DNA Restriction Fragment Length Polymorphisms in *Mycosphaerella* Species That Cause Banana Leaf Spot Diseases

J. Carlier, X. Mourichon, D. González-de-Léon, M. F. Zapater, and M. H. Lebrun

First, second, and fourth authors: Centre de Coopération International en Recherche Agronomique pour le Développement (CIRAD)/ Departement Fruits Legumes et Productions Horticoles (FLHOR), Laboratoire de Pathologie Végétale, BP 5035, 34032 Montpellier; third author: CIRAD/Analyse du Genome des Plantes Tropicales (AGETROP), 34032 Montpellier; and fifth author: Institut de Génétique et Microbiologie, Université de Paris-Sud, CNRS-URA 1354, Bât. 400, 91405 Orsay, France.

Present address of third author: CIMMYT, Lisboa 27, Apdo. Postal 6-641, 06600 México, D. F. México.

We thank C. Lanaud and laboratory CIRAD/AGETROP for assistance, X. Perrier and C. Dubois (CIRAD/FLHOR) for their help in data analysis, B. A. Fullerton (Horticulture and Food Research Institute, New Zealand) and R. H. Stover (La Lima, Honduras) for supplying isolates, and Y. Brygoo and M. Dron (Université Paris-Sud) for discussions.

This research was supported by grants STD2 and STD3 from the European Community project on tropical agriculture.

Accepted for publication 5 April 1994.

ABSTRACT

Carlier, J., Mourichon, X., González-de-Léon, D., Zapater, M. F., and Lebrun, M. H. 1994. DNA restriction fragment length polymorphisms in *Mycosphaerella* species that cause banana leaf spot diseases. Phytopathology 84:751-756.

Restriction fragment length polymorphisms (RFLPs) of leaf spot pathogens of bananas, *Mycosphaerella* spp., were examined with low-copy nuclear DNA probes isolated from a *M. fijiensis* cosmid library. The two related fungi, *M. musicola* and *M. fijiensis,* displayed different RFLP patterns and hybridization intensities, suggesting considerable interspecific genetic divergence. The synonymy of *M. fijiensis* var. *difformis* with *M. fijiensis* was supported because RFLP patterns were similar and did not reflect morphological variations between these fungi. Fifty-seven isolates of *M. fijiensis* with different geographic origins were assayed for RFLPs. The highest level of genetic diversity in this pathogen was found in Southeast Asia, the region where it probably originated according to the chronological spread of *M. fijiensis* in the world. In other areas, isolates formed genetically homogeneous groups specific for each region (Africa, Pacific islands, and Latin America). Such geographical differentiation suggests that there was limited introduction of *M. fijiensis* into each of these regions.

Sigatoka leaf spot diseases of bananas (genus *Musa*) involve two related ascomycetous fungi: *Mycosphaerella fijiensis* Morelet, which causes black leaf streak disease (BLSD), and *M. musicola* Leach ex Mulder, which causes Sigatoka disease. BLSD causes more severe defoliation on a broad range of banana cultivars and is more difficult to control with fungicides than Sigatoka disease (35,37).

The geographical distribution of the two species was recently reviewed (18,26). *M. musicola* was first identified in Java in 1902; and since 1962, it has been reported in most of world's banana-growing areas. *M. fijiensis,* described in Fiji since 1964, is supposed to have originated in Papua New Guinea and Solomon islands (33). This species has spread throughout Australia, the Pacific islands, Africa, and Latin America, probably by importation of infected plant material (18,26). In most places, it has replaced *M. musicola* as the dominant banana leaf spot pathogen (18).

The sexual stages of these two pathogens are morphologically similar. *M. fijiensis* and *M. musicola* can be distinguished by their symptoms on banana and the morphology of their anamorphs, *Paracercospora fijiensis* and *Pseudocercospora musae,* respectively (10,34,35). BLSD was described in Latin America as a new leaf spot disease (black Sigatoka) caused by *M. fijiensis* var. *difformis* (28), which was classified as a variety of *M. fijiensis* because it produced conidia on sporodochia and simple conidiophores. Sporodochia are normally observed only for the *M. musicola* anamorph. Although the synonymy of *M. f. difformis* with *M. fijiensis* was proposed (30), its classification as a variety is still in use (16). This atypical anamorph morphology is also observed among *M. fijiensis* isolates in New Caledonia during the cold season and in highland plantations of Costa Rica (X. Mourichon, *unpublished data*). Thus, the taxonomic criterion based on the presence or absence of sporodochia is somewhat ambiguous. Also, *M. fijiensis* and *M. musicola* attack some common hosts. These observations raise questions about the taxonomy of these pathogens and possible interspecific hybridization (23,37).

