MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 4, No. 3, pp. 293-299, 1991

Genetic Diversity and Host Range in Strains of *Erwinia chrysanthemi*

Martine Boccara,[1] Regine Vedel,[1] Dominique Lalo,[2] Marc-Henri Lebrun,[3] and Jean François Lafay[3]

[1]Laboratoire de Pathologie Végétale, INRA, 75005 Paris, France; [2]IRAT-CIRAD Martinique, 97204 Fort de France, Martinique; and [3]Cryptogamie, Universite Paris 11, 91405 Orsay, Cedex France.
Received 5 December 1990. Accepted 26 February 1991.

Restriction fragment length polymorphism (RFLP) analysis was used to study the relationships among 52 strains of *Erwinia chrysanthemi* differing in such factors as original host, geographical origin, and year of isolation. The strains were distributed in 10 RFLP groups. The similarity coefficients obtained with a probe encoding pathogenicity genes of *E. chrysanthemi* gave similar results compared to those obtained with randomly selected probes from a cosmid library of *E. chrysanthemi* 3937. Comparison with other classification systems, such as biovars and pectinolytic enzyme profiles, showed that RFLP analysis was the most discriminative. However, good correlation between these methods was observed when very homogenous groups of strains were analyzed. A sample of strains from different RFLP groups was assayed for pathogenicity on different plant species. Some host plants, such as potato, tomato, lettuce, and saintpaulia, were susceptible to all the tested strains of *E. chrysanthemi*. Others, such as dieffenbachia and philodendron, were differential hosts because they were only attacked by members of particular RFLP groups.

Additional keywords: pectate lyase, pectin methyl esterase, soft rot.

Erwinia chrysanthemi Burkholder *et al.* is a causal agent of soft rot disease on a wide range of plant species in tropical, subtropical, and temperate regions of the world (Bradbury 1984). Several aspects of *E. chrysanthemi* pathogenicity have been investigated. In addition to pectic and cellulolytic enzymes (Aymeric *et al.* 1990; Boccara *et al.* 1988), iron acquisition and outer membrane components (Enard *et al.* 1988; Schoonejans *et al.* 1987) have been shown to be important in pathogenicity. However, little is known about host specificity of this bacterial species. Recently, different bacterial genes involved in host specificity have been described, including host species-specific genes and avirulence genes (reviewed in Gabriel 1989). Such genes have not been identified in *E. chrysanthemi*; however, this species, unlike the other pectinolytic *Erwinia* sp., exhibits a certain degree of host specificity (Lemattre 1977; Dickey 1981).

Originally, *E. chrysanthemi* strains were divided into pathovar groups according to the hosts from which they were isolated (Young 1978). *E. chrysanthemi* has since been isolated from more than 50 different plant species, and another classification system using biochemical and physiological criteria such as biovars has been proposed (Samson and Nassan-Agha 1978). The characteristics that were considered included growth at 39° C, production of arginine dihydrolase, and the ability to catabolize several sugars. However, biovar grouping did not correlate with pathovars (Samson and Nassan-Agha 1978).

Molecular biology provides techniques to estimate the relatedness among bacterial strains (Cook *et al.* 1989; Gabriel *et al.* 1988; Gabriel *et al.* 1989). In this study, we have reinvestigated *E. chrysanthemi* classification by analyzing the restriction fragment length polymorphisms (RFLPs) of 52 strains of *E. chrysanthemi* isolated from different ornamental and crop plant species and from different geographic areas. In addition, the results of this analysis have been compared with other classification systems such as biovars (Ngwira 1989) and pectinolytic enzyme profiles (Ried and Collmer 1986). Pathogenicity tests have also been conducted on several hosts with a limited number of strains from different RFLP groups, and these data are discussed in terms of a possible correlation between the genetic diversity of *E. chrysanthemi* strains and their host specificity.

Address correspondence to Martine Boccara, Laboratoire de Pathologie Végétale, INRA, 16 rue Claude Bernard 75005 Paris, France.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The origins of all strains of *E. chrysanthemi* are listed in Table 1. The strains were grown at 30° C in Luria broth medium (L medium) (Miller 1972). For pectinase induction (Reverchon *et al.* 1986), sodium polygalacturonate (0.5%, Sigma Chemical Co., St. Louis, MO) was added to M9 medium (Miller 1972).

