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Diagnostic Microbiology and Infectious Disease 58 (2007) 427–433

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE

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Multilocus sequence typing of *Candida tropicalis* shows clonal cluster enriched in isolates with resistance or trailing growth of fluconazole

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Received 24 November 2006; accepted 20 March 2007

Abstract

Multilocus sequence typing (MLST) was used to characterize the genetic profiles of 52 *Candida tropicalis* isolates collected from 10 hospitals in Taiwan. A total of 33 diploid sequence types (DSTs) were differentiated among the 52 isolates and all were novel to the Internet *C. tropicalis* DST database (http://pubmlst.org/ctropicalis/). Eleven (33.3%) of the 33 DSTs could be assigned to 3 major clonal clusters (1–3) based on eBURST calculation. Only clonal cluster 1 co-clustered isolates from UK and the United States. Clonal cluster 2 was enriched with isolates with resistance or trailing growth of fluconazole (14/20, 70%). Furthermore, there are a number of pulsed-field gel electrophoresis subtypes associated with the isolates in this cluster, demonstrating that possibly more than one clone with resistant or trailing property have emerged and spread in Taiwan in 1999.

Keywords: Molecular typing; *Candida tropicalis*; MLST; PFGE

1. Introduction

Invasive *Candida* infections continue to cause major problems of morbidity and mortality in a diverse range of debilitated and immunocompromised hosts (Tortorano et al., 2003; Hajjeh et al., 2004; Wisplinghoff et al., 2004; Kao et al., 1999; Gudlaugsson et al., 2003). The infections caused by *Candida* spp. result in increased length of hospital stay and medical costs that constitute an important public health problem (Sheng et al., 2005; Rentz et al., 1998). In Taiwan, *Candida tropicalis* ranked the second most frequently isolated *Candida* species and was the species most frequently resistant to fluconazole (Yang et al., 2003; Yang et al., 2004). To gain an insight into the reasons for the high prevalence of *C. tropicalis* as well as to establish a surveillance network, understanding of the molecular epidemiology of fungal infections is needed.

Pulsed-field gel electrophoresis (PFGE) is a well-established subtyping method for outbreak investigation and for the study of the epidemiology of *C. tropicalis* (Jang et al., 2005; Rho et al., 2004; Roilides et al., 2003). Recently, multilocus sequence typing (MLST) has been developed to meet the increasing need for global surveillance and comparison of genotypes in a central database via the Internet (Chen et al., 2006; Tavanti et al., 2005). MLST is based on the sequencing of selected housekeeping genes and the identification of polymorphic nucleotide sites. Combination of the alleles at the different loci results in unique diploid sequence types (DSTs) that can be used to discriminate strains. MLST for typing of pathogenic fungal species has been developed, including *Candida albicans* (Bougnoux et al., 2002; Bougnoux et al., 2003) and *Candida glabrata* (Dodgson et al., 2003). A set of 6 genes encoding housekeeping functions comprising the fragments *ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a* is recommended for MLST with *C. tropicalis* (http://pubmlst.org/ctropicalis/) (Tavanti et al., 2005).

C. tropicalis isolates with “trailing growth” phenotype exhibit reduced but persistent growth at drug concentrations above the MIC in broth dilution tests with azole antifungal agents, such as fluconazole. Isolates show trailing with an MIC at 48 h approximately 4-fold higher than that at 24 h. Thus, the trailing growth can make an isolate that appears susceptible (MIC <64 mg/L) at 24 h of incubation to

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0732-8893/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.diagmicrobio.2007.03.014

become resistant (MIC ≥64 mg/L) at 48 h (Arthington-Skaggs et al., 2002). In this article, we use MLST to assess the clonality of *C. tropicalis* and to ascertain whether different characteristics such as fluconazole susceptibility, hospital origin, and anatomic source can be attributed to certain specific MLST DST types in Taiwan.

