Curr Genet (1995) 28: 173–176

ORIGINAL PAPER

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Genetic duplication in *Fusarium oxysporum*

Received: 28 July 1994 / 20 January 1995

Abstract Genomic clones hybridizing to anonymous, single-copy sequences were used to probe chromosome-sized DNAs of *Fusarium oxysporum* f. sp. *cubense* separated by pulsed-field gel electrophoresis. As expected, most clones hybridized to single chromosome bands. However, two of eight „single-copy" clones hybridized to two chromosomes in some, but not all, of 14 isolates examined. This observation suggests a degree of genetic duplication in the fungus and is consistent with recent electrophoretic karyotype analysis indicating that intraspecific differences in genome size and chromosome number may be explained, at least in part, by persistent genetic duplication.

Key words Chromosome · Electrophoretic karyotype · Filamentous fungus · Plant pathogen

Introduction

Isolates of the fungus *Fusarium oxysporum* display variation in chromosome number and total genome size based on analysis of their electrophoretic karyotype (Momol and Kistler 1992; Kim et al. 1993; Boehm et al. 1994). Deletion, duplication, translocation, and the presence of dispersable chromosomes all have been suggested to be a source of such genomic variation in filamentous fungi (Kistler and Miao 1992). Duplication associated with aneuploidy may play a role in determining genome size in *F. oxysporum* f. sp. *cubense* (FOC) because of the strong positive linear relationship between chromosome number and genome size (Boehm et al. 1994). Such a relationship would be expected if the haploid genome had increased in size either by the retention of full or partial disomes or by the accumulation of unique supernumerary chromosomes of variable size.

For this investigation, we sought an approximation of genetic duplication in FOC. Specifically we wished to determine if "single-copy" DNA sequences could be found on different-sized chromosomes resolved by pulsed-field electrophoresis. Tandemly repeated genes for ribosomal RNAs (rDNAs) have been localized on more than one chromosome-sized DNA in some, but not all, isolates of FOC (Boehm et al. 1994) and other *Fusarium* species (Fekete et al. 1993). These observations prompted our preliminary investigation of the distribution of single-copy sequences to the chromosomes presented here.

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Communicated by O. C. Yoder

Materials and methods

Fungal isolates. Fourteen FOC isolates were chosen for study; one isolate was selected from each vegetative compatibility group (VCG) of FOC previously investigated (Boehm et al. 1994). This fungus causes the disease known as *Fusarium* wilt, or Panama disease, of banana. The origin and biological diversity of the isolates have been reported previously (Ploetz and Correll 1988; Ploetz 1990; Boehm et al. 1994). Members of a single VCG in *F. oxysporum* appear to be clonally derived, based on RFLP analysis of total genomic DNAs (Elias et al. 1993; Koenig and Kistler, unpublished).

DNA preparation and manipulation. Chromosome preparations and conditions for pulsed-field electrophoresis were as previously defined (Boehm et al. 1994) except that SeaKem Gold agarose (FMC BioProducts, Rockland, Me.) was used for all gels. Two additional run conditions were employed for the analysis of electrophoretic karyotype. To measure chromosomes larger than 5 Mb, pulse time was ramped from 1200 to 6000 s over a 240-h run. To measure chromosomes less than 2.0 Mb, 0.8% agarose gels were run at 40 V for 84 h using a linear, ramped, 40–480-s pulse. The chromosome size standards, *Saccharomyces cerevisiae* strain YNN295, *Schizosaccharomyces pombe* strain 972h- (BioRad Laboratories, Hercules, Calif.) and *Neurospora crassa* strain 74-OR23-1A (Orbach 1992), ranged from 2.45×10^5 to 1.03×10^7 bp. Genomic clones were obtained from isolate FRC 0-1078 of *F. oxysporum* f. sp. *lycopersici* (FOL), race 2 (Elias et al. 1993). Genomic DNA was partially digested with *Sau*3AI and cloned in the *Bam*HI site of pBluescript + SK (Stratagene, La Jolla, Calif.). The DNA insert size ranged from 0.5 to 2 kb. Based on Southern-hybridization analysis of clones, the genome of *F. oxyspo-*

