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Comparison of Electrophoretic Karyotypes in *Fusarium*

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The electrophoretic karyotypes of 6 species in different *Fusarium* sections were examined by using contour-clamped homogeneous electric field (CHEF) gel electrophoresis. Intact chromosomal DNA was prepared from protoplasts and up to 9 distinct bands were separated on 0.7% or 0.8% agarose gel under several different conditions. Putative chromosome numbers varied from 6 to 9 and polymorphic karyotypes were observed in different *Fusarium* sections. Using *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* chromosomes as standards, the sizes of the *Fusarium* spp. chromosomes were estimated. The electrophoretic karyotypes of *F. moniliforme* and *F. subglutinans* (section Liseola) were similar. Unidentified filamentous fungi, *F. beomiforme* was much closer to *F. oxysporum* (section Elegans) in karyotype and the karyotypes of *F. napiforme* were more similar to those of section Liseola than any other sections. *F. graminearum* (section Discolor) had a distictive electrophoretic karyotype.

Key words: *Fusarium*, **CHEF, electrophoretic karyotype**

The genus *Fusarium* is comprised of a wide and heterogeneous group of fungi, many of considerable importance in industry as food contaminants and food plant pathogens (8). Taxonomically, the species are notorious for their variability, especially in culture, to the extent that different species are morphologically identical (18).

Attempts have been made to classify *Fusarium* on the basis of soluble protein electrophoretic patterns (3), immunoelectrograms (1), isozyme comparison (15), and monoclonal antibody reactions (7). Recently, restriction fragment length polymorphism (RFLP) (10) and random amplified polymorphic DNA (RAPD) (21, 22) have also been used to determine molecular variation at the DNA level. However, a broadly applicable technique for electrophoretic separation of chromosomes of *Fusarium* species would provide an additional tool for karyotyping analysis. This could be useful in taxonomic studies as well as in a variety of molecular genetic applications such as genetic mapping and localization of specific genes on chromosomes.

Cytological studies of fungal chromosomes have usually been done with material from the sexual stage (13, 20) and the lack of a sexual stage has often inhibited such studies. Most fungal chromosomes are quite small, and it is difficult to accurately count chromosomes and construct karyotypes for these organisms by using classical staining and light microscopy.

Since the introduction of pulsed-field gel electrophoresis (PFGE) in 1984 (19), rapid advance has been made even in the resolution of large DNA molecules and PFGE has become an indispensible tool for determining karyotypes of many organisms, particularly lower eukaryotes like fungi with chromosomes too small to be isolated.

Here, we report the use of contour-clamped homogeneous electric field (CHEF) gel electrophoresis to obtain separation or partial separation of chromosomes of six different *Fusarium* species and to compare the resolved chromosomes of each species to those of already reported by using light microscopic techniques and PFGE.

Materials and Methods

Fungal strains and culture condition

F. beomiforme Nelson, Toussoun 9758, *F. napiforme* Marasas, Nelson and Robie 6129, *F. nygamai* Burgess and Trimboli 5668, *F. oxysporum* Schlecht 7500, *F. moniliforme* Scheldon 7150, *F. subglutinance* (Wollenw and Reinking) Nelson, Toussoun and Marasas 1082 were received from Dr. Burgess of Sydney University and *F. graminearum* was povided by Dr. Lee of Dongkuk University, Korea respectively.

Cultures were maintained on potato dextrose agar (PDA, Difco) plates at 28°C. Conidia harvested from agar plates were suspended in sterile distilled water and an aliquot (1.5×10^5 conidia/ml) of each species was transfe-

rred to 200 ml of potato dextrose broth (PDB, Difco) in a 500 ml Erlenmeyer flask. The flask was placed on a rotary shaker (180 rpm) and then incubated for 18 hr at 28℃.

Preparation of intact megabase-sized chromosomal DNA

To produce a high-quality chromosomal DNA sample for pulsed-field gel electrophoresis, intact chromosomal DNA was prepared from protoplasts executed by using the procedure of Ha *et al.* (5).

