco*RI fragments of the other strains.

A 4.5-kb *Eco*RI plasmid fragment of *X. c.* pv. *malvacearum* strain N was cloned into the vector pUCD5 and used as a hybridization probe against plasmid DNA from other strains of the same pathovar (Fig. 3). This plasmid DNA fragment hybridized to plasmids from all but one strain of *X. c.* pv. *malvacearum*. This probe hybridized to *Eco*RI fragments of equivalent size (lanes B–J, M, and N, Fig. 3) in several other strains of *X. c.* pv. *malvacearum*. Additionally, the probe hybridized to more than one of the *Eco*RI plasmid fragments in these strains, suggesting that some sequences on the cloned DNA are repeated in other parts of the plasmid DNA. The pUCD5 vector alone did not hybridize to any *X. c.* pv. *malvacearum* plasmid fragments. Because pUCD5 is a cosmid vector and contains the *cos* site of lambda phage DNA, hybridization of the vector to the corresponding DNA fragment of the molecular weight marker containing the *cos* site was observed. Plasmid DNA from *X. c.* pv. *malvacearum* strain Su2 did not hybridize to the 4.5-kb probe and did not hybridize to any other plasmid fragments from *X. c.* pv. *malvacearum* strain N. Another *X. c.* pv. *malvacearum* strain (Ch2), which did not have a 4.5-kb *Eco*RI plasmid DNA fragment, did have two other fragments (about 8 and 10 kb) that hybridized strongly to the 4.5-kb probe. In two other *X. c.* pv. *malvacearum* strains (FL79 and TX84), which appeared to have multiple plasmids, the hybridization signal was weak for the 4.5-kb fragments as compared with larger *Eco*RI fragments (about 23 kb).

Similar hybridization studies were done with cloned plasmid fragments from other pathovars. When the plasmid fragments

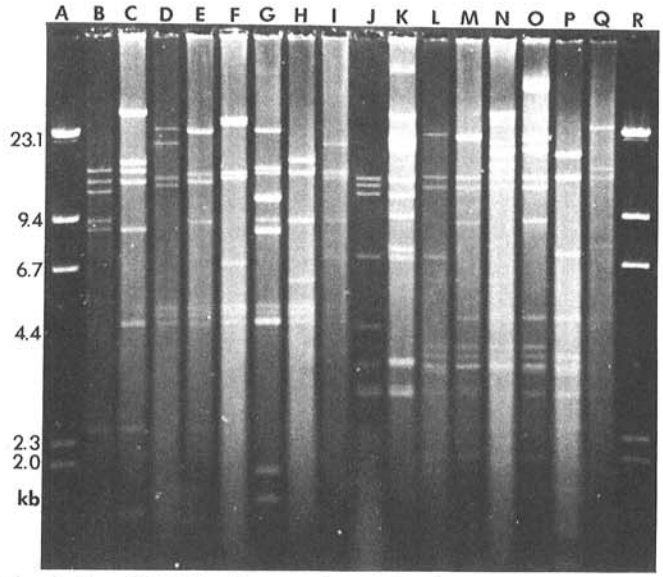

Fig. 1. Plasmid DNAs from strains of *Xanthomonas campestris* pv. *malvacearum* (*Xcm*) digested with restriction endonucleases *Eco*R I (lanes B–I) and *Bam*HI (lanes J–Q). Lanes shown above contain: A and R, lambda *Hind*III; B and J; *Xcm* J; C and K, *Xcm* N; D and L, *Xcm* H; E and M, *Xcm* V; F and N, *Xcm* Z; G and O, *Xcm* Q; H and P, *Xcm* X; and I and Q, *Xcm* D.

