Genomic Fingerprinting of Two Pathovars of Phytopathogenic Bacteria by Rare-Cutting Restriction Enzymes and Field Inversion Gel Electrophoresis

Donald A. Cooksey and James H. Graham

Associate professor, Department of Plant Pathology, University of California, Riverside 92521, and associate professor, Citrus Research and Education Center, University of Florida, Lake Alfred 33850.

J. H. Graham's research was supported by a Faculty Development Program grant from the University of Florida. We thank H. E. Stone for technical assistance and P. V. Oudemans for assistance in data analysis.

Accepted for publication 21 February 1989.

ABSTRACT

Cooksey, D. A., and Graham, J. H. 1989. Genomic fingerprinting of two pathovars of phytopathogenic bacteria by rare-cutting restriction enzymes and field inversion gel electrophoresis. Phytopathology 79:745-750.

Several restriction endonucleases cut infrequently in the genomes of *Pseudomonas syringae* and pathovars of *Xanthomonas campestris*, and distinctive patterns of large DNA fragments were generated using field inversion gel electrophoresis. The resolution of genomic DNA fragments by this method permitted standard analyses of genetic relationships between strains by means of dissimilarity coefficients and cluster analyses. We examined 10 strains of *P. s. tomato* and identified two groups that could be readily distinguished from each other. Strains of other pathovars of *P. syringae* had genomic fingerprints very different from those of the strains of *P. s. tomato*. The strains of *X. c. vesicatoria* all had similar genomic fingerprints, but distinctive subgroups within the pathovar could be identified. Strains of other pathovars of *X. campestris* also had very different genomic fingerprints from the strains of *X. c. vesicatoria*.

Several methods of DNA analysis have been used to compare and identify plant-pathogenic bacteria; these include DNA-DNA hybridization (38), restriction fragment length polymorphism (RFLP) analysis (12,14,15,27,29), hybridization with specific DNA probes (11), plasmid profile analysis (7,10,28), and genomic fingerprinting (13,19,29,32). Each method has its benefits and limitations, depending on the specificity of comparisons desired and the technical complexity of procedures required to perform the analysis.

We were especially interested in comparing strains within pathovars and in identifying pathovars at the DNA level. RFLP analysis and specific DNA probes are appropriate tools for these purposes, but both require hybridization techniques, usually involving radionucleotides, which could impede the extension of this technology to many diagnostic laboratories. Bacterial genomes can be examined more directly and easily by DNA fingerprinting, in which profiles of DNA fragments produced by digestion with restriction enzymes can be examined directly after staining gels with ethidium bromide. However, the common restriction enzymes traditionally used for this purpose, such as *Eco*RI, produce several hundred to more than 1,000 fragments, which makes resolution of individual fragments and analysis of the results difficult.

With recently developed pulsed field electrophoresis methods (5,6,16,39) and the discovery of restriction enzymes that cut rarely in bacterial genomes, less complex patterns of large DNA fragments can now be generated, making this modified DNA fingerprinting an attractive method for comparing plant-pathogenic bacteria. In addition, very simple methods for preparing bacterial DNA for pulsed field electrophoresis have been developed; these methods eliminate the need for DNA purification steps involving extractions with organic reagents and purification by cesium chloride-ethidium bromide density gradient centrifugation.

Pulsed field gel electrophoresis of *Escherichia coli* DNA digested with restriction enzymes (*Not*I and *Sfi*I) that recognize rare eight base pair sequences produces distinctive profiles with less than 30 DNA fragments (40). However, because the recognition sequences for these two enzymes consist entirely of G and C nucleotides, these enzymes cut frequently in GC-rich genomes (18,34) and would not be very useful for most plant-pathogenic bacteria. No other restriction enzymes with octanucleotide recognition sequences are available, but several enzymes that recognize six base pair sequences were recently shown to cut infrequently in GC-rich genomes; these include enzymes with recognition sequences consisting entirely of A and T nucleotides, such as *Dra*I and *Ssp*I, and enzymes with CTAG in their recognition sequences, such as *Xba*I, *Spe*I, *Avr*II, and *Nhe*I (18,34).

In the present study, we describe the use of rare-cutting enzymes for generating genomic fingerprints of plant-pathogenic bacteria and the use of this method for analyzing genetic relationships within two pathovars.