Information on genetic diversity and structure of these fungal populations is required for breeding resistant banana. *M. fijiensis* and *M. musicola* are heterothallic (27,32) and produce perithecia on infected plants (34). Both conidia and ascospores are involved in disease development caused by *M. musicola,* whereas ascospores play a major role in disease caused by *M. fijiensis* (34). However, the importance of sexual reproduction on the genetic variability of both fungi is unknown.

DNA restriction fragment length polymorphisms (RFLPs) can be used to address these problems (7,24). These genetic markers have been used to study inter- and intraspecific variations in many phytopathogenic fungi (5,6,8,9,15,19,21,22,25). In this paper, we report the results of a study in which RFLPs were used to examine the genetic variability of *Mycosphaerella* spp. From a *M. fijiensis* genomic cosmid library, we selected random clones corresponding to single or low-copy nuclear DNA sequences as probes to detect RFLPs. Given that BLSD is the most destructive and widespread leaf spot disease, we analyzed *M. fijiensis* isolates with different geographic origins. RFLPs were also used to evaluate the genetic relatedness between *M. fijiensis, M. f. difformis,* and *M. musicola.*

MATERIALS AND METHODS

Fungal isolates. Species, morphological types, locations, and sources of the isolates used in this study are presented in Table 1. Five *M. musicola* isolates and 57 *M. fijiensis* isolates were selected from a wide range of geographical origins. All cultures were derived from single spores.

DNA extraction. Isolates were grown in 50 ml of V8 broth (300 ml of V8 juice and 3 g of $CaCO_3$ per liter, pH 6) for 4–6

TABLE 1. Geographic origin, genomic group of banana host, year of collection, and source of *Mycosphaerella* isolates used in this study

Isolate	Area Country	Location	Isolated from[a]	Year isolated	Source[b]
M. fijiensis	Africa				
009	Gabon	Ntoum	Unknown	Unknown	1
142	Congo	Unknown	Unknown	1990	1
059	Cameroon	Yaounde	AAB	1987	1
075	Cameroon	Bafia	AAA	1988	1
089	Cameroon	Unknown	Unknown	1988	1
273	Cameroon	Obala	AAB	1991	1
275	Cameroon	Obala	AAB	1991	1
278	Cameroon	Nyombe	AAA	1991	1
030	Nigeria	Unknown	Unknown	1986	1
301	Nigeria	Onne	AAA	1992	2
302	Nigeria	Onne	AAA	1992	2
141	Tanzania	Unknown	AAA	1990	1
140	Burundi	Unknown	AAB	1990	1
124	Rwanda	Bugarama	AAA	1989	1
	Southeast Asia				
135	Indonesia	Sumatra	AAA	1990	1
173	China	Unknown	Unknown	1988	1
299	Philippines	Davao	AA	1988	2
300	Philippines	Laguna	AAA	1988	2
119	Papua New Guinea	Unknown	AA	1988	2
165	Papua New Guinea	Unknown	AAB	1988	2
286	Papua New Guinea	Rabaul	AAB	1988	2
287	Papua New Guinea	West Sepik	AAA	1988	2
291	Papua New Guinea	Morobe	AAB	1988	2
292	Papua New Guinea	Mt. Hagen	AAA	1988	2
293	Papua New Guinea	Goroka	AAA	1988	2
294	Papua New Guinea	Madang	AAA	1988	2
295	Papua New Guinea	Koipa	AAA	1988	2
296	Papua New Guinea	Embi	AAA	1988	2
297	Papua New Guinea	OK Mart	AA	1988	2
	Australia				
118	...	Unknown	AAA	1989	2
163	...	Unknown	AAA	1989	2
	Pacific Islands				
285	Vanuatu	Port Vila	AA	1989	2
195 at[c]	New Caledonia	Do Neva	AAA	1991	1
196 at	New Caledonia	Do Neva	AAA	1991	1
197 at	New Caledonia	Do Neva	AAA	1991	1
199	New Caledonia	Koumac	AAA	1991	1
211	New Caledonia	Kong	AAA	1991	1
234	New Caledonia	Do Neva	AAA	1991	1
235	New Caledonia	Do Neva	AAA	1991	1
236	New Caledonia	Do Neva	AAA	1991	1
237	New Caledonia	Do Neva	AAA	1991	1
120	Tonga	Unknown	AAA	1989	2
283	Tonga	Tongatapu	AAAA	1990	2
133	West Samoa	Anadale	AAA	1986	2
284	Samoa	Arp	AAA	1982	2
134	Cook Island	Totokoitu	AAA	1986	2
281	Cook Island	Totokoitu	AA	1989	2
	Latin America				
269 di[d]	Mexico	Labna Yucatan	AAB	1991	1
271 di	Mexico	Tabasco	AAA	1991	1
011 di	Honduras	Unknown	Unknown	Unknown	3
147 di	Costa Rica	La Lola	AAB	1990	1
303 di	Costa Rica	San Jose	AAA	1989	2
092 di	Panama	Unknown	AAB	1990	1
128 di	Colombia	Pueblo Rico	AA	1989	1
259 di	Colombia	Risaralda	Unknown	1991	1
091 di	Ecuador	Timbre	AAA	1988	1
M. musicola	Africa				
038	Ivory Coast	Unknown	Unknown	1986	1
060	Cameroon	Yaounde	AAB	1987	1
	Southeast Asia				
131	Indonesia	Java	AA	1989	2
	West Indies				
102	Martinique	Rivière Lezarde	Unknown	1988	2
	Latin America				
250	Costa Rica	Turrialba	AAA	1991	2