selected were smaller, they were much more specific. Two different probes were constructed from 2.0- and 2.3-kb *Eco*RI plasmid fragments derived from *X. c.* pv. *phaseoli* (strain XP2). When hybridized against *X. c.* pv. *cyamopsidis*, *X. c.* pv. *glycines*, *X. c.* pv. *phaseoli*, and *X. c.* pv. *vignicola*, each of these probes only hybridized to *X. c.* pv. *phaseoli* (Fig. 4). The 2.3-kb plasmid probe hybridized to similar-sized fragments in other *X. c.* pv. *phaseoli* strains, including *X. c.* pv. *phaseoli* var. *fuscans* (strains SC3-B (not shown) and Xpf11). These *X. c.* pv. *phaseoli* var. *fuscans* strains differ from typical *X. c.* pv. *phaseoli* strains in that they produce an extracellular dark brown, melanin-like pigment in culture; otherwise, they are considered similar. However, the 2.0-kb plasmid probe did not hybridize to the *X. c.* pv. *phaseoli* var. *fuscans* strains, which did not have the corresponding 2.0-kb *Eco*RI fragment in their plasmid profile [lane C, Fig. 4, and strain SC3-B (not shown)]. Repeated hybridizations with Southern transfers containing these strains had the same results.

Dot-blot hybridization. DNA probes were also hybridized against total DNA of other *X. campestris* pathovars fixed onto a nitrocellulose membrane by use of a dot-blot manifold apparatus. Radiolabeled total plasmid DNA from *X. c.* pv. *malvacearum* strain N, which carries two plasmids, hybridized to DNA of 13 out of 23 *X. campestris* pathovars tested (Table 3). Of these 13, seven cross-hybridized to the 4.5-kb subcloned fragment of *X. c.* pv. *malvacearum* strain N. Plasmids were not detected in some of the pathovars that hybridized to the probe. A 2.3-kb subcloned plasmid fragment from *X. c.* pv. *vignicola* hybridized to only six of the 23 pathovars tested. Plasmids were present in all six of those pathovars that hybridized to the probe. A 2.3-kb cloned plasmid fragment of *X. c.* pv. *phaseoli* hybridized strongly to other strains of the same pathovar, and weakly to two of three different *X. c.* pv. *citri* strains tested. The *X. c.* pv. *phaseoli* probe appears to have hybridized to chromosomal DNA of *X. c.* pv. *citri* in this case, as the probe did not hybridize against Southern transfers of *Eco*RI digested plasmid fragments of *X. c.* pv. *citri* strains (not shown).

DISCUSSION

The results suggest that there is extensive conservation of plasmid DNA sequences (as represented by conserved restriction fragments) within, but not usually among pathovars of *X. campestris*. Plasmid DNA fragment patterns will be identical if there is no rearrangement of the DNA sequence at the restriction enzyme recognition site (a six-base pair sequence for *Eco*RI and *Bam*HI), if no new restriction fragments are created within the fragment, and if there are no major additions or deletions causing a change in fragment size. Given these possibilities, it was surprising to find so little restriction fragment length polymorphism of plasmids within a pathovar, especially when strains obtained from different continents were compared. Southern hybridization analyses confirmed that plasmid DNA fragments of similar size