MATERIALS AND METHODS

Preparation of bacterial DNA embedded in agarose. Bacterial strains (Table 1) were grown overnight on a rotary shaker at 28 C in 5 ml of nutrient-yeast-glucose broth (37) for *Xanthomonas* or mannitol-glutamate-yeast extract broth (23) for *Pseudomonas*. The bacteria were then treated essentially as described by Grothues and Tümmler (18). Cells were harvested by centrifugation, washed once with SE buffer (75 mM NaCl, 25 mM EDTA, pH 7.5), and resuspended in SE buffer to about 10^9 cfu/ml by adjusting to a Klett-Summerson colorimeter reading of 100 (blue filter). A 0.5-ml sample of the bacterial suspension was mixed with 0.5 ml of 2% SeaPlaque agarose (FMC Corporation). The 2% agarose was dissolved by boiling in 10 mM Tris, 10 mM $MgCl_2$, and 0.1 mM EDTA at pH 7.5 and cooled to 37 C before being mixed with the bacterial suspension. The mixed suspension was drawn into about 25 cm of Tygon tubing (2.4 mm i.d.) and allowed to solidify. There was no need to sterilize the tubing, and we successfully reused tubing for subsequent preparations after rinsing it with water.

The 25-cm-long cylinder of agarose was then removed from the tubing and placed in a 15-ml screw-cap tube with 2 ml of lysis solution consisting of proteinase K (0.5 mg/ml), 1% *N*-lauroylsarcosine, and 0.5 mM EDTA at pH 9.5. After incubation at 56 C overnight, the agarose cylinder was washed five times with 10 ml of TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA) over a 1-hr period. For each wash, the tube was gently inverted two or three times, the wash solution was decanted through a screen, and fresh buffer was added. Some breakage of the agarose occurred during this process, resulting in cylinders about 0.5–5

cm long. The washed cylinders were then used for restriction of the DNA contained in the agarose or stored in TE buffer at 4 C until needed.

Restriction endonuclease digestion. A portion about 2.5 cm long of each agarose cylinder was placed in a 1.5-ml microcentrifuge tube and incubated for several hours at 37 C with an appropriate restriction buffer containing 1.0 mM dithiothreitol, bovine serum albumin (0.13 mg/ml), and five to 10 units of enzyme in a final volume of 0.5 ml. The restriction enzymes *Not*I, *Sfi*I, and *Xba*I were from Promega Corporation; *Dra*I, *Ssp*I, and *Spe*I were from New England Biolabs, Inc.

Field inversion gel electrophoresis. Agarose cylinders were cut to 6-mm lengths and placed in 6- × 2-mm wells in a 1% agarose gel (20 × 20 cm) made with TBE running buffer (88.9 mM Tris, 8.9 mM boric acid, 2.5 mM EDTA). Agarose blocks containing concatemers of lambda DNA (FMC Corporation) were placed in the outer wells of the gel as size standards. The agarose cylinders and blocks were embedded in the wells with 1% SeaPlaque agarose in TBE buffer containing tracking dye. Electrophoresis was for 17 hr at 5.7 V/cm with a pulse time of 8 sec forward and 2 sec reverse. A standard horizontal gel apparatus was used (Model H4, Bethesda Research Laboratories, Gaithersburg, MD) with a switching device constructed in our laboratory that inverted the electric field at desired intervals; similar devices have been described (5,26). Gels were then stained with ethidium bromide.

Analysis of DNA fingerprints. Analysis of DNA fragment profiles was similar to that used for RFLP analysis (12,14,15). The number of fragments larger than 100 kilobases (kb) was recorded for each strain, and the number of fragments shared between strains was recorded. A dissimilarity coefficient was calculated for each pair of strains by the following equation:

$$D = 1 - [2n_{xy}/(n_x + n_y)],$$

where n_{xy} is the number of fragments shared between two strains and n_x and n_y are the number of fragments larger than 100 kb for strain x and strain y, respectively. Cluster analysis was done by the unweighted pair-group method with averages (41) by using the NTSYS-pc program (Applied Biostatistics, Inc.).

RESULTS

Genomic DNAs from several plant pathogens were digested with different enzymes and analyzed by field inversion gel electrophoresis. We initially used the enzyme *Not*I, with an octanucleotide recognition sequence consisting entirely of G and C nucleotides. This enzyme generally produced fragments smaller than 50 kb for the *Xanthomonas* species tested (*data not shown*). However, *Not*I digestion of DNA from *P. syringae, P. fluorescens, P. corrugata, Erwinia chrysanthemi,* and *E. herbicola* produced 10–20 fragments between 50 and 150 kb. The only other enzyme with an octanucleotide recognition sequence, *Sfi*I, cut more frequently than *Not*I in the DNA from *P. syringae* and *X. campestris* strains tested. The enzymes *Dra*I and *Ssp*I, with hexanucleotide recognition sequences consisting entirely of A and T nucleotides, produced several fragments between 50 and 200 kb in *Xanthomonas* but cut frequently in *P. syringae,* producing fragments generally smaller than 50 kb. The most generally useful enzymes tested were *Xba*I and *Spe*I, with CTAG in their recognition sequences. Fragments produced by these enzymes were mostly between 20 and several hundred kilobases.

