The Journal of Microbiology, December 2001, p.265-272
Vol. 39, No. 4
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Variation of the Intergenic Spacer (IGS) Region of Ribosomal DNA among *Fusarium oxysporum* formae speciales

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(Received August 21, 2001 / Accepted September 27, 2001)

Variation within the intergenic spacer (IGS) of the ribosomal DNA gene for twenty-two strains of *F. oxysporum* and its formae speciales was examined by PCR, coupled with RFLP analysis. The length of the amplified IGS region was about 2.6 kb in all strains except *F. oxysporum* f. sp. *cucumerinum* from Korea and *F. oxysporum* f. sp. *niveum*. Those two strains were 2.5 kb long. Restriction digestion of IGS-RFLP regions by *Eco*RI, *Nru*I, *Hinc*II, *Sal*I, *Sma*I, *Bgl*II, *Hind*III, *Xho*I, and *Kpn*I gave rise to nine IGS haplotypes among all strains. Cluster analysis based on the presence or absence of comigrating restriction fragments show the two groups based on 44% genetic similarity. These results demonstrated that analysis of IGS showed some difference within and between *F. oxysporum* formae speciales.

Key words: *Fusarium oxysporum,* formae speciales, Intergenic spacer (IGS), PCR-RFLP

Fusarium oxysporum Schlect. has one of the broadest host ranges of many plant pathogenic fungi. However, individual pathogenic strains within the species have a limited host range (13). Strains with a similar or identical host range are assigned to an intraspecific group, called formae speciales. The forma specialis concept proposed by Snyder and Hansen (21) for *F. oxysporum* is based on the strict host specificity of the strains. The pathogenic diversity of *F. oxysporum* has been interpreted as suggesting extreme genetic diversity within the species despite its highly conserved morphology (12).

Nuclear rDNA provides useful inter- and intra-specific polymorphisms in eukaryotic organisms. There are multiple copies of the ribosomal genes, which are arranged as head-to-tail repeats separated by non-coding spacers. The larger intergenetic spacer (IGS) or non-transcribed spacer (NTS) lies between the large subunit and small subunit coding regions of consecutive cistrons (20). The IGS, which separates rDNA repeat units, appears to be the most rapidly evolving spacer region. Closely related species may show considerable diversity in IGS, often reflecting both length and sequence variation (10).

Recently, polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) of the IGS region have been applied to the identification of the species of *Armillaria* (4, 9, 24, 23), *Saccharomyces* (18), *Laccaria* (22), *Hebeloma* (8). A few studies using IGS as a molecular marker for the genus *Fusarium* have been done so far, restricted within *F. oxysporum* isolates (1, 2, 3, 7) and *F. graminearum* (6).

The purpose of the current study was to assess the extent of variability in IGS and to determine whether or not the observed variation served to clarify the intraspecific relationship in *Fusarium oxysporum* and its formae speciales.

Materials and Methods

Strains, culture condition, and DNA extraction

The 22 strains of 20 *Fusarium oxysporum* and its formae speciales belonging to section *Elegans* were used in this study (Table 1). Each strain was grown on PDA (potato dextrose agar, Difco) at 28°C and maintained at 4°C. Mycelia for DNA extraction were grown in 250 ml of PDB (potato dextrose broth) in a rotary shaker at 180 rpm for 24 h at 28°C. After vacuum filtration, the mycelia were lyophilized, ground with sea sand and stored at -20°C. Genomic DNA for PCR was extracted according to Lee *et al.* (16).

