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Isolation of an Insertion Sequence (IS*1051*) from *Xanthomonas campestris* pv. dieffenbachiae with Potential Use for Strain Identification and Characterization

YVETTE BERTHIER,[1] DOMINIQUE THIERRY,[2] MONIQUE LEMATTRE,[1] AND JEAN-LUC GUESDON[2]*

Laboratoire de Prédéveloppement des Sondes, Institut Pasteur, 75724 Paris Cedex 15,[2] and Station de Pathologie Végétale, Institut National de la Recherche Agronomique, 78026 Versailles,[1] France

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A new insertion sequence was isolated from *Xanthomonas campestris* pv. dieffenbachiae. Sequence analysis showed that this element is 1,158 bp long and has 15-bp inverted repeat ends containing two mismatches. Comparison of this sequence with sequences in data bases revealed significant homology with *Escherichia coli* IS*5*. IS*1051*, which detected multiple restriction fragment length polymorphisms, was used as a probe to characterize strains from the pathovar dieffenbachiae.

Xanthomonas campestris pv. dieffenbachiae (McCulloch and Pirone) Dye is the causal agent of *Anthurium* blight, which is widely distributed throughout *Anthurium*-growing countries, and is the limiting factor of plant production. Hayward reported the first observations of this disease in Hawaii in 1972. First identified as a pathogen of *Dieffenbachia* species, *X. campestris* pv. dieffenbachiae can infect a broad range of aroid hosts. Different approaches recently used to characterize and detect *X. campestris* pv. dieffenbachiae strains (2, 3, 12) have demonstrated the heterogeneity of this pathovar. In an attempt to understand the genetic variability of *X. campestris* pv. dieffenbachiae strains, our approach was to find a repetitive DNA sequence and use it as a probe. Numerous repetitive DNA sequences including insertion sequences have been reported and proven to be valuable tools for molecular genetic studies and bacterial strain characterization (13, 20). Few repetitive sequences have been found in *Xanthomonas* spp. (5, 8, 9, 11). The repetitive sequence from *Xanthomonas oryzae* pv. oryzae was used to study the genetic diversity of 98 strains of this pathovar (10). By using this probe, it was possible to distinguish *X. campestris* pv. zeae from other pathovars capable of infecting maize and to identify *X. campestris* pv. vasculorum strains from different geographical origins (15, 16).

We describe here the isolation of a repetitive DNA fragment from the genome of *X. campestris* pv. dieffenbachiae. Sequence analysis of this DNA fragment revealed the presence of an insertion sequence-like element given the name IS*1051* by the Central Plasmid Registry (Stanford, Calif.). IS*1051* has significant homology with *Escherichia coli* IS*5* (4). In this report, the entire nucleotide sequence of IS*1051* is given, and its potential use for *X. campestris* pv. dieffenbachiae strain characterization and detection is proposed.

The origins of bacterial strains used in this study are listed in Tables 1, 2, and 3. Strains from the epiphytic flora on *Anthurium* leaves were recovered by washing the samples in sterile water and were then grown on YDA medium (5 g of yeast extract, 5 g of Bacto Peptone, 10 g of glucose, 20 g of agar [each per liter]).

A cosmid library of *X. campestris* pv. dieffenbachiae strain 11044 genomic DNA was constructed by a procedure described in detail elsewhere (21). Cosmid DNA was digested to completion with *Sal*I and electrophoresed through 0.8% agarose gels. Gels were incubated once for 15 min in 0.25 M HCl, twice for 15 min each time in 1.5 M NaCl containing 0.5 M NaOH, and twice for 15 min each time in 1 M CH_3COONH_4 and transferred to Hybond N filters (Amersham, Les Ulis, France). Filters were probed with [α-^{32}P]dCTP-labeled genomic DNA from *X. campestris* pv. dieffenbachiae strain 11044. A 2-kb *Sal*I fragment which hybridized strongly with DNA from *X. campestris* pv. dieffenbachiae strain 11044 was electroeluted from the agarose gel and cloned in pUC18. After sequential digestions with restriction endonucleases *Sal*I and *Bam*HI, a 1,574-bp fragment was subcloned in pUC18 and sequenced by the chain termination method of Sanger et al. (18), using the Sequenase sequencing kit (United States Biochemical Corp., Cleveland, Ohio). To complete the sequence, we synthesized and used specific internal oligonucleotides.

