Electrophoretic karyotype of *Fusarium solani*

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INTRODUCTION

*Fusarium* species have gained importance because of their involvement in plant pathogenesis (Leary & Endo, 1971; Kuhn & Smith, 1978; Smith et al., 1982), production of plant hormones (Jeffreys, 1973), and lignin biodegradation (Norris, 1980; Sutherland et al., 1983). *Fusarium solani* (Mart.) Sac., ATCC 64023, degrades ferulic acid, one of the monomeric lignin model compounds (Crawford, 1981), via decarboxylation to 4-vinylguaiacol, prior to its further oxidation (Nazareth & Mavinkurve, 1986). This pathway is not commonly reported to occur in fungi. Little is known genetically about the species, which offers a wide scope for study; the determination of its genomic composition would be of significance.

CHEF gel electrophoresis has been successfully applied in resolution of the large chromosomal DNA (ChDNA) molecules of a number of fungi, such as *Aspergillus* spp. (Brody & Carbon, 1989; Debets et al., 1990), *Septoria nodorum* (Cooley & Caten, 1991), *Neurospora crassa* (Orbach et al., 1988), *Saccharomyces cerevisiae*, *Candida albicans* and *Schizosaccharomyces pombe* (Vollrath & Davis, 1987; Magee & Magee, 1987). ChDNA of *Nectria haematococa* was partially resolved by Miao et al. (1991), who reported the chromosomal locations of the cytochrome P-450 gene family in the organism. Using this technique, we report here the first complete electrophoretic karyotype of *F. solani*.

METHODS

**Organism.** *Fusarium solani* ATCC 64023 (Nazareth & Mavinkurve, 1986), was cultured routinely on potato dextrose agar (PDA) or broth (PDB).

**Preparation of intact chromosomal DNA.** The culture was grown in PDB and conidia were harvested by filtration through cheesecloth. Conidia (4 × 10⁹ as counted by a haemocytometer) were resuspended in PDB (125 ml), and incubated on a rotary shaker at 30 ºC, 200 r.p.m., for 3–4 h, until the conidia had germinated with germ tubes 1–4 times the length of the conidium.

ChDNA was isolated by the agarose-spheroplast method of Orbach et al. (1988), with slight modifications. Germinated conidia (about 2 × 10⁸) were mixed with molten 1% (w/v) low-melting-point agarose prepared in 0·125 M sodium citrate (pH 5·7) containing Lysing Enzymes (Sigma), so that the final cell density was 2·0 × 10⁶ ml⁻¹, agarose concentration 0·6%, and Lysing Enzymes 2·7 mg ml⁻¹. Lysing enzymes from *Trichoderma harzianum*, containing cellulase, protease and chitinase, substituted the Novozym 234 preparation of Orbach et al. (1988). The cell/agarose mixture was pipetted out with an Eppendorf tip (1000 µl) into the plug mould (Bio-Rad), and kept at 4 ºC to gel. The plugs were then removed, put into 0·05 M sodium citrate (pH 5·7)/0·05 M EDTA (pH 8·0)/7·5% (v/v) 2-mercaptoethanol for spheroplast formation, and incubated at 37 ºC for 24 h. Plugs were rinsed three times with 0·4 M EDTA (pH 8·0) and incubated for 4 h at 50 ºC in NDS buffer [0·5 M EDTA (pH 8·0), 0·01 M Tris/ HCl (pH 9·5) and 1% (w/v) N-lauroylsarcosine] containing 2 mg proteinase K ml⁻¹, to lyse the cells. Plugs were rinsed three times with 0·05 M EDTA (pH 8·0) at room temperature and stored at 4 ºC. ChDNA stored thus was stable for over a year.

ChDNA was also prepared by the liquid spheroplast method of Orbach et al. (1988). However, the agarose spheroplast method was preferred for its ease of preparation, and was used for all further analysis.

**CHEF analysis.** CHEF gel electrophoresis was performed in a CHEF-DR II system (Bio-Rad) fitted with a buffer-circulating pump in a cold room at 4 ºC. ChDNA agarose plugs were cut

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**Abbreviations:** ChDNA, chromosomal DNA; CHEF, contour-clamped homogeneous electric field.
into blocks of 3 mm height (to fit into the gel well), each block containing approximately 10^7 lysed germinated conidial cells. Gels of 0.7% chromosomal-grade agarose (Sigma) were run in 0.5 x TBE at 9 °C, as indicated in the Bio-Rad manual, along with standard ChDNAs of Sch. pombe and Sacch. cerevisiae (Bio-Rad). The run conditions were varied as described in the results. The band sizes were determined by comparison with standard ChDNA samples.

RESULTS AND DISCUSSION

The molecular sizes of ChDNAs of F. solani extend over a fairly wide range and could not be resolved by any one set of electrophoretic conditions, but with appropriate run conditions the entire karyotype could be resolved by a combination of two runs. Resolution of large ChDNAs (Vollrath & Davis, 1987) can be obtained only with long switch intervals for a prolonged time at a lowered voltage, while ChDNA intermediate in size requires a shorter switch interval. Thus the ChDNA of F. solani was separated mainly by a combined switch interval of 45 min for 52 h followed by 30 min for 68 h, all at 60 V. Twelve bands were obtained, of molecular sizes 6.08, 4.95, 3.45, 3.22, 3.05, 2.72, 2.45, 2.39, 2.32, 2.17 and 2.08 Mb (Fig. 1a). The curving of lane 1 in Fig. 1(a) may be explained as being a result of the migration of bulky chromosomes, both of F. solani and of Sch. pombe in the adjacent lane. Hence the ChDNA migration pattern differs a little from that in lane 4. Replicates of F. solani ATCC 64023 gave identical electrophoretic patterns. Observations were recorded for a minimum of three runs.

Using run conditions employed for separation of Sacch. cerevisiae ChDNA, with a little modification, viz. a switch interval of 60 s for 15 h followed by 90 s for 9 h, at 150 V, a further band, corresponding to 0.42 Mb, was obtained (Fig. 1b). The above results indicate that F. solani has 13 chromosomes. Based on the ChDNA molecule sizes obtained, the total chromosomal DNA is calculated to be 39-90 Mb. Puhalla (1981) reported the chromosome number as being 4-7 in various Fusarium species, while Miao et al. (1991) described the presence of 10-15 ChDNAs in species of Nectria haematococca (the sexual state of Fusarium solani).

For many fungi there is little or no genetic data available, and electrophoretic karyotyping is one of the molecular tools, together with restriction fragment length polymorphism (RFLP), for analysis of variability/homology in fungi. Electrophoretic karyotyping is reported to show more variability than RFLP analysis (Magee & Magee, 1987). Thus the electrophoretic karyotype of F. solani holds much scope for further genetic analysis and a better understanding of the genetic makeup of this organism.

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REFERENCES


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