Polymerase chain reaction (PCR)

The IGS region of the ribosomal DNA was amplified with the primer CNL12 (5'-CTGAACGCCTCTAAGTCAG-3') and CNS1 (5'-GAGACAAGCATATGACTACTG-3') with priming sites indicated in Fig. 1. (25). Amplification was performed in 100 μl of reaction mixture containing 10 μl

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Table 1. List of *Fusarium oxysporum* formae speciales used in this study

Strains	Isolate number	Source
F. oxy. f. sp. *chrysanthemi* Luttrell *et al.*	52422	ATCC[a]
F. oxy. f. sp. *cucumerinum* Owen	16416	ATCC
F. oxy. f. sp. *cucumerinum*	-	Korea
F. oxy. f. sp. *cyclaminis* Gerlach	16016	ATCC
F. oxy. f. sp. *cyclaminis* Gerlach	34371	ATCC
F. oxy. f. sp. *dianthi* (Prillieux et Delacroix) Snyder et Hansen	11939	ATCC
F. oxy. f. sp. *lilii* Imle	15642	ATCC
F. oxy. f. sp. *lycopersici* (Saccardo) Snyder et Hansen	34298	ATCC
F. oxy. f. sp.*radicis-lycopersici* Jaris et Shoemaker	52429	ATCC
F. oxysporum Schlecht.	7500	Australia
F. oxy. f. sp. *conglutinans* (Wollenweber) Snyder et Hansen	744001	MAFF[b]
F. oxy. f. sp. *fragariae* Winks et Williams	744009	MAFF
F. oxy. f. sp. *raphani* Kendrick et Snyder		Korea
F. oxy. f. sp. *niveum* (Smith) Snyder et Hansen	305608	MAFF
F. oxy. f. sp. *lini* (Bolley) Snyder et Hansen	305120	MAFF
F. oxy. f. sp. *tulipae* Apt	235110	MAFF
F. oxy. f. sp. *batatas* Snyder et Hansen	103071	MAFF
F. oxy. f. sp. *melongenae* Matuo et Ishigami	103051	MAFF
F. oxy. f. sp. *lagenariae* Matuo et Yamamoto	103008	MAFF
F. oxy. f. sp. *gladioli* (Massey) Snyder et Hansen	305610	MAFF
F. oxy. f. sp. *spinaciae* (Shorbakoff) Snyder et Hansen	103063	MAFF
F. oxy. f. sp. *melonis* (Leach et Currence) Snyder et Hansen	305544	MAFF

[a]American Type Culture Collection
[b]Ministry of Agriculture, Forestry and Fisheries, Japan

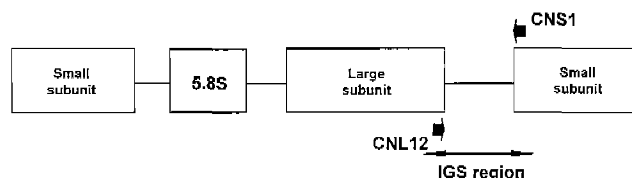

Fig. 1. Schematic diagram of the ribosomal DNA(rDNA) repeat unit showing the CNL12 and CNS1 sites used in PCR amplification of the intergenic spacer (IGS) region.

of 10 × PCR buffer (500 mM KCl, 100 mM Tris HCl (pH 9.0), 1% Triton X-100), 2 mM $MgCl_2$ 0.4 μg of template DNA, 200 μM of each dNTPs, 2.5 units Taq DNA polymerase (BioBasic) and 10 pmol of both primers, CNL12 and CNS1. The mixture was subjected to PCR in an MWG Biotech (Germany). An initial denaturation step for 5 min at 94°C was followed by 30 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 54°C and extention at 72°C for 2 min. A final extension of 72°C for 5 min was incorporated into the program. PCR products were separated by electrophoresis through 1.5% agarose gels. Gels were stained with ethidium bromide and photographed under a UV transilluminator.

