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Rapid Identification of *Candida dubliniensis* with Commercial Yeast Identification Systems

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Candida dubliniensis* is a newly described species that is closely related phylogenetically to *Candida albicans* and that is commonly associated with oral candidiasis in human immunodeficiency virus-positive patients. Several recent studies have attempted to elucidate phenotypic and genotypic characteristics of use in separating the two species. However, results obtained with simple phenotypic tests were too variable and tests that provided more definitive data were too complex for routine use in the clinical laboratory setting. The objective of this study was to determine if reproducible identification of *C. dubliniensis* could be obtained with commercial identification kits. The substrate reactivity profiles of 80 *C. dubliniensis* isolates were obtained by using the API 20C AUX, ID 32 C, RapID Yeast Plus, VITEK YBC, and VITEK 2 ID-YST systems. The percentages of *C. dubliniensis* isolates capable of assimilating or hydrolyzing each substrate were compared with the percentages from the *C. albicans* profiles in each kit's database, and the results were expressed as percent *C. dubliniensis* and percent *C. albicans*. Any substrate that showed >50% difference in reactivity was considered useful in differentiating the species. In addition, assimilation of methyl-α-D-glucoside (MDG), D-trehalose (TRE), and D-xylose (XYL) by the same isolates was investigated by the traditional procedure of Wickerham and Burton (L. J. Wickerham and K. A. Burton, J. Bacteriol. 56:363–371, 1948). At 48 h (the time recommended by the manufacturer for its new database), we found that the assimilation of four carbohydrates in the API 20C AUX system could be used to distinguish the species, i.e., glycerol (GLY; 88 and 14%), XYL (0 and 88%), MDG (0 and 85%), and TRE (15 and 97%). Similarly, results with the ID 32 C system at 48 h showed that XYL (0 and 98%), MDG (0 and 98%), lactate (LAT; 0 and 96%), and TRE (30 and 96%) could be used to separate the two species. Phosphatase (PHS; 9 and 76%) and α-D-glucosidase (23 and 94%) proved to be the most useful for separation of the species in the RapID Yeast Plus system. While at 24 h the profiles obtained with the VITEK YBC system showed that MDG (10 and 95%), XYL (0 and 95%), and GLY (26 and 80%) could be used to separate the two species, at 48 h only XYL (6 and 95%) could be used to separate the two species. The most useful substrates in the VITEK 2 ID-YST system were TRE (1 and 89%), MDG (1 and 99%), LAT (4 and 98%), and PHS (83 and 1%). While the latter kit was not yet commercially available at the time of the study, it would appear to be the most valuable for the identification of *C. dubliniensis*. Although assimilation of MDG, TRE, and XYL proved to be the most useful for species differentiation by the majority of commercial systems, the results with these carbohydrates by the Wickerham and Burton procedure were essentially the same for both species, albeit following protracted incubation. Thus, it is the rapidity of the assimilation achieved with the commercial systems that allows the differentiation of *C. dubliniensis* from *C. albicans*.

Candida dubliniensis, first described in 1995 by Sullivan et al. (27), has been isolated from various geographic locations (24, 25), most commonly from oral specimens from human immunodeficiency virus (HIV)-infected and AIDS patients (5, 24). *C. dubliniensis* has been implicated as an agent of oral candidiasis (5, 23) and can develop resistance to fluconazole (10, 16, 17, 19), a common antifungal drug used for the treatment of mycoses in AIDS patients. The yeast has also been recovered from vaginal, fecal, lung, sputum, and blood specimens from HIV-negative individuals (15, 16, 18, 20). While only recently described, one isolate (formerly misidentified as *C. stellatoidea*) dates back to 1957, and another (formerly misidentified as *C. albicans*) dates back to 1952 (15, 24, 27). Retrospective studies of two yeast stock collections showed that approximately 2% of isolates originally identified as *C. albicans* were actually *C. dubliniensis* (5, 18). A similar investigation of a collection of oral yeast isolates from HIV-infected individuals originally identified as *C. albicans* revealed that 16.5% were *C. dubliniensis* (5).

C. dubliniensis is closely related phylogenetically to *C. albicans* and the former species *C. stellatoidea* (5), now considered to be a synonym of *C. albicans* (1, 11). Consequently, phenotypically, *C. dubliniensis* and *C. albicans* have many similarities, including their microscopic morphology and ability to form germ tubes in serum. Since many laboratories use the germ tube test (28) as their sole method for the identification of *C. albicans*, isolates of *C. dubliniensis* have been overlooked and misidentified as the former species (5, 18). In addition, both species typically produce blastoconidia with pseudohyphae, true hyphae, and chlamydospores. Some investigators have noted more abundant production and different arrangements

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(pairs, triplets, or clusters) of chlamydospores with *C. dubliniensis* (8, 27), but this can be somewhat subjective for clear separation of the two species (10).

