Genome organization of *Magnaporthe grisea*: genetic map, electrophoretic karyotype, and occurrence of repeated DNAs

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Abstract. A genetic map of *Magnaporthe grisea* (anamorph = *Pyricularia oryzae* and *P. grisea*), the causal agent of rice blast disease, was generated from segregation data utilizing 97 RFLP markers, two isoenzyme loci and the mating type locus among progeny of a cross between parental strains Guy 11 and 2539. Of the seven chromosomes of *M. grisea*, three were resolved by contour-clamped homogeneous electric field (CHEF) electrophoresis, while the remaining four migrated as two doublet bands. By utilizing differences between CHEF mobilities of unresolved chromosomes from the parental strains, Southern analysis with selected markers allowed the chromosomal assignment of all linkage groups. A small translocation involving 1 marker was found in the parental strains used to produce the segregating population from which the map was constructed. Nine classes of repetitive DNA elements were found in the genome of a fungal isolate pathogenic to rice. These occurred only a few times or not at all in the genomes of isolates showing reduced virulence on rice. One repetitive DNA was shown to have structural similarity to the *Alu* sequences found in primates, a sequence similarity to the *copia-like* elements of *Drosophila*, and peptide similarity to transposable elements found in *Drosophila*, other fungi, and higher plants.

Key words: Rice blast – RFLP – Retrotransposon

Introduction

*Magnaporthe grisea* (Hebert) Barr comb. nov. is the teleomorphic stage of *Pyricularia oryzae* and *P. grisea*, fungi causing rice blast and diseases of many other grass species, respectively. The teleomorphic stage of *P. grisea* was first described in 1971 as *Ceratosphaeria grisea*, the sexual stage of crabgrass-infecting isolates of the fungus (Hebert 1971). Five years later it was reported that *P. grisea* and *P. oryzae*, which are morphologically identical, could be mated to produce perithecia, although the level of fertility was low (Yaegashi and Hebert 1976). Later, *P. grisea* was transferred to the teleomorph *Magnaporthe grisea* Hebert (Yaegashi and Udagawa 1978).

The rice blast fungus is considered to be one of the most important plant pathogens in the world (Ou 1985). Because of its economic importance, considerable efforts have been made to understand the genetics and molecular biology of this fungus. A previous genetic study of *M. grisea* defined five linkage groups based on 11 auxotrophic and fungicide resistance markers (Nagakubo et al. 1983). Other markers have been described (Leung and Williams 1985; Leung and Taga 1988), although little is known about the linkage relationships of those markers. A RFLP map utilizing preliminary data from this study has been reported (Skinner et al. 1990; Budde et al. 1993), and a genetic map utilizing a repetitive DNA sequence as a genetic marker has been constructed (Romao and Hamer 1992) and has yielded eight large linkage groups. Another map has been constructed using cloned genes,
cosmid clones, repeated DNA, and a telomeric repeat (Sweigard et al. 1993).

Several studies have centered on the processes involved in the interaction with the host plant (Chumley and Valent 1990; Hamer et al. 1988; Howard and Ferrari 1989; Leung et al. 1988). Transformation systems based on auxotrophic complementation (Parsons et al. 1987) and a dominant, selectable marker (Leung et al. 1987) and a dominant, selectable marker (Leung et al. 1987) have been developed for M. grisea, greatly facilitating the cloning and study of genes. To further facilitate the cloning of genes, we have undertaken the construction of a genetic map of M. grisea that is based on restriction fragment length polymorphisms (RFLPs) to provide starting points for “chromosome-walks” from RFLP markers to linked genes of interest. The principle of gene isolation based on saturated maps has been discussed elsewhere (Botstein et al. 1980; Michelmore and Hultbert 1987), and the utility of marker-based cloning in fungi has been demonstrated in several studies (Froeliger and Leong 1989; Glass et al. 1988; Giasson et al. 1989; Mutasa et al. 1990; Tzeng et al. 1991; May et al. 1991; Romao and Hamer 1992).

Historically, the construction of conventional genetic maps of fungi has been based solely on the segregation analysis of genetic markers. The routine assignment of linkage groups to fungal chromosomes was not possible. However, the development of pulsed-field electrophoretic techniques has allowed the separation of fungal chromosomes and the assignment of DNA markers to chromosomes by hybridization studies. The resolution of fungal chromosomes was first reported for yeast chromosomes that range in size from about 0.2 megabase pairs (Mbp) to about 3 Mbp (Schwartz and Cantor 1984). The larger chromosomes of Neurospora crassa, ranging in size from about 4 to 12 Mbp, have also been resolved (Orbach et al. 1986) using CHEF electrophoresis (Chu et al. 1992). We report here the use of the CHEF system to resolve the chromosomes of M. grisea and the subsequent use of the resolved chromosomes for the construction of chromosome-enriched plasmid libraries. Southern analysis of CHEF gels allowed the assignment of all linkage groups to electrophoretically separated chromosomes.