Restriction enzyme digestion

The size of the amplified products was estimated based on comparison to size marker, 1 kb DNA ladder (Promega). The reaction mixture was precipitated by adding 0.1 vol. of 3M sodium acetate and 2.5 vol. of absolute ethanol to remove excess dNTPs and primers, and resuspended in sterilized distilled water. Twenty-one restriction enzymes were used to digest amplified DNA: *Alu*I, *Bgl*II, *Eco*RI, *Eco*RV, *Hae*III, *Hinc*II, *Hind*III, *Hinf*I, *Kpn*I, *Msp*I, *Nru*I, *Pst*I, *Sal*I, *Sac*I, *Sau*3A, *Sma*I, *Sph*I, *Stu*I, *Taq*I, *Xba*I, and *Xho*I according to the manufacturer's instruction. Electrophoresis of the restriction fragments was carried out on 1.5% agarose gel for 4 h at 4 v/cm. Gels were stained with ethidium bromide and photographed under a UV transilluminator.

PCR-RFLP analysis

The molecular size of each fragment was estimated using a standard curve of migration versus the log of the molecular size of the 1 kb DNA ladder. Fragments which migrated the same distance during agarose gel electrophoresis were considered to be fragments in common. A data matrix was constructed from the restriction analysis and the presence or absence of restriction fragments was indicated as 1 or 0, respectively.

The computer program NTSYS-PC (version 1.70) was used to analyze the relationship among the *Fusarium oxysporum* and its formae speciales. A similarity matrix was generated using the Qualitative program. This matrix was subjected to the unweighted pair group method with arithmetical averages (UPGMA). Cluster analysis was performed on the similarity matrix with the SAHN program

using UPGMA and a dendrogram was produced with the TREE program.

Results

Amplification of IGS region

The fragments amplified by PCR were of about 2.6 kb in all strains tested except two, *F. o.* f. sp. *cucumerinum* Korea and *F. o.* f. sp. *niveum*, in which they were 2.5 kb in size (Fig. 2a). The 2.6 kb corresponded with the expected size as reported by Appel and Gordon (2). But Alves-Santos *et al.* (1) reported that there are two IGS haplotypes in *F. oxysporum* isolates, the smaller IGS (2.55 kb) and the larger IGS (2.60 kb) following the size of the IGS fragment.