[a] Genomic group of banana host.
[b] 1 = CIRAD/FLHOR; 2 = B. A. Fullerton (Horticulture and Food Research Institute, New Zealand); 3 = R. H. Stover (La Lima, Honduras).
[c] *M. fijensis* isolate with atypical anamorph morphology.
[d] *M. f. difformis* isolate.

days at 25 C on an orbital shaker at 100 rpm. Mycelium was harvested by centrifugation, rinsed with distilled water, and immediately dried for 2 days in an oven at 50 C. Approximately 300 mg of dried mycelia was ground with sand, and total DNA was extracted by the CTAB (cetyltrimethylammonium bromide) procedure (13). Total DNA was extracted from *M. fijiensis* isolate 009GAB, and mitochondrial DNA was separated from chromosomal DNA by ultracentrifugation on bisbenzimide-cesium chloride gradients by the method of Garder and Yoder (11).

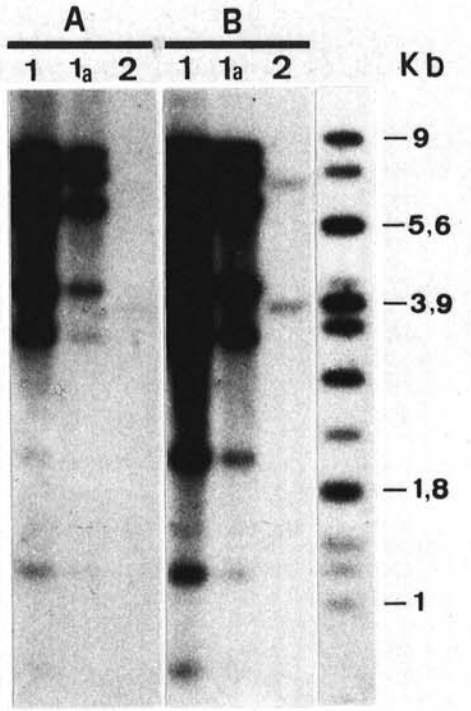

Fig. 1. Hybridization patterns of genomic DNA from *Mycosphaerella fijiensis* and *M. musicola* isolates. **A,** Three micrograms and **B,** 5 μg of genomic DNA from *M. fijiensis* isolate 009GAB (lane 1) and *M. musicola* isolate 102MQT (lane 2) were digested with *Bam*HI, fractionated by agarose gel (0.8% in Tris-acetate-EDTA) electrophoresis (1 V/cm for 15 h), and hybridized with cosmid probe SC9.188. For each experiment, one-half the amount of DNA from the *M. fijiensis* isolate was also loaded (lane 1a).

