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Clonal Population Structure of *Xanthomonas campestris* and Genetic Diversity Among Citrus Canker Strains

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Restriction fragment length polymorphism (RFLP) analyses of the bacterial plant pathogen *Xanthomonas campestris* reveal a generally clonal population structure, with each pathovar comprised of one or more clonal groups (Lazo, G. R., Roffey, R., and Gabriel, D. W. 1987, Int. J. Syst. Bacteriol. 37:214-221). Those pathovars with a narrow host range exhibit little polymorphism within clonal groups, and those with a wide host range exhibit moderate polymorphism. The population structure is likely the result of linkage disequilibrium between the RFLP marker loci and highly selected virulence factors. RFLP analyses of *X. c.* pv. *citri* strains revealed three clonal groups. One of the clonal groups corresponded to the previously described *X. c.* pv. *citri* "A" strains; another included the previously described "B," "C," and "Mexican bacteriosis" strains. The third group consisted of strains recently discovered in Florida citrus nurseries. These showed moderate polymorphism between strains of the group, a characteristic of strains included in pathovars with a wide host range. Furthermore, strains of this new group appeared to be related to *X. c.* pv. *alfalfae* by RFLP analyses, and strains of one subclone of the group had a host range similar to that of *X. c.* pv. *alfalfae* in limited pathogenicity tests. We propose that: 1) diverse *X. campestris* strains may have been selected on citrus because they independently carried genes with selective value on these hosts; 2) related strains may have different combinations of virulence factors conditioning host range specificity; and 3) RFLP analyses are a generally useful method to indicate wide versus narrow host range specificity of pathogenic populations without pathogenicity tests.

Additional key words: genetic structure, host range, pathovars, polymorphism, RFLP, specificity.

Microbial plant pathogens are often classified below the species rank into pathovars, biovars, or forma speciales on the basis of limited pathogenicity tests. Such classifications are suspect because they are based on a single characteristic and that characteristic is the result of an interaction with another organism. The phenotype of the interaction may depend as much on the host as on the organism being classified. *Xanthomonas campestris* strains are always found in association with plants and are differentiated into at least 125 pathovars (Bradbury 1984). Although the species as a whole is pathogenic to a broad range of plant hosts, strains isolated from one host species are usually thought to be pathogenic to just that host species and perhaps a limited number of others. Exactly how many other plant species may serve as host to a given strain is difficult to determine, and there are no well-accepted physiological or biochemical tests that consistently distinguish *X. campestris* pathovars. Ten of the 125 *X. campestris* pathovars are listed as having a host range on plants in different families (Bradbury 1984). The rank of pathovar is often assigned on the basis of the "host from which first isolated" convention (Starr 1983). Such classifications may be arbitrary (Keyser *et al* 1982) and imply a higher degree of host specificity and uniformity than may exist among strains within pathovars.

A survey of 93 strains representing 26 pathovars of *X. campestris* by restriction fragment length polymorphism (RFLP) analyses revealed that the population structure of this species is essentially clonal (Lazo *et al* 1987). Although the data base is limited, most pathovars examined are comprised of one to several clonal groups, and the highest level of polymorphism is observed within pathovars having the broadest host range. Presumably, the clones are selected from a variety of genetic backgrounds, and fitness on any one host is a function of one or more highly selected virulence factors. Therefore, it would not be expected *a priori* that a wide host range clone would be an equally effective parasite on all hosts or that different clones would be equally effective on a common host. In fact, strains from different pathovars usually induce reproducibly different disease symptoms in common hosts. For example, *X. c.* pv. *phaseoli* and *alfalfae* both have a host range on *Phaseolus vulgaris* (common bean), but *X. c.* pv. *phaseoli* appears much more pathogenic (in terms of water-soaking and lesion spread) on this host (unpublished observations). If strains of *X. c.* pv. *alfalfae* had been originally isolated from bean and the host range on alfalfa was unknown, these strains would be classified as *X. c.* pv. *phaseoli*. They would, however, be distinguishable as a distinct "form" by pathogenicity tests and as a separate clonal "group" by RFLP analyses. If moderate levels of polymorphism were observed among strains within the group, a wider host range would be suspected. The purpose of this study was to characterize *X. campestris* strains found in Florida and recently classified as *X. c.* pv. *citri* by RFLP analyses and, if indicated, by pathogenicity tests.