2. Materials and methods

2.1. Fungal strains

A total of 52 *C. tropicalis* clinical isolates collected from 10 hospitals in different geographic origins in Taiwan were used in this study. The fluconazole MIC, geographic origin,

Table 1
Details of *C. tropicalis* isolates tested by MLST and PFGE

Strain no.	Hospital	Source	MIC—48 h	Collection date	DST	MLST clonal cluster	PFGE types
YM020273	E2	Sputum	4	2002/7/31	140	2-2	
YM020274	E2	Sputum	0.3	2002/7/31	153	1-2	III-1
YM020287	M1	Urine	0.3	2002/7/3	154		
YM020291	M1	Sputum	0.3	2002/7/6	155	1-4	III-5
YM020294	M1	Urine	16	2002/7/11	144	2-1	II-1
YM020309	M1	Urine	4	2002/8/3	140	2-2	
YM020311	M1	Urine	16	2002/8/5	90	1-3	
YM020649	S1	others	8	2002/8/19	156		I-4
YM020659	S1	Sputum	0.1	2002/8/25	157		V-2
YM020671	S1	Blood	8	2002/7/17	158		IV-2
YM020693	S1	Blood	0.5	2002/8/26	90	1-3	III-4
YM020709	S2	Sputum	8	2002/8/6	159		
YM020715	S2	Urine	0.3	2002/8/23	160		V-1
YM020725	S2	Urine	8	2002/9/3	161		II-12
YM020743	S2	Blood	0.1	2002/8/9	134	1-5	III-2
YM020919	S3	Sputum	1	2002/7/16	140	2-2	II-14
YM020948	S3	Blood	1	2002/9/2	162		I-5
Ym990131	E2	Urine	64	1999/5/10	98	2-4	II-7
Ym990135	E2	Sputum	64	1999/5/14	98	2-4	II-3
Ym990136	E2	Blood	64	1999/5/15	133		IV-1
Ym990138	E2	Sputum	0.5	1999/5/18	134	1-5	II-9
Ym990144	E2	Urine	64	1999/5/20	135		
Ym990145	E2	Sputum	64	1999/5/21	136		II-5
Ym990148	E2	Urine	0.5	1999/5/24	137	2-3	
Ym990236	N3	Urine	64	1999/5/11	138	3-1	VI-2
Ym990268	S2	Urine	0.5	1999/5/15	139	3-2	I-2
Ym990275	S2	Urine	64	1999/5/24	140	2-2	II-1
Ym990323	M1	Sputum	64	1999/4/26	141		
Ym990329	M1	Urine	1	1999/4/7	142		IV-3
Ym990444	N1	Blood	2	1999/5/1	143		II-10
Ym990458	N1	Blood	64	1999/5/10	144	2-1	II-8
Ym990488	S1	Urine	0.3	1999/5/12	163		I-3
Ym990490	S1	Urine	64	1999/5/13	140	2-2	II-6
Ym990533	E1	Sputum	0.5	1999/5/17	145	1-1	I-1
Ym990537	E1	Urine	64	1999/6/5	140	2-2	II-3
Ym990553	N4	Urine	1	1999/4/21	146		
Ym990554	N4	Urine	64	1999/4/23	147		II-13
Ym990574	N4	Blood	2	1999/5/12	148		V-3
Ym990577	N4	Sputum	64	1999/5/21	140	2-2	II-4
Ym990579	N4	Urine	64	1999/6/1	149		VI-1
Ym990592	S3	Blood	64	1999/5/5	140	2-2	II-1
Ym990593	S3	Wound	0.5	1999/5/7	150		
Ym990598	S3	Sputum	64	1999/5/19	90	1-3	III-3
Ym990599	S3	Urine	2	1999/5/20	151		V-1
Ym990603	S3	Sputum	64	1999/6/8	140	2-2	II-2
Ym990645	N2	Urine	64	1999/4/2	140	2-2	II-11
Ym990647	N2	Urine	0.1	1999/4/8	140	2-2	II-11
Ym990649	N2	Urine	64	1999/4/13	140	2-2	II-11
Ym990652	N2	Urine	1	1999/4/23	152		VI-3
Ym990659	N2	Blood	64	1999/5/17	140	2-2	II-11
Ym990660	N2	Urine	64	1999/5/20	140	2-2	II-11
Ym990662	N2	Urine	64	1999/5/31	140	2-2	II-11

N, north; S, south; M, middle; E, east.