rum was estimated to consist of 68% single-copy sequences, 12% multiple-copy sequences and 20% repetitive sequences (Elias et al. 1993). Insert DNAs were considered single-copy sequences if hybridization occurred to a single DNA fragment for one-to-four tested restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hae*III). Multiple-copy sequences hybridized to a minimum of two bands for all enzymes tested while repetitive sequences hybridized to a minimum of ten restriction fragments for one or more enzyme. Many of the multiple-copy or dispersed repetitive sequences in *F. oxysporum* may be transposable elements (Daboussi and Langin 1994). Plasmid DNAs were isolated by the alkaline-lysis method (Sambrook et al. 1989) and were enumerated and labelled with the prefix "FG". Southern analysis was by either random primer digoxigenin-11-dUTP incorporation into probes and ELISA detection (GENIUS, Boehringer Mannheim) or by random primer fluorescein dUTP incorporation and chemiluminescent immunodetection (RENAISSANCE, DuPont NEN).

Results

Probes for Southern hybridization were obtained from a previous study of genetic diversity in the fungus *F. oxysporum* f.sp. *lycopersici* (Elias et al. 1993). The eight single-copy clones selected, hybridized to DNA from all isolates of *F. oxysporum* tested. Therefore isolate-specific or population-specific sequences were intentionally avoided. Figure 1 (top) shows the electrophoretic karyotype for 14 isolates of FOC. The estimated chromosome sizes, chromosome numbers, and derived genome sizes are listed in Table 1. These values generally are consistent with previously published data (Boehm et al. 1994; given in Table 1 for comparison) except for isolates 22994.96 and 8624.170 where additional 8.3- and 6.3-Mb chromosomes, respectively, were resolved by the different running conditions used in this study. The results of the current study are likely to be more accurate than the previous published values because three different gel-running conditions were used to determine chromosome size and number and because, here, chromosomes of *N. crassa* strain 74-OR23-1A (Orbach 1992) were used as size standards to obtain estimates for large chromosomes. Previously, the largest FOC chromosomes exceeded the size of the largest molecular-size standard (the 5.7-Mb *S. pombe* chromosome) and size estimates were based on extrapolation. (The primary representative isolate for VCG 0124 listed in Table 1 is STJ1.27, although isolate FCJ2.23 is illustrated in Fig. 1.)

As expected, most single-copy clones hybridized to single chromosome-sized bands [Fig. 1 (middle); results summarized in Table 1]. Hybridizing bands frequently differed in size, reflecting the polymorphic nature of presumed homologous chromosomes among the isolates. Curiously, the genomic clones, chosen in the manner described, hybridized primarily only to three chromosomes. These linkages are conserved for most isolates. The "linkage groups" include those defined by clones FG30, FG187 and pUF8-3 (C, G, I in Table 1), those defined by FG4, FG162 and FG260 (A, F, H), and those defined by FG7 and FG65 (B, D). This non-random distribution of probes to chromosomes may suggest that the chosen sequences, those hybridizing to all isolates of the fungus, are found only on a particular subset of chromosomes.

