Available online at www.sciencedirect.com
ScienceDirect
Fungal Genetics and Biology 44 (2007) 920–931
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

Lower filamentation rates of *Candida dubliniensis* contribute to its lower virulence in comparison with *Candida albicans*

C. Stokes [a], G.P. Moran [a], M.J. Spiering [a], G.T. Cole [b,1], D.C. Coleman [a], D.J. Sullivan [a,*]

[a] *Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental School and Hospital, Trinity College Dublin, Dublin 2, Ireland*
[b] *Department of Medical Microbiology and Immunology, Medical College of Ohio, Toledo, OH 43614-5808, USA*

Received 27 October 2006; accepted 28 November 2006
Available online 23 January 2007

Abstract

Candida albicans and *C. dubliniensis* are very closely related yeast species. In this study, we have conducted a thorough comparison of the ability of the two species to produce hyphae and their virulence in two infection models. Under all induction conditions tested *C. albicans* consistently produced hyphae more efficiently than *C. dubliniensis*. In the oral reconstituted human epithelial model, *C. dubliniensis* isolates grew exclusively in the yeast form, while the *C. albicans* strains produced abundant hyphae that invaded and caused significant damage to the epithelial tissue. In the oral-intragastric infant mouse infection model, *C. dubliniensis* strains were more rapidly cleared from the gastrointestinal tract than *C. albicans*. Immunosuppression of *Candida*-infected mice caused dissemination to internal organs by both species, but *C. albicans* was found to be far more effective at dissemination than *C. dubliniensis*. These data suggest that a major reason for the comparatively low virulence of *C. dubliniensis* is its lower capacity to produce hyphae.

Keywords: *Candida albicans*; *Candida dubliniensis*; Hyphae; Morphogenesis; Infection models; Virulence

1. Introduction

Candida albicans is routinely reported as the most common cause of superficial and systemic candidiasis, indicating that it is the most pathogenic *Candida* species (Edmond et al., 1999; Eggimann and Pittet, 2001; Garber, 2001; Asmundsdottir et al., 2002; Ellis, 2002; Kibbler et al., 2003). *Candida dubliniensis*, which was first identified as a separate species in 1995, is the most closely related species to *C. albicans* (Sullivan et al., 1995). Due to this close phylogenetic relatedness the two species are phenotypically very similar and exhibit a similar range of putative virulence factors (Gilfillan et al., 1998; Hannula et al., 2000; Vilela et al., 2002). In particular, *C. dubliniensis* has the capacity to produce hyphae, pseudohyphae and chlamydospores, and thus like *C. albicans*, this species is polymorphic in nature (Gilfillan et al., 1998). Interestingly, despite the fact that the two species are genotypically and phenotypically so similar, one clear difference between them is their capacity to cause disease. *Candida dubliniensis* is only rarely identified as a cause of systemic infection (Kibbler et al., 2003; Sullivan et al., 2004), suggesting that *C. dubliniensis* may be less virulent than *C. albicans*. That this may indeed be the case appears to be corroborated by the data obtained in two studies that compared the comparative virulence of the two species in the murine model for systemic infection. In these studies, mice infected with *C. dubliniensis* showed significantly longer survival times, apparently confirming that it is less virulent than *C. albicans* (Gilfillan et al., 1998; Vilela et al., 2002).

In a previously published comparison of the phylogeny and virulence factors expressed by *C. albicans* and *C. dubliniensis*, Gilfillan et al. (1998) investigated the ability of the two species to produce hyphae, a trait widely

* Corresponding author. Fax: +353 1 6127295.
E-mail address: derek.sullivan@dental.tcd.ie (D.J. Sullivan).
[1] Present address: Department of Biology, University of Texas at San Antonio, Margaret Batts Tobin Building, Room 1.308E, 6900 North Loop 1604 West, San Antonio, TX 78249, USA.

