

Differentiation of *Candida dubliniensis* from *Candida albicans* on Staib Agar and Caffeic Acid-Ferric Citrate Agar

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The methods currently available for the identification of the pathogenic yeast *Candida dubliniensis* all have disadvantages in that they are time-consuming, expensive, and/or, in some cases, unreliable. In a recent study (P. Staib and J. Morschhäuser, *Mycoses* 42:521–524; 1999) of 14 *C. dubliniensis* and 11 *C. albicans* isolates, it was suggested that the ability of *C. dubliniensis* to produce rough colonies and chlamydoconidia on Staib agar (SA) provided a simple means of differentiating it from its close relative *C. albicans*. In the present investigation, we examined the colony morphology and chlamydoconidia production of 130 *C. dubliniensis* and 166 *C. albicans* isolates on SA and on the related defined medium caffeic acid-ferric citrate agar (CAF). All of the *C. dubliniensis* and *C. albicans* isolates produced chlamydoconidia on the control medium, i.e., rice-agar-Tween agar. However, while none of the *C. albicans* isolates produced chlamydoconidia on either SA or CAF, 85.4 and 83.8% of the *C. dubliniensis* isolates produced chlamydoconidia on SA and CAF, respectively. All of the *C. albicans* isolates grew as smooth, shiny colonies on SA after 48 to 72 h of incubation at 30°C, while 97.7% of the *C. dubliniensis* isolates grew as rough colonies, many (65%) with a hyphal fringe. In contrast, 87.4% of the *C. albicans* and 93.8% of the *C. dubliniensis* isolates yielded rough colonies on CAF. Although the results of this study confirm that SA is a good medium for distinguishing between *C. dubliniensis* and *C. albicans*, we believe that discrimination between these two species is best achieved on the basis of colony morphology rather than chlamydoconidia production.

Due to the increasing incidence of fungal infections and the recent emergence of novel opportunistic fungal pathogens, there is a growing need for the development of simple, rapid, and accurate identification methods for potential fungal pathogens recovered in the clinical microbiology laboratory (18). This is particularly true of the newly described yeast species *Candida dubliniensis*. Although first associated with oral candidiasis in human immunodeficiency virus (HIV)-infected patients (25), it has more recently been recognized as a cause of superficial and systemic disease in HIV-negative individuals (3, 11, 16, 17, 24, 26). The close genotypic relationship between *C. dubliniensis* and *C. albicans* results in their sharing a broad range of phenotypic characteristics, which hampers the accurate and rapid differentiation of the two species (23). Although the majority of *C. dubliniensis* isolates are susceptible to currently used antifungal drugs, it has been shown that isolates of this species, unlike *C. albicans*, can rapidly develop stable resistance to fluconazole upon exposure in vitro (12, 13). This ability, the emergence of *C. dubliniensis* worldwide, its growing importance as a cause of systemic disease, and the introduction of novel antifungal agents all indicate that a thorough investigation of the incidence and epidemiology of *C. dubliniensis* is required. In order to be able to achieve this, simple and reliable tests for differentiating *C. dubliniensis* from *C. albicans*

need to be developed. The “gold standard” methods for the identification of *C. albicans* are based on its ability to produce germ tubes and chlamydoconidia on appropriate nutrient media. However, since *C. dubliniensis* also produces these structures, many isolates of *C. dubliniensis* have been misidentified as *C. albicans* (4, 14, 15). Therefore, in order to perform urgently required epidemiological studies of *C. dubliniensis* infections, there is a need to develop inexpensive, accurate, and easy to perform tests that will allow the differentiation of isolates of the two species which have been recovered from clinical samples. In this regard, a variety of procedures have been developed and assessed in laboratories around the world, including, among others, colony color on CHROMagar Candida medium (19), lack of growth at 45°C (16), immunofluorescence (1), carbohydrate assimilation profiles (15), β -glucosidase activity (2), coaggregation with *Fusobacterium nucleatum* (8), and PCR tests (5). Some of these tests (e.g., PCR) are very reliable but are not yet used routinely by many clinical microbiology laboratories, while others rely on reagents which are not widely available (e.g., immunofluorescence with anti-*C. dubliniensis* antibodies) and yet others (e.g., colony color on CHROMagar Candida medium) are unreliable. In the original description of *C. dubliniensis*, it was noted that this species produces much higher numbers of chlamydoconidia than *C. albicans* when grown on rice-agar-Tween agar (RAT; 25) but subsequent studies have shown that this trait does not provide a definitive means of differentiating between the two species (9). In a recent study of 14 *C. dubliniensis* and 11 *C. albicans* strains, Staib and Morschhäuser (21) reported that colony morphology and chlamydoconidia production by