Probes. We constructed a cosmid library from *M. fijiensis* isolate 009GAB. Genomic DNA of *M. fijiensis* was partially digested with the restriction enzyme *Bam*HI. DNA fragments of 30–40 kb were isolated by centrifugation in a sucrose gradient (2) and ligated into the *Bam*HI site of the vector LLC 5000 (4). A DNA packaging kit (Boehringer, Mannheim, Germany) was used for transduction of *Escherichia coli* strain DH5α according to the manufacturer's recommendations. Copy numbers were determined by colony hybridization on Hybond-N+ membranes (Amersham, Arlington Heights, IL) with total and mitochondrial *M. fijiensis* DNAs labeled with [α-^{32}P] dCTP from a random priming kit (Amersham). Clones showing weak hybridization or no hybridization with both DNAs were assumed to carry single or low copy number nuclear DNA sequences. Recombinant cosmids were extracted by a boiling lysis method (13). Clones with the simplest *Bam*HI restriction patterns were selected. *Not*I sites flanking the *Bam*HI cloning site allowed the whole insert to be isolated by electrophoresis of *Not*I restriction products on 0.6% low-melting-point agarose gels. Inserts in agarose plugs (20–30 ng) were individually spotted onto Hybond-N+ membranes with a standard dot blot apparatus. These dot blots were hybridized with each cosmid probe. Probes showing no cross-hybridization were selected for a preliminary Southern analysis of *Bam*HI-cut genomic DNA from a few *M. fijiensis* isolates. Seven cosmids were selected on the basis of the simplicity of their hybridization patterns: SC9-038, SC9-049, SC9-062, SC9-064, SC9-099, SC9-203, and SC9-213.

Southern blotting and hybridization. Total genomic DNA (4 μg) from each isolate was digested with *Bam*HI and separated in 0.8% agarose Tris-acetate-EDTA gels (13). Before the gels were loaded, 75 pg each of two internal standards, a 24.8- and a 1.5-kb lambda fragment, were mixed with the digested DNA (D. Hoisington and D. González-de-Léon, *unpublished data*). Capillary transfer of DNA to Hybond-N+ membranes was performed according to the manufacturer's procedures (Amersham). DNA probes were labeled radioactively by random priming (Amersham). The internal lambda standards were revealed with radioactively labeled lambda DNA as the probe. Prehybridization and hybridization reactions were performed in bottles at 42 C in a Hybed oven (Appligene) with 50% formamide. Membranes were washed at 68 C twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 20 min followed by washings in 2× SSC plus 0.1% sodium dodecyl sulfate (SDS) for 25 min and 0.1 SSC plus 0.1% SDS for 30 min. Membranes were placed on X-ray film (Eastman Kodak, Rochester, NY) with intensifying screens for 1–3 days at −80 C.

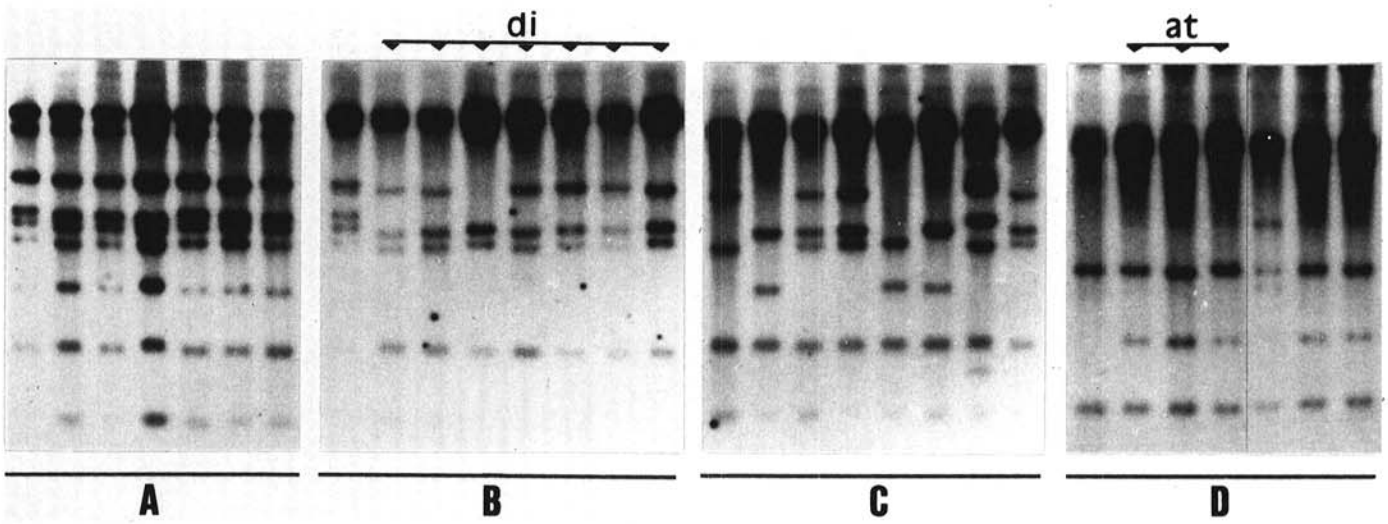

Fig. 2. Restriction fragment length polymorphism patterns of *Mycosphaerella fijiensis* isolates with different geographic origins: **A,** Africa; **B,** Latin America; **C,** Papua New Guinea; and **D,** Pacific islands. *Bam*HI-digested genomic DNA fragments of *M. fijiensis* isolates fractionated by agarose gel (0.8% in Tris-acetate-EDTA) electrophoresis (1 V/cm for 15 h) were hybridized with the cosmid probe SC9.038. *M. f. difformis* (di) and *M. fijiensis* isolates with atypical anamorph morphology (at) showed hybridization patterns similar to those of typical *M. fijiensis*.