Germinated spores were filtered and washed twice with 0.6 M KCl, 0.1 M potassium phosphate (pH 6.0) buffer. Washed germlings were suspended in 5~10 ml of the same buffer containing 3% driselase and incubated for 3 hr at 28℃ while being shaken at 180 rpm. Liberated protoplasts were filtered through sintered glass filter and collected by centrifugation at 4,000 rpm for 15 min and washed twice with 1 M sorbitol, 0.05 M EDTA (pH 8.0). Finally, protoplasts were pelleted by centrifugation and resuspended at a concentration of 1.0×10^9 protoplasts per milliliter. The protoplast suspension was warmed to 60℃, then mixed with an equal volume of 1% molten low-melting agarose at 60℃ in 1 M sorbitol, 0.05 M EDTA (pH 8.0), containing proteinase K (1 mg/ml). The molten mixture was poured into a mold, which was placed on ice for 1 hr. The gelled agarose plugs were then removed and incubated in NDS buffer (0.5 M EDTA, 10 mM Tris-HCl, pH 8.0, 1% N-lauryl sarcosine) containing proteinase K (1 mg/ml) for 48 hr at 50℃. Subsequently, the plugs were washed three times with 10 mM Tris-HCl (pH 8.0)/ 1 mM EDTA, and stored at 4℃ in 10 mM Tris-HCl (pH 8.0)/ 100 mM EDTA. Thus, samples for electrophoresis were prepared according to Schwartz and Cantor's method (19).

CHEF gel elcetrophoresis conditions

PFGE was performed with a CHEF system (CHEF Mapper, Bio-Rad, Richmond, CA), using 1X TBE as a running buffer. Buffer temperature was maintained at 14℃ in all experiments by using a circulator. To obtain optimal separation, three sets of parameters were applied depending upon the sizes of the DNA molecules. The agarose concentrations, field strengths, switching intervals, and run times are described in the figure legends.

After electrophoresis, the gels were stained in ethidium bromide (2 μg/ml) for 15 min, destained in TBE buffer for 40 min, and visualized on a UV transilluminator. To determine the size of each chromosome, well-defined size standards from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Bio-Rad) were co-electrophoresed. Altogether, several agarose plugs were prepared and electrophoretic separation was repeated to prove reproductibility.

Result analysis by image analyzer

The photograph of gels were scanned with a densitometer to measure the relative intensity of each stained band and were analyzed with a Cream-Image Analyzer.

Results and Discussion

Until recently, a few *Fusarium* species have been studied by cytological karyotyping with microscope (Table 1). Although karyotypes are different among the strains, especially even formae speciales, electrophoretic karyotyping has been regarded as a more accurate tool for researching the fungal chromosomes and the exact genome sizes.

McCluskey *et al.* (11) reported that chromosomes from ground mycelial cultures of *Ustilago hordei* and *U. maydis*

Table 1. Karyotypes of *Fusarium* spp.

Species	Cytological karyotypes		Electrophoretic karyotypes		
	Estimated no. chromosomes	Reference or source	Estimated no. chromosomes	Estimated length range (Mb)	Reference or source
F. moniliforme	5~8	Min 1989 (14)	12	0.7~12	Xu *et al.* 1995 (23)
	4	Howson *et al.* 1963 (6)	7~8	0.6~5.8	Migheli *et al.* 1993 (12)
	7	Punithaligon 1975 (17)/ Booth 1977 (2)			
	4	Puhalla 1981 (18)			
F. oxysporum	7	Min 1989 (14)	7~11	0.6~6.7	Migheli *et al.* 1993 (12)
	12	Punithaligon 1975 (17)/ Booth 1977 (2)	5~10	0.9~4.4	Kim *et al.* 1993 (9)
			8	0.6~6.7	Park and Min 1995 (16)
F. graminearum	4	Gordon 1953 (4)/ Howson *et al.* 1963 (6) Min 1989 (14)			