A 24-mer oligonucleotide (Y15) complementary to the inverted repeat sequence of IS*1051* with a *Bam*HI restriction site added to its 5′ extremity was used as the primer (AAAGGATCCGGAAGGTCTGAACAA), and *X. campestris* pv. dieffenbachiae strain 11044 genomic DNA (2 ng) was used as the template in a standard polymerase chain reaction (PCR) to synthesize IS*1051* DNA. The PCR product (1,158 bp) was cloned in pUC18, and the resulting plasmid was named pXC01. In addition, the PCR product was radiolabeled with [α-^{32}P]dCTP, using the Multiprime random-labeling system (Amersham), and used as a probe in restriction fragment length polymorphism (RFLP) analysis.

For Southern blot analysis (19), genomic DNA was extracted from bacterial cultures by a previously described protocol (2). DNA (1 μg) was then digested with 40 U of restriction endonuclease *Eco*RI in the appropriate buffer for 16 h at 37°C. DNA fragments were separated by an overnight electrophoresis through a 0.6% agarose gel, transferred to a Hybond N filter (Amersham), and hybridized with denatured ^{32}P-radiolabeled IS*1051*.

Primers designated Y5 (GGACGATCGTGGACGCCA

* Corresponding author. Mailing address: Laboratoire de Prédéveloppement des Sondes, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: 33-1-45688000. Fax: 33-1-45688639. Electronic mail address: guesdon@pasteur.fr.