We compared 10 strains of *P. s. tomato* and seven strains from five other *P. syringae* pathovars by examining the DNA fingerprints produced by *Xba*I digestion of genomic DNA (Fig. 1). Two distinct groups were recognized within *P. s. tomato* that were readily distinguished from the other pathovars of *P. syringae.* No differences were observed among the fingerprints of the five

TABLE 1. Sources and characteristics of strains of *Pseudomonas syringae* and *Xanthomonas campestris* used for genomic fingerprinting

Bacterium	Strain designation	Source[a]	Plant host	Copper sensitivity[b]
P. s. tomato	PT14	1, CA	Tomato	R
	PT15	1, CA	Tomato	R
	PT18	1, CA	Tomato	R
	PT23	1, CA	Tomato	R
	09885	1, MX	Tomato	R
	PT12	1, CA	Tomato	S
	PT17	1, CA	Tomato	S
	PT26	1, MX	Tomato	S
	PT30	1, CA	Tomato	S
	PT29	2, MI	Tomato	S
P. s. glycinea	4180	3	Soybean	
P. s. phaseolicola	02851	3	Common bean	
P. s. maculicola	08882	1, CA	Cauliflower	
P. s. apii	09881	1, CA	Celery	
P. s. syringae	06828	1, CA	Apricot	
	048510	1, CA	Citrus	
	048310	4, OR	Euonymous	
X. c. vesicatoria	10856	5, FL	Pepper	R
	10859	5, FL	Pepper	R
	10857	5, FL	Tomato	R
	10858	5, FL	Tomato	R
	108312	1, CA	Pepper	S
	09883	1, CA	Pepper	S
	10861	1, CA	Pepper	S
	07885	1, CA	Tomato	R
	048313	1, MX	Pepper	R
	098516	1, MX	Tomato	R
X. c. dieffenbachiae	078415	1, CA	Anthurium	
X. c. pelargonii	05876	1, CA	Geranium	
X. c. vitians	12827	6	Lettuce	
X. c. campestris	11882	1, CA	Cauliflower	

[a] 1 = Authors; 2 = D. Fulbright, Michigan State University; 3 = J. Leary, University of California, Riverside; 4 = L. Moore, Oregon State University; 5 = J. Jones, University of Florida, Bradenton; 6 = A. Alvarez, University of Hawaii; CA = California; MX = Mexico; MI = Michigan; OR = Oregon; FL = Florida.

[b] Strains were designated copper-resistant (R) if they grew confluently on MGY medium containing 1 mM $CuSO_4$ (2).

strains in the first group of *P. s. tomato,* although the strains were isolated from different locations in California and Mexico. Interestingly, these five strains were copper-resistant and carried the conserved copper resistance plasmid pPT23D (2,3,8). The five strains in the other group were copper-sensitive (2), and their DNA fingerprints showed some differences.

We also compared 10 strains of *X. c. vesicatoria* with single isolates of four other pathovars of *X. campestris* by DNA fingerprinting with *Xba*I (Fig. 2; data for strain 10856 not shown). All 10 strains of *X. c. vesicatoria* had recognizably similar DNA profiles, but some differences were evident between most strains; all were clearly distinguishable from the other pathovars of *X. campestris* examined.

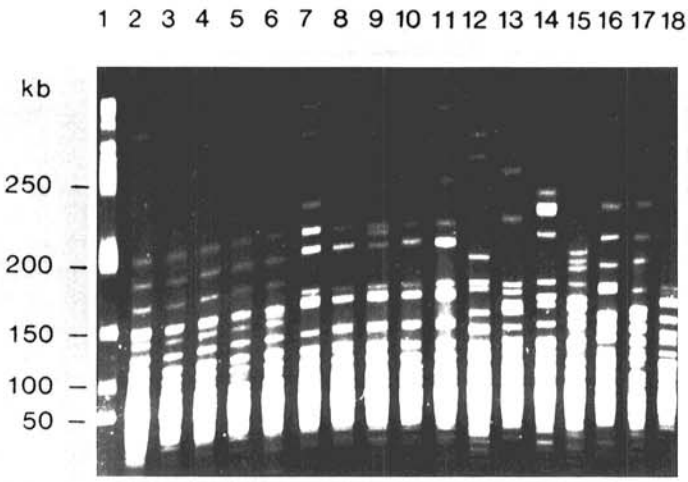

Fig. 1. Genomic fingerprints of strains of *Pseudomonas syringae*. DNA embedded in agarose was digested with *Xba*I, and the fragments were resolved by field inversion gel electrophoresis. Lane 1 contained lambda DNA concatemers with sizes indicated in kilobases; lanes 2–11 contained *P. s. tomato*, strains PT14, PT15, PT18, PT23, 09885, PT12, PT17, PT26, PT30, and PT29, respectively; lane 12 contained *P. s. glycinea* 4180; lane 13 contained *P. s. phaseolicola* 02851; lane 14 contained *P. s. maculicola* 08882; lane 15 contained *P. s. apii* 09881; lanes 16–18 contained *P. s. syringae* strains 06828, 048510, and 048310, respectively.