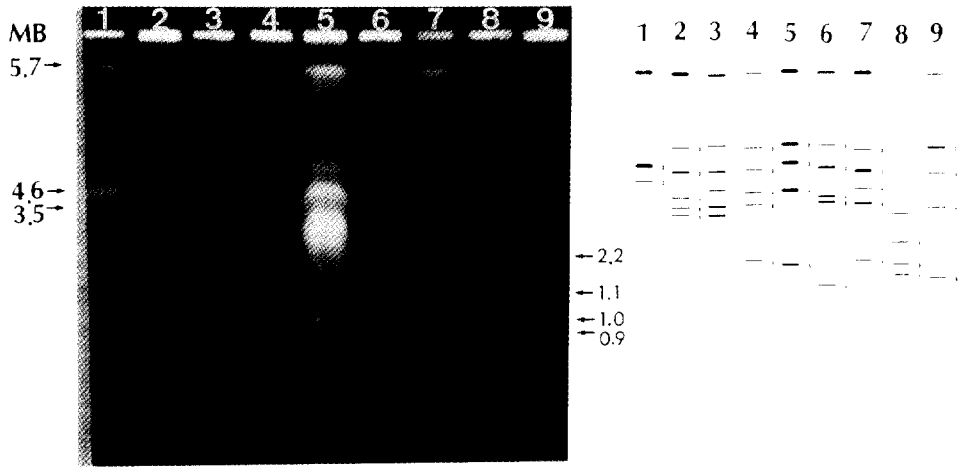

Fig. 1. Comparision of *Fusarium* karyotypes from the first condition. (1) *S. pombe;* (2) *F. oxysporum;* (3) *F. beomiforme;* (4) *F. napiforme;* (5) *F. nygamai;* (6) *F. moniliforme;* (7) *F. subglutinans;* (8) *S. cerevisiae;* (9) *F. graminearum.* Electrophoresis was done on 0.8% agarose gel. Three kinds of switching intervals were used: First at 2.0 v/cm, 30 min for 48 hr, second at 3.0 v/cm, 4 min for 25 hr and third using field inversion gel electrophoresis (FIGE); forward at 3.0 v/cm, 5 min/reverse at 0.7 v/cm, 30 sec for 12 hr. Right are computer-generated electrophoretic karyotypes with Cream Image Analyzer.