Strains of *X. c.* pv. *citri* were determined to be the causal agent of the recent citrus canker epidemic in Florida nurseries (Schoulties *et al* 1987). Once this was determined,

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state and federal regulatory agencies implemented an eradication program that resulted in the destruction of nearly 20 million citrus nursery trees (Schoulties *et al* 1987). Although a range of pathogenic variation was observed among these *X. c.* pv. *citri* strains, they have been considered as a group to cause a single new "E" form of citrus canker, distinct from the previously described A, B, or C disease forms (Schoulties *et al* 1987, Hartung and Civerolo 1987). Plasmid hybridization (Lazo and Gabriel 1987) and total DNA restriction analyses (Hartung and Civerolo 1987) reveal that not only are the new strains different from previously described strains, but some of the new strains are different from others. We report here that: 1) RFLP analyses characterized the E strains of canker as a moderately polymorphic group, distinguishable from previously described *X. c.* pv. *citri* forms A, B, and C; 2) within the E group, at least five unique strains and two highly clonal subgroups were identified and distinguished by RFLP analyses; 3) strains of the E group resembled *X. c.* pv. *alfalfae* strains by RFLP analyses; and 4) in limited pathogenicity tests, one of the E subclones, but not the other, exhibited a host range specificity similar to that of some available *X. c.* pv. *alfalfae* strains.

MATERIALS AND METHODS

Bacterial strains. The sources and references for all *X. c.* pv. *citri* strains used in this study are shown in Table 1. All citrus canker samples from Florida were isolated, identified as *X. campestris*, and confirmed to be pathogenic by the Florida Department of Agriculture, Division of Plant Industry (DPI). These isolates were maintained by DPI and rechecked for pathogenicity and species identification. All work in Florida with these bacterial strains was done at the BL-2 containment level. All citrus canker strains from sources other than Florida were maintained and provided by Dr. Ed Civerolo at the USDA-ARS Fruit Laboratory in Beltsville, MD. All work with these strains in Maryland was done at the BL-1 containment level. All other *X. campestris* strains used in this study were previously reported (Lazo *et al* 1987), except for four additional *X. c.* pv. *alfalfae* strains (L-142, L-234, L-334, and L-676), which were supplied by Dr. F. Lukezic of Pennsylvania State University.

DNA manipulations. All DNA extractions, restriction digests, agarose gel electrophoreses, Southern blots, hybridizations, autoradiographs, and densitometry scans were exactly as previously described (Lazo *et al* 1987). Four DNA probes were used: pUFT-1 (synonym XCT-1), pUFT-11 (synonym XCT-11), pUFT-41, and pUFA-704, containing 30 kb, 37 kb, 36 kb, and 32 kb chromosomal DNA inserts, respectively, cloned into the vector pUCD-5 (Close *et al* 1984) as previously described (Lazo *et al* 1987). The pUFT inserts were cloned from *X. c.* pv. *citri* strain 3401, and the pUFA insert was cloned from *X. c.* pv. *malvacearum* (Gabriel *et al* 1986).

In brief, the procedure was to extract total DNA from each strain, quantitate the DNA by spectrophotometry, digest the DNA overnight with approximately one unit of restriction endonuclease (*Eco*RI or *Bam*HI) per μg of DNA and separate approximately 8 μg of DNA from each strain by size on 0.65% agarose gels. The DNAs were visualized by staining with ethidium bromide, exposing the gel to ultraviolet light, and photographed. The total DNA extracts, now separated on the gels, were then denatured, transferred to nitrocellulose or nylon membranes, and incubated with ^{32}P-labeled, denatured probe. DNA bands from each strain which are homologous to the probes will hybridize and retain the probe under the high stringency conditions used (eg., 68°C, 15 mM NaCl, and 1.5 mM sodium citrate in water). Hybridizing bands were revealed by autoradiography on X-ray film.