The appearance of colonies on a chromogenic medium (i.e., CHROMagar Candida) has been considered a useful tool for recognition of *C. dubliniensis*, which is reported to develop dark green colonies after incubation at 37°C for 48 h, in contrast to the light green, light blue-green, or blue-green colonies seen with *C. albicans* (4, 5). However, the characteristic dark green colony pigment of *C. dubliniensis* may be lost after subculture or storage at −70°C, with colonies exhibiting the light green appearance of *C. albicans* (22, 24). It appears that incubation conditions are also important since colonies of *C. albicans* can have the same appearance as *C. dubliniensis* when they are grown for an extended period on CHROMagar Candida medium (18, 22). Although this method seems to have a high degree of sensitivity, specificity was lacking in some studies, suggesting that it should be used only as a screening tool for primary isolation of yeasts from clinical specimens and allows presumptive identification of *C. dubliniensis* in mixed cultures since it is often present with *C. albicans* (24).

Growth at 42°C was initially considered to be a useful test for differentiation of *C. dubliniensis* (no growth) from *C. albicans* (growth) in the original description of this new species (27). However, strains of *C. dubliniensis* which exhibit either poor (4, 5, 23–25) or good growth (10, 20, 22) at 42°C have been reported. In other temperature tolerance studies, 120 isolates of *C. dubliniensis* showed no growth, while 98 of 99 *C. albicans* strains grew at 45°C (20). However, in subsequent investigations only 18 of 28 *C. albicans* strains grew at 45°C (10).

Other phenotypic methods for the more definitive separation of *C. dubliniensis* from *C. albicans* have been described in the literature. Bikandi et al. (2) described the development of an immunofluorescence test based on the antibody detection of differential antigen expression on *C. dubliniensis* blastospores and *C. albicans* germ tubes. Furthermore, an intracellular β-glucosidase (βGLU) test was reported to indicate clear delineation between isolates of *C. albicans* and a cluster of "atypical *C. albicans*" (i.e., *C. dubliniensis*) isolates (3). A later study showed that all strains of *C. dubliniensis* were negative, but 11 of 481 *C. albicans* strains also tested negative (18). Although these methods provide other potential screening tests, they seem inefficient for routine use in clinical laboratories.

Several reports of studies that have used molecular methods to characterize the genetic difference between *C. dubliniensis* and *C. albicans* and their phylogenetic relationship (7, 26, 27) have appeared. These have included hybridization with *C. albicans*-specific probe 27A or Ca3 and *C. dubliniensis*-specific probe Cd2 (3, 4, 9, 10, 13, 18, 22, 25, 27), multilocus enzyme electrophoresis (3), oligonucleotide fingerprinting (4), electrophoretic karyotype analysis (4, 8, 25, 27), random amplified polymorphic DNA analysis (4, 25, 27), restriction fragment length polymorphism analysis (10, 14, 25–27), and rRNA sequencing analysis (4, 25–27). Data from those studies support *C. dubliniensis* as a new taxon separate from *C. albicans* and its synonym *C. stellatoidea*. However, these methods are labor-intensive and expensive and require special equipment, making them unsuitable for most clinical laboratories.

While several studies with *C. dubliniensis* have included data from the substrate assimilation-based ID 32 C or API 20C AUX yeast identification systems (3, 4, 8, 10, 13, 21, 22, 25, 27), this new species was not included in their respective databases when the studies were performed (before 1998). Thus, the biopatterns resulted in inconclusive identifications (not corresponding to any taxa included in the databases) or misidentifications (usually as *C. albicans* or *C. sake*).

Simple phenotypic tests are useful screening tools but are too variable for definitive identification of *C. dubliniensis*. Genotypic tests (i.e., molecular typing methods) provide definitive data and clear-cut differentiation of species but are too complex for routine use in the clinical laboratory setting. The present study was undertaken to determine if *C. dubliniensis* could be identified reproducibly with commercial identification kits. We studied the profiles of 80 *C. dubliniensis* isolates obtained with the API 20C AUX, ID 32 C, RapID Yeast Plus, VITEK YBC, and VITEK 2 ID-YST systems. The data obtained in this study support the use of commonly used commercial systems for the rapid and precise recognition of *C. dubliniensis* in the clinical setting.