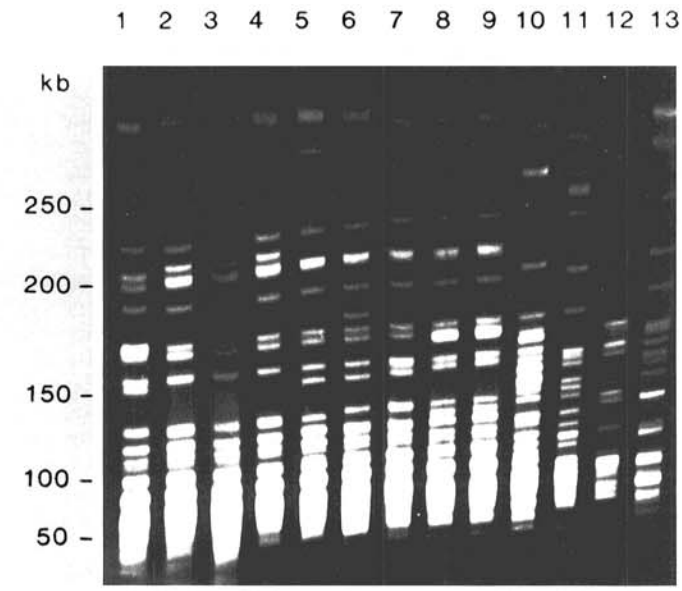

Fig. 2. Genomic fingerprints of strains of *Xanthomonas campestris*. DNA embedded in agarose was digested with *Xba*I, and the fragments were separated by field inversion gel electrophoresis. Lanes 1–9 contained *X. c. vesicatoria*, strains 10859, 108312, 09883, 10861, 048313, 098516, 07885, 10857, and 10858; lane 10 contained *X. c. dieffenbachiae* 078415; lane 11 contained *X. c. pelargonii* 05876; lane 12 contained *X. c. vitians* 12827; lane 13 contained *X. c. campestris* 11882.

The resolution of individual fragments permitted a numerical analysis of relationships between strains similar to that used for RFLP analysis. With pulse times that allowed resolution of fragments larger than 100 kb, 11–18 fragments larger than 100 kb were resolved in *P. s. tomato* and *X. c. vesicatoria.* Dissimilarity coefficients were calculated for each pair of strains within each pathovar (Tables 2 and 3), and these data were used to perform cluster analyses (Fig. 3).

The dendrogram derived from fingerprint analysis of *P. s. tomato* showed that the genetic distance of the copper-resistant group (PT14, PT15, PT18, PT23, 09885) from the copper-sensitive strains was substantial, with a dissimilarity coefficient of 0.54–0.56. The copper-sensitive strains (PT12, PT17, PT26, PT30, PT29) were isolated from California, Mexico, and Michigan but formed a cluster of relatively closely related bacteria.

For strains of *X. c. vesicatoria*, the cluster analysis suggested that genetic distance was more closely correlated with the source of isolation, with strains from Florida, California, and Mexico forming separate clusters. In addition, the analysis separated tomato isolates from pepper isolates within the geographical clusters, and copper-sensitive isolates were separated from copper-resistant isolates.

DISCUSSION

Several commercially available restriction enzymes predicted to be rare-cutting in GC-rich genomes (34) were used with plant-pathogenic bacteria to produce distinctive restriction fragment profiles that were resolved by field inversion gel electrophoresis (5). Other methods of pulsed field electrophoresis can also be used for this purpose (6,16,39), but each of these requires an unconventional electrophoresis apparatus in addition to a pulse controller. These other methods resolve much larger DNA fragments (up to 12,000 kb), but this is not necessary for analysis of bacterial DNA, which generally is cut by enzymes into fragments no larger than 1,000 kb. Commercially available pulse controllers with programmable pulse times can be used in field inversion electrophoresis to separate more fragments than the number reported here. By changing pulse times continuously during electrophoresis, a broader range of fragment sizes is resolved. Although we estimate that more than 50% of the bacterial

TABLE 2. Dissimilarity coefficients determined from *Xba*I genomic fingerprints of 10 *Pseudomonas syringae* pv. *tomato*

Strain	PT14	PT15	PT18	PT23	09885	PT12	PT17	PT26	PT30	PT29
PT14	0.00									
PT15	0.00	0.00								
PT18	0.00	0.00	0.00							
PT23	0.00	0.00	0.00	0.00						
09885	0.00	0.00	0.00	0.00	0.00					
PT12	0.54	0.54	0.54	0.54	0.54	0.00				
PT17	0.56	0.56	0.56	0.56	0.56	0.16	0.00			
PT26	0.54	0.54	0.54	0.54	0.54	0.20	0.10	0.00		
PT30	0.54	0.54	0.54	0.54	0.54	0.13	0.10	0.07	0.00	
PT29	0.52	0.52	0.52	0.52	0.52	0.20	0.13	0.10	0.03	0.00

