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The 1.6 Mb chromosome carrying the avirulence gene *AvrPik* in *Magnaporthe oryzae* isolate 84R-62B is a chimera containing chromosome 1 sequences

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ABSTRACT

A genetic map was constructed previously from a cross between *Magnaporthe oryzae* isolates 84R-62B and Y93-245c-2, and genetic markers closely linked to the cultivar-specific avirulence (*Avr*) gene, *AvrPik*, were assigned to a 1.6 Mb small chromosome of 84R-62B that is absent from Y93-245c-2. In the present study, the 1.6 Mb chromosome was characterized by using contour-clamped homogeneous electric fields (CHEF) electrophoresis and hybridization analysis. CHEF electrophoresis analysis showed that the 1.6 Mb chromosome was inherited in Mendelian fashion, and co-segregated with *AvrPik*. Southern hybridization analysis revealed that the 1.6 Mb chromosome carried sequences only distributed to the supernumerary chromosome in *M. oryzae* isolates, as well as sequences corresponding to those in the supercontig 17 of chromosome 1 in the *M. grisea* database. Thus, we conclude that the Mendelian 1.6 Mb chromosome is a chimera containing sequences from chromosome 1 and from supernumerary chromosomes in *M. oryzae*.

Introduction

Magnaporthe oryzae (formerly *M. grisea*; Couch & Kohn 2002) is the causal agent of rice blast, which is one of the most damaging diseases of cultivated rice (*Oryza sativa*). The development of contour-clamped homogeneous electric fields (CHEF) electrophoresis (Chu *et al.* 1986), has allowed the separation of intact chromosomal DNA molecules of organisms. Karyotyping using CHEF electrophoresis provided valuable information about the organization of the genomes in several filamentous fungi including *M. oryzae*. Talbot *et al.* (1993) examined the electrophoretic karyotypes of *M. oryzae* isolates representing a diverse collection of pathotypes of rice blast fungus in the USA. Orbach *et al.* (1996) further analysed chromosomes of *M. oryzae* from a wide variety of host species and laboratory cultures. Sone *et al.* (1997) investigated electrophoretic karyotypes of more than 20 Japanese rice-field isolates of *M. oryzae*, which belonged to 12 different races (pathotypes). In those studies, the presence of extra chromosomes, less than 2 Mb in size, termed mini-chromosomes has been demonstrated (Orbach *et al.* 1996; Sone *et al.* 1997; Talbot *et al.* 1993). Subsequently, the term of supernumerary chromosome was recommended for general use by Covert (1998) to designate the extra chromosome that is composed primarily of DNA

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not found in all representatives of the species, and some mini-chromosomes were characterized as supernumerary chromosomes in *M. oryzae* (Covert 1998). Such chromosomes are similar to B chromosomes in other eukaryotic organisms, which were described as small, extra, dispensable for growth, and inherited in a non-Mendelian manner (Jones 1995). Although the function of supernumerary chromosomes in most fungal species is still unknown, some studies have shown that the supernumerary chromosomes can carry functional genes and, in at least three fungal species, genes on such chromosomes play important roles in pathogenicity on a specific host (Ahn & Walton 1996; Hatta *et al.* 2002; Miao *et al.* 1991a). Supernumerary chromosomes that confer an adaptive advantage in certain habitats, such as the ability to cause disease on a specific host, may be referred to as 'conditionally dispensable' (CD) chromosomes in order to reflect their importance in some, but not all, growth conditions (Covert 1998).