MATERIALS AND METHODS

Test organisms. The 80 isolates of *C. dubliniensis* used in this study were from the culture collection of the Department of Oral Medicine and Oral Pathology, School of Dental Science and Dublin Dental Hospital, Trinity College, University of Dublin. All isolates were previously characterized by one or more of the molecular methods mentioned above. Cultures were maintained at −80°C in Protect cryovials (Technical Service Consultants Ltd., Lancashire, United Kingdom) or at −70°C in Trypticase soy broth with 15% (vol/vol) glycerol until the time of use. Frozen stocks were subcultured onto Sabouraud glucose agar (SGA; Oxoid, Poole, Dorset, United Kingdom, or Remel, Inc., Lenexa, Kans.) and were incubated at 30°C for 24 to 48 h before a second subculture to SGA. Colonies from the second subculture were used for testing. Incubation conditions (30°C for 18 to 48 h) for secondary SGA subcultures were as specified by the manufacturer for each of the respective commercial systems.

API 20C AUX system. The API 20C AUX system (bioMérieux, Marcy l'Etoile, France), a commercial kit for the evaluation of the assimilation of 19 carbon sources, was used according to the manufacturer's instructions. Since 48 to 72 h of incubation is recommended, test strips were incubated for both times to allow a comparative evaluation of the assimilation patterns. Numerical profiles were constructed from the reaction patterns and were used to obtain identification results with either the analytical profile index or the identification software program.

ID 32 C system. The ID 32 C system (bioMérieux) was used according to the manufacturer's instructions. The kit allows the evaluation of the assimilation of 29 carbon sources, growth in the presence of actidione (cycloheximide), and an esculin test (not used in the identification profile number). Test strips were incubated at 30°C for 48 h (24 to 48 h is recommended). Numerical profiles were constructed from the reaction patterns and were used to obtain identifications with the identification software program.

RapID Yeast Plus system. The RapID Yeast Plus system (Remel, Inc. [formerly Innovative Diagnostic Systems, Norcross, Ga.]) was used according to the manufacturer's instructions. This system permits investigation of the assimilation of five carbon sources and hydrolysis of 13 enzymatic substrates. The test panels were incubated at 30°C for 4 h. Numerical profiles were constructed from the reaction patterns and were used to obtain identifications with the code compendium.

VITEK YBC system. The VITEK YBC system (bioMérieux, Inc., Hazelwood, Mo.) was used according to the manufacturer's instructions. With this system, one may evaluate the assimilation of 23 carbon substrates, growth in the presence of cycloheximide, nitrate assimilation, and urea hydrolysis. Cards were read automatically at 24 h and again at 48 h for those that required additional incubation (flagged by the identification software). Numerical profiles were constructed automatically from the reaction patterns, and identifications were obtained with the instrument software.

VITEK 2 ID-YST system. The VITEK 2 ID-YST system (bioMérieux, Inc.) was used according to the manufacturer's instructions. This new system uses fluorogenic substrates to indicate the assimilation of 26 carbon substrates (adonitol, trehalose [TRE], *N*-acetyl-D-glucosamine, cellobiose, dulcitol, galactose, glucose, lactose, α-methyl-D-glucoside [MDG], maltose, mannitol, melibiose, melezitose, palatinose, raffinose, rhamnose, sucrose, salicin, sorbose, sorbitol, citrate, galacturonate, gluconate, lactate [LAT], mono-methyl ester succinate, and succinate), growth in the presence of actidione, nitrate assimilation, reactivity with 9 arylamidases (glycine, hydroxyproline, lysyl-alanine, γ-glutamic acid, glycyl-glycine, histidine, isoleucine, proline, and valine), and reactivity with 10 other enzymes (α-galactosidase, α-glucosidase [αGLU], α-mannosidase, β-galactosidase, βGLU, β-glucuronidase, *N*-acetyl-β-glucosaminidase, β-xylosidase, phosphatase [PHS], and urease). A yeast suspension was made in 0.45% (wt/vol) aqueous NaCl to achieve a turbidity equivalent to that of a no. 2 McFarland standard and was inoculated into the test card. The cards were incubated at 35°C for 15 h, and the test cards were read automatically at 15-min intervals. Biopat-

TABLE 1. API 20C AUX system tests useful for separation of *C. dubliniensis* from *C. albicans*

Test	% *C. dubliniensis* (80 isolates) positive at:		% Positive at 48 h with database (version 3.0)	
	48 h	72 h	*C. dubliniensis*	*C. albicans* 1[a]
GLY	88	98	96	14
XYL	0	0	0	88
MDG	0	0	0	85
TRE	15	99	0	97

[a] *C. albicans* 1, sucrose-positive *C. albicans*.

terns were constructed automatically, and reaction patterns were used to obtain identifications with the instrument software.