TABLE 3. Dissimilarity coefficients determined from *Xba*I genomic fingerprints of 10 strains of *Xanthomonas campestris* pv. *vesicatoria*

Strain	10856	10859	108312	09883	10861	048313	098516	07885	10857	10858
10856	0.00									
10859	0.37	0.00								
108312	0.41	0.20	0.00							
09883	0.35	0.20	0.00	0.00						
10861	0.35	0.20	0.00	0.00	0.00					
048313	0.29	0.31	0.18	0.18	0.18	0.00				
098516	0.24	0.31	0.18	0.18	0.18	0.18	0.00			
07885	0.31	0.22	0.14	0.14	0.14	0.20	0.20	0.00		
10857	0.37	0.22	0.20	0.20	0.20	0.31	0.26	0.22	0.00	
10858	0.37	0.22	0.20	0.20	0.20	0.31	0.26	0.22	0.00	0.00

genome was represented in the 11–18 fragments larger than 100 kb that our apparatus was able to resolve on a single gel, it should be possible to analyze the majority of the genome using programmable pulse controllers.

In addition to a qualitative assessment of bacterial relatedness based on the similarity of DNA fingerprint patterns, we have shown that the profiles can be analyzed numerically as in RFLP analysis (12,14,15); this is a distinct advantage over previous genomic fingerprinting methods using enzymes that cut frequently in bacterial DNA (13,19,29,32). Preliminary RFLP analysis with the same set of strains of *X. c. vesicatoria* suggested that the ability to measure genetic differences between strains by genomic fingerprinting was more sensitive than by RFLP analysis; no polymorphisms were observed among these strains using two cosmid clone probes in genomic Southern blots (D. Cooksey, R. Adams, C. Lim, J. Lorang, and H. Shen, *unpublished*). In the fingerprint analysis, the 11–18 large restriction fragments represented an average of about 15 enzyme recognition sites of six bases each, for a total of 90 bases. A mutation in any one of these 90 bases would cause a change in the profile. In RFLP analysis, a probe is used to detect changes in the restriction sites in the bacterial genome within the specific DNA sequence homologous to the probe. Typically, a cosmid clone of 20–30 kb is used as the probe, and a fragment this size is probably cut an average of seven times by commonly used enzymes, such as *Eco*RI, with a six base recognition sequence. A mutation in any one of the 42 bases that make up these restriction sites will result in a restriction fragment length polymorphism. Therefore, a single DNA fingerprint gel in effect monitors about twice as many bases in the genome as RFLP analysis with one cosmid probe. In addition, the restriction sites monitored by fingerprinting are theoretically randomly distributed throughout the genome, whereas restriction sites monitored by RFLP analysis are necessarily restricted to one small region of the genome. Assuming that the accumulation of mutations in the genome is random, a random distribution of restriction sites might be a more sensitive monitor of mutational events. The sensitivity of RFLP analysis is, of course, increased when multiple probes are used (12,14,15,29) but, as mentioned above, it should also be possible to resolve more restriction fragments by changing the pulse times during DNA fingerprinting. The number of restriction sites monitored by fingerprinting can also be doubled or tripled by using a second or third enzyme.