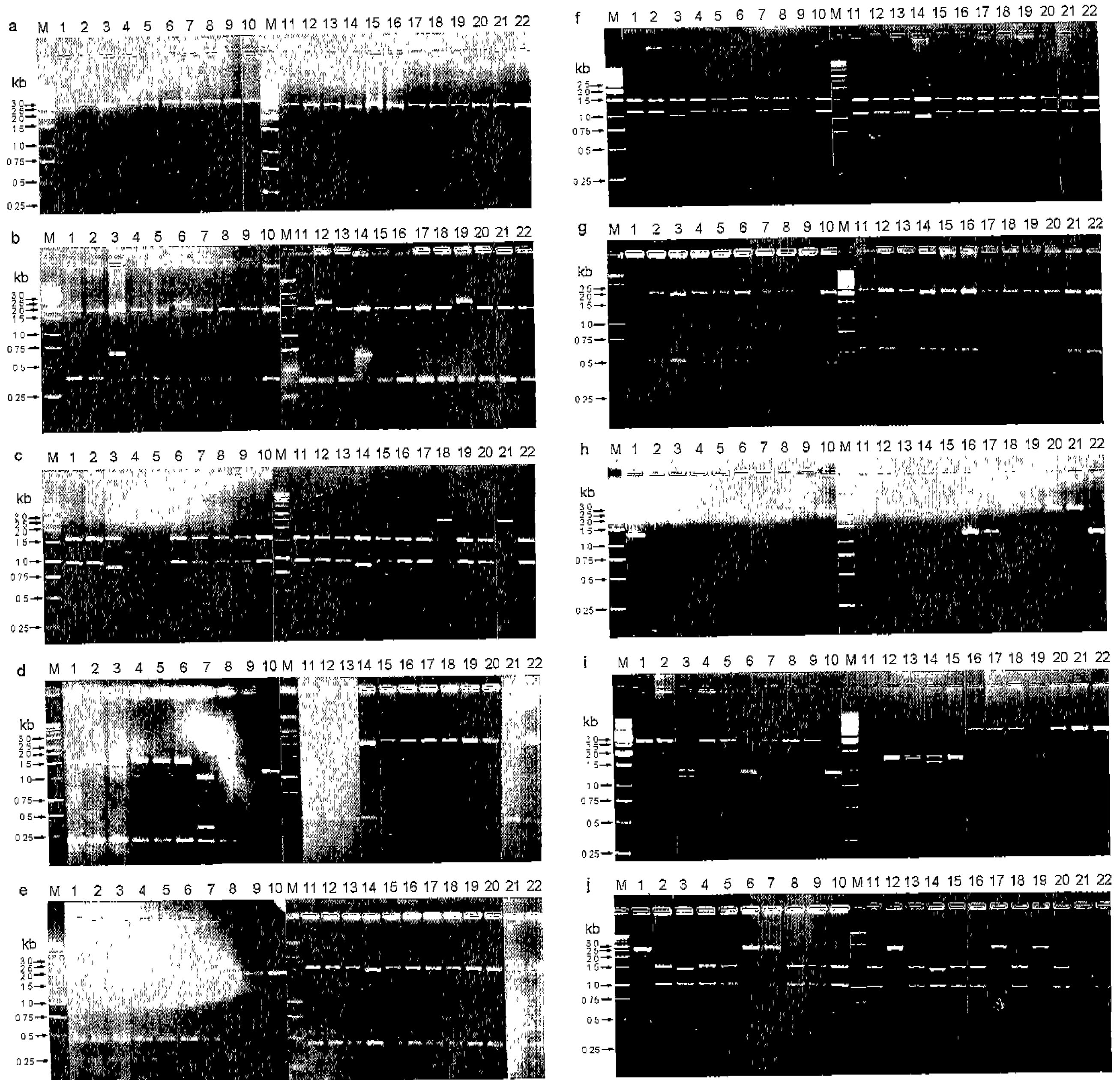

Fig. 2. Amplification products of the IGS region (a) and RFLPs of the PCR-amplified IGS region on EtBr-stained agarose gels (1.5%) from restriction endonuclease *Eco*RI (b), *Nru*I (c), *Hinc*II (d), *Sal*I (e), *Sma*I (f), *Bgl*II (g), *Hind*III (h), *Xho*I (i), *Kpn*I (j). M. DNA size marker of 1kb ladder, 1. *F. o.* f. sp. *chrysanthemi*, 2. *F. o.* f. sp. *cucumerinum* Owen, 3. *F. o.* f. sp. *cucumerinum* Korea, 4. *F. o.* f. sp. *cyclaminis* 16016, 5. *F. o.* f. sp. *cyclaminis* 34371, 6. *F. o.* f. sp. *dianthi*, 7. *F. o.* f. sp. *lilii*, 8. *F. o.* f. sp. *lycopersici*, 9. *F. o.* f. sp. *radicis-lycopersici*, 10. *F. o.* 7500, 11. *F. o.* f. sp. *conglutinans*, 12. *F. o.* f. sp. *fragariae*, 13. *F. o.* f. sp. *raphani*, 14. *F. o.* f. sp. *niveum*, 15. *F. o.* f. sp. *lini*, 16. *F. o.* f. sp. *tulipae*, 17. *F. o.* f. sp. *batatas*, 18. *F. o.* f. sp. *melongenae*, 19. *F. o.* f. sp. *lagenariae*, 20. *F. o.* f. sp. *gladioli*, 21. *F. o.* f. sp. *spinaciae*, 22. *F. o.* f. sp. *melonis*.