Similarity coefficients. Band locations on each track were determined by using a Gilford Response II integrating spectrophotometer equipped with an autoradiograph scanner. Comparisons were made of tracks run on the same gel. Autoradiographs were scanned, and the spectrophotometer automatically determined the origin plus the 11 most strongly hybridizing DNA fragments by peak height. In a few cases, tracks that were underloaded were amplified by increasing the photometer absorbance range. The threshold for a strongly hybridizing fragment peak was a difference between the peak height and the adjacent valley that exceeded 5% of the photometer absorbance range in all cases (Seadler 1984). Peak locations of the bands were compared manually by aligning the origins of the printed autoradiograph scans over a light box. The computer-selected, strongest hybridizing bands from each track were matched (± 1 mm absolute location on a 150-mm scale) against any bands (major or minor) appearing in the other track, and vice versa. The number of major bands that

Table 1. Isolates of *Xanthomonas campestris* pv. *citri* classified by pathogenicity, and restriction fragment length polymorphism (RFLP) pattern group

Strain[a]	Origin	Source[b]	Pathogenic Form[c]	Clonal Group[d]
XC59*	Brazil	E.L.C.	A	A
XC62*	Japan	E.L.C.	A	A
XC63*	Japan	E.L.C.	A	A
XCl43	Reunion Island	E.L.C.	A	A
XCl625	Reunion Island	E.L.C.	A	A
X86-3210	St. Petersburg, FL	J.W.M.	A	A
X86-3213	Holmes Beach, FL	J.W.M.	A	A
XC64*	Argentina	E.L.C.	B	B
XC69*	Argentina	E.L.C.	B	B
XCl4B*	Mexico	E.L.C.	Mexican bacteriosis	B
XC70*	Argentina	E.L.C.	C	B/C
X85-4600	Haines City, FL	J.W.M.	E1	E1
P84-3401	Haines City, FL	J.W.M.	E1	E1
P84-0329	Haines City, FL	J.W.M.	ND	E1
P84-3048	Polk County, FL	J.W.M.	E2	E2
P84-3162	Highlands County, FL	J.W.M.	E2	E2
P84-3294	Felda, FL	J.W.M.	E2	E2
P84-3328	LaBelle, FL	J.W.M.	ND	E2
X85-5436	Arcadia, FL	J.W.M.	E2	E2
X85-6774	Bradenton, FL	J.W.M.	ND	E3
X85-0634	Valrico, FL	J.W.M.	ND	E4
X85-6260	DeSoto City, FL	J.W.M.	ND	E5
X85-6572	Berea, FL	J.W.M.	ND	E6
X85-7364	Wahneta, FL	J.W.M.	ND	E7

[a] Strains marked with asteriks are considered type strains for the form.
[b] E.L.C. strains (Hartung and Civerolo 1987) were provided by Dr. E. L. Civerolo, USDA-SEA-ARS, Beltsville, MD 20705. J.W.M. strains are author's lab strains, this study.
[c] Pathogenicity groups A, B, C, and Mexican bacteriosis have been previously defined (Stall and Seymour 1983). Pathogenicity group E2 is defined by strains exhibiting moderate pathogenicity on alfalfa and citrus. Pathogenicity group E1 is defined by a moderate level of pathogenicity on citrus and no pathogenicity on alfalfa. ND = not determined.
[d] RFLP groups are defined by identical (or nearly so) banding patterns, regardless of the probe or restriction enzyme used. Strains of the E group are all moderately related.

matched and the total number of major bands in each pairwise comparison were tallied. Comparative data from each probe/enzyme combination were totaled, and similarity coefficients between two strains were calculated by using the following formula:

$$F = (n_{xy} + n_{yx}) / (n_x + n_y)$$

where n_x and n_y are the numbers of major fragments in strains X and Y, respectively, n_{xy} is the number of major fragments in strain X that match any fragments in strain Y, and n_{yx} is the number of major fragments in strain Y that match any fragments in strain X.

Pathogenicity tests. Cultures of *X. campestris* grown for 24–48 hr at 28°C on lima bean agar (Difco) plates were suspended in sterile tap water, adjusted to approximately 10^8 cfu/ml ($A_{600} = 0.1$), and dilutions ranging from 10^3 to 10^8 cfu/ml were inoculated into plants by syringe injections. These experiments were performed three times with selected strains under double-blind conditions. In these experiments, all cultures, including the positive and negative controls, were coded, and pathogenicity ratings were made on a relative scale of 0–4. In later experiments, the cultures were labeled. All cultures were tested at least twice. For citrus, inoculations of 6–8 spots per tender, newly flushed leaves of Duncan grapefruit or Swingle citrumelo were performed; tuberculin syringes were used to infiltrate the spongy mesophyl. For alfalfa, inoculations of 1–2 spots per leaf of cultivar Florida 77 were similarly performed.