In *M. oryzae*, it has been reported that the presence of the supernumerary chromosomes was correlated with female sterility (Orbach *et al.* 1996), but was not with any other obvious traits (Orbach *et al.* 1996; Talbot *et al.* 1993). In our previous study (Luo *et al.* 2005), a small chromosome of ~1.6 Mb was identified in the rice-field isolate 84R-62B by using CHEF electrophoresis. At the same time, a genetic map, including eight linkage groups, was constructed in a genetic cross of 84R-62B with another rice-field isolate Y93-245c-2, and the linkage groups were assigned to chromosomes of the parental isolates based on the hybridization of selected RFLP markers from these linkage groups to the CHEF-separated parental chromosomes. Interestingly, selected RFLP markers from the genetic linkage group including the avirulence (*Avr*) gene *AvrPik* (determining avirulence on *Pik*-containing rice cultivars) hybridized with chromosome 1 or the 1.6 Mb chromosome in 84R-62B. Furthermore, a RAPD marker clone pAE12AF12 produced two different dominant RFLP markers *AE12AF12-1* and *AE12AF12-2*, which hybridized with the 1.6 Mb chromosome in 84R-62B and chromosome 1 in Y93-245c-2, respectively. Thus, the 1.6 Mb chromosome was suggested as a fragment of chromosome 1 that arose when the larger chromosome was broken (Luo *et al.* 2005). Three RFLP markers, *AE6AF6-1*, *AI6AJ6-1* and *AK11AL11-1*, that were closely linked to *AvrPik*, all hybridized to the 1.6 Mb chromosome in 84R-62B, suggesting that *AvrPik* was located on this small chromosome (Luo *et al.* 2005). Conversely, these three RFLP markers did not hybridize to any chromosomes in Y93-245c-2. Furthermore, a BLAST search revealed that these RFLP markers carried unique sequences that showed no significant homology to any sequences in the *M. grisea* database v2.3 (http://www.broad.mit.edu/annotation/fungi/magnaporthe/index.html) (Luo *et al.* 2005). These data suggested that the 1.6 Mb chromosome possessed its own unique or specific sequences, and led us to further identify the origin of the 1.6 Mb chromosome.

In the present study, to obtain firmer genetic evidence for the location of *AvrPik*, co-segregation between the 1.6 Mb chromosome and *AvrPik* was directly investigated using CHEF electrophoresis. Also, we surveyed the distribution of the 1.6 Mb chromosome specific sequences to the supernumerary chromosomes in other Japanese rice-field isolates for inferring the origin of the 1.6 Mb chromosome. At the same time, in order to identify the chromosome 1-derived region on the 1.6 Mb chromosome, we performed hybridization analysis using selected genes sequences from all of the known supercontigs of chromosome 1 in the *M. grisea* database v2.3 as probes.

Materials and methods

Fungal isolates

Parental isolates, 84R-62B (*MAT1-1*, Japan) and Y93-245c-2 (*MAT1-2*, China), a subset of 60 F_1 progeny isolates used in this study were described previously (Luo *et al.* 2004, 2005). To investigate the relatedness of the 1.6 Mb chromosome in 84R-62B and supernumerary chromosomes in other rice-field isolates, 18 Japanese rice-field isolates were used (Table 1).

Hybridization probes

The insert in pAK11AL11 (DDBJ accession no. AB175832) was shown to contain 1.6 Mb chromosome specific sequences (Luo *et al.* 2005). pAE6S and pAI6S are sub-clones obtained from pAE6AF6 and pAI6AJ6 (DDBJ accession no.s AB175829 and AB175831), respectively. pAE6AF6 and pAI6AJ6 also carry specific sequences to the 1.6 Mb chromosome, but contain short sequences less than 200 bp homologous to the larger chromosomes (Luo *et al.* 2005). Therefore, the sequences that exist only on the 1.6 Mb chromosome were amplified from those clones using primer pairs of T7

Table 1 – Rice-field isolates of *Magnaporthe oryzae* used in this study

Isolate[a]	Locality (Prefecture in Japan)	Year	Race[b]
Ina 93-3	Yamaguchi	1993	301.0
Ina 168	Aichi	1958	101.1
Naga 69-150	Nagano	1969	007.0
Hoku 1	Hokkaido	1948	007.0
Shin 83-34	Niigata	1983	005.0
SA98-41A	Saga	1998	001.0
Ina 72	Nagano	1956	031.1
Ai74-134	Aichi	1974	477.1
Ai 79-142	Aichi	1979	037.3
Mu-183	Kumamoto	1995	337.3
TH68-140	Yamagata	1968	035.1
SA98-48A	Saga	1998	033.0
Ken 53-33	Aichi	1953	137.1
Ken 54-20	Yamaguchi	1954	003.0
Ina 86-137	Aichi	1986	007.0
GFOS8-1-1	Gifu	1993	303.0
TH69-8	Fukushima	1969	071.7
SA98-44A	Saga	1998	000.0

a SA98-41A, SA98-48A and SA98-44A are stock cultures in our laboratory, other isolates are stock cultures in National Agricultural Research Center for Kyushu Okinawa Regions.
b Race codes (Yamada *et al.* 1976) are obtained from our previous study (SA98-41A, SA98-48A and SA98-44A) (Fukuta *et al.* 2000) or Microorganisms Section of the Ministry of Agriculture, Forestry and Fisheries (MAFF) Genebank, National Institute of Agrobiological Sciences (other isolates).