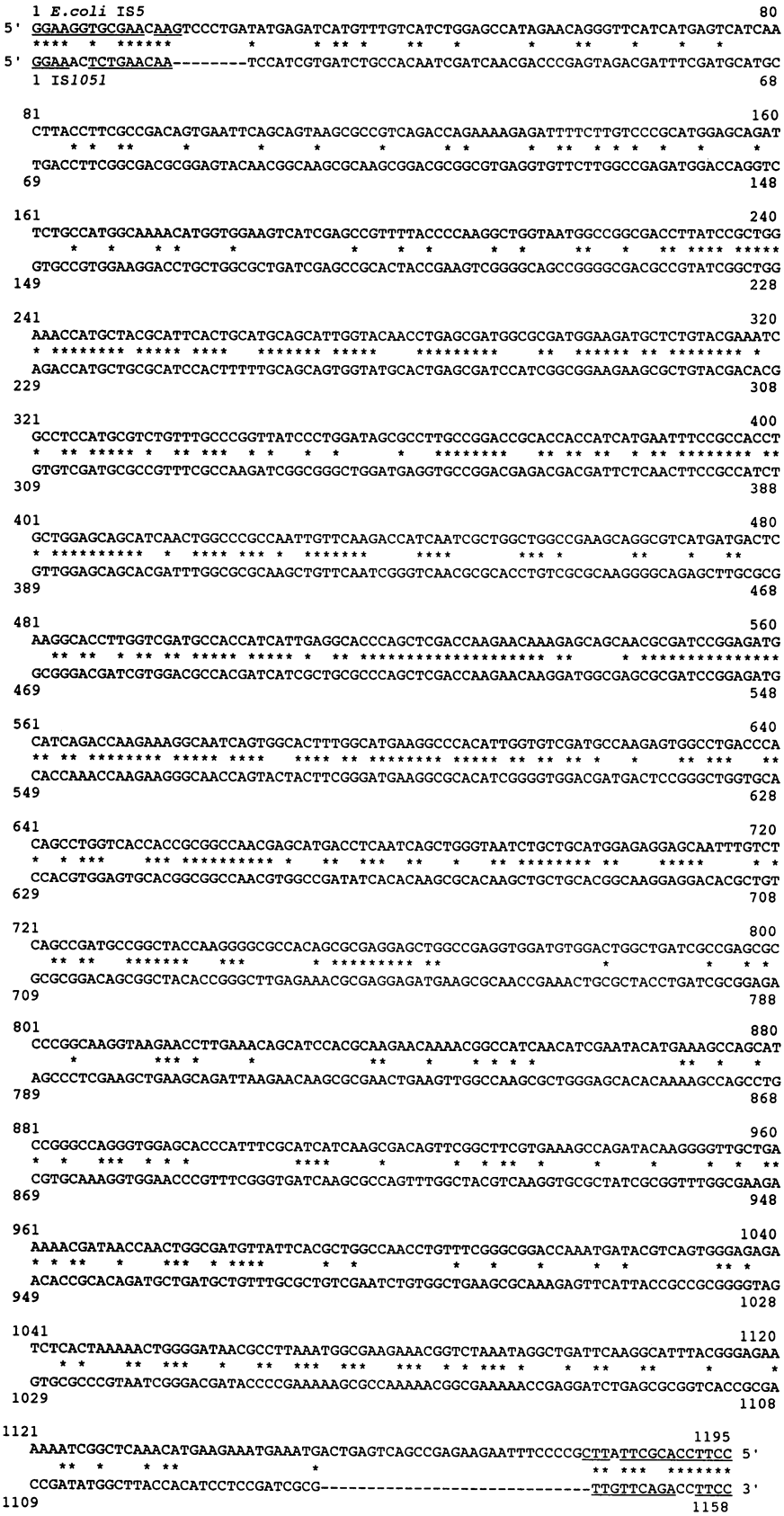

FIG. 1. Nucleotide sequences of *X. campestris* pv. dieffenbachiae IS*1051* (lower line) and *E. coli* IS*5* (upper line). The sequences are numbered from the outer end of the inverted repeat sequences. Inverted repeats are underlined. Asterisks indicate homology between the two sequences. Dashes were added to allow the inverted repeats to be aligned.

TABLE 1. *X. campestris* pv. dieffenbachiae strains studied

CRA strain[a]	Source strain[b]	Host	Location	Hybridization banding pattern
11044		*Anthurium* sp.	Guadeloupe	1
11001		*Anthurium* sp.	Guadeloupe	1
11003		*Anthurium* sp.	Guadeloupe	1
11005		*Anthurium* sp.	Guadeloupe	1
11006		*Anthurium* sp.	Guadeloupe	1
11008		*Anthurium* sp.	Guadeloupe	1
11010		*Anthurium* sp.	Guadeloupe	1
11019		*Anthurium* sp.	Martinique	1
11014		*Anthurium* sp.	Venezuela	1
11060	AV 945	*Anthurium* sp.	Venezuela	1
11022		*Anthurium* sp.	Hawaii	2
11053	PR 58	*Anthurium* sp.	Puerto Rico	3
11054	PR 63	*Anthurium* sp.	Puerto Rico	3
11056	PR 197	*Anthurium* sp.	Puerto Rico	3
11002		*Anthurium* sp.	Guadeloupe	4
11007		*Anthurium* sp.	Guadeloupe	4
11020		*Anthurium* sp.	Guadeloupe	4
11004		*Anthurium* sp.	Guadeloupe	5
11063	400/2	*Anthurium* sp.	Mauritius	6
11064	400/4	*Anthurium* sp.	Mauritius	7
11052	NCPPB 1833[c]	*Anthurium* sp.	Brazil	8
11059	X 326	*Anthurium* sp.	Florida	9
11050	NCPPB 985	*Dieffenbachia* sp.	United States	10
11051	NCPPB 986	*Dieffenbachia* sp.	United States	10
11208	X 265	*Philodendron oxycardium*	Florida	11
11215	X 696	*Philodendron oxycardium*	Florida	12
11216	X 736	*Xanthosoma malanga*	Florida	13
11217	X 742	*Xanthosoma malanga*	Florida	14
11218	X 745	*Xanthosoma malanga*	Florida	14
11219	X 807	*Caladium bicolor*	Florida	15

[a] CRA, Institut National de la Recherche Agronomique, Versailles, France.
[b] NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, England; AV 945, Y. Guevara; PR 58, PR 63, and PR197, M. Zapata; 400/2 and 400/4, N. Sinaretty; X 265 to X 807, A. Chase.
[c] Pathovar reference strain.

CG) and Y9 (GTTGGCCGCCGTGCACTCCA) capable of specifically amplifying a 200-bp fragment of the insertion sequence IS*1051* were synthesized on a Cyclone plus DNA synthesizer (Millipore-Waters, Saint Quentin Yvelines, France) by the phosphoramidite coupling method. Generation of primers and subsequent purification were performed according to the manufacturer's instructions.