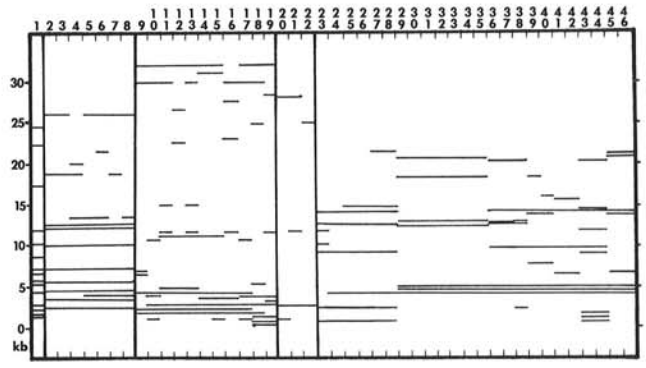

Fig. 2. Graphic representation of plasmid restriction fragment profiles for *Xanthomonas campestris* pvs. *cyamopsidis* (*Xcc*; lane 1), *vignicola* (*Xcv*; lanes 2–8), *phaseoli* (*Xcp*; lanes 9–17), *phaseoli* var. *fuscans* (*Xcpf*; lanes 18–19), *glycines* (*Xcg*; lanes 20–22), and *malvacearum* (*Xcm*; lanes 23–46) digested with *Eco*RI. Lanes shown above contain 1, *Xcc* 13D5; 2, *Xcv* SN2; 3, *Xcv* A81-331; 4, *Xcv* C-1; 5, *Xcv* CB5-1; 6, *Xcv* Xv19; 7, *Xcv* 82-38; 8, *Xcv* 432; 9, *Xcp* EK11; 10, *Xcp* 82-2; 11, *Xcp* Xpa; 12, *Xcp* Xp11; 13, *Xcp* XP2; 14, *Xcp* XP-JF; 15, *Xcp* XP-DPI; 16, *Xcp* Xph25; 17, *Xcp* 82-1; 18, *Xcpf* Xpf11; 19, *Xcpf* SC-3B; 20, *Xcg* B-9-3; 21, *Xcg* 17915; 22, *Xcg* 1717; 23, *Xcm J*; 24, *Xcm L*; 25, *Xcm C*; 26, *Xcm O*; 27, *Xcm K*; 28, *Xcm N*; 29, *Xcm A*; 30, *Xcm B*; 31, *Xcm E*; 32, *Xcm F*; 33, *Xcm G*; 34, *Xcm H*; 35, *Xcm S*; 36, *Xcm W*; 37, *Xcm I*; 38, *Xcm V*; 39, *Xcm D*; 40, *Xcm M*; 41, *Xcm X*; 42, *Xcm U*; 43, *Xcm Q*; 44, *Xcm R*; 45, *Xcm Y*; and 46, *Xcm Z*. DNA fragment sizes are represented by a linear scale, whereas migration of DNA fragments under electrophoresis conditions approximates a logarithimic scale that is inversely proportional to the molecular weight of the DNA fragment.