Wickerham and Burton procedure. The conventional broth tube method of Wickerham and Burton (29) was used to test assimilation of methyl-α-D-glucoside, D-trehalose, and D-xylose. A white card with 3-mm-wide ink lines was used to obtain turbidity measurements. Tubes were held against the card, and turbidity was considered 1+ if the lines were clear but indistinct, 2+ if the lines were diffused, and 3+ if the lines were obliterated. Assimilation and negative control tubes were inoculated with 100 μl of a dilute (1+ turbidity) yeast suspension in water, incubated at 30°C for 28 days, and read weekly for turbidity. Assimilation tubes were compared to negative control tubes for each isolate and were recorded as positive if 2+ or 3+ turbidity was observed. A 1+ turbidity reaction was considered very weak if the negative control tube was clear.

Nomenclature. The API 20C AUX and ID 32 C system databases list two biotypes of *C. albicans* (*C. albicans* 1 and *C. albicans* 2). *C. albicans* 1 represents the sucrose-positive isolates of *C. albicans* that are phenotypically similar to *C. dubliniensis*, whereas *C. albicans* 2 represents the sucrose-negative isolates formerly known as *C. stellatoidea*. One should note that these biotypes have been artificially created by the vendor for its databases and do not reflect actual taxonomic designations. For more information, the reader is referred to the work of Kwon-Chung et al. (12).

RESULTS

API 20C AUX system. High separation values were found with four carbohydrates in the API 20C AUX system (Table 1). Following 48 h (recommended for most yeast species) and 72 h of incubation, none of the 80 *C. dubliniensis* isolates tested were found to assimilate xylose (XYL) or MDG, whereas the database percentages for *C. albicans* 1 for these substrates are 88 and 85%, respectively. Positive assimilation of TRE was seen with 15% of the *C. dubliniensis* isolates after 48 h of incubation, but 99% were positive after 72 h of incubation. In contrast, the database indicates that 97% of *C. albicans* 1 can assimilate TRE after 48 h. Positive glycerol (GLY) assimilation was seen with 88% of the *C. dubliniensis* strains after 48 h of incubation, while all but two strains (98%) were positive after 72 h of incubation. However, the GLY assimilation results for *C. albicans* 1 in the database indicate that only 14% can assimilate this substrate. The numerical profiles constructed from *C. dubliniensis* reaction patterns after 48 and 72 h of incubation are shown in Table 2. Identification to the species level could be achieved after 48 h of incubation for 74% of the isolates, and an additional 11% were identified accurately to the genus level (low level of discrimination of *C. dubliniensis* and *C. albicans* 1). Misidentification of 11% of the *C. dubliniensis* isolates as *C. albicans* 1 was found, and 4% were unidentified. After 72 h of incubation, 95% of the 80 *C. dubliniensis* isolates were misidentified as *C. albicans* 1, 4% were unidentified, and 1% gave a correct identification of *C. dubliniensis*.

ID 32 C system. High separation values were again found with four carbohydrates in the ID 32 C system (Table 3). After 48 h of incubation, none of the 80 isolates of *C. dubliniensis* tested with this system assimilated LAT, MDG, or XYL, whereas the database percentages for these substrates with *C. albicans* 1 are 96, 98, and 98%, respectively. Thirty percent of the *C. dubliniensis* strains assimilated TRE, whereas 97% of *C. albicans* 1 isolates assimilated TRE, as indicated in the database. Identification was correct to the species level for 70% of the 80 strains tested (Table 4). The remaining isolates (30%) were unidentified, giving a doubtful profile result. The only difference between these profiles and the ones that gave very good identification to the species level was an additional positive reaction for TRE assimilation. The current database percentage for TRE assimilation is 0% for *C. dubliniensis*.

RapID Yeast Plus system. The results obtained with the RapID Yeast Plus system showed that two tests had the potential to separate *C. albicans* and *C. dubliniensis*. After 4 h of incubation, results for αGLU and PHS with *C. dubliniensis* strains were 23 and 9%, respectively, whereas the database percentages for *C. albicans* are 94 and 76%, respectively. This product's database does not include data for *C. dubliniensis*, so correct identification was not possible. Thirty-two different profiles were associated with testing of the 80 *C. dubliniensis* isolates (Table 5). Test results indicated that 5% of the isolates had three profiles that gave a misidentification as *C. zeylanoides* and 4% of the isolates had one profile that gave a misidentification as *C. stellatoidea*. Eight profiles that gave no identification (not found in the code compendium) were seen for 10% of isolates tested, and the remaining isolates (81%)