Amplification reactions were performed in a total volume of 50 μl of a mixture containing 50 mM Tris-HCl (pH 8.5), 2 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 100 pmol of each primer, 200 μM (each) deoxyribonucleoside triphosphate, 2 ng of template DNA (in 5 μl), and 2 U of *Thermus aquaticus* DNA polymerase (Perkin-Elmer Corp., Norwalk, Conn.). The amplification mixture in each tube was overlaid with 50 μl of mineral oil and subjected to 39 cycles of amplification as follows: samples were incubated at 95°C for 2 min to denature the DNA, at 60°C for 2 min to anneal the primers, and at 72°C for 2 min to extend the annealed primers. Thermal cycling was performed in a programmable heat block (Gene ATAQ controller; Pharmacia). Each amplification experiment included a negative-control sample without DNA and a positive-control sample with 2 ng of DNA from *X. campestris* pv. dieffenbachiae strain 11044. In order to verify the specifity of the amplified fragment, 1/10th of the amplification reaction was analyzed by electrophoresis on a 2% agarose gel, using ϕX174 DNA digested by *Hin*cII (Pharmacia) as a size marker. Gels were stained with ethidium bromide, photographed with a UV transilluminator, and analyzed by Southern blotting using ^{32}P-labeled IS*1051* as the probe. The exposure time was 5 min.

Hybridizations were performed at 68°C for 16 h (17) in a mixture composed of 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate buffer [pH 7]), 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), 100 μg of denatured salmon sperm DNA per ml, and the DNA probe (10^6 cpm/ml). After hybridization, filters were washed twice with 2× SSC at 65°C for 10 min, once with 2× SSC–0.1% SDS at 65°C for 30 min, and once with 0.1× SSC at 65°C for 10 min. Filters were briefly air dried and exposed to Kodak XAR5 film with intensifying screens at −80°C.

With the aim of finding a repetitive DNA sequence in the genome of *X. campestris* pv. dieffenbachiae, we constructed a cosmid library representative of the complete *X. campestris* pv. dieffenbachiae strain 11044 genome. A set of 200 recombinant clones was analyzed by Southern blotting, using *X. campestris* pv. dieffenbachiae strain 11044 total DNA as the probe after *Sal*I digestion. Several clones contained DNA fragments that hybridized strongly with *X. campestris* pv. dieffenbachiae DNA. Among them, clone *I9* contained a 2-kbp fragment that met our initial goals of desired size and strong hybridization. This fragment was isolated from an agarose gel, radiolabeled, and used as a probe in preliminary Southern blot experiments with different *X. campestris* DNA samples. The results (not shown) clearly demonstrated that this 2-kbp fragment was present in multiple copies in the *X. campestris* genome, thus fulfilling our third criterion of repeats within the genome.