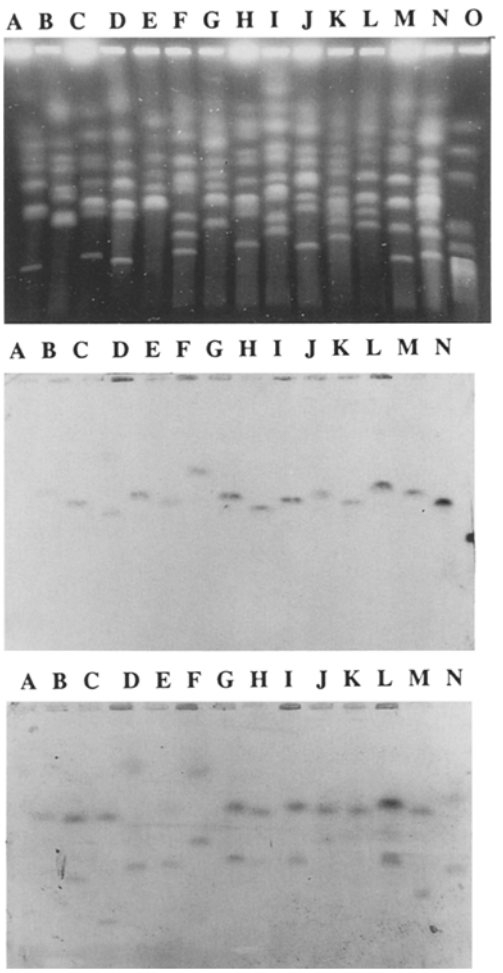

Fig. 1 Chromosome-sized DNAs of *F. oxysporum* f.sp. *cubense* separated by pulsed-field electrophoresis and visualized by ethidium bromide staining (top). Southern transfers of DNAs were probed with clones FG 7 (middle, clone B in Table 1) or FG 120 (bottom, clone E). Chromosome preparations were from isolates 22425.113 (*A*), GM.149 (*B*), PW4.09 (*C*), DAVAO.151 (*D*), FCJ2.23 (*E*), JLTH17.213 (*F*), STPA3.93 (*G*), STA2.158 (*H*), 22994.96 (*I*), 8624.170 (*J*), JC1.189 (*K*), STNP1.98 (*L*), 1–2.119 (*M*), and MW40.104 (*N*). Molecular-size markers (*O*) are chromosomes from *S. pombe* and *S. cereviseae*

Surprisingly, for two of eight "single-copy" clones, hybridization occurred in some instances to two different-sized chromosome bands (e.g., Fig. 1, bottom). Clones FG 30 and FG 120 each hybridize to two chromosomes in some, but not all, isolates. The chromosomes hybridizing to the two probes are usually different. For example, for isolate STPA3.93, clone FG30 (C) hybridizes to a 6.3- and 5.7-Mb chromosome whereas clone FG 120 (E) hybridizes to a 5.0- and 2.85-Mb chromosome (Fig. 1, bottom).

Clones FG 30 and FG 120 do not appear to contain more than a single *Sau*3AI DNA insert. FG 30 contains a 0.5-kb sequence that hybridizes to a single restriction fragment in total DNA digests of 102 isolates of *F. oxysporum* when tested with any of three restriction enzymes (Elias et al. 1993; Katan, Koenig and Kistler, unpublished). Similarly, FG 120 contains a 2-kb insert that hybridizes to a single,

Table 1 Estimated chromosome size, chromosome number and genome size for 14 isolates of FOC

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Isolate #	22425.113	GM.149	PW4.09	DAVAO.151	STJ1.27	JLTH17.213	STPA3.93	STA2.158	22994.96	8624.170	JC1.189	STNP1.98	1–2.119	MW40.104
VCG	0120	0121	0122	0123	0124	0124-5	0125	0126	0128	0129	01210	01212	01213	01214
Chromosome size (Mb)	8 5.4 5 C, G, I 5 4.4 A, E, F 4.1 3.4 B, D 3.4 2.8 2.8 1.23	6.3 C, G, I 5 4.2 A, E, F 3.8 3.25 3.1 B, D 2.65 2.55 E 2.55	5.7 C, G, I 5.1 4.2 A, E, F 3.9 3.3 2.8 B, D 2.8 2.6 1.42 E	7.1 C 6.3 C, E, G 5 4.3 A, F, H 3.35 3.3 B, D 2.7 E 2.7 2.55 1.27	6.9 6.9 5.9 C, G 5.9 4.85 E 4.2 A, F 4.2 3.5 3.25 B, D 3.25 2.75 E 2.75 2.65	6.3 C 5.7 C, E, G 5.1 4.2 A, F, H 4.2 B, D 3.4 3.35 3.1 E 2.4 1.85 1.52	6.3 C 5.9 5.7 C, G 5.5 5.5 5 E 4 A, F 3.5 B, D 3.2 2.85 E 2.85 2.25	6.3 5.4 C, G 5.2 C, E 4.5 4.1 A, F 3.4 2.95 B, D 2.95 E 2.7 2.55 1.67	8.3 6.3 C 5.4 C, G 4.9 E 4.2 A, F 3.4 B, D 3.1 3.1 2.9 E 2.35 2	6.3 C 5.7 C, G 5.4 4.6 E 4 A, F, H 3.4 B, D 3.4 E 2.9 2.6 1.55	6.3 C 5.4 C, G 5 4.5 E 4 A, F 3.6 3.1 B, D 2.8 2.8 2.4 2.25 2	6.7 C 5.9 C, G 5.4 4.7 E 4.2 A, F 3.5 B, D 2.9 E 2.9 2.6 2.15	6.5 C 5.7 C, G 5.1 4.35 E 4.1 A, F 3.3 B, D 3.3 2.75 2.75 2.2 E 1.27	6.7 C 5.3 C, I 5 E, G 5 3.9 A, F, H 3.25 3.05 B, D 2.85 2.85 2.4 E 2.1 1.38
Total size (Mb)	45.53	33.4	31.8	38.57	57	41.12	52.55	41.72	45.95	39.9	44.15	40.95	41.32	43.78
Chromosome number	11	9	9	10	13	11	12	11	11	10	12	10	11	12
Total size (Mb)	42.3	37.28	32.1	40.5	57.8	40.75	44.6	41.71	38.42	33.5	45.75	36.4	41.65	43.85
Chromosome number (Boehm et al., 1994)	11	9	9	10	13	11	12	11	10	9	12	10	11	12