TABLE 2. Identification results for *C. dubliniensis* with API 20C AUX system (version 3.0 database)

Incubation time	Profile no.	% Isolates	Identification	Identification level[a]
48 h	2172134	11	*C. dubliniensis* or *C. albicans* 1[b]	VGG
	2172174	1	*C. albicans* 1	G
	6152134	3	*C. dubliniensis*	E
	6162134	3	*C. dubliniensis*	V
	6172034	5	*C. dubliniensis*	E
	6172074	4	No identification	D
	6172134	64	*C. dubliniensis*	E
	6172174	10	*C. albicans* 1	G
72 h	2172174	3	*C. albicans* 1	G
	6162134	1	*C. dubliniensis*	V
	6172074	4	No identification	D
	6172174	93	*C. albicans* 1	G

[a] Abbreviations: VGG, very good to genus level; G, good; V, very good; E, excellent; D, doubtful profile.
[b] *C. albicans* 1, sucrose-positive *C. albicans*.

TABLE 3. ID 32 C system assimilation tests useful for separation of *C. dubliniensis* from *C. albicans*

Test	% *C. dubliniensis* (80 isolates) positive at 48 h	% Positive at 48 h with database (version 2.0)	
		C. dubliniensis	*C. albicans* 1[a]
LAT	0	10	96
TRE	30	0	97
MDG	0	0	98
XYL	0	0	98

[a] *C. albicans* 1, sucrose-positive *C. albicans*.

had 20 different profiles that gave a misidentification as *C. albicans*.

VITEK YBC system. Results with the VITEK YBC system indicated that three test substrates (Table 6) had high separation values after 24 h of incubation. Results for MDG, XYL, and GLY assimilation were 10, 0, and 26%, respectively, for *C. dubliniensis* strains, whereas they are 95, 95, and 80%, respectively, for *C. albicans*. For 47 of 80 isolates tested, reincubation was required since *C. dubliniensis* (the database does not include data for this species) was recognized by the database as being the closest match to an underreactive strain of *C. albicans*. For the 47 isolates reincubated, the result for MDG assimilation was 64%, that for GLY assimilation was 51%, and that for XYL assimilation was 6%. This reincubation (to 48 h) resulted in increased positivity for tests that had the highest separation values when the results were more negative after 24 h of incubation. Thus, separation values for MDG and GLY assimilation decreased significantly, while XYL assimilation retained its high separation value. The bionumbers constructed from the *C. dubliniensis* reaction patterns after 24 and 48 h of incubation are shown in Table 7 along with the identification results. The majority of *C. dubliniensis* isolates (59%) were unidentified at 24 h. After 48 h of incubation, 99% were misidentified as *C. albicans* and 1% remained unidentified.

VITEK 2 ID-YST system. Four substrates were found to have high separation values in the VITEK 2 ID-YST system (Table 8). After 15 h of incubation, the results for TRE, MDG, LAT, and PHS assimilation were 1, 1, 4, and 83%, respectively, whereas database percentages for *C. albicans* are 89, 99, 98, and 1%, respectively. Our tests showed percentages in line with those in the database except for PHS. The current database percentage of positivity for PHS for *C. dubliniensis* is 1%, but we found a higher level of positivity (83%). Correct identification to the species level was highest (98%) with the VITEK 2 ID-YST system compared to those for the other two products (API 20C AUX and ID 32 C) that include data for *C. dubliniensis* in their databases. The identification algorithm used for the VITEK 2 ID-YST system does not allow bionumber (numerical profile) construction since occasional questionable test results can arise from data in uncertainty zones near test thresholds. These questionable results were not included in the totals used to calculate percentages. Identification results are shown in Table 9. Identification was correct to the species level for 98% of the 80 isolates tested, with 1% of the isolates unidentified and 1% of the isolates misidentified.

Wickerham and Burton procedure. Table 10 shows the results of conventional tests for assimilation of MDG, TRE, and XYL that were eventually positive for all 80 isolates of *C. dubliniensis* over the extended incubation period. Assimilation of MDG was slow for 16 isolates (20%) and was delayed for 7 isolates (9%), while TRE assimilation was slow for 10 isolates (12%). The most remarkable differences were seen with XYL assimilation, which was slow for 8 isolates (10%) and delayed for all but 9 isolates (11%), requiring 3 weeks of incubation for 63 isolates (79%) and 4 weeks of incubation for 6 isolates (8%). The remaining two isolates (2%) had very weak positive reactions even after the incubation period was prolonged to 31 days.