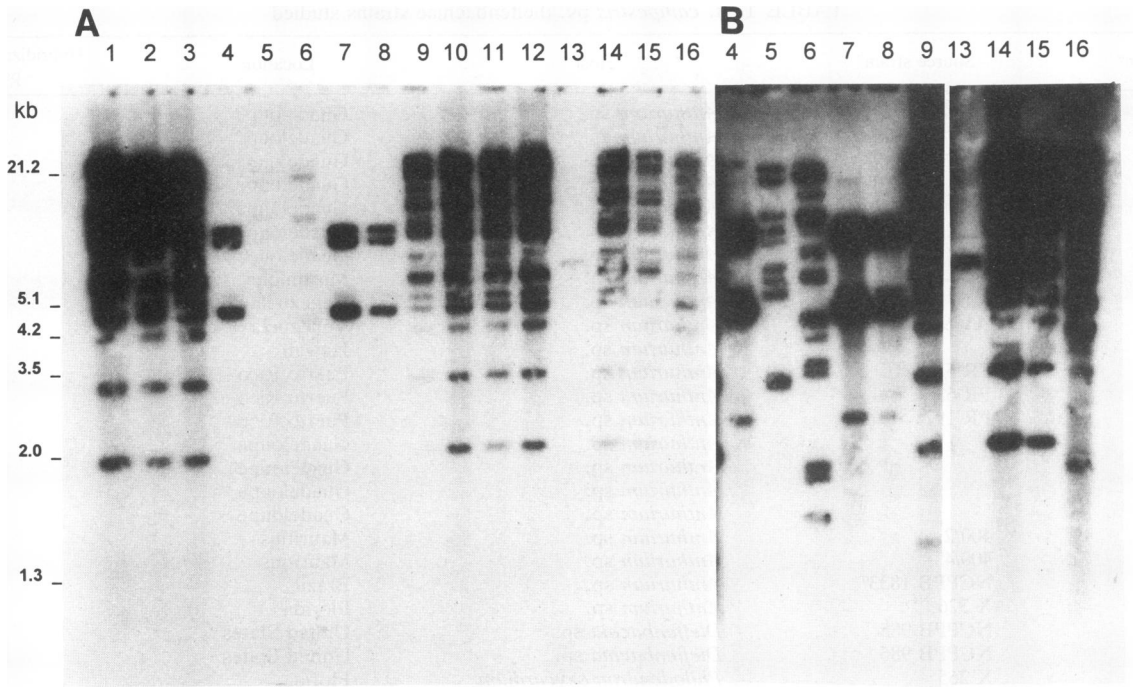

FIG. 2. Southern blot analysis of *Eco*RI-cleaved total DNA from strains of *X. campestris* pv. dieffenbachiae, representing different geographical areas, probed with ^{32}P-labeled IS*1051*. The gels were exposed for 90 min (A) or 48 h (B). Lanes 1 to 3, strains 11001, 11010, and 11019, respectively, exhibiting pattern 1; lane 4, strain 11022 exhibiting pattern 2; lane 5, strain 11002 exhibiting pattern 4; lane 6, strain 11063 exhibiting pattern 6; lane 7, strain 11053 exhibiting pattern 3; lane 8, strain 11056 exhibiting pattern 3; lanes 9 to 12, strains 11044, 11006, 11008, and 11014, respectively, exhibiting pattern 1; lane 13, strain 11052 exhibiting pattern 8; lanes 14 and 15, strains 11060 and 11005, respectively, exhibiting pattern 1; lane 16, strain 11050 exhibiting pattern 10.

The DNA fragment of interest was cloned in pUC18 plasmid, and after *Sal*I and *Bam*HI digestion, a 1,574-bp fragment was subcloned and its nucleotide sequence was determined. The presence of an almost perfect inverted repeat (13 of 15 matching bases) at the ends of this sequence indicated the presence of a potential insertion sequence of 1,158 bp. This insertion sequence-like element was named IS*1051*. Its complete nucleotide sequence is shown in Fig. 1, and its GC content is 60.19%. A search of the EMBL and GenBank data bases revealed significant homologies with insertion sequences belonging to the IS*5* family (for example, 47% homology between this fragment and the *E. coli* insertion element IS*5* [Fig. 1] previously described [4]). Moreover, the inverted repeats of IS*1051* are similar to the inverted repeats of the IS*5* family isolated from various gram-negative bacteria (1).

For RFLP analysis, we examined 22 strains of *X. campestris* pv. dieffenbachiae isolated from *Anthurium* species representative of different production areas and 8 strains isolated from other aroids (Table 1). Nine hybridization banding patterns were observed for *Anthurium* strains. Commonly obtained patterns are shown in Fig. 2. In the strains studied, the number of fragments containing IS*1051* varied from 1 to more than 20. Strains isolated from *Anthurium* species from Guadeloupe, Martinique, and Venezuela all exhibited pattern 1, while one strain from Hawaii exhibited pattern 2 and three strains isolated from Puerto Rico exhibited pattern 3; patterns 2 and 3 were similar but distinguishable. Three strains from Guadeloupe (11002, 11007, and 11020) expressed pattern 4, while the fourth strain (11004) expressed pattern 5, which is closely related to pattern 10 of two American strains (11050 and 11051) isolated from *Dieffenbachia* species. The two strains isolated from *Anthurium* species from Mauritius exhibited patterns 6 and 7. Most *Anthurium* strain patterns appeared clearly after 30 min of exposure, whereas those of strains 11002, 11063, and 11052 required 2 days of exposure. Extensive variability was observed in the patterns of the few strains isolated from other aroids: eight strains isolated from *Dieffenbachia* sp., *Philodendron oxycardium*, *Xanthosoma malanga*, and *Caladium bicolor* gave six different patterns (i.e., patterns 10 to 15) (Table 1). In addition, DNA samples from 18 strains recovered from the epiphytic flora on *Anthurium* leaves were analyzed by Southern blotting with the IS*1051* probe; no hybridization was observed (data not shown). Twenty strains from other families and species were tested (Table 2). Hybridization banding patterns were obtained only with the DNA samples from two strains each of *Xanthomonas axonopodis*, *Xanthomonas fragariae*, and *X. oryzae* pv. oryzae and from one strain of *X. oryzae* pv. oryzicola (Table 2). Genomic DNAs from 33 strains of 15 other *X. campestris* pathovars were analyzed with radiolabeled IS*1051*. Hybridization was observed for 11 of the 15 pathovars (Table 3). For pathovar citri, IS*1051* hybridized with strain 11508 (LMG 9181, Civerolo XC70, pathogenicity group C) but not with the pathovar reference strain, 11502 (NCPPB 409). Hybridization patterns are shown in Fig. 3.

