Rapid Differentiation of *Candida dubliniensis* from *Candida albicans* by Early D-Xylose Assimilation

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Key Words

D-Xylose · *Candida albicans* · *Candida dubliniensis*

Abstract

Objective: To determine if D-xylose (XYL) and/or α -methyl-D-glucoside (MDG) assimilation can be used reliably as a rapid test to differentiate *Candida dubliniensis* from *Candida albicans* at an earlier time point such as 2 h after inoculation.

Materials and Methods: Thirty isolates of *C. albicans* and *C. dubliniensis* recovered from anatomical sites and clinical specimens were used. Isolates were inoculated into the API 20C AUX yeast identification system, and incubated at 30°C. XYL and MDG assimilations were read at 2-hour intervals beginning 2 h after the initial inoculation and up to 24 h of incubation; thereafter, results were read after 48 and 72 h. **Results:** Twenty-nine (97%) *C. albicans* isolates had assimilated XYL at 16 h and, by 24 h, all isolates were positive for XYL assimilation. None of the *C. dubliniensis* isolates assimilated XYL. The MDG assimilation revealed that 24, 40, 92 and 100% of *C. albicans* isolates became positive after 16, 24, 48 and 72 h of incubation, respectively, whereas only 3% of *C. dubliniensis* isolates assimilated MDG after 72 h. **Conclusions:** The findings showed that it is possible to rapidly differentiate *C. albicans* from *C. dubliniensis* isolates using the API 20C AUX carbohydrate assimilation kits after 16 h of incubation at 30°C based on the XYL assimilation.

Introduction

Candida dubliniensis is now well recognized as an opportunistic pathogen associated with recurrent oral candidiasis in AIDS patients as well as other compromised patient groups [1–4]. Resistance as well as diminished susceptibility to fluconazole has also been observed in *C. dubliniensis* isolates obtained from HIV-infected patients [5, 6]. Recently a breakthrough in *C. dubliniensis* fungemia occurred in a patient during prolonged exposure to voriconazole [7], and it was revealed that *C. dubliniensis* isolates from HIV-infected patients may acquire itraconazole resistance, even in the absence of prior azole therapy [8]. Development of such resistance may have important implications for antifungal therapy and indicate a need for a method to distinguish *C. dubliniensis* from *Candida albicans* as well as from other *Candida* species.

C. dubliniensis is closely related to *C. albicans*, sharing its properties of commensalism and opportunistic infection. For instance, 96% of genes are $\geq 60\%$ homologous between these species, with the vast majority of genes being approximately 90% homologous [9]. Given this genetic similarity, it is not surprising that these species share many phenotypic traits which present difficulties in accurately discriminating between *C. albicans* and *C. dubliniensis*.

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Several assays based on phenotypic characteristics have been used in an attempt to distinguish *C. dubliniensis* from *C. albicans*, including characteristic chlamydospore formation, growth at elevated temperatures, carbohydrate assimilation patterns, colony color on CHROMagar Candida medium, and β -glucoside production. However, in multiple publications variable success using each of these methods has been reported because of the phenotypic similarities shared by these two species [10–13]. To circumvent these difficulties, molecular methods have been developed [14, 15]. Latex agglutination tests, fluorescent in situ hybridization with peptic nucleic acid probes and immunochromatographic assays have also been used for this purpose [16–18]. However, most of these procedures have not been widely used or are not well-suited to the routine clinical microbiology laboratory.

Whereas molecular identification is the most specific method to differentiate these species, an easy-to-perform phenotypic test, if reliable, would be a valuable tool for this purpose. Perhaps the most convenient and common method for *Candida* species identification consist of strips or plates containing substrates for carbohydrate assimilation and/or enzyme detection. These phenotypic tests are commercially available in a variety of formats. One such phenotypic test is the API 20C AUX yeast identification system [10–13]. This system uses an increase in turbidity in each of a series of wells containing different substrates to produce a biochemical profile. The biochemical profile produced is then translated into a numerical code that is deciphered using the manufacturer's reference manual.

