Microbiological Research **160** (2005) 361—366

Available online at www.sciencedirect.com

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Microbiological Research

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Subtypes of genotype A *Candida albicans* isolates determined by restriction endonuclease and sequence analyses

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Accepted 28 February 2005

KEYWORDS
Candida albicans;
Candida dubliniensis;
25s intron analysis;
Fungal pathogen;
Mycosis

Summary

Systemic yeast infections are the leading cause of mortality and morbidity in immunocompromized patients. *Candida albicans*, being the most frequently isolated fungal pathogen in these patients, can be divided into three genotypes (genotypes A, B and C) by 25S intron analysis. In our study, we found that molecular sizes of genotype A *C. albicans* isolates were heterogeneous. In order to determine the molecular basis of this difference, *HaeIII* digestion was applied, and strains forming different band patterns were analyzed by automated sequence analysis. As a result of sequence analysis, eight different subtypes (a→h) were found among genotype A *C. albicans* strains and an easy differentiation scheme consisting of *HaeIII* and *MspI* digestions was constructed.

Introduction

The increasing incidence of acquired immunodeficiency syndrome (AIDS) and recent development of new and more aggressive treatment procedures for patients with malignancies and organ transplants have resulted in an increase in the number of immunocompromized patients (Tamura et al., 2001). Systemic yeast infections are the major cause of morbidity and mortality in these patients. Although the number of fungi isolated from these infections is increasing, *Candida* species are still the most frequently isolated pathogens. During the past 15 years, there has been an increase in the number of infections caused by non-*albicans* *Candida* species, but the leading agent is still *Candida albicans* (McCullough et al., 1999; Tamura et al., 2001; Vrioni-Bernard, 2001). Rapid identification of the etiological agent is important for the outcome of these patients, and molecular methods

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doi:10.1016/j.micres.2005.02.010

are increasingly used to shorten identification time and to determine species specific characteristics (Khan and Mustafa, 2001; De Baere et al., 2002).

Molecular typing of the infectious agent is important for epidemiological studies and for development of appropriate infection control strategies (McCullough et al., 1999; Pfaller, 1995; Soll, 2000). Owing to the importance of *C. albicans* and the need for better understanding of its epidemiology, new molecular techniques have been developed to provide strain characteristics (Bretagne et al., 1997; Soll, 2000). These techniques include genomic sequencing, multilocus enzyme electrophoresis, restriction enzyme digestion, pulsed field gel electrophoresis and randomly amplified polymorphic DNA (RAPD) analysis (Soll, 2000; Vrioni-Bernard, 2001). The method preferred to be used must distinguish between identical, related, and unrelated strains. It must be easy to perform, and be able to differentiate appropriate number of strains. It also must give clear and reliable results in large number of isolates (Dalle et al., 2000; Warnock, 1984). Although an ideal epidemiological typing technique applicable to a wide range of fungal pathogens is not yet available, several molecular-typing methods promise rapid, simple, and sensitive discrimination of specific strains (Pfaller, 1995; Soll, 2000).

Using ribosomal sequences for genetic typing meets most of the mentioned criteria and is being used for typing of many fungal pathogens (Maleska and Clark-Walker, 1993; Tamura et al., 2001). The method developed by McCullough et al. (1999) uses a pair of PCR primers designed to flank the region that includes the transposable intron of the 25S rRNA gene (rDNA) and can be used to classify *C. albicans* strains into three genotypes on the basis of the amplified PCR product length: Genotype A (~450 bp product), genotype B (~840 bp product), and genotype C (~450 and ~840 bp products). Studies using this method have confirmed that the two other observed genotypes; genotypes D (~1080 bp product) and E (~1400 bp product), belong to the same taxon as *Candida dubliniensis* (McCullough et al., 1999; Tamura et al., 2001).

Using the method of McCullough et al. (1999), we investigated the genotypic distribution of *C. albicans* isolates obtained from patients hospitalized in three different care units in Turkey. We found that genotype A isolates had slightly different molecular sizes. On the basis of this finding we further investigated these different isolates by restriction endonuclease and sequence analyses and found eight subtypes in genotype A *C. albicans* strains. From the sequence data, we constructed a differentiation scheme consisting of *HaeIII* and *MspI* digestions to easily differentiate these subtypes.

Materials and methods

Candida albicans strains

A total of 144 genotype A *C. albicans* isolates were included in this study. The isolates were obtained from various colonization and infection sites of different patients hospitalized in various departments (surgery, adult and pediatric hematology and oncology, intensive care, burn and newborn units) of three different hospitals (Ankara University Medical Faculty Hospitals [AUH], Ankara Numune Education and Investigation Hospital [ANH], and Istanbul University Medical Faculty Hospital Department of Foundation of Children with Leukemia [IUH]) from two cities in Turkey. These isolates were collected in a 5 year period. All patients had predisposing factors such as solid tumors, hematological malignancies, diabetes mellitus, burns, and immaturity/prematurity. Distribution of these isolates according to their sites of recovery is shown in Table 1. *C. albicans* strains were identified on the

Table 1. Distribution of subtypes of genotype A *C. albicans* isolates according to the sites of recovery

Site of recovery	Subtypes (*n* (%))								Total
	a	b	C	d	e	f	g	h	
Blood and body fluids	12 (33.3)	3 (8.3)	11 (30.6)	9 (25)	—	—	—	1 (2.8)	36
Sputum	12 (42.8)	3 (10.7)	11 (39.3)	—	1 (3.6)	1 (3.6)	—	—	28
Throat-nose and ear	8 (53.3)	5 (33.3)	—	—	2 (13.3)	—	—	—	15
Wound	10 (71.4)	1 (7.1)	1 (7.1)	—	—	2 (14.3)	—	—	14
Urine	5 (55.6)	1 (11.1)	1 (11.1)	—	—	—	2 (22.2)	—	9
Feces	8 (57.1)	4 (28.6)	—	—	1 (7.1)	1 (7.1)	—	—	14
Vagina	20 (71.4)	4 (14.3)	4 (14.3)	—	—	—	—	—	28
Total %	75 (52.1)	21 (14.6)	28 (19.4)	9 (6.2)	4 (2.8)	4 (2.8)	2 (1.4)	1 (0.7)	144