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TABLE 1. *C. dubliniensis* and *C. albicans* isolates used in this study

Yeast species and country of isolation	No. of isolates	Chlamyospore production (no. of isolates)						Source or reference
		SA		CAF		RAT		
		+	-	+	-	+	-	
<i>C. dubliniensis</i> ^a								
Argentina	1	1	0	1	0	1	0	24
Australia	1	0	1	0	1	1	0	25
Belgium	5	5	0	5	0	5	0	16
Canada	6	4	2	5	1	6	0	16
Finland	1	1	0	1	0	1	0	16
France	9	5	4	8	1	9	0	This study
Germany	4	4	0	3	1	4	0	16
Greece	1	1	0	1	0	1	0	16
India	1	1	0	1	0	1	0	This study
Israel	3	1	2	3	0	3	0	17
Ireland	41	36	5	36	5	41	0	16, 25, this study
Japan	1	1	0	1	0	1	0	This study
Malta	3	3	0	3	0	3	0	This study
Norway	5	4	1	0	5	5	0	This study
Spain	5	5	0	4	1	5	0	1
Switzerland	3	3	0	2	1	3	0	23
United Kingdom	18	16	2	16	2	18	0	16
United States	22	20	2	19	3	22	0	15, this study
Total	130	111	19	109	21	130	0	
<i>C. albicans</i> ^a								
Australia	1	0	1	0	1	1	0	16
Hong Kong	6	0	6	0	6	6	0	16
Ireland	61	0	61	0	61	61	0	16, this study
United Kingdom	2	0	2	0	2	2	0	16
United States	96	0	96	0	96	96	0	15
Total	166	0	166	0	166	166	0	

^a *C. dubliniensis* type strain CD36 (25) produced abundant chlamyospores on all three media, whereas *C. albicans* reference strains 132A (serotype B) (6) and 179A (serotype A) (6) only produced chlamyospores on RAT.

C. dubliniensis on Staib agar (SA; a medium originally developed for the identification of *Cryptococcus neoformans*) could form the basis of a simple and accurate test for distinguishing this species from *C. albicans*.

In the present study, we evaluated the usefulness of colony morphology and chlamyospore production on SA and on caffeic acid-ferric citrate agar (CAF; a defined medium also developed to aid in the identification of *C. neoformans*) as a means of differentiating *C. albicans* from *C. dubliniensis* using a large collection of *C. dubliniensis* isolates recovered from individuals in 18 different countries around the world.

MATERIALS AND METHODS

Yeast isolates. The yeast isolates and reference strains used in this investigation are listed in Table 1. A total of 296 isolates were studied, including 130 *C. dubliniensis* isolates and 166 *C. albicans* isolates. All isolates were from the culture collection of the Microbiology Research Laboratory, Department of Oral Medicine and Oral Pathology, School of Dental Science, Trinity College, University of Dublin, Dublin, Republic of Ireland. Each isolate was originally recovered from one or more specimens from a separate individual, and its identity was confirmed using the API ID 32C (bioMérieux, Marcy l'Étoile, France) yeast identification system, growth at 45°C, and PCR analysis with *C. dubliniensis*-specific primers (5, 15, 16).

Chemicals, enzymes, and oligonucleotides. Analar-grade or molecular biology grade chemicals were purchased from Sigma-Aldrich or BDH (Poole, Dorset, United Kingdom). Enzymes were purchased from Roche Diagnostics Ltd. (Lewes, East Sussex, United Kingdom) or the Promega Corporation (Madison, Wis.) and used in accordance with the manufacturer's instructions. Custom-

synthesized oligonucleotides were purchased from Sigma-Genosys Biotechnologies (Pampisford, Cambridge, United Kingdom).