D-xylose (XYL) is one of the substrates present in the API 20C AUX yeast identification system. Assimilation of XYL has been reported to be a reproducible and reliable test for the differentiation of *C. dubliniensis* from *C. albicans* [10–13]. In addition assimilation of α -methyl-D-glucoside (α -MDG) has also been somewhat helpful for the differentiation of these two species [11]. These findings were based upon the use of API 20C AUX test strips that were incubated for 72 h at 30°C as recommended by the manufacturer [10, 12, 13]. It was of interest, therefore, to determine if XYL and/or α -MDG assimilation could be used to rapidly differentiate *C. dubliniensis* from *C. albicans* at an earlier time point, rather than waiting for 72 h to determine the outcome. Hence, the main aim of this study was to determine the time course of XYL and α -MDG assimilation beginning as early as 2 h after inoculation of *C. dubliniensis* and *C. albicans* isolates into API 20C AUX yeast identification test strips in an attempt to determine the utility of such a test system for the early differentiation of these two species.

Materials and Methods

Microorganisms

Thirty isolates each of *C. albicans* and *C. dubliniensis* recovered from various anatomical sites and clinical specimens were used for this study. All *C. albicans* and 23 *C. dubliniensis* isolates were oral isolates obtained from patients attending the Kuwait University Dental Clinic. The remaining *C. dubliniensis* isolates were from blood ($n = 1$) and urine samples ($n = 6$). All yeast isolates were tested for germ tube formation as previously described [19]. Thereafter a presumptive identification of *Candida* isolates was performed on the basis of the characteristics of colony color on CHROMagar Candida medium (Becton Dickinson Co., Sparks, USA). All isolates produced green colonies. All the isolates were further identified based on their carbohydrate assimilation pattern by API 20C AUX yeast identification system (bioMérieux, France). The identity of *C. dubliniensis* was confirmed by production of rough colonies with hyphal fringes and chlamydospores on simplified sunflower seed agar and by using seminested PCR amplification of internal transcribed spacer (ITS)-2 region of rDNA followed by direct DNA sequencing of the ITS region of rDNA as described previously [20].

Carbohydrate Assimilation

For each isolate two sets of API 20C AUX yeast identification kits were used. The carbohydrate assimilation pattern of all of the isolates was examined after growth in the API 20C AUX yeast identification system at 30°C for 72 h as recommended by the manufacturer (bioMérieux Vitek, Inc., Hazelwood, Mo., USA). Before inoculation into the API 20C AUX yeast identification kits, all isolates were subcultured on Sabouraud dextrose agar Emmons plates (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md., USA) and incubated at 36°C for 24 h. Single colonies were used for the preparation of each inoculum as suggested by the manufacturer. The API 20C AUX yeast identification strips were filled with inoculated medium and incubated for 72 h at 30°C. In the same time, to determine the earliest time point at which *C. albicans* and *C. dubliniensis* isolates might be differentiated based on XYL and/or α -MDG assimilation, the patterns were observed at 2-hour intervals beginning at 2 h after initial inoculation up to 24 h; thereafter, strips were observed at 48 and 72 h. Results were read independently by two laboratory staff members. Cupules demonstrating significant turbidity relative to the negative control were considered to be positive and there was no discrepancy in the readings reported by the two laboratory staff members.

Results

Of the 30 *C. albicans* isolates, 29 (97%) had assimilated XYL at 16 h and, by 24 h, all *C. albicans* isolates were positive for XYL assimilation. Although all wells containing *C. albicans* isolates began to develop some turbidity by 8–12 h after incubation, positive turbidity, as defined by the manufacturer, was observed as early as 16 h for 29 of 30 isolates (97%) and at 24 h for the remain-

ing 1 isolate. None of the *C. dubliniensis* isolates were positive for XYL assimilation at any time point studied (table 1). *C. albicans* progressively became positive for α -MDG assimilation, at 16 h of incubation (table 1). In contrast, only 1 of 30 (3%) *C. dubliniensis* isolates assimilated α -MDG after 72 h of incubation (table 1).