Repeated DNA species occur in all organisms. Some are of known function (e.g. the genes encoding the ribosomal subunits), while others have no known function. DNA elements capable of transposition can be reiterated throughout the genome, hence repeated DNA elements are of interest as possible transposable elements. We report here the occurrence of several unique classes of repeated DNA in the genome of M. grisea and the relationship of one class of this DNA to known transposable elements from other organisms.

### Materials and methods

#### Fungal isolates

*M. grisea* isolate Guy11 (Mat 1-2), a rice pathogen that was originally discovered in French Guyana was obtained from Dr. J. L. Nottjeghem, Institute de Recherche Agronomiques Tropicales, Montpellier, France. Isolate 2539 (Mat 1-1) was developed in the laboratory as described previously (Leung et al. 1988). Isolates AR-4, CH40-1, CH104-3, and 6-28 were provided by A. H. Ellingboe (Kolmer and Ellingboe 1988). Isolates O-135 and 4091-5-8 were provided by B. Valent (Valent et al. 1991).

The mapping population was derived from a single cross between Guy11 and 2539 from which 68 random ascospores and ten complete or partial tetrads were obtained (Leung et al. 1988). From this cross, 61 random ascospore progeny were used to determine the segregation of RFLPs and linkage relationships. The segregation of mating type and two lactate dehydrogenase loci (LDH1 and LDH3) were determined as described previously (Leung and Williams 1985).

#### DNA isolation

DNA was extracted from mycelium grown in 100 ml liquid complete medium (CM) (Valent et al. 1986) in 250-ml Erlenmeyer flasks, at 25°–30 °C with shaking at 100 rpm on an orbital shaker. The flasks were inoculated with mycelia from PDA or oatmeal plate cultures and were harvested when mycelial density reached its maximum but before dark pigments were produced, usually about 3 days after inoculation. Mycelial harvest and DNA extraction was by the CTAB method (Manicom et al. 1987).

**Electrophoresis and Southern transfer of genomic DNA**

Restriction endonuclease-digested DNA was electrophoresed in 0.7%, SeaKem LE agarose gels (1.5 mg/lane) using standard conditions (Maniatis et al. 1982). The electrophoresed DNA was transferred to a positively charged nylon membrane (Schleicher and Schuell, Keene, N.H.) according to standard protocol (Southern 1975).

#### Preparation of intact chromosomes in agarose microbeads and separation by CHEF electrophoresis

The preparation of intact chromosomes in microbeads was accomplished following the protocol of Koob and Szybalski (1992) with modification. Mycelial cultures were established at 25°–30 °C in 50 ml CM Erlenmeyer flask with shaking at 100 rpm on an orbital shaker. After 72 h of growth, the cultures were ground briefly at full speed in a blender and then added to 200 ml of CM in a 1-l Erlenmeyer flask. The culture was grown for 18 h and was then harvested by filtration through Miracloth filters (Calbiochem, San Diego, Calif.). The mycelium was suspended in SEC buffer (1 M sorbitol, 50 mM NaCl, EDTA, 50 mM Na citrate, final pH 6.2) and centrifuged at 1600 g for 5 min. It was then resuspended in fresh SEC buffer containing 1.5 mg/ml Novozyme 234 (Calbiochem, San Diego, Calif.) and incubated at 30 °C for 2–3 h. The protoplasts were harvested by filtration through Miracloth, rinsed with SEC buffer, and then pelleted by centrifugation (200 g, 10 min). A hemacytometer was used to determine that the pellet contained approximately 10^9 protoplasts/ml. The pellet was diluted ten fold (to a maximum volume of 2 ml) in SEC to give a concentration of approximately 1 × 10^9 protoplasts/ml. To this was added an equal volume of 1.2% InCert agarose (FMC, Rockland, Me.) in SEC at 55 °C. The solution was mixed by pipetting and then added to 5 ml of mineral oil at 55 °C in a 25 ml Erlenmeyer flask. This solution was mixed vigorously for 1 min at full speed on a vortex mixer...