Data analysis. A translucent digitizing tablet (Numonics, Montgomeryville, PA) interfaced to a Macintosh computer was used to record restriction fragment positions. These positions were analyzed with the HyperBlot Program V1.0.0 (D. Hoisington and D. González-de-Léon, *unpublished data*). This program allows the classification of restriction fragments by using the internal lambda standard for normalization and cluster analysis algorithms, which reduces the risk of classifying two similar bands as different. Each gel included three control lanes. Lanes on both sides of the gels were loaded with DNA from reference isolates 009GAB and 119PNG. These two isolates showed distinct hybridization patterns with the seven cosmid probes used. The middle lane was loaded with a mixture of DNA from these two reference isolates. With this procedure, it was possible to compare restriction fragments within and between gels. Fragments of less than 1.5 kb and more than 15 kb were eliminated from the analysis. Each restriction fragment was treated as a unit character and scored as present (coded 1) or absent (coded 0) for all isolates (binary matrix). A pairwise distance index matrix was calculated with the Nei and Li distance index (29):

$$D_{xy} = 1 - 2\,N_{xy}/(N_x + N_y)$$

in which N_{xy} is the number of fragments shared between the pair of isolates, and N_x and N_y are the numbers of fragments present in isolates x and y, respectively. Hierarchical cluster analysis was performed by average linkage clustering (unweighted paired group method with arithmetic means [UPGMA]) (12) with the ADDAD program on a Data General mainframe computer.