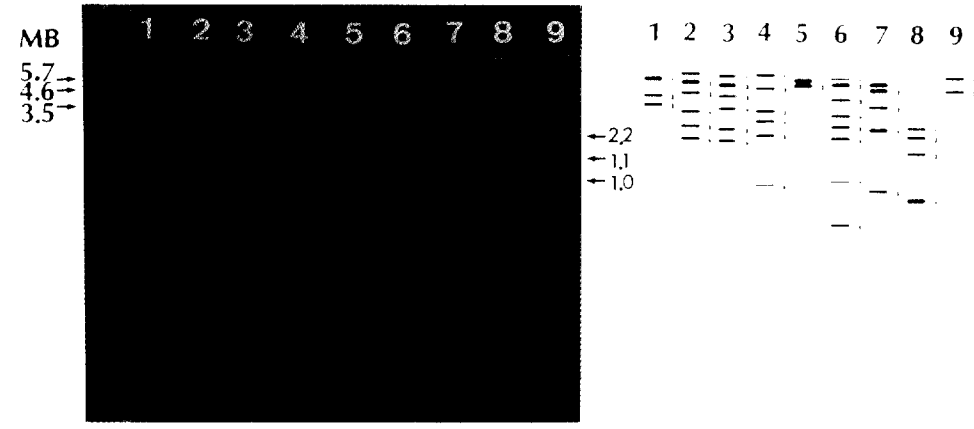

Fig. 2. Comparision of *Fusarium* karyotypes from the second condition. (1) *S. pombe;* (2) *F. oxysporum;* (3) *F. beomiforme;* (4) *F. napiforme;* (5) *F. nygamai;* (6) *F. moniliforme;* (7) *F. subglutinans;* (8) *S. cerevisiae;* (9) *F. graminearum.* Electrophoresis was done on 0.8% agarose gel. Two kinds of switching intervals were used: First at 2.0 v/cm, 30 min for 48 hr and second at 3.0 v/cm, 4 min for 25 hr. Right is a computer-generated analysis of electrophoretic karyotypes with Cream Image Analyzer.

were clearly resolved and produced karyotypes. Other fungi, including strains of *Rhizopus, Botrytis, Fusarium, Pseudocercosporella herpotrichoides, Cephalosporium gramineum,* as well as the unicellular green alga *Chlorella* have proven amenable to this simplified technique. However, according to our previous experimental results (16), the mycelium-grinding method is not suitable for chromosomal DNA preparation from *Fusarium* spp., so protoplasts were isolated and used for CHEF gel electrophoresis.

Several running conditions for the CHEF gel, such as agarose concentrations, field strengths, switching intervals, and run times were tested to resolve *Fusarium* spp. megabase-sized chromosomes.

After CHEF gel electrophoresis on 0.8% agarose gel for 72 hr and field inversion gel electrophresis (FIGE) for 12 hr, the DNA bands of each *Fusarium* spp. were resolved to three size classes: small (2.2 Mb), medium (2.2~4.8 Mb), and large (4.8 Mb) chromosomes (Fig. 1). The majority of *Fusarium* chromosomes fell within the medium size class and the largest chromosome was separated near the largest band of *S. pombe.* The resolution of small DNAs was slightly different in each strain and chromosomes belonging to the small class were observed only in *F. napiforme, F. nygamai, F. subglutinans,* and *F. graminearum.* Therefore, these conditions were regarded as suitable for identifying low molecular weight chromosomal DNAs. Although similar results were obtained by running without FIGE for 12 hr, the largest chromosome migrated slower than the largest chromosome of *S. pombe* and was estimated to be over 6.0 Mb in

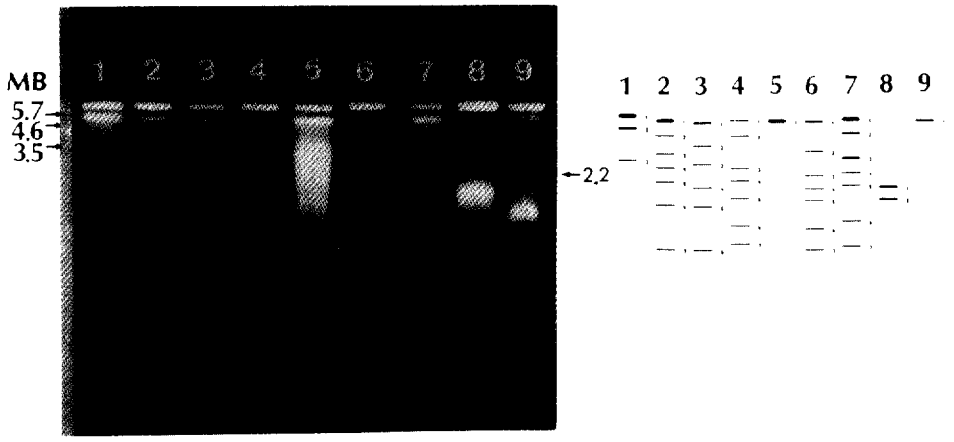

Fig. 3. Comparision of *Fusarium* karyotypes from the third condition. (1) *S. pombe;* (2) *F oxysporum;* (3) *F. beomiforme;* (4) *F. napiforme;* (5) *F. nygamai;* (6) *F. moniliforme;* (7) *F. subglutinans;* (8) *S. cerevisiae;* (9) *F. graminearum.* Electrophoresis was done on the 0.7% agarose gel, initially at 1.3 v/cm increasing to 2.0 v/cm and the switching interval was initially 60 min decreasing to 30 min during 70 hr. Right is computer-generated analysis of electrophoretic karyotypes with Cream Image Analyzer.