Culture media and growth conditions. Stock cultures of yeast isolates were maintained on plastic beads in Protect cryovials (Technical Service Consultants Ltd., Lancashire, United Kingdom) at -80°C. For each isolate, two or three plastic beads were removed from their respective cryovials using a sterile plastic loop, allowed to thaw, and then used to inoculate potato dextrose agar (PDA; Oxoid) medium. Forty-eight-hour-old PDA medium cultures grown at 37°C were used as the source of inocula for subsequent experiments with SA and CAF. SA (20) and CAF (7) were prepared fresh as described previously and used immediately. SA was prepared by first making an aqueous extract of *Guizotia abyssinica* seed (Power Seeds, Kildare, Republic of Ireland) by pulverizing 50 g of seed in a Moulinex B57 domestic blender for 2.5 min and then adding the ground seeds to 1 liter of distilled water, followed by boiling for 30 min. The seed extract was cooled and filtered, and the following ingredients were added: glucose, 1 g; KH₂PO₄, 1 g; creatinine, 1 g. The pH was adjusted to 5.5, the volume was readjusted to 1 liter, and 15 g of agar (Difco) was then added before autoclaving. The composition of CAF (per liter) was as follows: NH₄SO₄, 5 g; glucose, 5 g; yeast extract, 2 g; K₂HPO₄, 0.8 g; MgSO₄ · 7H₂O, 0.7 g; caffeic acid, 0.18 g; chloramphenicol, 0.05 g; ferric citrate, 0.002 g.

A 48-h-old single colony from a PDA medium plate culture of each isolate to be tested was separately streak inoculated with a sterile wire loop onto SA and CAF, respectively, contained in 90-mm-diameter petri dishes (25 ml of agar per plate) and incubated at 30°C for 72 to 120 h. Gross colony morphologic features were examined visually on both media at 24-h intervals, and the data were recorded. Yeast colonies were also evaluated microscopically to detect the presence or absence of chlamyospore formation following 48 to 120 h of incubation on SA and on CAF. For each isolate, 10 well-separated single colonies were chosen at random and stained by the addition of 1 drop of 1% (wt/vol) lactophenol cotton blue stain (10) to enhance the detection of chlamyospores. Lactophenol cotton blue preferentially stains chlamyospores more intensely than spore cells, pseudomycelium, and blastospores (blastoconidia; 25). Colonies were allowed to stain for 5 min and then covered with sterile glass coverslips and examined microscopically under bright-field illumination using a ×40 objective. Plates containing isolates that did not exhibit detectable chlamyospore formation within 120 h were re-examined following further incubation at intervals of 24 h for up to 3 weeks in total. All isolates were also tested for chlamyospore formation on RAT (bioMérieux) as described previously (25).

All of the isolates included in this study were examined on SA and CAF on at least two separate occasions with different batches of medium prepared from different lots of reagents.

RESULTS AND DISCUSSION

Growth of *Candida* isolates on SA and CAF. All 296 yeast isolates grew on both SA and CAF and yielded grey-white colonies. On SA, all of the 166 *C. albicans* isolates tested produced smooth, shiny colonies after 48 h of incubation (Fig. 1a). In all but three isolates, the colonies were found upon microscopic examination to be composed only of blastospores. Colonies of the three isolates consisted mainly of blastospores with a few pseudohyphal elements after 48 and 72 h of incubation. Similar findings were observed after 96 and 120 h of incubation.

In contrast, 127 (97.7%) of the 130 *C. dubliniensis* isolates tested on SA yielded rough colonies, the majority (84; 64.6%) of which also exhibited a hyphal halo or fringe around the colonies visible to the naked eye after 72 h of incubation (Fig. 1b). The rough colonies were composed of mycelial forms (predominantly pseudohyphae) and blastospores. The three remaining *C. dubliniensis* isolates (two from Norway and one from Canada) produced smooth, shiny colonies on SA that were similar in appearance and composition to those of *C. albicans* isolates, even after 2 weeks of incubation. The identity of these isolates as *C. dubliniensis* was reconfirmed using PCR with *C. dubliniensis*-specific primers, by carbohydrate assimilation profile analysis with the API ID 32C system, and by absence of