To guard against the possibility of contamination or mixed cultures giving false results in the host range tests, most *X. c.* pv. *citri* strains tested and all *X. c.* pv. *citri* E2 strains were reisolated from inoculated plants, single-colony purified, and inoculated onto citrus plants to confirm pathogenicity on citrus. Furthermore, purified colonies from *X. c.* pv. *citri* E2 and several other *X. c.* pv. *citri* strains inoculated onto alfalfa plants were strain-checked by RFLP analyses.

RESULTS AND DISCUSSION

The proportion of DNA fragments (F) shared by any two strains is expected to be positively correlated with their degree of genetic relatedness, and may be estimated from RFLP data using the formula:

$$\hat{F} = 2n_{xy} / (n_x + n_y)$$

where n_x and n_y are the numbers of fragments in strains X and Y, respectively, and n_{xy} is the number of fragments shared by the strains (Nei and Li 1979). When the number of hybridizing bands on a blot was few (no evidence of repeated sequence hybridization or partial digests), this estimator appeared to discriminate well between pathovars. In practice, however, certain probes hybridized against a large number of fragments from strains of certain pathovars, making it difficult to discriminate valid from spurious matching fragments and increasing the F values of those tracks with the highest total numbers of fragments. Furthermore, because we simply added the data from each probe/enzyme combination, those blots with the most bands influenced the combined totals more than blots with only a few bands. One alternative was to compare only the strongest hybridizing bands from each track, but this method resulted in a loss of data. Another alternative was to calculate the similarity coefficients for each blot and then average the combined data, but this method of calculation gave undue weight to blots with few bands.

The method we chose was to compare the strongest hybridizing bands from each track against any bands appearing in the other track, and vice versa. This method essentially provides a way to give double weight to heavily hybridizing bands that match other heavily hybridizing bands, and single weight to heavily hybridizing bands that match lighter hybridizing bands. Similarity coefficients were estimated by using the following formula, which we propose as an alternative estimator of F:

$$\hat{F} = (n_{xy} + n_{yx}) / (n_x + n_y)$$

where n_x and n_y are the numbers of major fragments (not total fragments) in strains X and Y, respectively, n_{xy} is the number of major fragments in strain X that match any fragment (major or minor) in strain Y, and n_{yx} is the number of major fragments in strain Y that match any fragment in strain X.

Comparisons of RFLPs of previously described *X. c.* pv. *citri* strains (Stall and Seymour 1983), and new *X. c.* pv. *citri* E strains are shown in Figure 1 and quantified in Table 2. Strain distinctions based on RFLP groups corresponded to forms based on pathogenicity (Table 1). Different strains of disease form A, regardless of source, were highly clonal at the loci examined. As expected from previous studies comparing RFLP groups and pathogenicity forms of other pathovars (Lazo *et al* 1987), strains of clonal group A found in Florida exhibited pathogenic symptoms identical to those described for other A forms from Reunion Island, Brazil, or Japan. Similarly, and confirming a previous report (Hartung and Civerolo 1987), strains of disease form B and a strain causing Mexican bacteriosis were genetically very similar, suggesting that these are clonally related. The one extant strain of disease form C (XC70) appeared to be related to the B clonal group (up to 64% homology) by RFLP analyses, which is consistent with serological tests on these same strains (Civerolo and Fan 1982). The relative lack of polymorphism among strains of the A and B groups indicated a narrow host range, and there is no evidence of a host other than citrus for these strains. It is unlikely that the B strains are attenuated forms of an A strain, as suggested (Aubert *et al* 1982), because these *X. c.* pv. *citri* groups did not appear to be clonally related. Taken together, these results provide evidence that strains of *X. c.* pv. *citri*, described prior to the 1984 Florida epidemic, form two

Table 2. Similarity coefficients of the combined data from Figure 1, expressed as percent

	A							B		MX	C	E	
	59	**62**	**63**	**143**	**1625**	**3210**	**3213**	**64**	**69**	**14B**	**70**	**3401**	**3048**
59	···	**100**	**96**	**100**	**96**	**89**	**83**	13	21	18	15	17	24
62		···	**96**	**100**	**96**	**89**	**83**	13	21	18	15	17	24
63			···	**96**	**93**	**86**	**89**	16	20	15	17	28	29
143				···	**96**	**89**	**83**	13	21	18	15	17	24
1625					···	**93**	**87**	19	13	24	11	17	17
3210						···	**93**	19	17	29	23	28	26
3213							···	12	9	17	16	24	26
64								···	**100**	**95**	**64**	25	32
69									···	**97**	**49**	29	38
14B										···	**41**	31	29
70											···	9	24
3401												···	**40**
3048													···

distinct clonal groups: the A group and the B/C/Mexican bacteriosis group.

