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Electrophoretic karyotype of *Fusarium solani*

Sarita W. Nazareth† and Carlo V. Bruschi

Author for correspondence: Carlo V. Bruschi. Tel: +39 40 375 7304. Fax: +39 40 375 7343.
e-mail: bruschi@icgeb.trieste.it

Department of Microbiology, International Centre for Genetic Engineering and Biotechnology, Padriciano 99, I-34012 Italy

The electrophoretic karyotype of *Fusarium solani* was determined by contour-clamped homogeneous electric field (CHEF) gel electrophoresis. Thirteen chromosomal bands were obtained. The size of these bands, based on their migration relative to the chromosomal DNA of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*, is estimated to be between 0·42 and 6·08 Mb, the total molecular size of the DNA being 39·90 Mb.

Keywords: *Fusarium solani*, electrophoretic karyotype, CHEF, chromosomes

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.) Sacc., 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 haematococca* 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*.

† **Present address:** Department of Microbiology, Goa University, Taleigao Plateau, Bambolim, Goa-403202, India.

Abbreviations: ChDNA, chromosomal DNA; CHEF, contour-clamped homogeneous electric field.

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 (4×10^8 as counted by a haemocytometer) were resuspended in PDB (125 ml), and incubated on a rotary shaker at 30 °C, 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 2×10^8) were mixed with molten 1% (w/v) low-melting-point agarose prepared in 0·125 M EDTA/0·05 M sodium citrate (pH 5·7) containing Lysing Enzymes (Sigma), so that the final cell density was $2{\cdot}0 \times 10^8$ 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 °C to gel. The plugs were then removed, put into 0·05 M sodium citrate (pH 5·7)/0·4 M EDTA (pH 8·0)/7·5% (v/v) 2-mercaptoethanol for spheroplast formation, and incubated at 37 °C for 24 h. Plugs were rinsed three times with 0·4 M EDTA (pH 8·0) and incubated for 24 h at 50 °C 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 °C. 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 °C. ChDNA agarose plugs were cut

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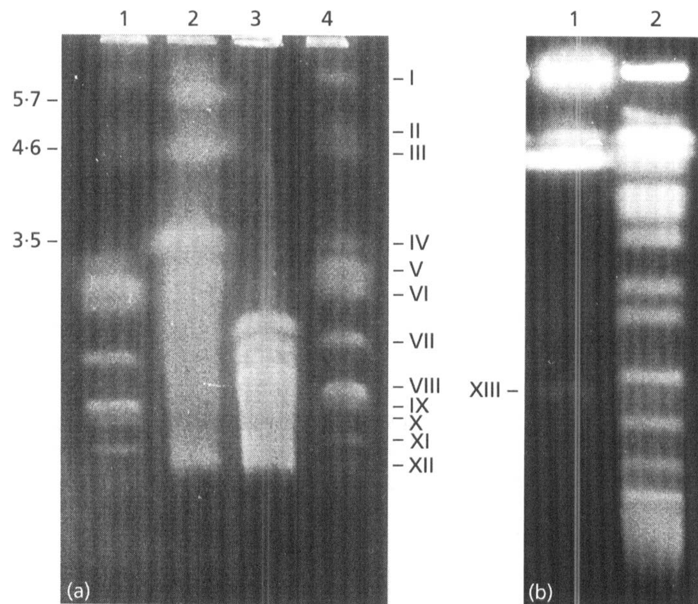

Fig. 1. **Electrophoretic karyotype of *F. solani* by CHEF gel electrophoresis with run conditions of: (a) 60 V, with switch intervals of 45 min for 52 h, followed by 30 min for 68 h; (b) 150 V, with switch intervals of 60 s for 15 h, followed by 90 s for 9 h. Lanes: (a) 1 and 4, *F. solani*; 2, *Sch. pombe*; 3, *Sacch. cerevisiae*. The numbers on the left indicate the DNA size of the *Sch. pombe* chromosome standard in megabase pairs. (b) 1, *F. solani*; 2, *Sacch. cerevisiae*. The numbers on the right indicate the DNA size of two of the *Sacch. cerevisiae* chromosome standards in megabase pairs. Roman numerals indicate the individual chromosomal bands of *F. solani*.**

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 × TBE at 9 °C, as indicated in the Bio-Rad manual, along with standard ChDNA samples 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, 4·60, 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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