In order to detect *X. campestris* strains, oligonucleotides derived from IS*1051* were used as primers for PCR analysis. Two 20-mer primers, Y5 and Y9, were selected for amplification experiments. When genomic DNA samples from *Anthurium* strains of different geographical origins and representative of different patterns were analyzed by PCR with internal oligonucleotides Y5 and Y9, a 200-bp fragment was

TABLE 2. Strains tested from the families *Enterobacteriaceae* and *Pseudomonadaceae*

Species	CRA strain[a]	Source strain[b]	Host	Location	Hybridization[c]
Erwinia chrysanthemi	30503		*Lycopersicon esculentum*	Martinique	–
	31502		*Nicotiana tabacum*	United States	–
	30407		*Solanum tuberosum*	Peru	–
Pseudomonas solanacearum	1153	ORST 1153b	*Solanum melongena*	Congo	–
	1000		*Lycopersicon esculentum*	French Guiana	–
Pseudomonas syringae pv. pisi	202		*Pisum sativum*	United States	–
	870		*Pisum sativum*	United States	–
	974		*Pisum sativum*	United States	–
Xylophilus ampelinus	16004	NCPPB 2220	*Vitis vinifera*	Greece	–
	16006	NCPPB 3026	*Vitis vinifera*	Italy	–
		CFBP 2098	*Vitis vinifera*	France	–
Xanthomonas albilineans	15004	HV 5	*Saccharum* sp.	Burkina Faso	–
	15005	R 8	*Saccharum* sp.	Réunion	–
Xanthomonas fragariae	17010	NCPPB 1469	*Fragaria ananassa*	United States	+
	17012	NCPPB 1822	*Fragaria ananassa*	New Zealand	+
Xanthomonas axonopodis	18001	NCPPB 457	*Axonopus scoparius*	Colombia	+
	18002	NCPPB 2375	*Axonopus scoparius*	Colombia	+
X. oryzae					
pv. oryzae	11606	LMG 634	*Oryza sativa*	Colombia	+
	11609	NCPPB 3002[d]	*Oryza sativa*	India	+
pv. oryzicola	11802	LMG 654	*Oryza sativa*	Philippines	+

[a] CRA, Institut National de la Recherche Agronomique, Versailles, France.
[b] NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, England; LMG, Laboratorium voor Microbiologie Ghent Culture Collection, Ghent, Belgium; ORST, ORSTOM (Institut Français de Recherche Scientifique pour le Développement en Coopération), V. Verdier; HV 5 and R 8, P. Rott; CFBP, Collection Française des Bactéries Phytopathogènes, Angers, France.
[c] –, no hybridization; +, hybridization.
[d] Pathovar reference strain.

reproducibly amplified. The PCR product of 200 bp was also observed in strains of pathovars begoniae, cassavae, and vasculorum.

Insertion sequences, like other repetitive DNA sequences, can be used as probes for RFLP analysis and strain characterization (10, 11). In this study, we examined genetic variability within the pathovar dieffenbachiae. In a previous investigation (2), we analyzed genetic variability by ribotyping, using acetylaminofluorene-labeled 16S and 23S rRNA from *E. coli* as the probe. We were able to differentiate between strains according to the host plant; however, a correlation between the pattern obtained and the geographical origin of the strains could not always be clearly established. Using IS*1051* as the probe, we could distinguish, within rRNA pattern 24 (described in our previous work [2]), between strain 11022 from Hawaii and the three isolates from Puerto Rico. rRNA pattern 25 (2) was obtained with two strains isolated from *Dieffenbachia* species (11050 and 11051) and four strains isolated from *Anthurium* species (11002, 11004, 11007, and 11020) which were pathogenic only in *Dieffenbachia* species. By using IS*1051* as the probe, three different patterns were observed in this group; the two strains isolated from *Dieffenbachia* species shared hybridization pattern 10, three strains isolated from *Anthurium* species exhibited pattern 4, and strain 11004 showed pattern 5 (not shown), which is similar to but distinguishable from pattern 10. With IS*1051*, the host plant could be clearly distinguished (Table 1). It will be interesting to use this probe to analyze a larger collection of *X. campestris* pv. dieffenbachiae strains from aroids. We postulate that a certain degree of variability must exist in the sequences of the insertion sequences within the pathovar dieffenbachiae, as reflected by the different intensities of hybridization banding patterns; some strains gave a strong signal after a 30 min exposure, while others required 2 to 3 days of exposure to become visible, despite the application of the same amount (1 μg) of DNA in each well.