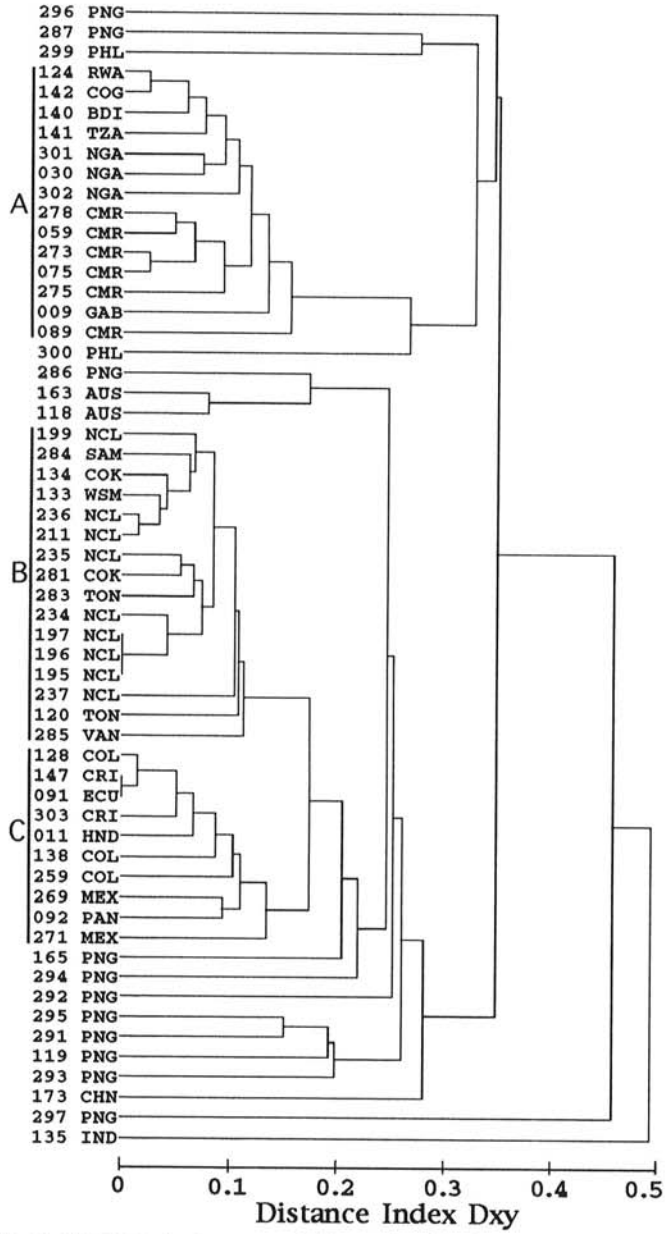

Fig. 3. UGPMA dendrogram of *Mycosphaerella fijiensis* isolates with different geographic origins: group A, Africa (RWA = Rwanda; COG = Congo; BDI = Burundi; TZA = Tanzania; NGA = Nigeria; CMR = Cameroon; and GAB = Gabon); group B, Pacific islands (NCL = New Caledonia; SAM = Samoa; COK = Cook Island; WSM = West Samoa; TON = Tonga; and VAN = Vanuatu); and group C, Latin America (COL = Colombia; CRI = Costa Rica; ECU = Ecuador; HND = Honduras; MEX = Mexico; and PAN = Panama). Other isolates are from Australia (AUS), Papua New Guinea (PNG), Philippines (PHL), China (CHN), and Indonesia (IND).

RESULTS

Colony hybridization of cosmid clones from the *M. fijiensis* genomic library with labeled *M. fijiensis* genomic and mitochondrial DNA showed that most clones carried single or low copy number nuclear DNA sequences. These cosmid probes were hybridized with genomic DNA digested by *Bam*HI, revealing six to 15 restriction fragments per *M. fijiensis* isolate (Fig. 1).

We analyzed five *M. musicola* isolates sampled from diverse geographic areas where they coexisted with *M. fijiensis.* Four cosmid probes from the *M. fijiensis* library weakly hybridized to *M. musicola* genomic DNA, revealing restriction fragments that differed from all *M. fijiensis* RFLP patterns (Fig. 1). We obtained different results for morphologically atypical *M. fijiensis* and *M. f. difformis* isolates. In all experiments, hybridization signals of atypical *M. fijiensis* and *M. f. difformis* isolates with *M. fijiensis* cosmid probes had intensities similar to those observed with typical *M. fijiensis* isolates. RFLP patterns of atypical *M. fijiensis* were closely related to those of typical *M. fijiensis* isolates from the same geographic area (Fig. 2). The RFLP patterns of *M. f. difformis* isolates were related to those of typical *M. fijiensis* isolates from other geographic areas (Fig. 2), since all Latin American isolates are classified under *M. f. difformis.*

The seven cosmid probes revealed a high level of intraspecific polymorphism in *M. fijiensis;* only three of 96 fragments were shared by all isolates. A UGPMA dendrogram was constructed from all pairwise Nei and Li distance indexes (29) to visualize relatedness between isolates (Fig. 3). The same results were obtained with a factor analysis of correspondence (3) from binary matrix (data not show).

Three main groups of related isolates with $D_{xy} < 0.15$ (more than 85% similarity) were revealed (Fig. 3). Group A was composed exclusively of isolates from Africa. Group B corresponded to the Pacific islands isolates and group C to isolates from Latin America. While there were few polymorphisms within African, Pacific islands, and Latin American groups of isolates, distances between each of these groups were important, except Pacific islands and Latin American groups, which seemed to be related. Two isolates from Australia, which were also closely related, have been included in this analysis for preliminary study. A high degree of polymorphism was detected between isolates from Papua New Guinea. These isolates were not clearly related to each other or to isolates from other geographic areas. The few isolates from other countries in Southeast Asia (Philippines, China, and Indonesia) were also unrelated to other *M. fijiensis* isolates.

DISCUSSION

Repetitive DNA sequences are rare in some fungi (20,36) but common in others (9,15). *M. fijiensis* appears to belong to the first group of fungi, since 98% (94 of 96) of the cosmid clones tested contained single-copy DNA. These cosmid probes detected many polymorphisms either among *M. fijiensis* isolates or between *M. musicola* and *M. fijiensis* isolates (Fig. 1). The hybridization signal of *M. fijiensis* cosmids to *M. musicola* DNA was faint. The RFLP and hybridization signal differences observed between these isolates are likely to be caused by a high nucleotidic diver-

gence between genomes of these two species. This genetic divergence supports the classification of these pathogens under two separate species. However, because of the small number of *M. musicola* isolates analyzed, we cannot rule out the existence of *M. musicola* isolates more closely related to *M. fijiensis*.

Morphological differences observed between anamorphs of atypical *M. fijiensis* or *M. f. difformis* and typical *M. fijiensis* isolates were not correlated to differences in RFLP patterns (Fig. 2). Atypical *M. fijiensis* and *M. f. difformis* isolates, which belong to the same anamorph group, were clearly related to typical *M. fijiensis* isolates. This observation supports the synonymy of *M. f. difformis* with *M. fijiensis,* as has already been proposed (30). The presence of sporodochia is not an accurate taxonomic criterion to classify *M. f. difformis* as a new variety.