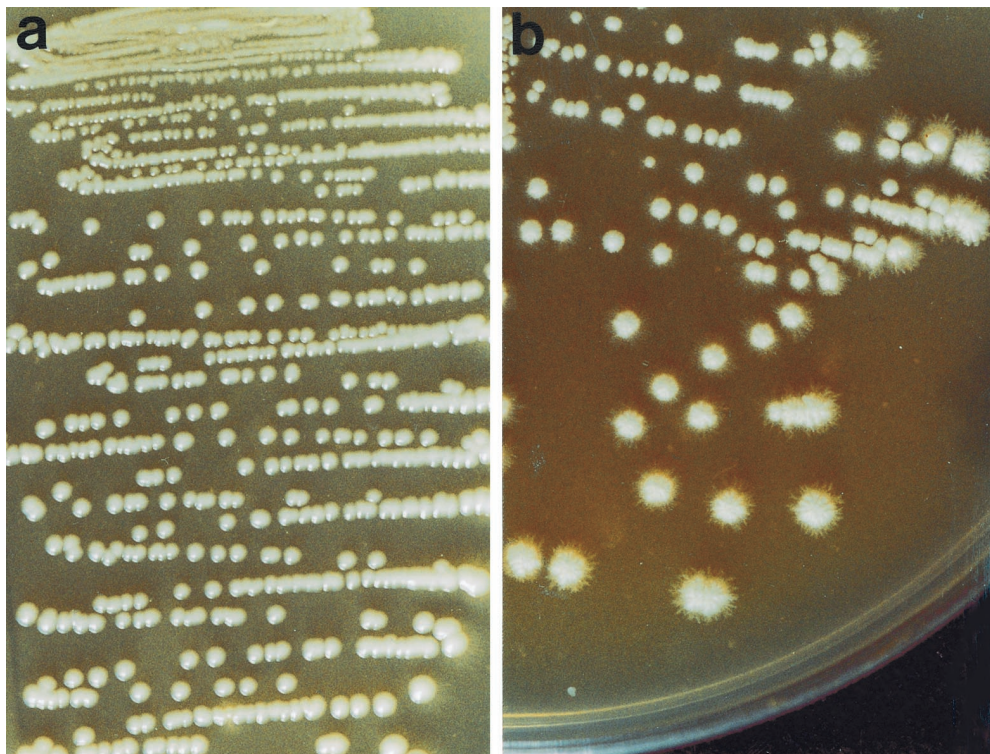


FIG. 1. Morphological appearance of *C. dubliniensis* and *C. albicans* colonies on SA following 72 h of incubation at 30°C. (a) Smooth, shiny colonies exhibited by *C. albicans* strain 132A (6). (b) Rough colonies exhibited by *C. dubliniensis* strain CD36 (25) displaying a hyphal fringe or halo.

growth at 45°C. Apart from these three isolates, the difference between the *C. dubliniensis* and *C. albicans* isolates was particularly evident on SA after 48 h of incubation, and became further enhanced after 72 h of incubation, in the area of heavy culture growth where the primary inoculum was streaked (i.e., the *C. dubliniensis* culture growth appeared rough and the *C. albicans* culture growth appeared smooth and shiny). These findings indicated that the different colony morphologies exhibited by isolates of *C. dubliniensis* and *C. albicans* were due predominantly to the production of mycelial forms by *C. dubliniensis* isolates. These results confirm the findings recently reported by Staib and Morschhäuser (21) that SA can be used as a useful means to discriminate between isolates of *C. dubliniensis* and *C. albicans*. However, the method is not absolute, as a small minority (3 [2.3%]) of the 130 *C. dubliniensis* isolates tested were indistinguishable from *C. albicans* based on colony morphology on SA.

Several isolates each of *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* were also tested on SA in order to determine whether they could be distinguished from isolates of *C. albicans* and *C. dubliniensis*. The five oral *C. glabrata* isolates tested yielded smooth, grey-white colonies similar to those of *C. albicans* on this medium. Furthermore, of the five *C. tropicalis* isolates tested, three yielded rough colonies similar to those of *C. dubliniensis* and two yielded smooth colonies similar to those of *C. albicans*. Of the six *C. parapsilosis* isolates tested, four yielded smooth, shiny colonies similar to those of *C. albicans* and two yielded rough colonies similar to those of *C. dubliniensis*. These findings indicated that it would not be possible to iden-

tify colonies of *C. albicans* and *C. dubliniensis* on SA based on colony morphology alone following primary isolation from a clinical specimen. Therefore, we propose that SA be used to assess clinical isolates which have been shown to be germ tube positive or to confirm the identity of *C. dubliniensis* isolates presumptively identified following primary isolation on CHROMagar Candida medium or by other means. The use of SA to differentiate *C. dubliniensis* and *C. albicans* isolates has a number of advantages over carbohydrate assimilation profile analysis. Firstly, the use of SA is considerably less expensive. Secondly, it is amenable to the analysis of large numbers of isolates. Finally, whereas the databases used with many of the commonly used commercial yeast identification systems (e.g., the bioMérieux API 20C AUX and ID 32C systems) have been updated in recent years to include *C. dubliniensis* profiles, they are far from comprehensive. In this regard, recent studies have highlighted the necessity to revise the databases to improve the accuracy of identification of *C. dubliniensis* (15).