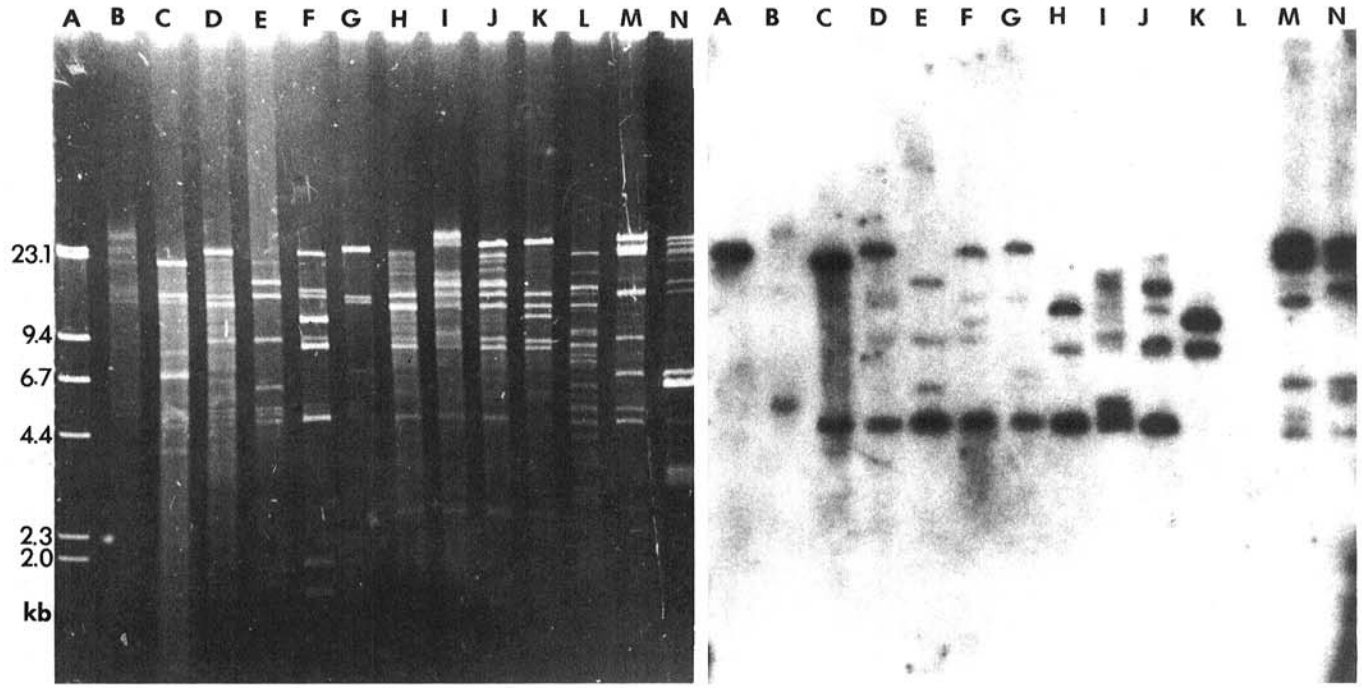

Fig. 3. Plasmid DNAs from strains of *Xanthomonas campestris* pv. *malvacearum* (*Xcm*) digested with restriction endonuclease *Eco*RI (left) and autoradiograph of plasmid DNAs probed with a clone containing a 4.5-kb *Eco*RI plasmid fragment from *Xcm* strain N (right). Lanes shown above contain: A, lambda *Hind*III; B, *Xcm H*; C, *Xcm W*; D, *Xcm V*; E, *Xcm X*; F, *Xcm Q*; G, *Xcm Y*; H, *Xcm L*; I, *Xcm N*; J, *Xcm* HV25; K, *Xcm* Ch2; L, *Xcm* Su2; M, *Xcm* FL79; and N, *Xcm* TX84.

were in fact highly homologous. For example, *X. c.* pv. *malvacearum* strain N, isolated in North America, had a restriction digest pattern identical to one of the African strains (K) (Fig. 2). All North American strains except strain N form a distinctive pattern subgroup and the African strains form a somewhat different subgroup. These data suggest that either all the African strains are derived from strain N or that strain N was introduced to North America from Africa. The latter possibility seems more likely because the African strains were isolated from more than one location in Africa. The conservation of overall restriction fragment profiles of plasmids from geographically isolated populations and the extent of homology seen in Southern hybridizations strongly suggests that plasmid sequences are both highly conserved and stable.

TABLE 3. Hybridization of radiolabeled plasmid probes to total DNA of pathovars of *Xanthomonas campestris* and one other *Xanthomonas* species

Bacterium (no. tested)	Probes[a]			
	N80	N4.5	V2.3	P2.3
Xanthomonas campestris pathovar				
alfalfae (1)	+	−	−	−
argemones (1)	−	−	−	−
begoniae (1)	−	−	−	−
campestris (1)	−	−	−	−
carotae (1)	+	−	−	−
citri (3)	+	+/−[b]	+/−[b]	+/−[b]
cyamopsidis (1)	+	+	+	−
dieffenbachiae (1)	−	−	−	−
esculenti (1)	−	−	−	−
glycines (1)	+	−	+	−
hederae (1)	+	−	−	−
holcicola (1)	+	−	−	−
maculifoliigardeniae (1)	−	−	−	−
malvacearum-cotton (6)	+/−[c]	+/−[c]	+	−
malvacearum-hibiscus (2)	−	−	−	−
mangiferaeindicae (1)	−	−	−	−
phaseoli (1)	+	+	−	+
poinsettiicola (1)	−	−	−	−
pruni (1)	+	+	−	−
translucens (1)	+	−	−	−
vesicatoria (1)	+	+	+	−
vignicola (1)	+	+	+	−
vitians (1)	−	−	−	−
zinniae (1)	−	−	−	−
X. albilineans (1)	−	−	−	−

[a] + = hybridization observed, − = no hybridization observed. N80 = plasmid DNA derived from *X. c.* pv. *malvacearum* strain N (about 80 kb); N4.5 = cloned *Eco*RI plasmid fragment (4.5 kb) from strain N; V2.3 = cloned *Eco*RI plasmid fragment (2.3 kb) from *X. c.* pv. *vignicola* strain SN2; P2.3 = cloned *Eco*RI plasmid fragment (2.3 kb) from *X. c.* pv. *phaseoli* strain XP2.

[b] Strain FL-11 was negative, only plasmid DNA used.