Probes and DNA manipulations. The five probes used in this study are described in Table 2. Plasmid DNA was made from a clear lysate method and was purified by caesium chloride ethidium bromide density gradient centrifugation (Maniatis *et al.* 1982). Genomic DNA from each strain was obtained from 10 ml of overnight culture by the method of Klotz and Zimm (1972). Briefly, the bacteria were resuspended in TE_{50} buffer (50 mM Tris, pH 7.5, 10 mM EDTA) and lysed by incubation in 1% Sarkosyl at 37° C until clear. The mixture was extracted twice with phenol and once with chloroform. The DNA was ethanol-precipitated and resuspended in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA). Chromosomal DNA

(4 μg) was hydrolyzed with one unit of restriction endonuclease (*Eco*RI or *Bam*HI) per microgram of DNA, and then electrophoresed on 0.8% agarose gels. The DNA was denatured, transfered bi-directionally onto biodyne nylon membrane (Pall Biosupport Division, Glen Cove, NY) and hybridized with the probe (^{32}P-labeled by nick translation using a Bethesda Research Laboratories, Gaithersburg, MD, kit). Hybridization and washing were conducted under stringent conditions (68° C, 15 mM NaCl, 1.5 mM sodium citrate). The hybridized fragments were detected by autoradiography on X-ray film. Each manipulation was repeated at least twice. Standardization between gels was accomplished by including, in all the gels, molecular weight markers and genomic DNA from several reference strains.

Similarity coefficients. Sizes of the hybridizing fragments from each strain were determined and similarity coefficients

Table 1. List of the fifty-two strains of *Erwinia chrysanthemi* used for restriction fragment length polymorphisms (RFLPs) analysis

Host	Number	Geographical origin/year	Source[a]	RFLP group	Biovar[b]	PL profile[c]	PME profile[c]
Dieffenbachia	3665	France 1974	2	2	2	1	4
	2051	USA 1957	1	2	2	1	4
	1870	Ivory Coast 1976	1	2	2	1	4
	1152	Italy 1968	1	2	2	1	4
	1345	Italy 1969	1	2	2	1	4
	1237	Germany 1962	1	2	2	1	4
	3642	France 1974	2	6	3	1	4
	2014	France 1974	1	6	3	1	4
	ED1	Martinique 1987	3	6	3	1	4
Philodendron	3805	France 1976	2	6	3	1	2
	1245	USA 1959	1	6	3	1	4
	EP2	Martinique 1987	3	6	3	1	4
Pelargonium	B374	Comores 1960	1	3	3	1	4
Tomato	722	France 1981	1	1	1	3	3
	G3	Guadeloupe 1988	3	9	3	1	3
	G4	Guadeloupe 1988	3	3	3	1	4
	ET1	Martinique 1987	3	3	3	1	4
	ET2	Martinique 1987	3	3	3	1	4
	ET3	Martinique 1987	3	6	3	1	4
Saintpaulia	3937	France 1977	2	3	3	1	4
	3908	France 1973	2	3	3	1	4
	3912	France 1973	2	3	3	1	4
Corn	1268	USA 1966	1	7	3	1	2
	2052	USA 1970	1	7	3	1	2
	1271	Egypt 1961	1	7	3	1	2
	1522	Colombia 1973	1	7	3	1	2
	1499	France 1973	1	7	8	1	2
	1596	France 1974	1	8	8	1	2
Banana	1445	Colombia 1972	1	4	4	2	1
	1451	Colombia 1972	1	4	4	2	1
	EM1	Martinique 1987	3	4	NC[d]	2	1
	1871	Ivory Coast 1976	1	10	3	1	2
Kalanchoe	3728	France 1978	2	1	1	3	3
	3716	France 1978	2	1	1	3	3
	1805	Denmark 1977	1	1	9	3	3
Dahlia	3367	France 1977	2	1	1	3	3
	3310	France 1977	2	1	1	3	3
	2013	France 1974	1	1	1	3	3
Potato	86.14.2y	France 1986	4	1	1	3	3
	2288	France 1980	1	1	1	3	3
	1888	France 1978	1	1	1	3	3
	CH37	Switzerland 1987	5	1	1	3	3
	2015	France 1975	1	1	7	3	3
	2267	Australia 1978	1	8	3	1	2
	CIPO32	Peru	4	3	3	1	4
Carnation	1200	England 1956	1	1	1	3	3
	795	France 1965	1	1	1	3	3
	1151	Italy 1967	1	1	1	3	3
	1984	France 1972	1	1	1	3	3
	1441	USA	1	5	5	4	3
	1275	England 1961	1	5	5	4	3
Chrysanthemum	2048	USA 1956	1	5	5	4	3

[a]Source of the considered strains. 1: Collection française des bacteries phytopathogènes, INRA Angers, France. 2: M. Lemattre, INRA Versailles, France. 3: IRAT-CIRAD Martinique, France. 4: B. Jouan, INRA Rennes, France. 5: O. Cazelles, Station Fédérale de Recherches Agronomiques Changins, Switzerland.

[b]The distribution of *E. chrysanthemi* into nine biovars was established with the criteria given in Table 3.

[c]The pectate lyase (PL)/pectin methyl esterase (PME) profiles are those defined in Figure 3, A and B.

[d]Nonclassified in the nine defined biovars.