At least seven different Florida nursery strains or strain groups (*X. c.* pv. *citri* E form [Hartung and Civerolo 1987]) were found to be moderately related by RFLP analyses (Tables 1–3 and Fig. 2; some data not shown). Of these, strains designated as belonging in clonal subgroups E-1 and E-2 were independently isolated at several different nurseries; strains within each respective subgroup were identical by RFLP analyses (data not shown). We interpreted the moderate level of polymorphism found among strains exhibiting similar field symptoms as indicating a wider host range than that of citrus alone.

RFLP comparisons of the E strains with those from strains of 26 other pathovars of *X. campestris* revealed moderate similarity to strains of *X. c.* pv. *alfalfae, phaseoli, cyamopsidis,* and *dieffenbachiae* (Lazo *et al* 1987). A representative sampling of these strains, a few others that attack leguminous hosts, and *X. c.* pv. *malvacearum* were analyzed together, using two enzymes and two probes, and are presented in Figure 2. In Table 3 are similarity coefficients calculated from the combined data in Figure 2. Similarity coefficients of strains considered highly clonal (e.g., *X. c.* pv. *phaseoli*) ranged from 74 to 96%. Similarity coefficients of strains considered moderately clonal (e.g., the E group) ranged from 54 to 63%. Interestingly, the two strains chosen of *X. c.* pv. *alfalfae* exhibited only 43% correspondence in banding pattern and appeared to be more related to some *X. c.* pv. *citri* strains (50–68%) than to each other.

The congruence of the RFLP banding patterns of the E strains and *X. c.* pv. *alfalfae* strains led to the double-blind pathogenicity tests of these strains on alfalfa and citrus plants. Coded samples at 10^3, 10^5, and 10^8 cfu per milliliter of water were injected into plants and relative pathogenic responses were recorded. Selected strains were reisolated from citrus and checked by RFLP analyses for the possibility of contamination. These experiments were repeated at least two more times for some strains, but not under blind testing conditions. Pathogenic variability on