SA was originally developed as a means of identifying colonies of *C. neoformans*, which, unlike other members of this genus, develops dark pigmentation on this medium. Strachan et al. (22) demonstrated that similar results could be obtained on a growth medium containing caffeic acid extracted from *G. abyssinia* seeds. Since SA was found to be excellent for distinguishing between *C. dubliniensis* and *C. albicans*, we investigated the usefulness of the more defined CAF to differentiate between isolates of these species. Of the 166 *C. albicans* isolates included in the study, 145 (87.4%) yielded rough colonies with a mycelial halo visible to the naked eye after 5 days

TABLE 2. Chlamyospore production by *C. dubliniensis* isolates^a on SA and CAF

Medium and chlamydo- spore production	No. of isolates
SA	
+	111 ^c
-	19 ^d
CAF	
+	109 ^e
-	21 ^f
SA + CAF ^b	
+	83
-	3

^a 130 isolates were studied.

^b Refers to isolates that were chlamyospore positive on both SA and CAF or chlamyospore negative on both media.

^c 28 isolates were negative on CAF.

^d 16 isolates were positive on CAF.

^e 26 isolates were negative on SA.

^f 18 isolates were positive on SA.

incubation. The remaining 21 (12.6%) isolates yielded smooth, nonshiny colonies on this medium. In comparison, 122 (93.8%) of the 130 *C. dubliniensis* isolates, including the 3 isolates which exhibited a smooth-colony phenotype on SA, yielded rough colonies with a mycelial halo after 5 days of incubation. The remaining eight (6.2%) isolates yielded rough colonies without a mycelial halo. Colonies of the *C. dubliniensis* isolates were noticeably smaller (~2 mm in diameter) than the *C. albicans* colonies (~3 mm) after 5 days of incubation on CAF. These findings indicated that, unlike that on SA, colony morphology on CAF could not be used to differentiate between *C. dubliniensis* and *C. albicans* isolates.

Chlamyospore production on SA and CAF. None of the 166 *C. albicans* isolates tested produced chlamyospores on SA or CAF, even after prolonged incubation periods of up to 3 weeks (Table 1). In contrast, all isolates produced chlamyospores within 48 to 72 h on RAT. Similarly, all 130 of the *C. dubliniensis* isolates formed chlamyospores on RAT but not all produced chlamyospores on either SA or CAF (Tables 1 and 2). A total of 111 (85.4%) of the 130 *C. dubliniensis* isolates formed chlamyospores on SA, and 100 (90.1%) of these 111 produced abundant chlamyospores within 72 h, whereas 11 (9.9%) of them produced relatively few chlamyospores, which were only detected following incubation periods of up to a week. Similarly, 109 (83.8%) of the 130 *C. dubliniensis* isolates produced chlamyospores on CAF, all but 5 within 120 h (Tables 1 and 2). Eighty-three (63.9%) of the *C. dubliniensis* isolates produced chlamyospores on both SA and CAF, whereas the remainder produced chlamyospores on one or the other medium only (Table 2).

On the basis of these data, we agree with Staib and Morschhäuser that growth on SA is an efficient means of discriminating between *C. dubliniensis* and *C. albicans*. However, our results suggest that colony morphology, rather than chlamyospore formation, is a more accurate criterion for species identification, since a significant proportion of *C. dubliniensis* isolates failed to produce chlamyospores on SA. The disparity between our data and that of Staib and Morschhäuser could be due to either different supplies of *G. abyssinica* seed, to seed of different ages, or to seed storage conditions. However, the

most likely explanation lies in the larger and more diverse group of *C. dubliniensis* isolates examined in the present study.

