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Evaluation of a Method for Identification of *Candida dubliniensis* Bloodstream Isolates

Banu Sancak,[1]* John H. Rex,[2] Victor Paetznick,[2] Enuo Chen,[2] and Jose Rodriguez[2]

Department of Clinical Microbiology and Microbiology, Hacettepe University Medical School, Ankara 06100, Turkey,[1] and Division of Infectious Diseases, Department of Internal Medicine, Center for the Study of Emerging and Reemerging Pathogens, University of Texas Medical School, Houston, Texas 77030[2]

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To evaluate methods for differentiating *Candida albicans* and *Candida dubliniensis*, 772 putative *C. albicans* bloodstream isolates were tested for growth at 37 and 42°C. Isolates showing no growth at 42°C, abundant chlamydospore production, and the sugar assimilation pattern of the type strain were confirmed by DNA-based procedures to be *C. dubliniensis*.

Candida dubliniensis shares many of the phenotypic characteristics of *Candida albicans*, such as production of chlamydospores and germ tubes. *C. dubliniensis* also characteristically fails to assimilate xylose (XYL) or α-methyl-D-glucoside (MDG) (9; I. F. Salkin, W. R. Pruitt, A. A. Padhye, D. Sullivan, D. Coleman, and D. H. Pincus, Letter, J. Clin. Microbiol. **36:**1467, 1998.). However, these characteristics are not definitive, and the similarity between the two species causes problems (7, 12, 14). A number of supplemental phenotypic approaches for the separation of the two species have recently been described; these include determining relative growth at 42 and 45°C (2, 10, 12, 15, 18), β-glucosidase activity (12, 15), and colony color on CHROMagar (2, 12). In an effort to develop a routine approach to identifying this species, we studied the utility of identification based on selected phenotypic features (ability to grow at 42°C, ability to produce chlamydospores on cornmeal-Tween 80 agar, and sugar assimilation reactions as measured by the API 20 AUX system. We have confirmed the validity of this approach by PCR and restriction enzyme analysis of genomic DNA (REAG).

During screening of >2,000 sequential bloodstream isolates collected from multiple centers in the United States as part of a multicenter study between 1995 and 1999, 772 isolates were tentatively identified as *C. albicans* at the referring center by different methods and were subsequently confirmed as *C. albicans* by the *C. albicans* CA50 kit (Murex Diagnostics, Inc., Norcross, Ga.) in our laboratory. In accordance with the National Committee for Clinical Laboratory Standards M27-A inoculum preparation procedure (6), isolates were prepared at a density of 1×10^3 to 5×10^3 cells/ml. For growth studies 100 μl of fungal inoculum was combined with 100 μl of double-strength RPMI 1640 in the wells of a 96-well round-bottom microdilution tray. Duplicate wells were used for each isolate, and identical trays for each target temperature were prepared. Growth was monitored visually after 24 and 48 h of incubation. Isolates with reduced growth relative to that of a known *C. albicans* isolate (approximately 50% less turbidity, similar to the MIC-2 end point used for antifungal susceptibility testing [6]) or no growth after 24 h at a temperature higher than 37°C were considered suspicious and were examined by determining assimilation profiles with the API 20C AUX system (bioMerieux Vitek, Inc., Hazelwood, Mo.) and chlamydospore production on cornmeal-Tween 80 agar.

PCR and REAG were used to confirm the identification of putative *C. dubliniensis* isolates. DNA was extracted by an adaptation of the methods of Scherer and Stevens (11). For restriction enzyme analysis of genomic DNA, DNA was digested with *Hin*fI (Invitrogen, Carlsbad, Calif.) according to the manufacturer's instructions and then separated by electrophoresis. PCR was performed with the previously described *C. dubliniensis*-specific ACT-1-associated primer pairs DUBF and DUBR, which yield an amplimer of 288 bp (4).

C. dubliniensis type strain CD36 (courtesy of Derek Sullivan; lodged with the British National Collection of Pathogenic Fungi under accession no. NCPF 3949) and *C. albicans* ATCC 90028 (American Type Culture Collection) were used as positive and negative controls, respectively.

Of 772 candidate isolates, 149 showed reduced growth or no growth at 42°C (preliminary testing failed to identify any isolates that grew at 45°C). By API 20C AUX, 22 of the 149 isolates were identified as *C. dubliniensis*, 113 were identified as *C. albicans*, 13 were identified as *Candida parapsilosis*, and 1 was identified as *Candida guilliermondii*. Of the 113 *C. albicans* isolates, 11 showed no growth at 42°C but were capable of using both XYL and MDG. However, 9 of the 113 isolates identified as *C. albicans* failed to utilize XYL, and 1 of these 9 isolates also failed to assimilate MDG in the API 20C AUX system. There were thus 31 isolates with one or more sugar assimilation characteristics suggestive of *C. dubliniensis* (Table 1).