(F) were calculated for all pairwise combinations by means of the following formula:

$$F = 2n_{xy}(100)/(n_x + n_y)$$

in which n_x and n_y are the numbers of restriction fragments describing strains X and Y, and n_{xy} is the number of fragments shared by the strains X and Y (Nei and Li 1978). The similarity coefficients were used to estimate genetic distances according to the formula of Nei and Miller (1990). These distances allowed the construction of phylogenic trees using the method of Fitch and Margoliash (1967) from the Phylip software package (Selsenstein 1990) and the Neighbor Joining method (Saitou and Nei 1987).

Biovar determination. The criteria for biovar classification are those described by Samson *et al.* (1978) and Ngwira and Samson (1990). They included growth at 39° C, arginine-dihydrolase activity (ADH), acidification or alkalinization of D(−)-arabinose, 5-ketogluconate, inulin, mannitol, melibiose, raffinose, and D(−)-tartrate in mineral basal medium. The nine biovars of *E. chrysanthemi* are listed in Table 3.

Electrofocusing. Pectate lyase isozymes were analyzed from the supernatant of stationary-phase cell cultures that had grown in pectinase-inducing medium. Fifteen microliters of supernatant were layered on a thin polyacrylamide gel, and electrofocusing was done in a pH gradient from 3 to 10 or 8 to 10.5 at a constant power of 30 W. Pectate lyase or pectin methyl esterase activities were assessed directly on the gel according to the method of Bertheau *et al.* (1984).

Pathogenicity tests. Pathogenicity tests were performed on the following test plant species: *Solanum tuberosum* L. (potato; cultivar Bintje), *Lycopersicum esculentum* L. (tomato; cultivar Caraibo), *Lactuca sativa* L. (lettuce, seven to eight leaves; cultivar Minetto), *Saintpaulia ionantha* H. Wendl. (2-mo-old; cultivar Blue Rhapsody), *Dieffenbachia amoena* W. Bull (dieffenbachia, six to eight leaves; cultivar Tropic Snow), and *Philodendron* sp. (six to eight leaves; cultivar Burgundy). Leaf parenchyma were infiltrated with

Table 2. Probes used in this study

Probe	Nature	*Erwinia chrysanthemi* DNA insert size (kb)	Function	Source
C1B4	Cosmid	35	Unknown	Our collection
C4F9	Cosmid	40	Unknown	Our collection
B41	Plasmid	13	Pectate lyase a,d,e	S. Reverchon *et al.* 1986
pMH18	Plasmid	3	Endoglucanase y	A. Guiseppi[a]
PNL5	Plasmid	2	Exopectate lyase	A. Collmer[b]

[a] Laboratoire de chimie bactérienne, CNRS, Marseille.
[b] Cornell University, Ithaca, NY.

Table 3. Definition of nine biovars within *Erwinia chrysanthemi*

Biovar	Growth at 39° C	ADH	D(−)-arabinose	5-Ketogluconate	Inulin	Mannitol	Melibiose	Raffinose	D(−)-tartrate
1	−	+	−	−	+	+	+	+	+
2	+	−	+	−	−	+	−	−	−
3	+	−	+	−	−	+	+	+	−
4	+	−	+	+	−	−	+	+	+
5	+	+	−	−	+	+	+	+	−
6	+	−	−	−	−	+	+	+	−
7	−	+	−	−	+	+	−	−	+
8	+	+	+	−	−	+	+	+	−
9	−	−	−	−	+	+	−	−	+

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
	RFLP 1	RFLP 4	RFLP 5	RFLP 2	RFLP 6	RFLP 6	RFLP 6	RFLP 6	RFLP 6	RFLP 6	RFLP 6	RFLP 3	RFLP 3	RFLP 3	RFLP 3	RFLP 3	RFLP 3	RFLP 3	RFLP 7	RFLP 7	RFLP 7	RFLP 7	RFLP 7	RFLP 8	RFLP 8
	3728	1445	2048	2051	3642	2014	ED1	1245	3805	EP2	ET3	B374	3937	3908	G4	ET1	ET2	CIP032	1268	2052	1271	1522	1499	1596	2267
3728		24	42	29	16	21	12	21	29	17	26	22	27	21	27	20	21	20	20	12	33	22	32	20	33
1445			24	22	6	13	7	6	13	13	6	22	27	32	26	31	13	40	25	14	34	20	20	5	11
2048				43	27	27	6	16	29	5	22	33	38	33	34	27	28	26	17	18	30	30	18	10	28
2051					60	56	50	50	54	48	51	41	56	56	57	55	52	56	30	27	27	37	35	33	36
3642						74	68	66	60	66	62	35	45	45	52	33	41	45	29	25	25	31	32	26	17
2014							70	85	68	62	82	35	45	47	48	40	36	47	22	19	12	25	27	22	18
ED1								81	62	89	77	32	38	51	46	37	40	38	18	14	13	20	10	11	13
1245									84	78	91	34	34	40	41	33	35	40	23	18	18	25	25	15	11
3805										62	81	25	28	31	25	30	25	37	25	26	13	27	21	17	19
EP2											68	31	37	43	45	30	38	37	19	13	20	20	21	11	13
ET3												29	21	41	36	34	30	41	17	13	6	18	27	16	18
B374													70	58	66	64	66	58	24	38	38	19	26	16	12
3937														62	72	65	78	64	24	37	38	19	26	21	27
3908															66	74	66	81	24	37	45	32	26	16	32
G4																64	75	60	31	38	46	26	34	27	34
ET1																	70	68	23	36	31	18	19	21	23
ET2																		64	31	45	46	26	27	22	25
CIP032																			18	23	30	25	18	6	10
1268																				64	55	67	62	44	43
2052																					69	75	66	41	37
1271																						66	60	41	45
1522																							62	29	33
1499																								48	38
1596																									62
2267																									