Table 2. Restriction fragment size (in base pairs) and pattern analysis of *Fusarium oxysporum* IGS region digested with restriction endonuclease

Strains	*Eco*R I	*Nru* I	*Hinc* II	*Sal* I	*Sma* I	*Bgl* II	*Hind* III	*Xho* I	*Kpn* I	IGS type	IGS band pattern
chrysanthemi	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	1300, 1300	2600	2600	V	A A A A A A C A A
cucumerinum Owen	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	I	A A A A A A A A B
cucumerinum Korea	1850, 670	1600, 880	2050, 450	2050, 450	1500, 1000	2000, 520	2500	1350, 1170	1450, 1050	VII	B B B B B B B C C
cyclaminis 16016	1850, 380, 380	2600	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	II	A C A A A A A A B
cyclaminis 34371	1850, 380, 380	2600	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	II	A C A A A A A A B
dianthi	2200, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	1350, 1250	2600	IV	C A A A A A A B A
lilii	1850, 380, 380	1600, 980	1550, 600, 450	2150, 450	1500, 1100	2100, 520	2600	2600	2600	VIII	A A D A A A A A A
lycopersici	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	I	A A A A A A A A B
radicis-lycopersici	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	I	A A A A A A A A B
oxysporum	1850, 380, 380	1600, 980	1750, 450, 390	2150, 450	1500, 1100	2100, 520	2600	1350, 1250	1540, 1050	IX	A A C A A A A B B
conglutinans	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	1350, 1250	1540. 1050	III	A A A A A A A B B
fragariae	2200, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	1350, 1250	2600	IV	C A A A A A A B A
raphani	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	1350, 1250	1540, 1050	III	A A A A A A A B B
niveum	1850, 670	1600, 880	2050, 450	2050, 450	1500, 1000	2000, 520	2500	1350, 1170	1450, 1050	VII	B B B B B B B C C
lini	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	1350, 1250	1540, 1050	III	A A A A A A A B B
tulipae	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	1300, 1300	2600	1540, 1050	VI	A A A A A A C A B
batatas	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	1300, 1300	2600	2600	V	A A A A A A C A A
melongenae	1850, 380, 380	2600	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	II	A C A A A A A A B
lagenariae	2200, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	1350, 1250	2600	IV	C A A A A A A B A
gladioli	1850, 380, 380	1600. 980	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	I	A A A A A A A A B
spinaciae	1850, 380, 380	2600	2150, 450	2150, 450	1500, 1100	2100, 520	2600	2600	1540, 1050	II	A C A A A A A A B
melonis	1850, 380, 380	1600, 980	2150, 450	2150, 450	1500, 1100	2100, 520	1300, 1300	2600	1540, 1050	VI	A A A A A A B A B

IGS-RFLP Analysis

The PCR products were digested with each of the twenty-one restriction enzymes. Among twenty-one restriction enzymes, six enzymes, *Eco*RV, *Sph*I, *Xba*I, *Stu*I, *Sac*I, and *Pst*I had no restriction site in the amplified IGS. Results of digestion with *Hae*III, *Hinf*I, *Alu*I, *Taq*I, *Sau*3A, and *Msp*I were not used in analysis because they produced a large number of small fragments and very small fragments could not be detected on the gels. These results coincided with those of Appel and Gordon (2) digesting with *Sau*3A and Alves-Santos *et al.* (1) digesting with *Msp*I. Nine restriction enzymes, *Bgl*II, *Eco*RI, *Nru*I, *Hinc*II, *Hind*III, *Sal*I, *Sma*I, *Kpn*I, and *Xho*I were sufficient to resolve the haplotype among the 22 strains. The fragment sizes of the IGS region produced by these nine restriction enzymes are listed in Table 2. We identified polymorphism following analysis of restriction enzyme fragments.

