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Complete Sructure of Nuclear rDNA of the Obligate Plant Parasite *Plasmodiophora brassicae*: Intraspecific Polymorphisms in the Exon and Group I Intron of the Large Subunit rDNA

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Plasmodiophora brassicae **is a soil-borne obligate intracellular parasite in the phylum Cercozoa of the Rhizaria that causes clubroot disease of crucifer crops. To control the disease, understanding the distribution and infection routes of the pathogen is essential, and thus development of reliable molecular markers to discriminate geographic populations is required. In this study, the nuclear ribosomal RNA gene (rDNA) repeat unit of *P. brassicae* was determined, with particular emphasis on the structure of large subunit (LSU) rDNA, in which polymorphic regions were expected to be present. The complete rDNA complex was 9513 bp long, which included the small subunit, 5.8S and LSU rDNAs as well as the internal transcribed spacer and intergenic spacer regions. Among eight field populations collected from throughout Honshu Island, Japan, a 1.1 kbp region of the LSU rDNA, including the divergent 8 domain, exhibited intraspecific polymorphisms that reflected geographic isolation of the populations. Two new group I introns were found in this region in six out of the eight populations, and the sequences also reflected their geographic isolation. The polymorphic region found in this study may have potential for the development of molecular markers for discrimination of field populations/isolates of this organism.**

Key words: Cercozoa; divergent domain in LSU rDNA; group I intron; intraspecific polymorphism; nuclear rDNA; *Plasmodiophora brassicae*.

Introduction

Plasmodiophora brassicae is a soil-borne plant pathogen that causes clubroot disease of crucifers. Although it was traditionally classified as a fungus, recent molecular phylogenetic analyses of the small subunit (SSU) ribosomal RNA (Castlebury and Domier 1998; Cavalier-Smith and Chao 1997), actin and polyubiquitin genes (Archibald and

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Keeling 2004) suggest that it belongs to the protist phylum Cercozoa in the kingdom Rhizaria (Archibald and Keeling 2004; Cavalier-Smith and Chao 2003). Within the Cercozoa, the order Plasmodiophorida has attracted particular attention because it includes not only *P. brassicae* but also several plant pathogens of worldwide importance, such as *Spongospora subterranea*, which causes potato powdery scab disease, and *Polymyxa* spp., which transmit various plant viruses (Braselton 1995; Bulman et al. 2001). Information about the genomic structure of plasmodiophorids, however, is limited due to their nature as obligate intracellular parasites.

Pathotypes of *P. brassicae* have been characterized from sets of different hosts (Buczacki et al. 1975; Hatakeyama et al. 2004; Kuginuki et al. 1999; Somé et al. 1996; Williams 1966), but this process is time-consuming, labor-intensive, and subject to varying environmental conditions. Identification of the geographic populations/isolates of *P. brassicae* is also important to understand the distribution and infection routes. Therefore, development of reliable molecular markers that enable rapid identification of the pathotypes and geographic populations is required. For this purpose, molecular characterization of the genome is essential. Recently, Bulman et al. (2007) compared the nucleotide sequences of 24 genes in the *P. brassicae* genome with those of the corresponding cDNA and found that the genome was rich in spliceosomal introns. Random amplified polymorphic DNA (RAPD) analysis on *P. brassicae* populations that differed in pathogenicity revealed that the genome was highly polymorphic among the populations, although correlations between the pathogenicity and RAPD patterns were not clear (Manzanares-Dauleux et al. 2001; Osaki et al. 2008). In *Polymyxa graminis*, on the other hand, sequence variation in the two transcribed spacer (ITS1 and ITS2) regions of the rDNA were observed among the geographical populations and also among those with different host ranges (Legrève et al. 2002). In contrast, there was no sequence variation in the ITS sequences of *Spongospora subterranea* among Australasian and European populations, except for one from Scotland (Bulman and Marshall 1998). Among *P. brassicae* populations, however, little information about sequence variation among geographic populations of particular genes is available so far.

In eukaryotes, large variation in length and sequence have been reported in the large subunit (LSU) rDNA. Twelve divergent domains that showed sequence variability in the LSU rDNA were first identified in mouse (Hassouna et al. 1984). These domains have been employed for identification and/or detailed characterization of species/isolates in a range of organisms (e.g., Chenuil et al. 2008; Lachance et al. 2000). The intergenic spacer (IGS) region of rDNA repeats in eukaryotes also shows extensive sequence variation and has been used widely as a molecular marker in the population biology of pathogenic fungi (e.g., Chang et al. 2008; Jackson et al. 1999; Pantou et al. 2003; Pramateftaki et al. 2000). These observations led us to expect that several polymorphic regions would be present in the unexplored region of rDNA of *P. brassicae*, although no sequence information is currently available. In the present study, the complete structure of the rDNA repeat unit of *P. brassicae* was determined, with particular emphasis on polymorphisms among field populations of different geographic origin.

Results

Structure of the Ribosomal RNA Gene Repeat Unit

Clubroot galls formed on *B. rapa* var. *pekinensis* grown in an experimental field of Nagoya University were harvested and designated as population NGY. To determine the complete sequence of the rDNA repeat unit of this population, spores were purified from several galls, and then genomic DNA was extracted. Seven DNA fragments were amplified with the primer pairs Pbr1/Pbr2r, Pb121/Pbr4r, NS7/ITS4, Pbr4/NDL22, NDL22f/IGS1r, V282/Pbr1r, and IGSa-10f/Pb121r (Supplementary Table S1 and Fig. 1a), cloned, sequenced from both ends of each PCR product, and assembled. The total length of the rDNA repeat unit was 9513 bp. The lengths of the SSU rDNA, internal transcribed spacer 1 (ITS1)-5.8S rDNA-ITS2 region, LSU rDNA, and IGS region were 3105, 465, 3611, and 2332 bp, respectively, based on sequence alignment and comparisons to those of other eukaryotes. Three introns in the SSU rDNA that were described in Castlebury and Domier (1998) were named Pbr.S516, Pbr.S943, and Pbr.S1506, with reference to the corresponding positions in *Escherichia coli* rDNA. To examine the presence of introns in the LSU rDNA, four PCR fragments that covered 3145 bp of the LSU rRNA were amplified with the primer pairs LRP4/NDL22, NDL22f/28s1r, 28s1/28s3r, and 28s3/P282r using cDNA that was reverse-transcribed from RNA as template and sequenced (Supplementary Table S1 and Fig. 1b). Comparative analysis between