Fig. 1. Similarity coefficients among 25 restriction fragment length polymorphism (RFLP) haplotypes representative of eight RFLP groups of *Erwinia chrysanthemi*. A unique group representative was considered for highly related strains of RFLP groups 1, 2, 4, and 5 (similarity coefficients >90%). All the haplotypes from RFLP groups 3, 6, 7, and 8 have been examined. Data for the strains G3 and 1871, which were the unique representatives of RFLP groups 9 and 10, respectively, are not presented.

100 μl of a bacterial suspension (3–5.10^6 and 10^8 colony-forming units per milliliter). For potato and tomato, inoculations were made by stem puncture (stem length: 30–40 cm). Each strain was inoculated into three to four plants, and each experiment was repeated at least twice. After inoculation, plants were placed at 29° C with a day-length period of 16 hr under saturating humidity. Results were scored as (+) for systemic maceration and (−) for water-soaked lesions leading to slight necrosis or chlorosis.

RESULTS AND DISCUSSION

RFLP analysis. The genetic structure of *E. chrysanthemi* populations has been deduced from the comparison of RFLP profiles of 52 strains differing in such factors as original host, geographical origin, and year of isolation (Table 1). We used two types of probes: two randomly selected clones from a cosmid library of *E. chrysanthemi* 3937 and three probes corresponding to clones containing hydrolytic enzymes encoding genes (Table 2). Genomic DNA samples were hydrolyzed either with *Eco*RI (probes C4F9, C1B4, B41, and pMH18) or by *Bam*HI (probe PNL5). Polymorphism was observed neither in DNAs hydrolyzed with *Bam*HI and hybridized to probe B41, nor when probe PNL5 was used to analyze DNA digested by *Eco*RI. Similarity coefficients were computed either from the patterns obtained with each probe (data not shown) or with all the probes (Fig. 1). For a given strain, a total of about 30 fragments hybridized with all the probes. The similarity coefficients obtained with the probe encoding PLa, d, e isozymes of pectate lyase (B41), which are virulence factors of *E chrysanthemi* (Boccara *et al.* 1988), were equivalent to those obtained with the random probes. The short probes pMH18 and PNL5 hybridized with fewer fragments than the large probes but represented one fifth of the variable fragments for a given DNA.

Cluster analysis allowed the distribution of the strains into 10 RFLP groups (Table 1), each group representing strains with similarity coefficients ranging from 60 to 100% (Figs. 1 and 2). Identical phylogenic trees were obtained using either the Fitch and Margoliash (Fig. 2) or the Neighbor Joining methods (not shown). No correlation was observed between the host of origin and the RFLP grouping for the majority of the strains.

RFLP group 1 included strains isolated from temperate regions and a diverse group of hosts, including crop plants (potato, tomato) and ornamental plants (dahlia, kalanchoe, and carnation). These strains formed a very homogenous