Digestion of the IGS amplification products with *Eco*RI (Fig. 2b) resulted in three patterns with one or two restriction sites. Digestion with *Nru*I (Fig. 2c) showed three patterns having one restriction site or no restriction site. Digestion with *Hinc*II (Fig. 2d) resulted in four patterns with one or two cleavage sites. Digestion patterns with *Sal*I (Fig. 2e), *Sma*I (Fig. 2f), and *Bgl*II (Fig. 2g) showed two patterns for all strains having one restriction site. *Hin-dIII* (Fig. 2h), *Xho*I(Fig. 2i), and *Kpn*I (Fig. 2j) had no site or one site in the amplified IGS region. Depending on the restriction enzymes, two or four distinct restriction patterns were obtained among the 22 strains. Each unique fragment pattern produced by an endonuclease was designated with a letter (A-D). We interpret each combination of size and RFLP pattern as an IGS haplotype. Nine different combinations of patterns representing IGS types were identified among the 22 strains of *F. oxysporum* and its formae speciales.

Based on restriction fragments, a genetic similarity coefficient matrix was obtained (Table 3). The similarity among 22 strains ranged from the highest, 100%, to the lowest, 44%. By the UPGMA method, we constructed a dendrogram based on similarity matrix. The cluster analysis separated the 22 strains into two major groups at the genetic similarity level of more than 44%. One group included the *F. o.* f. sp. *cucumerinum* Korea and *F. o.* f. sp. *niveum* with 100% genetic similarity. The other group included all the remaining strains. Variable levels of genetic relatedness were found among IGS types resolved among formae speciales. IGS type I showed a close relationship with IGS type VI and to a lesser extent, with other IGS types. On the other hand, IGS type VII, which included *F. o.* f. sp. *cucumerinum* and *F. o.* f. sp. *niveum* was distantly related to all of the other IGS types.

Discussion

In higher plants, as in animals, the length and sequence heterogeneities mostly occur in the IGS region (5). Previously published data for different fungal species indicate that intraspecific variability within the IGS is commonplace (8). But the structure of fungal IGS is not known for many species.

In our results, there are length and restriction site variations of IGS in *F. oxysporum* and its formae speciales. Amplification of the IGS region resulted in an interspecific size polymorphism, the size of the fragments being 2.5 kb for *F. o.* f. sp. *cucumerinum* Korea and *F. o.* f. sp. *niveum,* and 2.6 kb for the others. A total of nine IGS haplotypes were identified among of the IGS-RFLP data

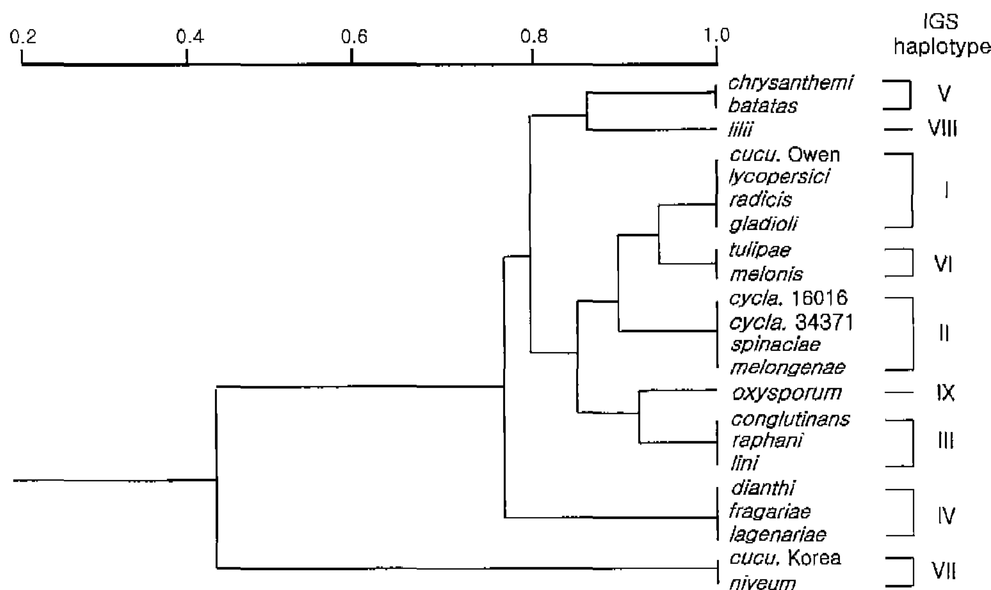

Fig. 3. UPGMA dendrogram showing relationships among the 22 strains of *Fusarium oxysporum* formae speciales based on PCR-RFLP analysis.