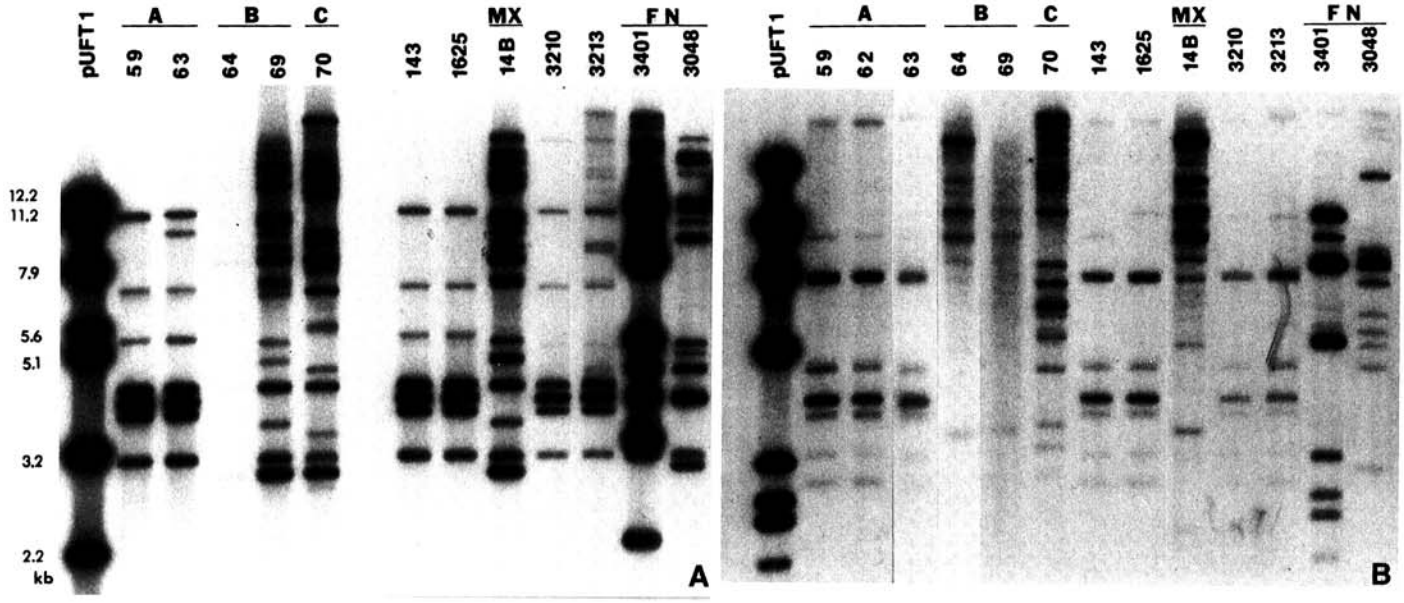

Fig. 1. *X. campestris* pv. *citri* total DNAs extracted from isolates described in Table 1, digested with restriction enzymes, and probed with cosmid clone pUFT1. **A,** DNAs cut with *Eco*RI. **B,** DNAs cut with *Bam*HI. Type strains A, B, C, and Mexican bacteriosis (MX) are labeled, as are two FN (Florida Nursery) or E strains. Isolate 3401 is of the E-1 group. Isolate 3048 is of the E-2 group.

Table 3. Similarity coefficients of the combined data from Figure 2, expressed as percent

					Xanthomonas campestris pv. *phaseoli*				*X. c.* pv. *citri* E			*X. c.* pv. *alfalfae*			
	mal	**dif**	**gly**	**cym**	**ph8**	**ph5**	**ph3**	**ph2**	**6572**	**6774**	**3401**	**alfF**	**alfK**	**pis**	**vig**
mal	...	19	36	28	26	22	22	28	22	22	21	17	18	9	12
dif		...	16	25	20	24	22	16	43	46	45	49	46	15	15
gly			...	23	20	12	15	15	26	24	24	17	19	18	18
cym				...	17	25	18	14	35	43	47	35	29	7	21
ph8					...	**91**	**88**	**96**	24	20	10	14	17	2	38
ph5						...	**90**	**74**	22	28	10	16	16	11	24
ph3							...	**85**	17	23	24	20	20	2	32
ph2								...	26	22	13	15	17	6	27
6572									...	**63**	**54**	**50**	**53**	4	18
6774										...	**55**	**53**	**68**	17	19
3401											...	**71**	**45**	16	18
alfF												...	**43**	9	10
alfK													...	8	32
pisi														...	17
vig															...

citrus between different *X. c.* pv. *citri* strains was often difficult to assay, but was reproducible. Variation was observed in phenotypic reactions over time, between plants, and between leaves on the same plant. The most reliable comparisons were of strains inoculated at the same concentration on opposite sides of the same leaf. In terms of pathogenic symptoms observed in greenhouse tests, strains of the A group appeared much more pathogenic than the E strains. At least some strains of the E-1 group appeared slightly more pathogenic on citrus than the E-2 strains tested. When citrus leaf disks were cut from the plant, placed on water agar, and inoculated, E-2 strains appeared slightly more pathogenic than E-1 strains in repeated experiments. Comparative pathogenicity tests under greenhouse or laboratory conditions may be artifactual (for example, see Yuen *et al* 1987), because symptom expression by the host under these conditions may be a poor indicator of parasitic fitness (reproductive capacity) in field situations.

On alfalfa cultivar Florida 77, the A and E-1 strains tested were asymptomatic, whereas all E-2 strains appeared moderately pathogenic and were not distinguished from some *X. c.* pv. *alfalfae* strains. The E-1 and E-2 clones were therefore readily distinguished by pathogenicity tests on this alfalfa cultivar. Other cultivars of alfalfa were not tested, however, and these results could be due to race-specific avirulence and not host range-specific virulence differences (Gabriel 1986). Two out of six *X. c.* pv. *alfalfae* strains, one from Florida and one from Kansas, appeared somewhat pathogenic on citrus. These induced water-soaked, leaf-spotting symptoms that were not distinguished from the E-2 strains at 10^8 and 10^5 cfu per milliliter. At 10^3 cfu per milliliter, however, the *X. c.* pv. *alfalfae* strains induced fewer (about 50% fewer) water-soaked lesions than the E-2 strains in repeated experiments. We interpret the RFLP similarities and host range overlap to indicate a close genetic relationship between the pathogenicity determinants in some *X. c.* pv. *alfalfae* strains and some of the *X. c.* pv. *citri* E strains. Because the variation in the population of *X. campestris* strains on noncultivated, natural plant communities is unknown, the inoculum source for the E