Our results showed that complex hybridization banding patterns could be obtained from other *Xanthomonas* strains (Tables 2 and 3). Therefore, IS*1051* may be an adequate probe to study by RFLP the genetic variability of populations of strains of *Xanthomonas* species and *X. campestris* pathovars that hybridize with this probe. This insertion sequence seems to be widely distributed throughout the genus. Interestingly, no hybridization was obtained with *Xylophilus ampelinus*, which has been shown by different approaches to be genetically distant and which is no longer considered a *Xanthomonas* species (24). The distribution of IS*1051* that we observed does not directly correlate with the currently accepted taxonomy of the genus *Xanthomonas*, since the sequence is not characteristically present in all patterns of the species *X. campestris*. In light of the increasing amount of genetic data, in addition to other taxonomic criteria, the species now classified as *X. campestris* appears to be heterogeneous. From their analysis of DNA relatedness of 24 xanthomonad strains representing 23 *X. campes-*

TABLE 3. Strains of other *X. campestris* pathovars

X. campestris pathovar	CRA strain[a]	Source strain[b]	Host	Location	Hybridization[c]
begoniae	10125		*Begonia* sp.	France	+
	423		*Begonia* sp.	The Netherlands	+
	11054	NCPPB 3003	*Begonia* sp.	The Netherlands	+
vasculorum	11401	CFBP 1289	*Saccharum* sp.	Réunion	+
	11403	NCPPB 796[d]	*Saccharum officinarum*	Mauritius	+
	11413	NCPPB 795	*Saccharum officinarum*	Malagasy Republic	+
	11412	NCPPB 186	*Thysanolaena maxima*	Mauritius	+
manihotis	10504	ORST 1	*Manihot esculenta*	Congo	+
	10508	ORST 10	*Manihot esculenta*	Congo	+
	10509	ORST 18	*Manihot esculenta*	Congo	+
glycines	12401	NCPPB 554[d]	*Glycine max*	Sudan	+
	12402	LMG 7403	*Glycine max*	Zambia	+
poinsettiicola	12301	NCPPB 581[d]	*Euphorbia pulcherrima*	India	+
cassavae	12202	LMG 670	ND[e]	ND	+
	12201	NCPPB 101[d]	*Manihot esculenta*	Malawi	+
campestris	10415	NCPPB 196	*Brassica oleracea*	Mauritius	+
	10417	LMG583	*Brassica* sp.	Burundi	+
phaseoli	10703	LMG 821	ND	ND	+
	10702	NCPPB 3035[d]	*Phaseolus vulgaris*	ND	+
incanae	10905	NCPPB 937[d]	*Matthiola incana*	United States	+
	10906	NCPPB 990	*Matthiola* sp.	United States	+
pelargonii	13301	NCPPB 2269	*Pelargonium hortorum*	United Kingdom	+
citri	11508	LMG 9181	*Citrus aurantifolia*	Brazil	+
	11501	CFBP 1814	*Citrus* sp.	Réunion	−
	11502	NCPPB 409[d]	*Citrus limon*	New Zealand	−
juglandis	11301	CFBP 1023	*Juglans regia*	France	−
	11303	LMG 746	*Juglans regia*	United Kingdom	−
malvacearum	11706		*Gossypium hirsutum*	Sudan	−
	11736		*Gossypium hirsutum*	Costa Rica	−
	11761	NCPPB 633	*Gossypium* sp.	Sudan	−
vesicatoria	10607	LMG 904	ND	ND	−
	10603	NCPPB 422[d]	*Lycopersicon lycopersicum*	New Zealand	−
mangiferae indicae	11901	NCPPB 490	*Mangifera indica*	India	−

[a] CRA, Institut National de la Recherche Agronomique, Versailles, France.
[b] NCPPB, National Collection of Plant Pathogenic Bacteria, Harpenden, England; LMG, Laboratorium voor Microbiologie Ghent Culture Collection, Ghent, Belgium; ORST, ORSTOM, V. Verdier; CFBP, Collection Française des Bactéries Phytopathogènes, Angers, France.
[c] +, hybridization; −, no hybridization.
[d] Pathovar reference strain.
[e] ND, not determined.