The letters listed in the table indicate clones that hybidize to the adjacent chromosome-sized DNA. The letters represent the clones: (A) FG4, (B) FG7, (C) FG30, (D) FG65, (E) FG120, (F) FG162, (G) FG187, (H) FG260, and (I) pUF8-3, a clone containing the genes for the major ribosomal RNAs from *Nectria haematococca*. When hybridization was to a doublet band, it is uncertain if one or both chromosomes contained sequence similarity

polymorphic, restriction fragment for DNA digested with any of three restriction enzymes, for 55 isolates of *F. oxysporum* (Elias et al. 1993).

Discussion

The FOC genome contains a degree of genomic duplication. Out of a total of 112 combinations (14 isolates×8 arbitrarily chosen, single-copy probes) at least 21 show sequences localized to two chromosomes. While these results might imply an unexpectedly high level of duplication, the small number of loci examined prevents any generalization about overall duplication frequency. Also, for the present study, only conserved single-copy sequences (those hybridizing to all tested isolates of *F. oxysporum*) were chosen. By doing this, we had hoped to avoid unique, non-essential, genomic sequences, whose absence or duplication on supernumerary chromosomes might be expected to give a higher estimate of overall genetic variation. However, by examining only conserved sequences it appears that the loci tested were not randomly distributed on chromosomes. This also may have given a biased result.

Still it is possible that this study underestimates the extent of duplication in FOC because, for bands determined to be doublets by the intensity of ethidium bromide staining, it cannot be determined if hybridization occurs to one or both bands of the doublet. Also, clones that hybridize to a single chromosome band for the isolates described here may hybridize to two chromosome bands in other isolates. For example, FG162 hybridizes to two chromosome bands in FOC isolate MW53.67 (Benny and Kistler, unpublished), but to one band in all isolates used in this study.

Genetic duplication has been more readily detected in FOC than for similar filamentous fungi examined by the same method. By comparison, *Mycosphaerella graminicola* (McDonald and Martinez 1991) showed evidence for a single duplication in only one strain of seven examined, using nine single-copy probes. *Ustilago hordei* (McCluskey and Mills 1990) appears to contain no such duplications for 14 isolates and five single-copy probes while *Leptosphaeria maculans* (Morales et al. 1993) shows no evidence of duplication in nine strains for 11 presumably single-copy probes. In each case, isolates where chosen that represent different genetic variants VCGs, haplotypes or races).

Although some exceptions might exist (e.g., see Russell and Mills 1993), fungi with a meiotic reproductive strategy appear to have mechanisms for restricting the level of duplicated DNA sequences. For example, aneuploid strains of *Aspergillus nidulans* or *N. crassa* do not grow vigorously and rapidly revert to euploidy presumably by mitotic non-disjunction (literature summarized in Fincham et al. 1979). Premeiotic deletion of tandem duplications and the process of repeat-induced point mutation (RIP) also occur in *N. crassa* and other filamentous ascomycetes that undergo meiosis (Selker 1990). Strictly mitotically reproducing fungi, such as *F. oxysporum*, may lack some or all of the processes that serve to limit genome duplication in sexually reproducing organisms. Duplications resulting from translocation or aneuploidy presumably could contribute significantly to the variation in chromosome number and genome size within FOC detected by the analysis of electrophoretic karyotype (Boehm et al. 1994). It may be instructive to test whether, in general, there is a greater degree of duplication in strictly asexually reproducing fungi, than for field isolates of similar fungi that undergo meiosis in nature.

Acknowledgements This research was supported by Grant No. US-1812-90C from BARD, the United States-Israel Binational Agricultural Research and Development Fund. Additional support was provided by NSF/DOE/USDA grant 92-37310-7821.

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