By PCR (Fig. 1) and restriction enzyme analysis, 20 of these 31 isolates (isolates 2 to 21 in Table 1) were definitively identified as *C. dubliniensis*. All 20 of these isolates failed to assimilate XYL and MDG. In addition, 19 of these 20 isolates demonstrated abundant chlamydospores frequently arranged in triplets or in contiguous pairs on short branching pseudomycelia. The remaining isolate produced single and very rare chlamydospores. The two isolates that were identified by API 20C AUX as *C. dubliniensis* but that lacked the DNA signature

* Corresponding author. Mailing address: Hacettepe University Medical School, Ankara 06100, Turkey. Phone: 90 312 3051562. Fax: 90 312 3115250. E-mail: banusancak@yahoo.com.

TABLE 1. Analysis of isolates with a sugar assimilation pattern potentially consistent with *C. dubliniensis*[a]

Isolate(s)[c]	Growth[b] at 42°C at:		API 20C AUX identification	Assimilation of:		Chlamydospore formation	Identification as *C. dubliniensis* by:	
	24 h	48 h		XYL	MDG		REAG	PCR
1	LG	G	*C. dubliniensis*	−	−	None	−	−
2–20	NG	NG	*C. dubliniensis*	−	−	Abundant	+	+
21	NG	NG	*C. dubliniensis*	−	−	Few	+	+
22	NG	G	*C. dubliniensis*	−	−	None	−	−
23	LG	G	*C. albicans*	−	−	Few	−	−
24–25	NG	LG	*C. albicans*	−	+	Few	−	−
26	LG	G	*C. albicans*	−	+	Few	−	−
27–31	LG	LG	*C. albicans*	−	+	Few	−	−
32	NG	NG	*C. dubliniensis*	−	−	Abundant	+	+
33	G	G	*C. albicans*	+	+	Few	−	−

[a] Isolates 1 to 22 were identified as *C. dubliniensis* with API 20 AUX, whereas isolates 23 to 31 failed to assimilate XYL.
[b] G, good growth (isolates showing at least 50% of the growth of the *C. albicans* control isolate); LG, light growth (isolates showing less than 50% of the growth of the *C. albicans* control isolate); NG, no growth.
[c] Isolates 32 and 33 were *C. dubliniensis* type strain CD36 and *C. albicans* reference strain ATCC 90028, respectively.

of that species on the basis of PCR and restriction enzyme analysis failed to produce chlamydospores and were thus identified as *C. albicans*. The other *C. albicans* isolates formed longer filaments and produced fewer chlamydospores, with usually only one chlamydospore at the end of the pseudohyphae. None of the isolates identified as *C. albicans* with API 20C AUX produced abundant chlamydospores.

Of note, all 20 isolates that were identified as *C. dubliniensis* by molecular methods failed to show any evidence of growth at 42°C. The complete absence of growth at 42°C was seen with all isolates of *C. dubliniensis* and two *C. albicans* strains at 24 h, but this behavior was unique to *C. dubliniensis* after 48 h.

Our results show that the combination of an API 20C AUX identification as *C. dubliniensis* and the complete absence of growth at 42°C, particularly after 48 h of incubation, is strongly suggestive of *C. dubliniensis*. Production of chlamydospores by *C. dubliniensis* is very different from that by *C. albicans* (13, 14, 16). In our study, 19 of the 20 isolates which were confirmed as *C. dubliniensis* by PCR and REAG showed abundant chlamydospores on cornmeal-Tween 80 agar. We observed that the isolate that produced rare chlamydospores had smaller blastospores than other strains.

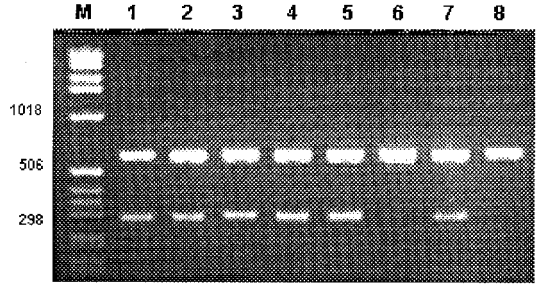

FIG. 1. Amplification of ACT1-associated 288-bp fragment by PCR. Lane M, molecular weight marker (1-kb DNA ladder); lane 2, *C. dubliniensis* type strain CD36; lane 8, *C. albicans* reference strain ATCC 90028; lanes 1, 3 to 5, and 7, *C. dubliniensis* isolates (Table 1, isolates 10, 12, 11, 19, and 20, respectively); lane 6, *C. albicans* isolate (Table 1, isolate 22).

Placing the results in overall context, *C. dubliniensis* was identified in 20 of 758 (2.6%) bloodstream isolates that might normally have been identified as *C. albicans*. To understand the clinical significance and epidemiological role of *C. dubliniensis*, it is very important to identify it correctly in clinical specimens. It has been suggested that the most reliable tests currently used for discriminating *C. albicans* and *C. dubliniensis* are based on molecular techniques (1, 3, 4, 5, 8, 16, 17). As these techniques require molecular biology equipment that may not be available in many mycology laboratories, our data suggest that a temperature study can be used as a screening test. For isolates showing no growth after 48 h at 42°C, carbohydrate assimilation profiles with the API 20C AUX yeast identification system and chlamydospore production on cornmeal agar should be investigated. Isolates that fit the entire profile are highly likely to be *C. dubliniensis* rather than *C. albicans*.

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