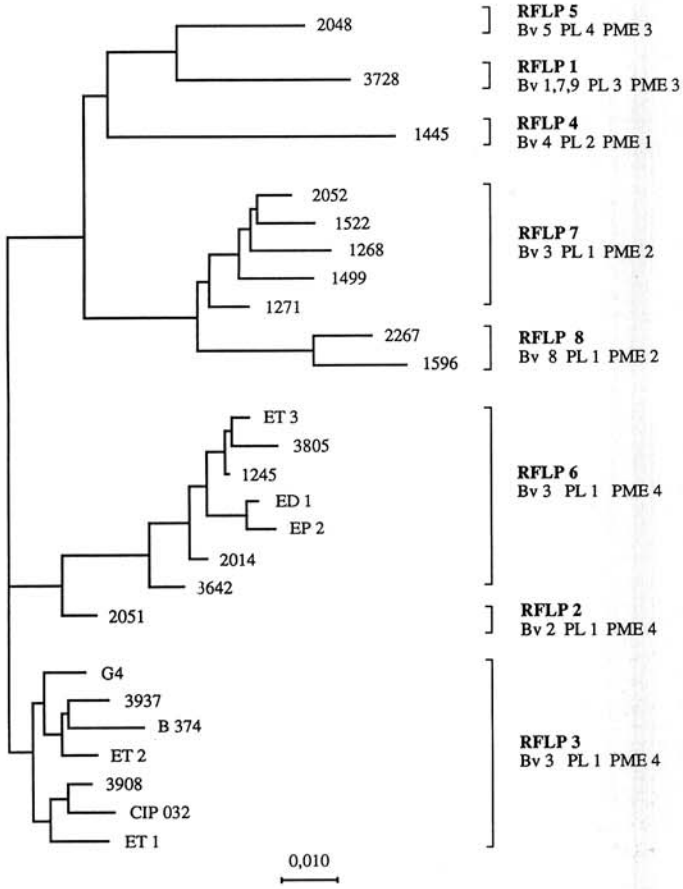

Fig. 2. Phylogenic relationship of 25 strains of *Erwinia chrysanthemi*. This phylogenic tree was calculated, from similarity coefficients given in Figure l, by the Fitch and Margoliash method (1967). The length of the branches are proportional to the genetic distances (1.5 cm = 1% nucleotide diversity).

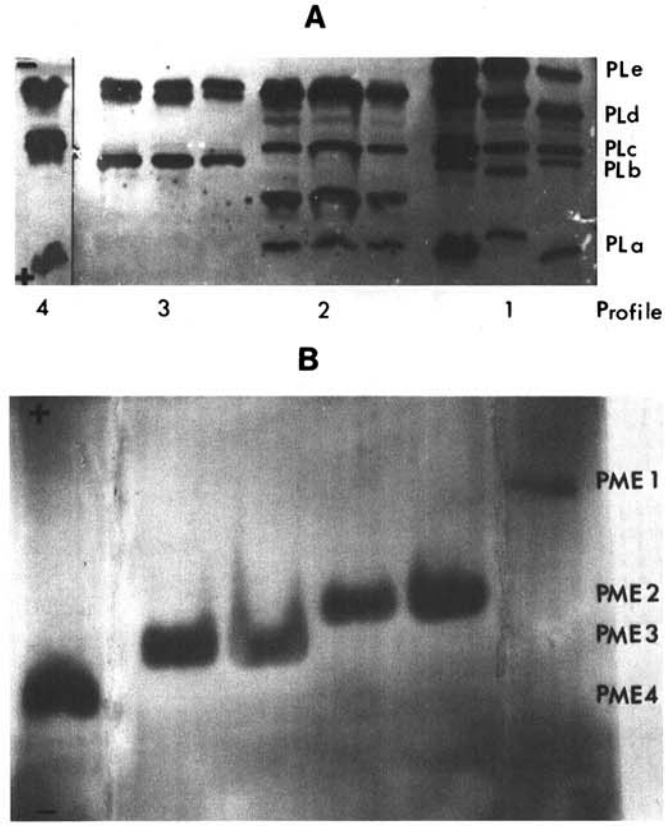

Fig. 3. Electrofocusing on a thin polyacrylamide gel of culture supernatant from *Erwinia chrysanthemi* strains from different restriction fragment length polymorphism (RFLP) groups. **A,** Pectate lyase (PL) detection (pH gradient 3–10). Lanes from right to left correspond to strains: 2048, 1888, 86.14.2Y, 2013, EM1, 1451, 1445, 1268, 3665, and 3937. **B,** Pectin methyl esterase (PME) detection (pH gradient 8–10.5). Lanes from right to left correspond to strains: EM1, 1268, 1596, 2267, 2048, and 3937.

RFLP group with similarity coefficients ranging from 90 to 100%. No correlation was observed between a particular type of cultural practice of the host plants (greenhouse, nursery, or open field) and RFLP group 1.

RFLP group 4 corresponded to strains isolated from banana in Columbia and Martinique. These strains were genetically homogenous (similarity coefficients within group 4: 80 to 90%) and were very different from the unique strain isolated from banana in the Ivory Coast (RFLP group 10). Furthermore, DNA from RFLP group 4 strains did not hybridize to the probe pMH18 (*celY*).

Some strains isolated from dieffenbachia constituted RFLP group 2. They were isolated from different geographical areas (Europe or Africa) and were shown to be very homogenous (similarity coefficient within RFLP group 2: >95%).

Strains isolated from corn were assigned to RFLP groups 7 and 8. They were distinct from other strains, and although only slightly related to one another (40% similarity), they were placed on the same branch of the phylogenic tree (Fig. 2).

The rest of the strains were distributed into three RFLP groups (RFLP groups 3, 5, and 6). The strains from RFLP groups 3 and 6 were isolated from crop plants (tomato and potato) in tropical or subtropical areas or from ornamental plants (saintpaulia, dieffenbachia, and philodendron). These ornamental plants were imported from subtropical areas and vegetatively propagated in greenhouses at high temperatures in temperate regions. The strains from RFLP groups 3 and 6 exhibited a high degree of polymorphism (within groups similarity coefficients ranged from 65 to 85%). The strains from RFLP group 5 included isolates from carnation and strain 2048, which was isolated from *Chrysanthemum morifolium* Ramat. (chrysanthemum).