Table 3. Genetic similarity coefficient matrix among 22 strains in genus *Fusarium* was calculated from RFLP patterns digested with 9 enzymes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	1.000																					
2	0.857	1.000																				
3	0.429	0.457	1.000																			
4	0.771	0.914	0.429	1.000																		
5	0.771	0.914	0.429	1.000	1.000																	
6	0.800	0.771	0.400	0.686	0.686	1.000																
7	0.857	0.829	0.400	0.743	0.743	0.771	1.000															
8	0.857	1.000	0.457	0.914	0.914	0.771	0.829	1.000														
9	0.857	1.000	0.457	0.914	0.914	0.771	0.829	1.000	1.000													
10	0.686	0.829	0.457	0.743	0.743	0.771	0.714	0.829	0.829	1.000												
11	0.771	0.914	0.486	0.829	0.829	0.857	0.743	0.914	0.914	0.914	1.000											
12	0.800	0.771	0.400	0.686	0.686	1.000	0.771	0.771	0.771	0.771	0.857	1.000										
13	0.771	0.914	0.486	0.829	0.829	0.857	0.743	0.914	0.914	0.914	1.000	0.857	1.000									
14	0.429	0.457	1.000	0.429	0.429	0.400	0.400	0.457	0.457	0.457	0.486	0.400	0.486	1.000								
15	0.771	0.914	0.486	0.829	0.829	0.857	0.743	0.914	0.914	0.914	1.000	0.857	1.000	0.486	1.000							
16	0.914	0.943	0.457	0.857	0.857	0.714	0.771	0.943	0.943	0.771	0.857	0.714	0.857	0.457	0.857	1.000						
17	1.000	0.857	0.429	0.771	0.771	0.800	0.857	0.857	0.857	0.686	0.771	0.800	0.771	0.429	0.771	0.914	1.000					
18	0.771	0.914	0.429	1.000	1.000	0.686	0.743	0.914	0.914	0.743	0.829	0.686	0.829	0.429	0.829	0.857	0.771	1.000				
19	0.800	0.771	0.400	0.686	0.686	1.000	0.771	0.771	0.771	0.771	0.857	1.000	0.857	0.400	0.857	0.714	0.800	0.686	1.000			
20	0.857	1.000	0.457	0.914	0.914	0.771	0.829	1.000	1.000	0.829	0.914	0.771	0.914	0.457	0.914	0.943	0.857	0.914	0.771	1.000		
21	0.771	0.914	0.429	1.000	1.000	0.686	0.743	0.914	0.914	0.743	0.829	0.686	0.829	0.429	0.829	0.857	0.771	1.000	0.686	0.914	1.000	
22	0.914	0.943	0.457	0.857	0.857	0.714	0.771	0.943	0.943	0.771	0.857	0.714	0.857	0.457	0.857	1.000	0.914	0.857	0.714	0.943	0.857	1.000