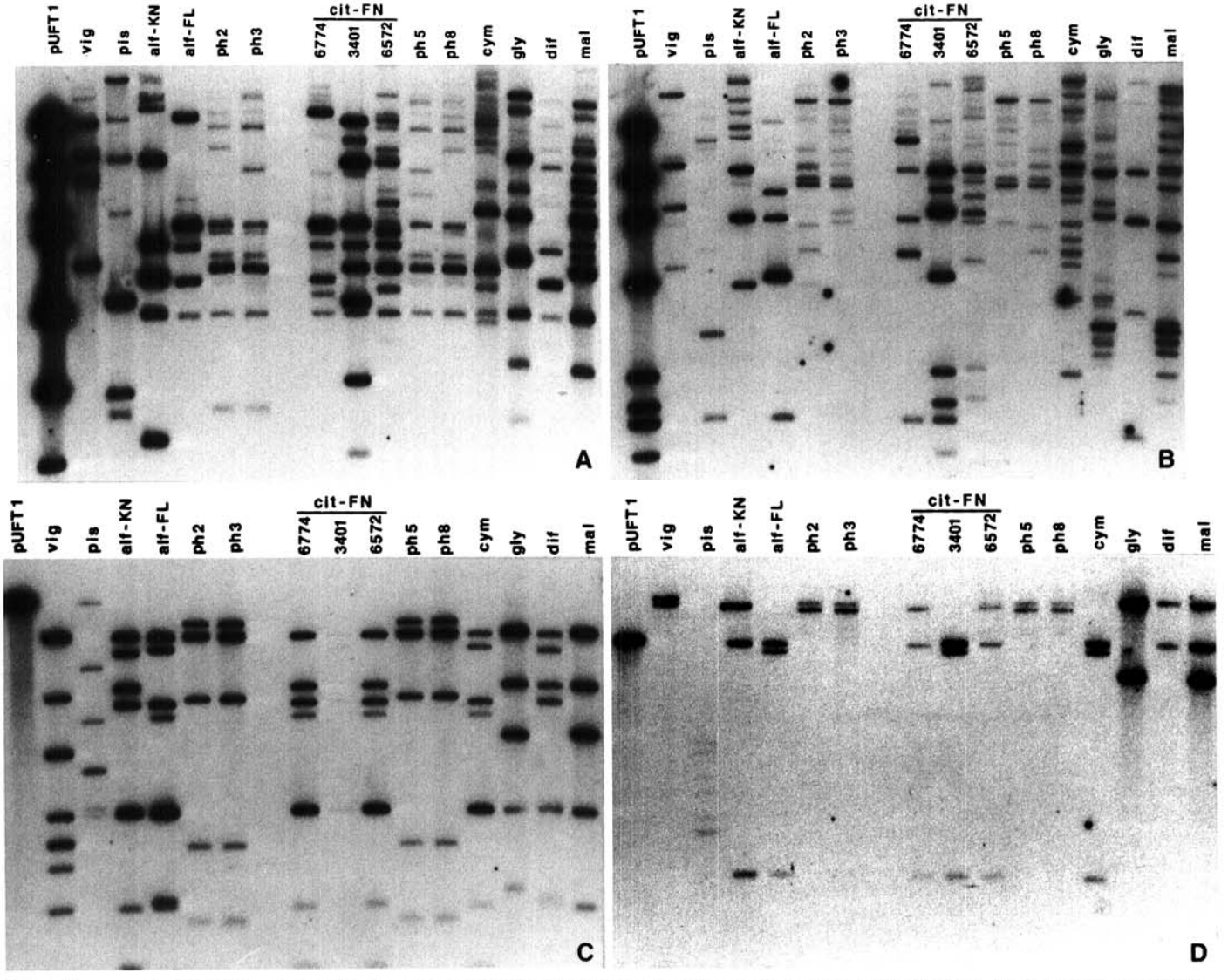

Fig. 2. *X. campestris* total DNAs extracted from strains representative of different pathovars, digested with restriction enzymes, and probed with pUFT1 or pUFA704. **A and C,** DNAs cut with *Eco*RI. **B and D,** cut with *Bam*HI. **A and B,** probed with pUFT1. **C and D,** probed with pUFA704. vig = *X. c.* pv. *vignicola* SN2; pis = *X. c.* pv. *pisi* XP1; alf-KN and -FL = *X. c.* pv. *alfalfae* KN and FL, from Kansas and Florida, respectively; ph2, -3, -5, and -8 = *X. c.* pv. *phaseoli* 82-2, Xpa, B5B, and JF, respectively; cit-FN = *X. c.* pv. *citri* strains as indicated from Florida nurseries; cym = *X. c.* pv. *cyamopsidis* X002; gly = *X. c.* pv. *glycines* B93; dif = *X. c.* pv. *diffenbachiae* 068-1163; and mal = *X. c.* pv. *malvacearum* N.

strains is unknown.

Surveys of natural populations of animal-associated bacteria (e.g., *Escherichia coli* [Selander and Levin 1980], *Haemophilus influenzae* [Musser *et al* 1985], *Legionella pneumophila* [Selander *et al* 1985], and *Bordetella* species [Musser *et al* 1986]) have revealed essentially clonal structures, implying low rates of recombination in bacteria in general (Whittam *et al* 1983). Surveys of natural populations of plant-associated fungi (e.g., *Puccinia graminis, P. recondita* [Burdon and Roelfs 1985, Watson 1981], and *Cochliobolus heterostrophus* [Luig and Watson 1970]) similarly suggest clonal populations within these species. Virulence factors appear to occur on a variety of genetic backgrounds and may be the selective elements responsible for the clonal structures (Selander 1985). Different clonal groups found on one host are most likely the result of independent infection events from external sources and not the result of recombination of strains already resident on that host (Caugant *et al* 1981). The most fit combination of virulence factors (for a given host) available in the species are therefore not likely to occur in a single strain.

The question of whether the E strains have been arbitrarily classified as *X. c.