tris pathovars and 1 *X. fragariae* strain, Hildebrand et al. (7) deduced that their results do not support the retention of *X. campestris* as a single species. In that study *X. campestris* pv. secalis was found to be more closely related to *X. fragariae* than to any other *X. campestris* pathovar. Vauterin et al. (22) agreed with the heterogeneity of the species and delineated six DNA homology groups within *X. campestris*. Among the *X. campestris* strains that we tested, some pathovars exhibited complex banding patterns and others exhibited no hybridization. Our results with the various strains demonstrate the potential of IS*1051* but are still too preliminary to allow generalizations. For *X. campestris* pv. citri, we observed no hybridization with strains 11501 and 11502, unlike strain 11508 from pathotype C, which gave a complex hybridization pattern. Ribotyping has previously enabled us to distinguish strains of *X. campestris* pv. citri from the different pathotypes (our unpublished results), thus confirming the heterogeneity of *X. campestris* pv. citri reported by other researchers (23). This result is also consistent with those of Pruvost et al. (14) and Hartung (6), who specifically detected strains of *X. campestris* pv. citri from pathotype A. Our results further confirm the promise that rapid diagnostic tools based on genomic relatedness can be developed. The RFLP detected indicates that a number of

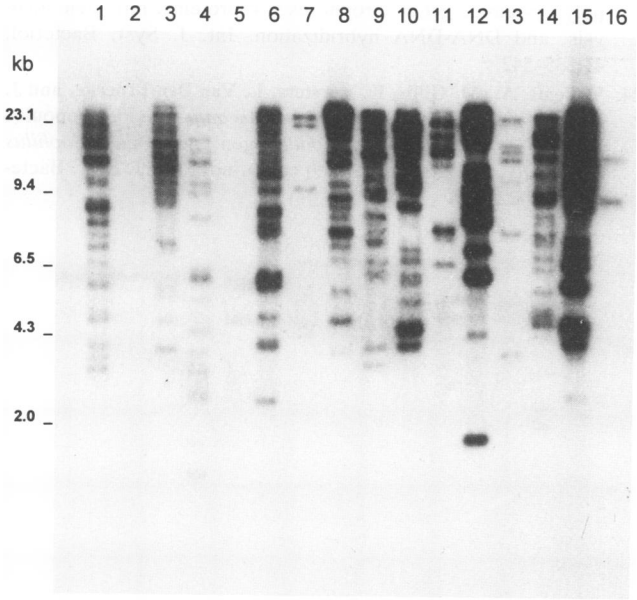

FIG. 3. Southern blot analysis of *Eco*RI-cleaved total DNA from strains of *Xanthomonas* species and *X. campestris* pathovars probed with ^{32}P-labeled IS*1051*. Lane 1, *X. fragariae* (17012); lanes 2 and 3, *X. campestris* pv. citri (11502 and 11508, respectively); lane 4, *X. campestris* pv. pelargonii (13301); lane 5, *X. campestris* pv. vesicatoria (10603); lane 6, *X. campestris* pv. begoniae (11054); lane 7, *X. campestris* pv. incanae (10905); lane 8, *X. campestris* pv. cassavae (12201); lane 9, *X. campestris* pv. manihotis (10504); lane 10, *X. oryzae* pv. oryzae (11609); lane 11, *X. oryzae* pv. oryzicola (11802); lane 12, *X. campestris* pv. phaseoli (10703); lane 13, *X. campestris* pv. campestris (10417); lanes 14 and 15, *X. campestris* pv. vasculorum (11403 and 11413, respectively); lane 16, *X. campestris* pv. glycines (12401).

copies of IS*1051* are inserted at different sites in different isolates of *X. campestris*. Moreover, IS*1051* is closely related to the characterized transposable IS*5* element from *E. coli*. Both observations suggest that IS*1051* is a functional transposable element. The availability of a transposable element, especially one so closely related to the characterized insertion sequence from members of the family *Enterobacteriaceae*, would be of considerable value for molecular studies of *Xanthomonas* strains.

In conclusion, the extensive polymorphism of *X. campestris* isolates evaluated with the IS*1051* probe could enable precise epidemiological investigations to be carried out by fingerprinting isolates. Indeed, from the results presented here it seems possible to track the spread of different strains of *X. campestris* through a geographical area or host plant community.

Nucleotide sequence accession number. The DNA sequence of IS*1051* has been entered in the EMBL data bank and assigned the accession number X70380.

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