SA is inexpensive and readily available in many clinical mycology laboratories and provides a simple test for the accurate differentiation of *C. dubliniensis* from *C. albicans*. We propose that colony morphology on SA is a reliable and inexpensive phenotypic test for confirming the identification of *C. dubliniensis* and will be of benefit for researchers interested in studying the incidence and epidemiology of this emerging pathogen. We suggest that germ tube-positive oral isolates from HIV-positive and AIDS patients, as well as germ tube-positive isolates from sterile sites from other immunocompromised groups, should be tested on SA in association with other phenotypic tests for *C. dubliniensis*.

The molecular basis of the phenotypic differences observed between *C. dubliniensis* and *C. albicans* following growth on these media has yet to be established. However, comparative analysis of gene expression on these media could prove helpful in increasing our understanding of dimorphism in these species.

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REFERENCES

- Bikandi, J., R. San Millán, M. D. Moragues, G. Cebas, M. Clarke, D. C. Coleman, D. J. Sullivan, G. Quindós, and J. Pontón. 1998. Rapid identification of *Candida dubliniensis* by indirect immunofluorescence based on differential localization of antigens on *C. dubliniensis* blastospores and *Candida albicans* germ tubes. *J. Clin. Microbiol.* **36**:2428–2433.
- Boerlin, P., F. Boerlin-Petzold, C. Durussel, M. Addo, J.-L. Pagani, J.-P. Chave, and J. Bille. 1995. Cluster of atypical *Candida* isolates in a group of human immunodeficiency virus-positive drug users. *J. Clin. Microbiol.* **33**:1129–1135.
- Brandt, M. E., L. H. Harrison, M. Pass, A. N. Sofair, S. Huie, R.-K. Li, C. J. Morrison, D. W. Warnock, and R. H. Hajjeh. 2000. *Candida dubliniensis* fungemia: the first four cases in North America. *Emerg. Infect. Dis.* **6**:46–49.
- Coleman, D. C., D. J. Sullivan, D. E. Bennett, G. P. Moran, H. J. Barry, and D. B. Shanley. 1997. Candidiasis: the emergence of a novel species, *Candida dubliniensis*. *AIDS* **11**:557–567.
- Donnelly, S. A., D. J. Sullivan, D. B. Shanley, and D. C. Coleman. 1999. Phylogenetic analysis and rapid identification of *Candida dubliniensis* based on analysis of *ACT1* intron and exon sequences. *Microbiology* **145**:1871–1882.
- Gallagher, P. J., D. E. Bennett, M. C. Henman, R. J. Russell, S. R. Flint, D. B. Shanley, and D. C. Coleman. 1992. Reduced azole susceptibility of *Candida albicans* from HIV-positive patients and a derivative exhibiting colony morphology variation. *J. Gen. Microbiol.* **138**:1901–1911.
- Hopfer, R. L., and F. Blank. 1975. Caffeic acid-containing medium for identification of *Cryptococcus neoformans*. *J. Clin. Microbiol.* **2**:115–120.
- Jabra-Rizk, M. A., W. A. Falkler, W. G. Merz, J. I. Kelley, A. A. Baqui, and T. F. Meiller. 1999. Coaggregation of *Candida dubliniensis* with *Fusobacterium nucleatum*. *J. Clin. Microbiol.* **37**:1464–1468.
- Kirkpatrick, W. R., S. G. Revankar, R. K. McAtee, J. L. Lopez-Ribot, A. W. Fothergill, D. I. McCarthy, S. E. Sanche, R. A. Cantu, M. G. Rinaldi, and T. F. Patterson. 1998. Detection of *Candida dubliniensis* in oropharyngeal samples from human immunodeficiency virus-infected patients in North America by primary CHROMagar *Candida* screening and susceptibility testing of isolates. *J. Clin. Microbiol.* **36**:3007–3012.
- Larone, D. H. 1993. Staining methods, p. 186–192. In D. H. Larone (ed.), *Medically important fungi: a guide to identification*, 2nd edition. American Society for Microbiology, Washington, D. C.
- Meis, J. F., M. Ruhnke, B. E. De Pauw, F. C. Odds, W. Siegert, and P. E. Verweij. 1999. *Candida dubliniensis* candidemia in patients with chemotherapy-induced neutropenia and bone marrow transplantation. *Emerg. Infect. Dis.* **5**:150–153.
- Moran, G. P., D. Sanglard, S. M. Donnelly, D. B. Shanley, D. J. Sullivan, and D. C. Coleman. 1998. Identification and expression of multidrug transporters