[c] Strain S2 was negative.

Conversely, it was surprising to find so much polymorphism of plasmids between pathovars. Homology among plasmids in different strains of one pathovar of *Pseudomonas syringae* (pv. *glycinea*) has been reported (6). However, identical plasmid profiles were found present in more than one pathovar of *P. syringae* (25). In the present study, similar plasmid profiles were not found in more than one pathovar. In addition, cloned plasmid fragments were identified that failed to hybridize to plasmids of different pathovars. For example, a cloned 2.3-kb plasmid fragment of *X. c.* pv. *phaseoli*, which hybridized to all strains of that pathovar, failed to hybridize to total DNAs of 22 other pathovars. This DNA fragment is highly conserved and is apparently pathovar-specific, with one exception (Table 3). Plasmid homology between *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* was revealed by hybridization that was not apparent by restriction digest patterns. There were distinct differences in plasmid digestion patterns of *X. c.* pv. *phaseoli* var. *fuscans* in comparison with typical *X. c.* pv. *phaseoli* strains. The fact that both *X. c.* pv. *phaseoli* and *X. c.* pv. *phaseoli* var. *fuscans* have an identical host range suggests that the homologous plasmid DNA regions that are conserved within some pathovars may encode host range specificity functions. Such functions have been described on plasmids within the Rhizobiaceae (19,20). This is a testable hypothesis that needs further experimental support.

In *X. c.* pv. *malvacearum*, plasmids were found in all 32 strains isolated from cotton and no plasmids were found in the eight strains isolated from hibiscus. Cotton and hibiscus each belong to the same plant family (Malvaceae), hence the *X. c.* pv. *malvacearum* designation. Atypical symptoms of cotton blight could be artificially produced in cotton using syringe inoculations with hibiscus strains and in hibiscus using cotton strains. The ability of these strains to be pathogenic on both hosts under natural conditions has not been established to our knowledge. Plasmid