DISCUSSION

Comparative growth at 42 to 45°C for the differentiation of *C. albicans* and *C. dubliniensis* has been shown to yield equivocal results and should be used only to obtain presumptive evidence for the identification of *C. dubliniensis*. Most incubators are controlled within 4°C, and this may explain the differences in the results of studies cited above. Another potential source for these differences is variation in the components of the growth media.

Features such as abundant chlamydospore production, a dark green colony pigment on CHROMagar Candida medium, and the absence of intracellular βGLU may be affected by phenotypic variation that can occur after subculture or long-term storage and are probably useful only for primary isolation (24). Since variability has been reported in the literature, the results obtained by these tests should be considered presumptive for *C. dubliniensis* and one or more confirmatory test methods should be used to obtain a definitive identification of this yeast.

Molecular methods are definitive but time-consuming and expensive and require special equipment not often available in many clinical laboratories. Our objective in this investigation was to determine if five commercially available yeast identification systems could be used to rapidly and accurately identify *C. dubliniensis* isolates recovered from clinical specimens.

C. dubliniensis is a recent addition to the API 20C AUX system database, and our results show that this system has the potential to be a means of identification of this yeast. Our data indicate that database modifications are suggested to avoid misidentification of *C. dubliniensis* as *C. albicans* 1 or no identification. The largest discrepancy that we observed was the positive TRE assimilation found for 15% of the *C. dubliniensis* isolates tested. It is reasonable to assume that incorporation of this variability in a future database would correctly identify all isolates that were misidentified or unidentified due to positive TRE reactions. According to the database, xylitol, *N*-acetyl-D-glucosamine, and sucrose should have had differential values, but our results after 48 h of incubation indicated similar percentages of assimilation (data not shown) for *C. dubliniensis* (98, 91, and 100%, respectively) and the expected reactions for *C. albicans* 1 (90, 99, and 97%, respectively).

Our data indicate that XYL assimilation was the most effective test in separating the two species. We also examined 100 isolates of *C. albicans* 1 with API 20C AUX kits (data not shown), and all but 4 (96%) were positive for XYL assimilation. Of the four isolates that were negative for XYL assimi-

TABLE 4. Identification results for *C. dubliniensis* with ID 32 C system (version 2.0 database)

Profile no.	% Isolates	Identification	Identification level[a]
7042 1000 11	4	*C. dubliniensis*	E
7142 1000 15	5	*C. dubliniensis*	E
7142 1400 15	61	*C. dubliniensis*	V
7143 1400 15	30	No identification	D

[a] Abbreviations: E, excellent; V, very good; D, doubtful profile.

TABLE 5. Identification results for *C. dubliniensis* with RapID Yeast Plus system (version 1.95 database)

Profile no.	% Isolates	Identification	Profile no.	% Isolates	Identification
000001	1	*C. zeylanoides*	300001	3	*C. albicans*
001001	1	*C. zeylanoides*	301000	4	*C. stellatoidea*
100000	1	No identification	301001	18	*C. albicans*
100001	3	*C. zeylanoides*	301003	6	*C. albicans*
101001	14	*C. albicans*	301013	3	*C. albicans*
101003	6	*C. albicans*	303001	1	*C. albicans*
101007	5	*C. albicans*	303003	3	*C. albicans*
101013	1	*C. albicans*	303007	4	*C. albicans*
103001	3	*C. albicans*	303017	1	*C. albicans*
103003	5	*C. albicans*	310001	1	*C. albicans*
103005	1	*C. albicans*	311003	3	*C. albicans*
103017	1	*C. albicans*	330003	1	No identification
107003	1	No identification	701001	1	No identification
107017	1	No identification	711001	1	*C. albicans*
111001	3	No identification	711013	1	No identification
117003	1	No identification	730001	1	*C. albicans*

lation, three gave profiles characteristic of *C. dubliniensis*. Molecular analysis of these three strains with *C. dubliniensis*-specific PCR primers complementary to *ACT1*-associated intron sequences (6) showed that two of the isolates were actually *C. dubliniensis* (data not shown). This is consistent with the frequency (>2%) observed in a retrospective study (18) of a stock collection of *C. albicans* isolates. Our data that showed 98% positivity for XYL assimilation for the 98 *C. albicans* isolates suggest that more than 88% (the API 20C AUX kit database percentage) of *C. albicans* 1 strains can assimilate XYL. It would seem worthwhile for the manufacturer to use molecular methods to reexamine the XYL-negative isolates of *C. albicans* 1 whose data were included in the database. A higher percentage of XYL positivity for *C. albicans* 1 would provide more definitive separation of *C. albicans* from *C. dubliniensis* and allow the option of 72 h of incubation.

