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Extensive chromosome rearrangements distinguish the karyotype of the hypovirulent species *Candida dubliniensis* from the virulent *Candida albicans*

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Abstract

Candida dubliniensis and *Candida albicans*, the most common human fungal pathogen, have most of the same genes and high sequence similarity, but *C. dubliniensis* is less virulent. *C. albicans* causes both mucosal and hematogenously disseminated disease, *C. dubliniensis* mostly mucosal infections. Pulse-field electrophoresis, genomic restriction enzyme digests, Southern blotting, and the emerging sequence from the Wellcome Trust Sanger Institute were used to determine the karyotype of *C. dubliniensis* type strain CD36. Three chromosomes have two intact homologues. A translocation in the rDNA repeat on chromosome R exchanges telomere-proximal regions of R and chromosome 5. Translocations involving the remaining chromosomes occur at the Major Repeat Sequence. CD36 lacks an MRS on chromosome R but has one on 3. Of six other *C. dubliniensis* strains, no two had the same electrophoretic karyotype. Despite extensive chromosome rearrangements, karyotypic differences between *C. dubliniensis* and *C. albicans* are unlikely to affect gene expression. Karyotypic instability may account for the diminished pathogenicity of *C. dubliniensis*.

Keywords: Chromosome translocations; Karyotype rearrangements; Major Repeat Sequence; Sfi digestion fragments; Genomic sequence

1. Introduction

The history of infectious disease is largely devoted to bacterial and viral infections, such as plague, tuberculosis, and smallpox, and it is only in the last 50 years or so that fungal infections have emerged as a serious medical problem. This emergence is due directly to the efficacy of medical care, which now has the resources to maintain highly debilitated patients with deficient immune systems. A significant number of these patients get infections with one or another candida species, and although *Candida albicans* is still the most commonly isolated pathogenic fungus, other species, such as *Candida dubliniensis* and *Candida glabrata*, are increasing in frequency among isolates (Kullberg and Filler, 2002; Maschmeyer, 2006).

Candida dubliniensis is the species most closely related to *C. albicans*, but the two differ significantly in virulence properties. *C. dubliniensis* is found mostly as an oral pathogen and only rarely in disseminated infections (Gilfillan and Sullivan et al., 1998). However, it has been estimated that more than 88% of the genes of these closely related species have nucleotide homology greater than 80% (Moran et al., 2004). In addition, the two species share the ability to grow as yeast, pseudohyphae, and hyphae

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and to make chlamydospores. Similarities have also been noted in their genomes: both are diploid and have a large repeated DNA sequence on most chromosomes (the Major Repeat Sequence or MRS). The origin and function of the MRS are not known, but it is found only in *C. dubliniensis* and *C. albicans*. It is the site of most of the translocations which have been mapped in *C. albicans*, and it is responsible for much of the chromosome length polymorphism which exists in that organism (Chibana et al., 2000).

The published genome sequence assembly of *C. albicans* released in 2004 (Assembly 19) was a diploid one, including separate alleles for many of the loci (Braun et al., 2005). Assembly 19 had 133 pairs of allelic contigs and 136 unique contigs and has been annotated by a large set of investigators (Jones et al., 2004). The genomic sequence of *C. dubliniensis* is virtually complete and is available (unpublished) at the Wellcome Trust Sanger Institute (SangerInstitute http://www.sanger.ac.uk/) but has not yet been annotated. Thus comparison between the two genomes is possible. The karyotype of *C. albicans*, determined using pulse-field electrophoresis and Southern blotting, demonstrated that there were 8 chromosomes (Magee et al., 1988; Lasker et al., 1989; Wickes et al., 1991). A major tool in the further characterization of chromosome substructure was the use of the 8-base-pair-specific restriction enzyme SfiI, which cleaves the various chromosomes into from 2 to 8 fragments, ranging in size from 90 to >2200 kb. This SfiI macro-restriction map allowed markers to be localized to specific parts of the chromosomes (Chu et al., 1993). The majority of the SfiI restriction sites are in the MRS, which ranges in size from 18 kb to as much as 80 kb (Iwaguchi et al., 1992; Chibana et al., 1994; Chindamporn et al., 1998). Furthermore, all of the reciprocal translocations analyzed in several strains seemed to take place between MRS regions (Chu et al., 1993; Navarro-Garcia et al., 1995). *C. dubliniensis* also has an MRS (Joly et al., 1999), but its role in chromosome organization and translocation has not been investigated.