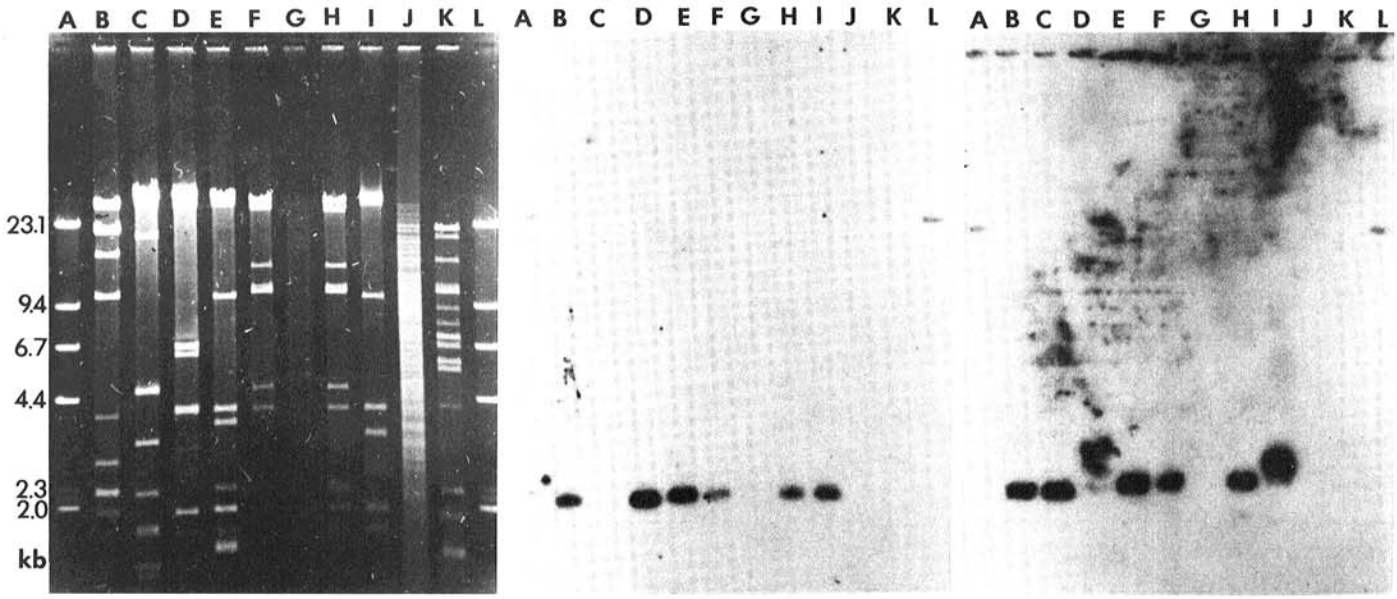

Fig. 4. Plasmid DNAs from strains of *Xanthomonas campestris* pvs. *phaseoli* (*Xcp*; lanes B, D–I), *phaseoli* var. *fuscans* (*Xcpf*; lane C), and *cyamopsidis* (*Xcc*; lane K) digested with restriction endonuclease *Eco*RI (left) and autoradiographs of plasmid DNAs probed with a clone containing a 2.0-kb *Eco*RI fragment (center) and a 2.3-kb *Eco*RI plasmid fragment (right) from *Xcp* strain XP2. Lane J contains chromosomal DNA of *X. campestris* pv. *alfalfae* (*Xca*) digested with *Eco*RI. Lanes shown above contain: A and L, lambda *Hind*III; B, *Xcp* Xph25; C, *Xcpf* Xpf11; D, *Xcp* EK11; E, *Xcp* 82-1; F, *Xcp* Xpa; G, *Xc* G65; H, *Xcp* Xp11; I, *Xcp* XP-JF; J, *Xca* FL; and K, *Xcc* 13D5. *Xc* G65 is a strain isolated from bean, which was determined to be nonpathogenic and contains no plasmid.

DNAs from cotton strains cross-hybridized with one another in Southern analyses, indicating extensive homology among the plasmids. As with *X. c.* pv. *phaseoli*, the cotton strains of *X. c.* pv. *malvacearum* appeared to carry highly conserved plasmid DNA sequences (similar in restriction digest sizes and by Southern analyses), which are unique to strains that have a host range on cotton. Plasmid DNA from the cotton strain (N), when radiolabeled, did not hybridize to chromosomal DNA derived from the hibiscus strain. Based on our limited pathogenicity tests and on the absence of plasmid DNA sequences in the hibiscus strains, there may be justification to differentiate the cotton and hibiscus strains into different pathovars.

Although some cross-hybridization between plasmids of strains from different pathovars was detected by dot-blot analyses, strains were readily differentiated by restriction digest profiles of the plasmid DNAs and by hybridization with selected DNA probes to identifiable restriction digested DNA fragments. Cross-hybridization may be the result of repetitive DNA sequences, insertion elements, or of conserved DNA sequences that are important for the stable maintenance of plasmids in *X. campestris*. Examples of plasmid functions that might be conserved are those involved with replication, incompatibility, or other host dependent factors. In some instances plasmid DNAs hybridized to total DNA of pathovars in which no plasmids were detected. This suggests that some sequences encoded on plasmids in some pathovars are located on chromosomes in other pathovars. It is also possible that those plasmids may integrate into the bacterial chromosome and excise again, in a manner similar to those in *P. syringae* pv. *phaseolicola* (26,29).

With interest in developing rapid diagnostic methods to identify bacterial pathogens, it is possible that the combined usage of plasmid restriction digest profiles and of plasmid DNA probes may be sufficient for the identification of some pathovars of *X. campestris*. This would require that a plasmid be stably associated with a given pathovar, that plasmid profiles for specific pathovars were known, and that an appropriate DNA probe consisting of conserved and unique DNA sequences were available. Of the few DNA probes constructed, it was apparent that plasmid DNA sequences were highly conserved within the pathovars studied. In some instances the DNA probes may prove sufficient for pathovar identification, provided they are extensively tested. This apparent stability of the plasmids provides a natural genetic marker that can be strain specific and perhaps useful in epidemiological investigations. In addition to aiding the identification for some pathovars of *X. campestris*, these observations may have taxonomic significance in differentiating these pathogens.

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