The genetic divergence between the two species, *M. fijiensis* and *M. musicola,* and the synonymy of *M. f. difformis* with *M. fijiensis* was recently confirmed by sequence analysis of the internal transcribed spacer (ITS1) region of nuclear ribosomal DNA (17).

Our purpose was to provide insights into the genetic diversity of *M. fijiensis* isolates sampled from most of the areas where the disease is endemic. We compared BLSD isolates from four major banana-producing areas (Papua New Guinea, Africa, Pacific islands, and Latin America) without investigating subpopulations from each country. For each area, we collected isolates from locations as diverse as possible (Table 1). A similar approach was used to study *Bremia lactucae* populations. Most variations were detected in one area, Europe, the center of diversity of the host genus *Lactucae* (15). Our data on genetic variation in *M. fijiensis* parallels that on *B. lactucae* evolution (Fig. 3). The history of BLSD caused by *M. fijiensis* suggests that it originated in Southeast Asia (18,26), which is the center of diversity of the host genus *Musa* (1,14,31). The pairwise distances between isolates from Papua New Guinea were in most cases greater than distances between isolates from other areas. This high level of diversity observed among isolates from Papua New Guinea supports the hypothesis that BLSD originated in this area (33). However, a larger sampling of BLSD isolates from all Southeast Asia is necessary to evaluate the amount and distribution of genetic diversity in this region. Such analysis would help localize the center of diversification of *M. fijiensis*.

The lower level of genetic diversity in *M. fijiensis* populations from Africa, the Pacific islands, and Latin America and distances between these populations suggest a founder effect for each of these geographic populations. The *M. fijiensis* populations from the Pacific islands and Latin America are slightly related. Since BLSD was observed earlier in the Pacific islands (26), the introduction of *M. fijiensis* into Latin America might have been caused by individuals from the Pacific islands.

This molecular study supports the current classification of banana leaf spot fungi into two different species (*M. musicola* and *M. fijiensis*). Our results show that RFLPs are useful tools for detecting genetic variation in *M. fijiensis*. These RFLPs show that *M. fijiensis* isolates from most areas are clustered according to their geographical origins. This geographic differentiation could be explained by the introduction of a few individuals into each area.