1087-1845/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.fgb.2006.11.014

regarded as one of the most important virulence factors of *C. albicans* (Gilfillan et al., 1998). This study, which was limited to two strains of each species and to a limited range of experimental conditions conducive to the induction of hyphae by *C. albicans*, revealed that while *C. dubliniensis* has the capacity to produce true hyphae, it appears that the hyphae are produced at a slower rate (Gilfillan et al., 1998). Since this original study a far greater range of *C. dubliniensis* isolates from various clinical sources have been identified. Population analysis using DNA fingerprinting has also revealed that *C. dubliniensis* is comprised of four distinct genotypes (Gee et al., 2002). Due to the importance of hyphae in candidal virulence, in the present study we have compared the dynamics and the levels of hypha-induction in a comprehensive range of *C. dubliniensis* isolates (representative of the four known genotypes and from a wide range of anatomic sites) with the production of hyphae by *C. albicans* under a broad range of in vitro hypha-induction conditions. In addition a further aim of this study was to compare the virulence of the two species in two additional infection models, namely the oral Reconstituted Human Epithelial (RHE) model of superficial infection (Rupniak et al., 1985; Korting et al., 1998; Schaller et al., 1998a,b) and the oral-intragastric infant mouse model which is a model for colonization of and dissemination from the gastrointestinal tract (Cole et al., 1990, 1993, 1996).

2. Materials and methods

2.1. Candida clinical isolates, strains and derivatives

All *Candida* clinical isolates, strains and derivatives were routinely cultured on Potato Dextrose Agar (PDA) medium (Oxoid, Basingstoke, Hampshire, UK) pH 5.6, at 37 °C for 18 h (Table 1). For routine liquid culture, isolates were grown in Yeast-Extract-Peptone-Dextrose (YPD) broth (10 g yeast extract (Oxoid), 20 g peptone (Difco, Becton Dickinson, Franklin Lakes, NJ, USA), 20 g glucose per liter, pH 5.5) at 37 °C in a Gallenkamp (Model G25) orbital incubator (New Brunswick Scientific Company Incorporated, Edison, NJ, USA) at 200 rpm.

2.2. Chemicals, enzymes and radioisotopes

All chemicals used were of analytical-grade or molecular biology-grade and were purchased from the Sigma–Aldrich Chemical Co. (Tallaght, Dublin, Ireland) or from Roche Diagnostics Ltd. (Lewes, East Sussex, UK). Restriction enzymes were purchased from the Promega Corporation (Madison, WI, USA) and from New England Biolabs (Beverley, MA, USA) and used according to the manufacturer's instructions. [α-^{32}P]dATP (6000 Ci/mmol; 222 TBq/mmol) was purchased from Amersham International Plc. (Little Chalfont, Buckinghamshire, UK).

2.3. Induction of germ tubes and hyphae

Production of hyphae by *Candida* isolates was induced by inoculation of cells grown for 18 h at 37 °C in YPD into media that promote the yeast-hypha transition. The media used included Medium-199, with and without 10% (v/v) newborn calf serum, RPMI-1640 medium, with and without 10% (v/v) newborn calf serum, YNB medium, with and without 10% (v/v) newborn calf serum, water with 10% (v/v) newborn calf serum, Lee's medium with pH/temperature shift (Buffo et al., 1984) and *N*-acetylglucosamine-yeast-nitrogen base-proline medium (NYP) medium (Schaude et al., 1990). Cells were inoculated to a cell density of 2×10^6 cfu/ml in 20 ml of each medium and incubated at 37 °C. In the case of NYP medium the cells were incubated in the presence of 5% (v/v) CO_2. The percentage of cells that produced germ tubes or hyphae was determined by counting one hundred cells every hour from $t = 0$ to 6 h and then again at $t = 24$ h by microscopic

Table 1
Candida isolates used in this study

Species	Strain	Genotype[a]	Body site	Reference
C. albicans	SC5314	N/A	Systemic	Gillum et al. (1984)
	132A	N/A	Oral	Gallagher et al. (1992)
	52.1	N/A	Oral	Pinjon et al. (1998)
	JP14CA	N/A	Oral	Pinjon et al. (1998)
C. dubliniensis	CD36	Genotype 1	Oral	Sullivan et al. (1995)
	CD57	Genotype 2	Vagina	Moran et al. (1997)
	CD514	Genotype 3	Oral	Gee et al. (2002)
	CD519	Genotype 3	Oral	Gee et al. (2002)
	CD539	Genotype 2	Oral	Pinjon et al. (1998)
	CD541	Genotype 2	Blood	Pinjon et al. (1998)
	CD96.34	Genotype 1	Oral	Pinjon et al. (1998)
	CS1	Genotype 3	Oral	This study
	H004	Genotype 2	Oral	Gee et al. (2002)
	P6265	Genotype 3	Sputum	Polacheck et al. (2000)
	P7718	Genotype 4	Wound	Gee et al. (2002)

[a] *Candida dubliniensis* genotype.