Data obtained with the ID 32 C system showed that TRE, MDG, and XYL assimilations were very useful in differentiating the two species. As noted with the API 20C AUX system, none of the *C. dubliniensis* isolates assimilated MDG or XYL, whereas 98% of the *C. albicans* 1 isolates whose data were included in the ID 32C database assimilated MDG and XYL. LAT assimilation (0% for *C. dubliniensis* and 96% for *C. albicans*) is another powerful substrate for the separation of these two species. Although 70% of isolates tested could be identified to the species level, the remaining isolates were unidentified due to a positive TRE reaction. We found that 30% of isolates assimilated TRE, in contrast to the expected 0% for *C. dubliniensis* in the database. It is clear that a modification to the TRE assimilation percentage for *C. dubliniensis* would result in a 100% correct identification to the species level. According to the database, palatinose assimilation should have had value in differentiating the two species, but our testing indicated similar percentages (data not shown) between *C. dubliniensis* (91%) and the expected percentage (100%) for *C. albicans* 1.

The RapID Yeast Plus system showed two tests (PHS and αGLU) with separation value, but a higher degree of variability was associated with them compared to useful tests from the other systems. Since 32 different profiles were observed for this 18-test system with 80 isolates of *C. dubliniensis*, it appears that the substrates used by the RapID Yeast Plus system are less useful for *C. dubliniensis* than tests present in the other two manual systems. It is difficult to predict the potential of database adjustments for inclusion of data for *C. dubliniensis*, but it seems likely that most strains of *C. dubliniensis* would not be identified to the species level. Of the most useful tests observed with the other commercial systems, only TRE assimilation and PHS tests are included in the RapID Yeast Plus system. Reactivity with TRE was nearly identical (data not shown) between *C. dubliniensis* (13%) and the expected percentage (14%) for *C. albicans*. While PHS showed some separation value, the difference between *C. dubliniensis* and *C. albicans* (9 and 76%, respectively) was not as remarkable as the differences seen with MDG and XYL assimilation present in all systems or LAT assimilation present in the ID 32 C and VITEK 2 ID-YST systems. While there is no single test in the RapID Yeast Plus system that clearly separates *C. dubliniensis* and *C. albicans*, a combination of test results, particularly negative PHS and αGLU results, may be useful for the identification of some strains of *C. dubliniensis*.

Assimilation of MDG, XYL, and GLY in the VITEK YBC system was found to be useful for the separation of *C. dubliniensis* from *C. albicans* after 24 h of incubation. As with the API 20C AUX and ID 32 C systems, the MDG and XYL substrates were significantly valuable, along with the GLY substrate in the API 20C AUX kit. Although differentiation with TRE was noted with the API 20C AUX and ID 32 C systems (Tables 1 and 3), it did not meet our criterion of a 50% difference with the VITEK YBC system. However, a lesser degree of separation (54% with *C. dubliniensis* compared to 90% with *C. albicans*; data not shown) was noted with this substrate, and TRE assimilation could have some utility if it were considered in combination with the other tests mentioned above. Since *C. dubliniensis* is not a taxon included in the VITEK YBC system database, a high percentage (59%) of the *C. dubliniensis* strains tested required reincubation. The YBC database recognized

TABLE 6. VITEK YBC tests useful for separation of *C. dubliniensis* from *C. albicans*

Test	% *C. dubliniensis* (80 isolates) positive at:		% Positive for *C. albicans* with database (version 5.03) at:	
	24 h	48 h	24 h	48 h
MDG	10	64	95	95
XYL	0	6	95	95
GLY	26	51	80	88

TABLE 7. Identification results for *C. dubliniensis* with VITEK YBC system (version 5.03 database)

Incubation time (no. of isolates)	Bionumber	% Isolates	Identification[a]
24 h (80)	110456411	1	No id-reinc
	510054411	1	No id-reinc
	510445411	13	No id-reinc
	510445451	1	No id-reinc
	510447411	1	No id-reinc
	510454011	1	No id-reinc
	510454411	3	No id-reinc
	510455011	6	No id-reinc
	510455411	8	No id-reinc
	510456411	1	No id-reinc
	510457411	9	*C. albicans*
	514055011	3	No id-reinc
	514055411	1	No id-reinc
	514454011	1	No id-reinc
	514454411	1	No id-reinc
	514455011	14	No id-reinc
	514455411	15	*C. albicans*
	514456411	1	No id-reinc
	514457011	1	No id-reinc
	514457411	8	*C. albicans*
	550417411	1	*C. albicans*
	554055411	1	*C. albicans*
	554455411	5	*C. albicans*
	554457411	3	*C. albicans*
48 h (47)	510445415	2	*C. alb*/*Cr. neo*
	510455415	6	*C. albicans*
	510456415	2	No id
	510457415	17	*C. albicans*
	511455415	2	*C. albicans*
	514455415	2	*C. albicans*
	514456415	2	*C. alb*/*C. zeyl*
	514457415	2	*C. albicans*
	554055415	2	*C. albicans*
	554057415	2	*C. albicans*
	554455415	32	*C. albicans*
	554455455	2	*C. albicans*
	554457415	21	*C. albicans*
	555457415	4	*C. albicans*