Table 2. Estimated numbers and sizes (Mb) of chromosomes from six *Fusarium* species according to three separate conditions

Chromosome number	*F. beo*	1	2	3	*F. nap*	1	2	3	*F. nyg*	1	2	3
I	>6.0	0	1	0	>6.0	0		0	>6.0	0	1	0
II	5.5	1	1	1	5.5	1		1	5.7	1	1	1
III	4.8	1	1	1	4.8	1		1	4.9	1	*	*
IV	3.8	1	1	1	3.6	1	0	1	4.6	1		
V	3.1	1	0	0	2.9	1		1	3.3	1		
VI	2.4	1	0	0	2.4	0	0	1	1.0	1		
VII	2.2	1	1	1	1.9	0		1				
VIII	1.8	0	1	1	1.0	1	0	1				
IX	1.1	0	0	1								
Total genome size		>30.7 Mb				28.1 Mb				25.5 Mb		

Chromosome number	*F. mon*	*1*	*2*	*3*	*F. sub*	1	2	3	*F. gra*	1	2	3
I	>6.0	0	1	0	>6.0	0		0	>6.0	0	1	0
II	5.5	1	1	1	5.5	1		1	5.7	1	1	1
III	4.8	1	1	1	4.8	1		1	4.8	1		*
IV	3.6	1	1	1	3.6	1	0	1	3.6	1		
V	3.0	1	1	1	3.1	1	0	1	2.3	1	*	
VI	2.6	1	1	1	2.6	1	0	1	0.8	1		
VII	2.0	0	1	1	2.0	0		1				
VIII	0.8	1	1	1	1.0	1		1				
Total genome size		>28.3 Mb				>28.6 Mb				>23.2 Mb		

Note. * multiple unresolved chromosomes.
Abbreviations: *F. beo, F. beomiforme; F. nap, F. napiforme; F. nyg, F. nygamai; F. mon, F. moniliforme; F. sub, F. subglutinans; F. gra, F. graminearum.*

size (Fig. 2). To improve the separation of unresolved chromosomes, we electrophoresed with the electric field reduced to 2.0 v/cm and increasing the switching interval up to 60 min (Fig. 3). In this condition, three bands of *S. pombe* were completely separated and only a 2.2 Mb-sized band of *S. cerevisiae* was resolved. By comparing and analysing the results shown in Fig. 1, 2, and 3, we obtained the approximate numbers and sizes of the DNA bands (Table 2). The chromosomes are numbered arbitrarily according to size, starting with the largest.

A significant karyotype variability was observed among six species. According to Table 2, similar band distributions were found, particularly in *F. moniliforme* and *F. subglutinans.* Because the two species belong to the same section, Liseola, it was suggested that they have

similar electrokaryotypes. The karyotypes demonstrate that members of the section Liseola contain chromosome sizes ranging about 0.8 Mb to over 6.0 Mb and a total genome size over 28.3 Mb. Taxonomically belonging to the intersection between sections Liseola and Elegans, *F. napiforme* has eight chromosomes ranging from 1.0 Mb to over 6.0 Mb. This resolved pattern was similar to *F. moniliforme* and *F. subglutinans* of the section Liseola, in chromosome number, size, and total genome size. After comparing the karyotypes of *F. beomiforme* to those of other species, it revealed that the *F. beomiforme* karyotype was much closer to that of *F. oxysporum* (lane 2 in three Figures) of the section Elegans. This is in accordance with the data previously obtained from isozyme banding patterns (14) and again from 2-D electrophoresis of detergent soluble proteins in *Fusarium* spp. (unpublished data, 1995).

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