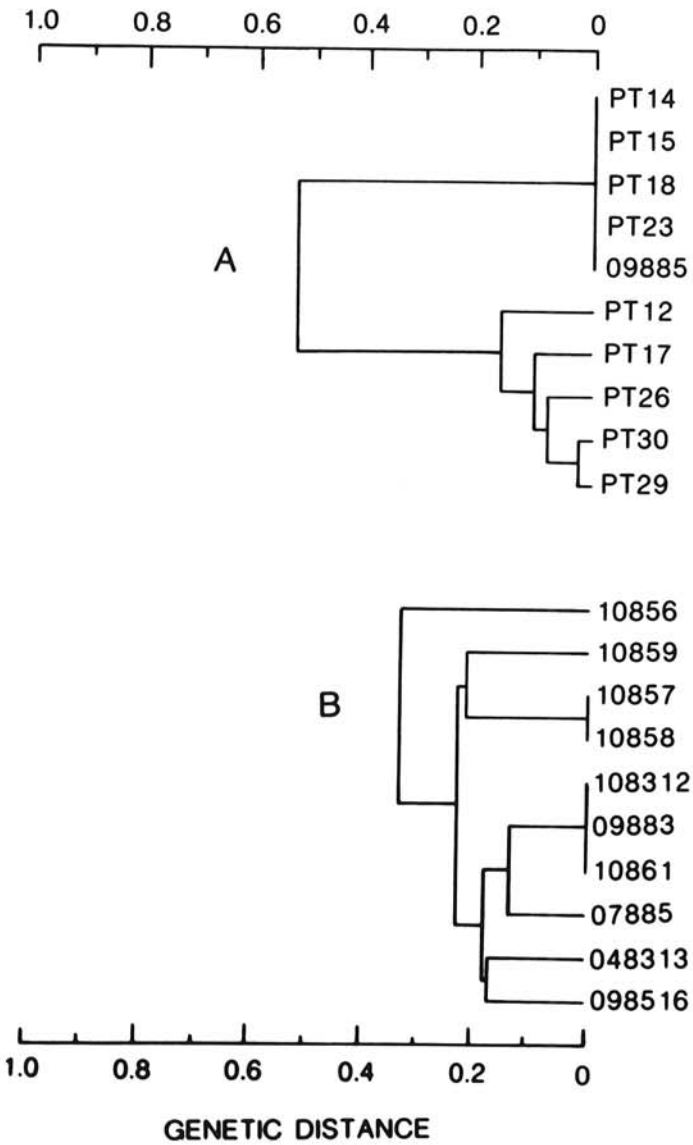

Fig. 3. Dendograms obtained by cluster analysis of dissimilarity coefficients determined from *Xba*I genomic fingerprints of **A,** 10 strains of *Pseudomonas syringae* pv. *tomato* and **B,** 10 strains of *Xanthomonas campestris* pv. *vesicatoria.*

The high specificity of DNA fingerprinting can also be a limitation, since apparently only very closely related bacteria can be compared. We did not attempt to compare strains of different pathovars numerically; the profiles looked very different, and there would be no reason to expect the few fragments that comigrated on gels to be related without confirmation by hybridization. For strains with obviously similar patterns, the assumption of relatedness for comigrating fragments was probably correct, but for strains as different as the copper-sensitive and copper-resistant groups of *P. s. tomato,* this assumption could be erroneous and could result in underestimation of genetic distance. Although the strains compared were different, our dissimilarity coefficients between strains of *P. s. tomato* were similar to those calculated by Denny et al (12) after RFLP analysis of this pathovar with three cosmid clones.

DNA fingerprinting and cluster analysis of strains of *P. s. tomato* provided the unexpected finding that the copper-resistant strains formed a separate and unusually uniform group of strains, in spite of their isolation from different parts of California and Mexico. It was previously speculated that the conservation of the copper resistance plasmid pPT23D in these strains was partly the result of conjugative transfer to indigenous strains (8), but our present results support the recent introduction and establishment of a single new copper-resistant strain carrying pPT23D. Such a strain could have been introduced from a common source of contaminated seed (1,33) or transplants (25).

Cluster analysis of genomic fingerprint data for strains of *X. c. vesicatoria* suggested that genetic distance within the group was related to the geographical source of the strains. Strains from pepper and tomato were separated within the geographical clusters, but no general clustering and separation of tomato strains from pepper strains was observed with strains from different geographical locations. These data suggest that specificity toward tomato or pepper could evolve separately after geographical distribution. The recent demonstration that the host specificity of this pathogen can be regulated by single avirulence genes (45), some of which are plasmid-borne (24,42,43), supports this hypothesis.

The present study used only 10 strains of each pathovar and was not intended to be an extensive examination of genetic diversity within the two pathovars, but it has demonstrated that DNA fingerprinting can now be used for this purpose. More genetic diversity would probably be identified within these pathovars if more strains from a larger geographical distribution were analyzed. Some pathovars, especially those with broad host ranges, will probably show greater diversity than the two pathovars examined here. Denny et al (12) have already shown a greater diversity within *P. s. syringae* than within *P. s. tomato* by RFLP analysis. Similarly, our preliminary examination of *X. c. dieffenbachiae* suggested that the pathovar is made up of several distinct groups with very different genomic fingerprints (J. H. Graham, D. A. Cooksey, and A. R. Chase, *unpublished*).

Genomic fingerprinting may be useful in the rapid identification of pathogen groups for regulatory purposes. The identification of *P. s. tomato* and its differentiation from *P. s. syringae* and *P. viridiflava,* which can also cause leaf spots of tomato, have generated considerable interest (9,11,17,21,22), and it should now be possible to accomplish this differentiation using genomic fingerprinting as described in this study. Tracing the origin of exotic pathogens, such as those causing citrus bacterial canker in Florida, might also be facilitated by genomic fingerprinting.

An additional use for this method is in genetic mapping, as demonstrated by the construction of a complete restriction map for *E. coli* (40). The ability to examine large DNA fragments, rather than being limited to the size of cosmid clone inserts, would facilitate the investigation of how pathogenicity and virulence genes are clustered in the genome of plant pathogens (4,20,30–32,35,36,44). For some pathogens with general conservation of restriction sites, such as *X. c. vesicatoria,* the construction of at least a partial physical and genetic map indicating the relative organization of pathogenicity, virulence, and avirulence genes is now feasible.