LITERATURE CITED

1. Argent, G. C. G. 1976. The wild bananas of Papua New Guinea. Notes R. Bot. Gard. Edinb. 35:77-114.
2. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, R. 1992. Current Protocols in Molecular Biology, vol. 1. John Wiley & Sons, New York.
3. Benzecri, J. P. 1973. L'Analyse de Données. Tome 2, L'Analyse des Correspondances. Dunod, Paris.
4. Binninger, D. M., Chevanton, L. L., Skrynia, C., Shubkin, C. D., and Pukkila, P. J. 1991. Targeted transformation in *Coprinus cinereus*. Mol. Gen. Genet. 227:245-251.
5. Braithwaite, K. S., Irwin, J. A. G., and Manners, J. M. 1990. Restriction fragment length polymorphisms in *Colletotrichum gloeosporioides* infecting *Stylosanthes* spp. in Australia. Mycol. Res. 94:1129-1137.
6. Brown, J. K. M., O'Dell, M., Simpson, C. G., and Wolfe, M. S. 1990. The use of DNA polymorphisms to test hypotheses about a population of *Erysiphe graminis* f. sp. *hordei*. Plant Pathol. 39:391-401.
7. Bruns, T. D., White, T. J., and Taylor, J. W. 1991. Fungal molecular systematics. Annu. Rev. Ecol. Syst. 22:525-564.
8. Carder, J. H., and Barbara, D. J. 1991. Molecular variation and restriction fragment length polymorphisms (RFLPs) within and between six species of *Verticillium*. Mycol. Res. 95:935-942.
9. Christiansen, S. K., and Giese, H. 1990. Genetic analysis of the obligate parasitic barley powdery mildew fungus based on RFLP and virulence loci. Theor. Appl. Genet. 79:705-712.
10. Deighton, F. C. 1979. Studies in *Cercospora* and allied genera. VII. New species and redisposition. Mycol. Pap. 144:47-52.
11. Garder, R. C., and Yoder, O. C. 1983. Isolation of DNA from filamentous fungi and separation into nuclear, mitochondrial, ribosomal and plasmid components. Anal. Biochem. 135:416-422.
12. Goor, M., Kesters, K., Mergaert, J., Ryckaert, C., Swings, J., Vantomme, R., Mooter, M. V. D., and Verdonck, L. 1990. Numerical analysis of phenotypic features. Pages 145-152 in: Methods in Phytobacteriology. Z. Klement, K. Rudolph, and D. C. Sands, eds. Akademiai Kiado, Budapest.
13. Hoisington, D. 1992. Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. CIMMYT, Mexico City, Mexico.
14. Horry, J. P. 1989. Chimiotaxonomie et organisation génétique dans le genre *Musa*. Fruits 44:455-475, 509-520, 573-578.
15. Hulbert, S. H., and Michelmore, R. W. 1988. DNA restriction fragment length polymorphism and somatic variation in the lettuce downy mildew fungus, *Bremia lactucae*. Mol. Plant-Microbe Interact. 1:17-24.
16. Jacome, L. H., and Schuh, W. 1992. Effects of leaf wetness duration and temperature on development of black Sigatoka disease on banana infected by *Mycosphaerella fijiensis* var. *difformis*. Phytopathology 82:515-520.
17. Johanson, A. 1993. Molecular methods for the identification and detection of the *Mycosphaerella* species that cause Sigatoka leaf spots of banana and plantain. Ph.D. thesis. University of Reading, Reading, England.
18. Jones, D. R. 1990. Black Sigatoka in the Southeast Asian-Pacific region. Musarama 3:2-5.
19. Koch, E., Song, K., Osborn, T. C., and Williams, P. H. 1991. Relationship between pathogenicity and phylogeny based on restriction fragment length polymorphism in *Leptosphaeria masculans*. Mol. Plant-Microbe Interact. 4:341-349.
20. Krumlauf, R., and Marzluf, G. A. 1979. Characterization of the sequence complexity and organization of the *Neurospora crassa* genome. Biochemistry 18:3705-3713.
21. McDonald, B. A., and Martinez, J. P. 1990. Restriction fragment length polymorphisms in *Septoria tritici* occur at a high frequency. Curr. Genet. 17:133-138.
22. McDonald, B. A., and Martinez, J. P. 1990. DNA restriction fragment length polymorphisms among *Mycosphaerella graminicola* (anamorph *Septoria tritici*) isolates collected from a single wheat field. Phytopathology 80:1368-1373.
23. Meredith, D. S. 1970. Banana leaf spot disease (Sigatoka) caused by *Mycosphaerella musicola* Leach. Phytopathological Papers, no. 11. Commonwealth Mycological Institute, Kew, England.
24. Michelmore, R. W., and Hulbert, S. H. 1987. Molecular markers for genetic analysis of phytopathogenic fungi. Annu. Rev. Phytopathol. 24:383-404.
25. Milgroom, M. G., Lipari, S. E., and Powell, W. A. 1992. DNA fingerprinting and analysis of population structure in the chestnut blight fungus, *Cryphonectria parasitica*. Genetics 131:297-306.
26. Mourichon, X., and Fullerton, R. A. 1990. Geographical distribution of the two species *Mycosphaerella musicola* Leach (*Cercospora musae*) and *M. fijiensis* Morelet (*C. fijiensis*), respectively agents of Sigatoka disease and black leaf streak disease in bananas and plantains. Fruits 45:213-218.
27. Mourichon, X., and Zapater, M. F. 1990. Obtention in vitro du stade *Mycosphaerella fijiensis* forme parfaite de *Cercospora fijiensis*. Fruits 45:553-557.
28. Mulder, J. L., and Stover, R. H. 1976. *Mycosphaerella* species causing banana leaf spot. Trans. Br. Mycol. Soc. 67:77-82.
29. 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.
30. Pons, N. 1987. Notes on *Mycosphaerella fijiensis* var. *difformis*. Trans. Br. Mycol. Soc. 89:120-124.
31. Simmonds, N. W. 1962. The Evolution of the Bananas. Longman, London.

32. Stover, R. H. 1963. Sexuality and heterothallism in *Mycosphaerella musicola*. Can. J. Bot. 41:1531-1532.
33. Stover, R. H. 1978. Distribution and probable origin of *Mycosphaerella fijiensis* in Southeast Asia. Trop. Agric. (Trinidad) 55:65-68.
34. Stover, R. H. 1980. Sigatoka leaf spots of bananas and plantains. Plant Dis. 64:750-756.
35. Stover, R. H. 1987. Diseases and disorders. Pages 281-323 in: Bananas. R. H. Stover and N. W. Simmonds, eds. Longman, New York.
36. Timberlake, W. E. 1978. Low repetitive DNA content in *Aspergillus nidulans*. Science 202:773-775.
37. Wardlaw, C. W. 1972. Banana Diseases Including Plantains and Abaca. Longman, London.