- responsible for fluconazole resistance in *Candida dubliniensis*. Antimicrob. Agents Chemother. **42**:1819–1830.
13. Moran, G. P., D. J. Sullivan, M. C. Henman, C. E. McCreary, B. J. Harrington, D. B. Shanley, and D. C. Coleman. 1997. Antifungal drug susceptibilities of oral *Candida dubliniensis* isolates from human immunodeficiency virus (HIV)-infected and non-HIV-infected subjects and generation of stable fluconazole-resistant derivatives in vitro. Antimicrob. Agents Chemother. **41**:617–623.
 14. Odds, F. C., L. Van Nuffel, and G. Dams. 1998. Prevalence of *Candida dubliniensis* isolates in a yeast stock collection. J. Clin. Microbiol. **6**:2869–2873.
 15. Pincus, D. H., D. C. Coleman, W. R. Pruitt, A. A. Padhye, I. F. Salkin, M. Geimer, A. Bassel, D. J. Sullivan, M. Clarke, and V. Hearn. 1999. Rapid identification of *Candida dubliniensis* with commercial yeast identification systems. J. Clin. Microbiol. **37**:3533–3539.
 16. Pinjon, E., D. Sullivan, I. Salkin, D. Shanley, and D. Coleman. 1998. Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from *Candida albicans*. J. Clin. Microbiol. **36**:2093–2095.
 17. Polacheck, I., J. Strahilevitz, D. Sullivan, S. Donnelly, I. F. Salkin, and D. C. Coleman. 2000. Recovery of *Candida dubliniensis* from non-human immunodeficiency virus-infected patients in Israel. J. Clin. Microbiol. **38**:170–174.
 18. Reiss, E., K. Tanaka, G. Bruker, V. Chazalet, D. Coleman, J. P. Debeaupuis, R. Hanazawa, J. P. Latge, J. Lortholary, K. Makimura, C. J. Morrison, S. Y. Murayama, S. Naoe, S. Paris, J. Sarfati, K. Shibuya, D. Sullivan, K. Uchida, and H. Yamaguchi. 1998. Molecular diagnosis and epidemiology of fungal infections. Med. Mycol. **36**(Suppl. 1):249–257.
 19. Schoofs, A., F. C. Odds, R. Colebunders, M. Ieven, and H. Goosens. 1997. Use of specialised isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. Eur. J. Clin. Microbiol. Infect. Dis. **16**:296–300.
 20. Staib, F., M. Seibold, E. Antweiler, B. Frollich, S. Weber, and A. Blisse. 1987. The brown colour effect (BCE) of *Cryptococcus neoformans* in the diagnosis, control and epidemiology of *C. neoformans* infections in AIDS patients. Zentralbl. Bakteriol. Microbiol. Hyg. A **266**:167–177.
 21. Staib, P., and J. Morschhauser. 1999. Chlamydospore formation on Staib agar as a species-specific characteristic of *Candida dubliniensis*. Mycoses **42**:521–524.
 22. Strachan, A. A., R. J. Yu, and F. Blank. 1971. Pigment production of *Cryptococcus neoformans* grown with extracts of *Guizotia abyssinica*. Appl. Microbiol. **22**:478–479.
 23. Sullivan, D., and D. Coleman. 1998. *Candida dubliniensis*: characteristics and identification. J. Clin. Microbiol. **36**:329–334.
 24. Sullivan, D., K. Haynes, J. Bille, P. Boerlin, L. Rodero, S. Lloyd, M. Henman, and D. Coleman. 1997. Widespread geographic distribution of oral *Candida dubliniensis* strains in human immunodeficiency virus-infected individuals. J. Clin. Microbiol. **35**:960–964.
 25. Sullivan, D. J., T. J. Westerneng, K. A. Haynes, D. E. Bennett, and D. C. Coleman. 1995. *Candida dubliniensis* sp. nov.: phenotypic and molecular characterisation of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology **141**:1507–1521.
 26. Willis, A. M., W. A. Coulter, D. J. Sullivan, D. C. Coleman, J. R. Hayes, P. M. Bell, and P.-J. Lamey. 2000. Isolation of *C. dubliniensis* from insulin-using diabetes mellitus patients. J. Oral Pathol. Med. **29**:86–90.