Biovars and RFLP groups. We compared biovar classification (Ngwira 1989; Ngwira and Samson 1990) of the 52 considered strains with the RFLP grouping (Table 1). In some cases, the distribution of the strains in a biovar corresponded to the distribution in RFLP groups: biovar 1 corresponded to RFLP group 1; biovar 2 to RFLP group 2; biovar 4 to RFLP group 4; and biovar 5 to RFLP group 5. However, the strains that belonged to biovar 3 were distributed in RFLP groups 3, 6, and 7, suggesting that RFLP analysis may be more discriminative than biovar analysis. On the other hand, in RFLP group 1 we found identical or very related RFLP profiles for strains belonging to biovars 1, 7, and 9. Thus, biovar determination could be used to identify some of the genetically homogenous groups (RFLP groups 2, 4, and 5). However, for strains that belong to biovar 3, RFLP analysis was necessary to differentiate the RFLP groups 3, 6, and 7. For RFLP group 1, one must consider that biovars 1, 7, and 9 may be too discriminating compared with the low genetic polymorphism of this RFLP group.

Isoenzymatic profiles and RFLP groups. Ried and Collmer (1986) have analyzed the enzymatic profiles of *E. chrysanthemi* strains isolated from different hosts and have suggested a possible mean of classification according to the original host. We examined the production of pectate lyase (PL) and pectin methyl esterase (PME) by the strains listed in Table 1 using electrofocusing methods (Fig. 3). The reference was strain 3937 (Fig. 3A; profile 1), which produced 5 PL isozymes: PLa which is acidic, PLb and PLc which are neutral, and PLd and PLe which are basic. Strains from RFLP groups 2, 3, 6, 7, and 8 had roughly this canonical profile with five pectate lyases. Strain 2014 does not produce the isozyme PLa in the supernatant nor in the cell, suggesting that this isolate contains a mutation in the corresponding gene. Strains from RFLP group 1 (profile 3) were very homogenous and produced only two isozymes of pectate lyase in the culture medium, one neutral and one basic. No other activity was found to be cell-associated. The strains from RFLP groups 4 and 5 also exhibited distinct profiles of pectate lyase (profiles 2 and 4 in Fig. 3A).

Polymorphism among PME activity in *E. chrysanthemi* strains has also been observed (Y. Bertheau and M. Boccara, unpublished), and the results obtained with pectate lyases could be extended to PME (see Table 1 and Fig. 3B). Interestingly, pectic isozyme and RFLP classifications could be correlated: RFLP groups 2, 3, and 6, which were found by cluster analysis to be slightly related, corresponded to PL 1 and PME 4 profiles (Fig. 2). However, although corn strains exibited a PL profile similar to strain 3937, they produced a PME with a different pI. Taken together, isozyme profiles like biovar characterization are less discriminative for classification purposes than RFLP, but they are in accordance with the clusters defined by the phylogenic tree (Fig. 2).

Host specificity of *E. chrysanthemi* strains. Pathogenicity tests were undertaken with a limited number of strains from RFLP groups 2, 3, and 6 on several test plant species to evaluate the possible correlation between RFLP grouping and host specificity (Table 4). All the strains considered incited disease on potato, lettuce, tomato, and saintpaulia leading to maceration of the whole plant. For example, the strains isolated from tomato in the French West Indies belonged to three different RFLP groups and were all pathogenic to tomato, potato, lettuce, and saintpaulia (D. Lalo, unpublished). Specific interactions were observed between some hosts, that is, dieffenbachia and philodendron and certain strains of *E. chrysanthemi*. Strain 2051 from RFLP group 2 was the only strain producing

Table 4. Pathogenicity of six *Erwinia chrysanthemi* strains for different test plant species

	Strains[a]					
Test plants	**3937**	**ET1**	**ET3**	**EP2**	**ED1**	**2051**
Potato	+[b]	+	+	+	+	+
Lettuce	+	+	+	+	+	+
Tomato	+	+	+	+	+	+
Saintpaulia	+	+	+	+	+	+
Philodendron	−	−	+/−[c]	+	+	−
Dieffenbachia	−	−	−	−	−	+

[a] Strain 3937 isolated from saintpaulia, RFLP 3, biovar 3; ET1 isolated from tomato, RFLP 3, biovar 3; ET3 isolated from tomato, RFLP 6, biovar 3; EP2 isolated from philodendron, RFLP 6, biovar 3; ED1 isolated from dieffenbachia, RFLP 6, biovar 3; 2051 isolated from dieffenbachia, RFLP 2, biovar 2.

[b] + Indicates systemic maceration; − indicates water-soaked lesions leading to slight necrosis or chlorosis.