[a] Abbreviations: No id-reinc, unidentified at 24 h and reincubated 24 h at 30°C; *C. alb*/*Cr. neo*, good confidence marginal separation between *C. albicans* and *Cryptococcus neoformans*. No id, unidentified; *C. alb*/*C. zeyl*, good confidence marginal separation between *C. albicans* and *C. zeylanoides*.

these isolates of *C. dubliniensis* as most closely resembling underreactive isolates of *C. albicans*. The normal occurrence of underreactive isolates of *C. albicans* that require reincubation is about 10% (unpublished data for isolates in the database). Since about 90% of *C. albicans* isolates are identified after 24 h of incubation and 0% of *C. dubliniensis* assimilate XYL after 24 h of incubation, it is likely that at least 90% of *C. dubliniensis* isolates would be accurately identified to the species level after 24 h of incubation if data for this new species were included in the VITEK YBC system database.

TABLE 8. VITEK 2 ID-YST tests useful for separation of *C. dubliniensis* from *C. albicans*

Test	% *C. dubliniensis* (80 isolates) positive at 15 h	% Positive at 15 h with database (version 1.01)	
		C. dubliniensis	*C. albicans*
TRE	1	1	89
MDG	1	1	99
LAT	4	1	98
PHS	83	1	1

TABLE 9. Identification results for *C. dubliniensis* with VITEK 2 ID-YST system (version 1.01 database)

No. of isolates	% Isolates	Identification	Identification level
9	11	*C. dubliniensis*	Excellent
49	61	*C. dubliniensis*	Very good
10	13	*C. dubliniensis*	Good
10	13	*C. dubliniensis*	Acceptable
1	1	No identification	Unidentified
1	1	*C. albicans*	Misidentified

The most encouraging results were seen with the VITEK 2 ID-YST system, which was not yet commercially available at the time of our study. This product is being introduced and has the best potential of the systems included in the study for correct identification of *C. dubliniensis* to the species level. The database already includes this taxon, and although our data showed much higher reactivity for PHS, this had no impact on the final identifications. As with TRE assimilation in the API 20C AUX and ID 32 C systems, this difference in PHS reactivity seems attributable to the larger data set used in our study. Since numerical profiles are not generated with this system, it was not feasible to determine how uniform reaction patterns were for the 80 isolates tested.

The most consistent test results were found with the ID 32 C system. Even though this system incorporates 30 tests, only four different profile numbers were seen at 48 h with the 80 isolates of *C. dubliniensis* that were tested. It is possible that incubation for 24 h would have resulted in more profile variation, as was the case when we compared data from the API 20C AUX kit after 48 and 72 h of incubation. Even considering this difference related to incubation time, the profile number variability was much less than that observed with the RapID Yeast Plus or the VITEK YBC system.

As mentioned above, our results indicate that database modifications are suggested for TRE assimilation with the API 20C AUX and ID 32 C systems to allow correct identification of 15 and 30%, respectively, of the *C. dubliniensis* isolates that gave either misidentification or no identification results. Our data indicate that the RapID Yeast Plus system may not have the optimal substrates present for differentiation of *C. dubliniensis*, but it has potential to identify *C. dubliniensis* to the genus level (but requires additional confirmatory testing for definitive separation from *C. albicans*). It appears that incorporation of our data into the VITEK YBC system database could allow accurate recognition of *C. dubliniensis*. It is clear that current users of these four products should be aware of the bionumbers

TABLE 10. Conventional assimilations of *C. dubliniensis* by Wickerham and Burton method

Incubation time (days)	MDG		TRE		XYL	
	No. of isolates positive	% Isolates positive	No. of isolates positive	% Isolates positive	No. of isolates positive	% Isolates positive
7	57	71	70	88	1	1
14	73	91	80	100	9	11
21	80	100			72	90
28					78	98
31					80[a]	100

[a] Two isolates showed a very weak positive reaction.

(profile numbers) shown in Tables 2, 4, 5, and 7 so that this important species can be recognized rapidly in today's clinical laboratory setting.

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