Assembly of the *C. dubliniensis* sequence at the Wellcome Trust Sanger Institute is based on the karyotype of SC5314, the sequenced *C. albicans* strain, and is similarly organized into 8 chromosomes, each nominally representing one of a pair of homologues. However unlike *C. albicans,* where approximately half the strains have a standard karyotype, in *C. dubliniensis* the karyotypes differ in every isolate we have examined (Magee and Chibana, 2002). Here we present the detailed karyotype of CD36, the *C. dubliniensis* strain whose genomic sequence was determined, and the pulse-field chromosome separations of some other *C dubliniensis* isolates compared to *C. albicans*. Although large blocks of DNA, corresponding in several cases to the SfiI restriction fragments in *C. albicans*, are similarly organized in the two species, they are much less frequently arranged as pairs of chromosome homologues in *C. dubliniensis*. As in *C. albicans*, the MRS in *C. dubliniensis* seems to be a major site for chromosome reorganization (Chibana et al., 2000) (Magee and Chibana, 2002).

2. Materials and methods

2.1. Strains

The strains of *C. albicans* and *C. dubliniensis* in this paper are listed in Table 1. Media and growth conditions were as previously described (Chu et al., 1993).

2.2. Pulse-field electrophoresis and Southern blotting

CHEF samples were made according to standard procedures Chibana et al., 1998. Gels were run using the CHEF DRIII System (Bio-Rad) at 14 C. Two separation protocols were used.

All chromosomes: 0.6% agarose (Amresco PFGE Grade III), in 0.5× TBE buffer (50 mM Tris, 50 mM boric acid, 1 mM EDTA, pH8.3) 60–300 s switch ramp, 24 h, 4.5 V/cm 120°; 720–900 s ramp, 12 h, 2.0 V/cm, 106° (Figs. 1 Center and Right, Fig. 3a, Fig. 5).

Lower chromosomes: 0.9% Bio-Rad Pulsed Field certified agarose, 60–120 s ramp 24 h, 6.0 V/cm; 120–360 s ramp, 15 h, 4.5 V/cm, 120° (Fig 1 Left).

SfiI and XhoI digests: 1.0% Bio-Rad Pulsed Field certified agarose, 7–100 s ramp, 20 h, 4.5 V/cm; 80–400 s ramp, 20 h, 3.5 V/cm, 120° (Fig. 2, Fig. 3b and c).

For Southern hybridization, the DNA on the gels was nicked (Bio-Rad Gene Linker) and vacuum blotted (Bio-Rad 785) to nylon membranes (MSI or Hybond) according to manufacturer's specifications. DNA probes were labeled using ^{32}P-dCTP with the Rediprime II kit (Amersham Pharmacia). Hybridizations were performed at 65 °C in modified Church/Gilbert Buffer (7% SDS, 0.5 M Na–Phosphate (pH 7.2), 1 mM EDTA). Blots were washed to medium stringency according to the membrane manufacturer (final washes at 37 °C).

Most *C. albicans* probes gave a poor signal with *C. dubliniensis* blots under standard conditions. We used DNA primers designed for *C. albicans* genes to amplify *C. dubliniensis* genomic DNA by PCR, using standard conditions. Most primers gave product. We also found that many *C. albicans* amplicons could be used directly

Table 1
List of strains used

Organism	Strain	Reference
Candida albicans		
	1006	Goshorn et al. (1992)
	SC5314	Jones et al. (2004)
Candida dubliniensis		
	CD36	Sullivan et al. (1995)
	R3b	Timmins et al. (1998)
	R1b	Timmins et al. (1998)
	16F	Sullivan et al. (1995)
	3225	Muller et al. (1999)
	3233	Joly et al. (1999)
	514	Gee et al. (2002)