[c] Symptom intensity was slightly reduced (50% of the inoculated plants were fully macerated).

systemic maceration on dieffenbachia. The strains from RFLP group 6 (EP2, ED1, and ET3) were the only strains pathogenic on philodendron. Recently, we observed that strains 3805, 1245 (RFLP group 6), and 2015 (RFLP group 1) were pathogenic to saintpaulia plants, but only strains 3805 and 1245 were also able to macerate philodendron plants (data not shown). Although a large number of test plant species have been screened to study the pathogenicity of RFLP group 3 strains (D. Lalo, in preparation), no differential host has been found for this group.

Several investigations have attempted to determine whether any relationship could be found between a given isolate and its pathogenicity on test plants, phenotypic properties, and the host of origin (Dickey 1981; Janse and Ruissen 1988). Dickey (1981) has established host specificity for strains isolated from dieffenbachia, and our results confirmed this observation. However, a strain (ED1) isolated from dieffenbachia was shown to be nonpathogenic (Table 4) on this plant species, suggesting that this strain may not have initiated the lesions from which it was isolated. Janse and Ruissen (1988) analyzed several strains of *E. chrysanthemi* isolated from crop and ornamental plants in the Netherlands. Interestingly, they found that potato plants were susceptible to all the analyzed strains. However, their strains from biovars 1, 5, and 7, which might correspond to RFLP groups 1 and 5, were not pathogenic on philodendron. Furthermore, they showed that kalanchoe might be a differential host for strains from biovar 7 (in our analysis, strains isolated in temperate areas and classified in RFLP group 1).

We and others (Dickey and Victoria 1980; Ngwira 1989) have observed a great heterogeneity in strains isolated from banana in Central America (i.e., the strains were placed into groups 3, 4, and 6; M. Boccara and R. Vedel, unpublished). We are studying the pathogenicity of these different strains on several genotypes of banana plants.

Conclusions. Using RFLP analysis, we have classified 52 *E. chrysanthemi* strains into 10 distinct groups. Cluster analysis allowed the construction of an unrooted phylogenic tree in which most of the RFLP groups converged to the same origin. This suggests that the strains are not derived from each other, but are more likely representative of divergent evolution from a common ancestor.

The homogeneity of the strains from RFLP group 1 based on genetic, biochemical, and physiological evidence strongly suggested a clonal origin for this group. It should also be noted that all these strains were shown to be unable to grow at 39° C. This characteristic might be related to their adaptation to crops grown in temperate climates. To test this hypothesis, we are currently analyzing *E. chrysanthemi* strains from other temperate regions of the world.

RFLP groups 2 and 6 are located on the same branch of the phylogenic tree and may be closely related (Fig. 2). The least related RFLP groups (similarity coefficients with other groups: <40%) might be considered as subspecies of *E. chrysanthemi*. To confirm this hypothesis, a larger number of strains should be analyzed or other phylogenic criteria, such as the sequence of specific regions from 16S or 23S rRNA, could be considered (DeParasis and Roth 1990).

RFLP determination is more precise and more discriminative than the other methods (biovars, pectinolytic enzymes), because it allowed the distinction of genetic groups within biovar 3 that were not resolved by other methods. Data of RFLP analysis showed that *E. chrysanthemi* is a highly polymorphic species and that it allows the characterization of genetically divergent groups, which were unexpected. RFLP group 6 was of particular interest because it corresponded to a group of strains pathogenic on philodendron.

The results presented here demonstrate that RFLP analysis can provide help not only in *E. chrysanthemi* classification but also in defining some host specificity within this species. This now gives the basis to identify the genes that control host range in *E. chrysanthemi*.

ACKNOWLEDGMENTS

We thank Régine Samson for providing most of the strains and for helpful and encouraging discussions during this work. We also thank Paul Gill and David Guest for help with the English translation. This work was funded by INRA (Action Incitative Programmée RFLP).

LITERATURE CITED

Aymeric, J. L., Enard, C., Renou, F., Neema, C., Boccara, M., and Expert, D. 1990. The requirement of depolymerizing enzymes and a high affinity iron transport system for pathogenicity of *Erwinia chrysanthemi*. Pages 679-683 in: Plant Pathogenic Bacteria. Part B. Z. Klement, ed. Proc. Int. Conf. Plant Pathog. Bact., 7th. Budapest. 1989. Akadémiai Kiadó, Budapest, Hungary. 397 pp.

Bertheau, Y., Madjidi-Erwan, E., Kotoujansky, A., Nguyen-The, C., Andro, T., and Coleno, A. 1984. Detection of depolymerase enzymes after electrophoresis or electrofocusing, or in titration curves. Anal. Biochem. 139:383-389.

Boccara, M., Diolez, A., Rouve, M., and Kotoujansky, A. 1988. The role of individual pectate lyases of *Erwinia chrysanthemi* 3937 in pathogenicity on saintpaulia plants. Physiol. Mol. Plant Pathol. 33:95-104.