LITERATURE CITED

1. Bashan, Y., Okon, Y., and Henis, Y. 1982. Long-term survival of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper seeds. Phytopathology 72:1143-1144.
2. Bender, C. L., and Cooksey, D. A. 1986. Indigenous plasmids in *Pseudomonas syringae* pv. *tomato*: Conjugative transfer and role in copper resistance. J. Bacteriol. 165:534-541.
3. Bender, C. L., and Cooksey, D. A. 1987. Molecular cloning of copper resistance genes from *Pseudomonas syringae* pv. *tomato*. J. Bacteriol. 169:470-474.
4. Boucher, C. A., Van Gijsegem, F., Barberis, P. A., Arlat, M., and Zeschek, C. 1987. *Pseudomonas solanacearum* genes controlling both pathogenicity on tomato and hypersensitivity on tobacco are clustered. J. Bacteriol. 169:5626-5632.
5. Carle, G. F., Frank, M., and Olson, M. V. 1986. Electrophoretic separations of large DNA molecules by periodic inversion of the electric field. Science 232:65-68.
6. Chu, G., Vollrath, D., and Davis, R. W. 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science 234:1582-1585.
7. Civerolo, E. L. 1985. Indigenous plasmids in *Xanthomonas campestris* pv. *citri.* Phytopathology 75:524-528.
8. Cooksey, D. A. 1987. Characterization of a copper resistance plasmid conserved in copper-resistant strains of *Pseudomonas syringae* pv. *tomato*. Appl. Environ. Microbiol. 53:454-456.
9. Cuppels, D. A. 1984. The use of pathovar-indicative bacteriophages for rapidly detecting *Pseudomonas syringae* pv. *tomato* in tomato leaf and fruit lesions. Phytopathology 74:891-894.
10. Denny, T. P. 1988. Phenotypic characterization of *Pseudomonas syringae* pv. *tomato* and its differentiation from *P. syringae* pv. *syringae*. J. Gen. Microbiol. 134:1939-1948.
11. Denny, T. P. 1988. Differentiation of *Pseudomonas syringae* pv. *tomato* from *P. s. syringae* with a DNA hybridization probe. Phytopathology 78:1186-1193.
12. Denny, T. P., Gilmour, M. N., and Selander, R. K. 1988. Genetic diversity and relationships of two pathovars of *Pseudomonas syringae*. J. Gen. Microbiol. 134:1949-1960.
13. Drahos, D., Brackin, J., and Barry, G. 1985. Bacterial strain identification by comparative analysis of chromosomal DNA restriction patterns. (Abstr.) Phytopathology 75:1381.
14. Gabriel, D. W., Hunter, J. E., Kingsley, M. T., Miller, J. W., and Lazo, G. R. 1988. Clonal population structure of *Xanthomonas campestris* and genetic diversity among citrus canker strains. Mol. Plant-Microbe Interact. 1:59-65.
15. Gabriel, D. W., Kingsley, M. T., Hunter, J. E., and Gottwald, T. 1989. Reinstatement of *Xanthomonas citri* (*ex* Hasse) and *X. phaseoli* (*ex* Smith) to species and reclassification of all *X. campestris* pv. *citri* strains. Int. J. Syst. Bacteriol. 39:14-22.
16. Gardiner, K., Laas, W., and Patterson, D. 1986. Fractionation of large mammalian DNA restriction fragments using vertical pulsed-field gradient gel electrophoresis. Somatic Cell Mol. Genet. 12:185-195.
17. Gitaitis, R. D., Jones, J. B., Jaworski, C. A., and Phatak, S. C. 1985. Incidence and development of *Pseudomonas syringae* pv. *syringae* on tomato transplants in Georgia. Plant Dis. 69:32-35.
18. Grothues, D., and Tümmler, B. 1987. Genome analysis of *Pseudomonas aeruginosa* by field inversion gel electrophoresis. FEMS Microbiol. Lett. 48:419-422.
19. Hartung, J. S., and Civerolo, E. L. 1987. Genomic fingerprints of *Xanthomonas campestris* pv. *citri* strains from Asia, South America, and Florida. Phytopathology 77:282-285.
20. Huang, H.-C., Schuurink, R., Denny, T. P., Atkinson, M. M., Baker, C. J., Yucel, I., Hutcheson, S. W., and Collmer, A. 1988. Molecular cloning of a *Pseudomonas syringae* pv. *syringae* gene cluster that enables *Pseudomonas fluorescens* to elicit the hypersensitive response in tobacco plants. J. Bacteriol. 170:4748-4756.
21. Jones, J. B., Gitaitis, R. D., and McCarter, S. M. 1986. Fluorescence on single-carbon sources for separation of *Pseudomonas syringae* pv. *syringae, P. syringae* pv. *tomato,* and *P. viridiflava* on tomato transplants. Plant Dis. 70:151-153.
22. Jones, J. B., McCarter, S. M., and Gitaitis, R. D. 1981. Association of *Pseudomonas syringae* pv. *syringae* with a leaf spot disease of tomato transplants in southern Georgia. Phytopathology 71:1281-1285.