* pv. *citri* may be moot, because the E-1 and E-2 subclones appeared to have been rapidly spreading in field situations and therefore emerging as some of the most fit selections of the polymorphic E group. The E strains have so far been found only on young citrus plants in nurseries and predominantly on the rootstock Swingle citrumelo (*Citrus paradisi* × *Poncirus trifoliata*) (Schoulties *et al* 1987). The reason these strains did not attract attention in Florida before 1984 is probably that Swingle was not available prior to 1974, but its use in nurseries gradually increased to 30.1% of the total by 1985 (Schoulties *et al* 1987). Over time, if an E strain reproduced better on citrus than on an alternate host, it would probably become the predominant strain found in a survey of susceptible citrus plants and possibly lose some of its original (wider) host range. Perhaps significantly, the E-1 strains did not appear pathogenic on alfalfa plants in greenhouse tests. Only long-term field testing and discovery of the inoculum source(s) could address questions of relative fitness and a more appropriate pathovar classification of these strains.

The clonal population structure of *X. campestris* (Lazo *et al* 1987) is evidence for limited genetic recombination in this species. The absence of citrus canker in Florida for more than 57 years and the sudden appearance of at least two genetically and pathologically distinct canker groups in the state (A and E) suggests that recombination of virulence factors in existing *X. c.* pv. *citri* strains did not occur. Because E-1 and E-2 were independently isolated at several locations, they may be somewhat more fit on citrus than other strains of the E group, which apparently were geographically localized. However, because all E strains were promptly eradicated upon discovery, no information on the comparative fitness of these strains is yet available. We conclude that the A, the B/C/Mexican bacteriosis, and the Florida E groups represent *X. campestris* strains with different genetic backgrounds that were selected on citrus plants, because they independently carried genes with some selective value on these hosts. Periodic selection among *X. campestris* strains from natural plant populations and perhaps other pathovars may result in epidemics caused by strains that are quite different from those previously reported to cause a disease. Such strains may have unusual pathogenic properties, because they will not likely carry the optimum combination of virulence factors, specific for a given host, available in the species.

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