Bradbury, J. F. 1984. Guide to Plant Pathogenic Bacteria. Pages 72-76 in: Commonw. Agric. Bur. Annot. Bibliogr. Int. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.

Cook, D., Barlow, E., and Sequeira, L. 1989. Genetic diversity of *Pseudomonas solanacearum*: Detection of restriction fragment length polymorphisms with DNA probes that specify virulence and the hypersensitive response. Mol. Plant-Microbe Interact. 2:113-121.

DeParasis, J., and Roth, D. A. 1990. Nucleic acid probes for identification of phytobacteria: Identification of genus-specific 16S rRNA sequences. Phytopathology 80:618-621.

Dickey, R. S. 1981. *Erwinia chrysanthemi*: Reaction of eight plant species to strains from several hosts and to strains of other *Erwinia* species. Phytopathology 71:23-29

Dickey, R. S., and Victoria, J. I. 1980. Taxonomy and emmended description of strains of *Erwinia* isolated from *Musa paradisiaca* Linnaeus. Int. J. Syst. Bacteriol. 30:129-134.

Enard, C., Diolez, A., and Expert, D. 1988. Systemic virulence in *Erwinia chrysanthemi* requires a functional high affinity iron assimilation system. J. Bacteriol 170:2419-2426.

Fitch, W. M., and Margoliash, E. 1967. Construction of phylogenetic trees. Science 155:279-284.

Gabriel, D. W. 1989. Genetics of plant parasite populations and host-parasite specificity. Pages 343-379 in: Plant-Microbe Interactions. Molecular and Genetic Perspectives. Vol. 3. T. Kosuge and E. Nester, eds. McGraw-Hill Inc., New York.

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.

Gabriel, D. W., Kingsley, M. T., Hunter, J. E., and Gottwald, T. 1989. Reinstatement of *Xanthomonas citri* (ex Hasse) and *X. phaseoli* to species and reclassification of all *X. campestris* pv. *citri* strains. Int. J. Syst. Bacteriol. 39:14-22.

Janse, J. D., and Ruissen, M. A. 1988. Characterization and classification of *Erwinia chrysanthemi* strains from several hosts in the Netherlands.

Phytopathology 78:800-808.
Klotz, L., and Zimm, B. H. 1972. Size of DNA determined by viscoelastic measurements: Results on bacteriophages, *Bacillus subtilis* and *Escherichia coli*. J. Mol. Biol. 72:779-800.
Lemattre, M. 1977. Etude du comportement sur *Saintpaulia ionantha* de quelques souches hétérologues d'*Erwinia carotovora* var. *chrysanthemi* (Burkholder) Dye. Pages 175-192 in: Travaux dédiés à Georges Viennot-Bourgin. Ed. Société française de phytopathologie. Paris, France.
Maniatis, T., Fritsch, E. F., Sambrook, J. 1982. Molecular cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 466 pp.
Nei, M., and Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269-5273.
Nei, M., and Miller, J. C. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. Genetics 125:873-879.
Ngwira, N. S. 1989. Diversité biochimique, sérologique et protéique de la bactérie phytopathogène *Erwinia chrysanthemi*. Ph.D. thesis. Université de Rennes, France.
Ngwira, N. S., and Samson, R. 1990. *Erwinia chrysanthemi*: Description of two new biovars (bv. 8 and bv. 9) isolated from kalanchoe and maize host plants. Agronomie 10:341-345.
Reverchon, S., Van Gijsegem, F., Rouve, M., and Kotoujansky, A. 1986. Organization of a pectate lyase gene family in *Erwinia chrysanthemi*. Gene 49:215-224.
Ried, J. L., and Collmer, A. 1986. Comparison of pectic enzymes produced by *Erwinia chrysanthemi*, *Erwinia carotovora* subsp. *carotovora* and *Erwinia carotovora* subsp. *atroseptica*. Appl. Environ. Microbiol. 52:305-310.
Saitou, N., and Nei, M. 1987. The Neighbor Joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.
Samson, R., and Nassan-Agha, N. 1978. Biovars and serovars among 129 strains of *Erwinia chrysanthemi*. Pages 547-553 in: Proc. Int. Conf. Plant Pathog. Bact., 4th. Station de Pathologie Végétale et Phytobactériologie, Angers, France. 1978. Ed. Gibert-Clarey, Tours, France.
Schoonejans, E., Expert, D., and Toussaint, A. 1987. Characterization and virulence properties of *Erwinia chrysanthemi* lipopolysaccharide-defective, PhiEC2-resistant mutants. J. Bacteriol 169:4011-4017.
Selsenstein, J. 1990. PHYLIP Manual version 3.3. University Herbarium. Univ. Calif., Berkeley.
Young, J. M., Dye, D. W., Bradbury, J. F., Panagopoulos, C. G., and Robbs, C. F. 1978. A proposed nomenclature and classification for plant pathogenic bacteria. N. Z. J. Agric. Res. 21:151-175.