23. Keane, P. J., Kerr, A., and New, P. B. 1970. Crown gall of stone fruit. II. Identification and nomenclature of *Agrobacterium* isolates. Aust. J. Biol. Sci. 23:585-595.
24. Kearney, B., Ronald, P. C., Dahlbeck, D., and Staskawicz, B. J. 1988. Molecular basis for evasion of plant host defense in bacterial spot disease of pepper. Nature (London) 332:541-543.
25. Kim, S. H. 1979. Dissemination of seed-borne *Pseudomonas tomato* by transplants. (Abstr.) Phytopathology 69:535.
26. Larson, J. J., Nicholson, A. W., and Siegel, A. 1987. Field inversion of large DNA fragments using an inexpensive unit. BioTechniques 5:228-231.
27. Lawson, E. C., Jonsson, C. G., and Hemming, B. C. 1986. Genotypic diversity of fluorescent pseudomonads as revealed by southern hybridization analysis with siderophore-related gene probes. Pages 315-329 in: Iron, Siderophores, and Plant Diseases. T. R. Swinburne, ed. Plenum Press, New York.
28. Lazo, G. R., and Gabriel, D. W. 1987. Conservation of plasmid DNA sequences and pathovar identification of strains of *Xanthomonas campestris.* Phytopathology 77:448-453.
29. Lazo, G. R., Roffey, R., and Gabriel, D. W. 1987. Pathovars of *Xanthomonas campestris* are distinguishable by restriction fragment-length polymorphism. Int. J. Syst. Bacteriol. 37:214-221.
30. Lindgren, P. B., Panopoulos, N. J., Staskawicz, B. J., and Dahlbeck, D. 1988. Genes required for pathogenicity and hypersensitivity are conserved and interchangeable among pathovars of *Pseudomonas syringae.* MGG Mol. Gen. Genet. 211:499-506.
31. Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. Gene cluster of *Pseudomonas syringae* pv. "*phaseolicola*" controls pathogenicity of bean plants and hypersensitivity on nonhost plants. J. Bacteriol. 168:512-522.
32. Malvick, D. K., and Moore, L. W. 1988. Population dynamics and diversity of *Pseudomonas syringae* on maple and pear trees and associated grasses. Phytopathology 78:1366-1370.
33. McCarter, S. M., Jones, J. B., Gitaitis, R. D., and Smitley, D. R. 1983. Survival of *Pseudomonas syringae* pv. *tomato* in association with tomato seed, soil, host tissue, and epiphytic weed hosts in Georgia. Phytopathology 73:1393-1398.
34. McClelland, M., Jones, R., Patel, Y., and Nelson, M. 1987. Restriction endonucleases for pulsed field mapping of bacterial genomes. Nucleic Acids Res. 15:5985-6005.
35. Niepold, F., Anderson, D., and Mills, D. 1985. Cloning determinants of pathogenesis from *Pseudomonas syringae* pathovar *syringae.* Proc. Natl. Acad. Sci. USA 82:406-410.
36. Peet, R. C., Lindgren, P. B., Willis, D. K., and Panopoulos, N. J. 1986. Identification and cloning of genes involved in phaseolotoxin production by *Pseudomonas syringae* pv. "*phaseolicola.*" J. Bacteriol. 166:1096-1105.
37. Schaad, N. W. 1988. Identification schemes. I. Initial identification of common genera. Pages 1-15 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd ed. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN.
38. Schleifer, K. H., and Stackebrandt, E. 1983. Molecular systematics of prokaryotes. Annu. Rev. Microbiol. 37:143-187.
39. Schwartz, D. C., and Cantor, C. R. 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37:67-75.
40. Smith, C. L., Econome, J. G., Schutt, A., Klco, S., and Cantor, C. R. 1987. A physical map of the *Escherichia coli* K12 genome. Science 236:1448-1453.
41. Sneath, P. H. A., and Sokal, R. R. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. W. H.

Freeman and Co., San Francisco.
42. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 76:240-243.
43. Swanson, J., Kearney, B., Dahlbeck, D., and Staskawicz, B. 1988. Cloned avirulence gene of *Xanthomonas campestris* pv. *vesicatoria* complements spontaneous race-change mutants. Mol. Plant-Microbe Interact. 1:5-9.
44. Turner, P., Barber, C., and Daniels, M. 1985. Evidence for clustered pathogenicity genes in *Xanthomonas campestris* pv. *campestris*. MGG Mol. Gen. Genet. 199:338-343.
45. Whalen, M. C., Stall, R. E., and Staskawicz, B. J. 1988. Characterization of a gene from a tomato pathogen determining hypersensitive resistance in non-host species and genetic analysis of this resistance in bean. Proc. Natl. Acad. Sci. USA 85:6743-6747.