Number: 1. *F. o.* f. sp. *chrysanthemi*, 2. *F. o.* f. sp. *cucumerinum* Owen, 3. *F. o.* f. sp. *cucumerinum* Korea, 4. *F. o.* f. sp. *cyclaminis* 16016, 5. *F. o.* f. sp. *cyclaminis* 34371, 6. *F. o.* f. sp. *dianthi*, 7. *F. o.* f. sp. *lilii*, 8. *F. o.* f. sp. *lycopersici*, 9. *F. o.* f. sp. *radicis-lycopersici*, 10. *F. o.* 7500, 11. *F. o.* f. sp. *conglutinans*, 12. *F. o.* f. sp. *fragariae*, 13. *F. o.* f. sp. *raphani*, 14. *F. o.* f. sp. *niveum*, 15. *F. o.* f. sp. *lini*, 16. *F. o.* f. sp. *tulipae*, 17. *F. o.* f. sp. *batatas*, 18. *F. o.* f. sp. *melongenae*, 19. *F. o.* f. sp. *lagenariae*, 20. *F. o.* f. sp. *gladioli*, 21. *F. o.* f. sp. *spinaciae*, 22. *F. o.* f. sp. *melonis*.

divided into two groups at the similarity level of about 44%. *F. o.* f. sp. *cucumerinum* Korea and *niveum* formed a distinct cluster with the lowest similarity. In *cucumerinum*, isolates from ATCC and from Korea showed different haplotypes, type I and type VII. These results are consistent with the finding of Kim *et al.* (11). Kim *et al.* (11) described that when thirty-nine isolates were examined for genetic similarity by restriction fragment length polymorphism analysis of mitochondrial DNA. *F. o.* f. sp. *cucumerinum* was the most diverse and may be the oldest forma specialis. In addition, our data on these fungi clearly demonstrate that a close relationship among the different formae speciales exists with a lot of genetic similarity. Appel and Gordon (2) identified 13 IGS haplotypes among a sample of 56 *F. oxysporum* isolates collected in Maryland and California. Alves-Santos *et al.* (1) found six different IGS haplotypes among one hundred and twenty-eight isolates of *F. oxysporum.* According to them, the diversity of the IGS haplotype within *F. oxysporum* suggests that sexual reproduction is infrequent or absent in this fungus. Nakimi *et al.* (19) reported that genetic differences between the two groups of formae speciales *melonis* which were divided into two different IGS groups could be due to geographic isolation and to their dispersal throughout the world.

Pathogenic strains of *F. oxysporum* have been studied for more than 100 years. The host range of these fungi is extremely broad (15). Over 90 different formae speciales of *F. oxysporum* are recognized and many are further divided into pathological races based on pathogenicity to a set of different host cultivars. Host specialization historically has been considered the most important trait in *F. oxysporum* and, because of its practical application, has been used as the basis of classification. However, some formae speciales have broader host ranges. The categorization of strains by host range may or may not lead to a natural subdivision within the species (14). Such pathogenic variation suggests an extreme genetic diversity within the species despite highly conserved morphology. Genetic diversity studies such as VCG, electrophoretic karyotyping (EK), DNA fingerprinting (FP), isozyme analysis, RFLP, random amplified polymorphic DNA (RAPD), and DNA sequencing have been studied for *F. oxysporum.* But most of the genetic diversity studies focus on single forma specialis, and very little data is available on the relationship among different formae speciales (11). In our previous paper, we reported there are different karyotypes and chromosomal length polymorphisms within and between formae speciales (17). Recent findings from genetic diversity studies suggest that certain isolates within a forma specialis may be less genetically similar to each other than to nonpathogenic isolates or isolates in other forma specialis. These results serve to emphasize the imprecision of the forma specialis naming system because it potentially obscures hidden genetic subdivisions and important biological differences among strains or even phylogenetically distinct species. In addition, genes for host specificity and the remainder of the genome may have separate evolution histories. This observation also has great significance for the forma specialis concept within *Fusarium* (15).

To understand the genetic relationship in *F. oxysporum* better and to identify *F. o.* f. sp. by PCR-RFLP analysis, it is necessary to investigate the IGS region of more isolates of *F. oxysporum* and to obtain the nucleotide sequence of the IGS region for *F. oxysporum*, which should provide more information than the digestion pattern by PCR-RFLP analysis.

Acknowledgment

We are grateful to MAFF for providing the *Fusarium* strains.

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