Discussion

In this study, XYL assimilation was positive for most of the *C. albicans* isolates after 16 h of incubation at 30°C and was negative for all *C. dubliniensis* isolates. Previous studies [10, 12, 13] had shown that characteristic assimilation patterns for XYL could be useful for the differentiation of *C. albicans* from *C. dubliniensis* after 72 h of incubation in the API 20C AUX carbohydrate assimilation test system [10, 12, 13]. However, our data showed that XYL assimilation could be used to differentiate 96% of all *C. albicans* isolates from *C. dubliniensis* isolates at as early as 16 h.

In the current study, while all *C. albicans* isolates assimilated α -MDG, 97% *C. dubliniensis* isolates did not assimilate α -MDG even after 72 h of incubation at 30°C (table 1). Assimilation of α -MDG has also been suggested to be a useful method for the differentiation of *C. albicans* from *C. dubliniensis* after 72 h of incubation at 30°C in the API 20C AUX system [10, 12, 13]. Our study confirmed that of Sancak et al. [10], who did not find any *C. dubliniensis* isolates capable of assimilating α -MDG whereas 99% of *C. albicans* isolates assimilated α -MDG. However, results for α -MDG assimilation were somewhat more equivocal in studies by Pincus et al. [13] and Gales et al. [12]. Pincus et al. [13] showed that 85% of *C. albicans* assimilated α -MDG whereas *C. dubliniensis* isolates did not. Gales et al. [12] reported that only 54% of the *C. albicans* isolates assimilated α -MDG while *C. dubliniensis* isolates did not assimilate α -MDG. Because of these discordant findings on *C. albicans*, α -MDG assimilation may not be a reliable method for differentiating these two species.

Hence these findings indicate that it is possible to differentiate a large majority of (97%) *C. albicans* from *C. dubliniensis* isolates as early as 16 h by comparing their XYL assimilation patterns. Comparing this method with other phenotypic identification methods such as incubation at different temperatures and growth in different culture media as done previously in our laboratory [21, 22] as well as other laboratories [23] will also help to determine the best possible easy-to-perform, early identification method to differentiate these two *Candida* species.

Table 1. Number (%) of *C. albicans* (CA) and *C. dubliniensis* (CD) isolates assimilating XYL and α -MDG at 30°C after progressive incubation intervals

Incubation time, h	XYL		α -MDG	
	CA	CD	CA	CD
2–6 ^a	0	0	0	0
8	*	0	*	0
12	*	0	*	0
16	29 (97)	0	7 (24)	0
24	30 (100)	0	12 (40)	0
48	30 (100)	0	28 (92)	0
72	30 (100)	0	30 (100)	1 (3)

^a None of the isolates showed any turbidity or growth at these time points (2–6 h).

*All isolates which eventually became positive for the assimilation of this carbohydrate demonstrated very mild turbidity at this time point and this turbidity progressed over time.

Early identification may be particularly useful in the management of *C. albicans* and *C. dubliniensis* infections in immunocompromised individuals because *C. dubliniensis* has been associated with invasive diseases in such hosts [24]. Although neither *C. albicans* nor *C. dubliniensis* has high rates of antifungal drug resistance among isolates derived from blood or other body sites, the rate of azole resistance among oral isolates of *C. dubliniensis* from HIV-infected patients is much greater [5–8]. Easy to use, rapid assimilation methods have been developed for the differentiation of *Candida glabrata* from other *Candida* species, for instance, the GLABRATA RTT test [25] (Fumouze Diagnostics, France) and the rapid-assimilation-of-trehalose test [26]. Hence, it would be of much interest if investigators could try to develop a similar rapid assimilation method using XYL. In fact, as cupules inoculated with *C. albicans* isolates elicited turbidity with XYL as early as 8 h after incubation (table 1), development of a colorimetric assay to determine XYL assimilation, similar to that of the GLABRATA RTT test, would be a valuable tool for early differentiation between *C. dubliniensis* from *C. albicans*.

Conclusions

Our findings showed that *C. albicans* can be rapidly differentiated from *C. dubliniensis* isolates using the API 20C AUX carbohydrate assimilation kits after as little as

16 h of incubation at 30°C based on XYL assimilation. This method, however, will be more reliable in germ-tube-positive *Candida* producing green colonies in CHROMagar *Candida* medium, and it is advisable to confirm the identification with molecular testing.

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