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Rapid Identification of *Candida glabrata* Based on Trehalose and Sucrose Assimilation Using Rosco Diagnostic Tablets

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We developed a simple method for the identification of *Candida glabrata* on the basis of the ability of this species to rapidly assimilate trehalose but not sucrose. After incubation of yeasts with Rosco diagnostic tablets containing sucrose or trehalose, identification of *C. glabrata* was achieved in 4 h with 100% sensitivity and specificity.

Historically, *Candida glabrata* has been considered a saprophyte of the normal flora of healthy individuals, rarely causing serious infection in humans. Within the past two decades however, non-*Candida albicans Candida* species have emerged as important opportunistic pathogens in immunocompromised patients. Although *C. albicans* remains the most frequent cause of severe candidiasis, *C. glabrata* is now recognized as an important nosocomial agent (2, 10). In our laboratory, this species accounts for 16% of all *Candida* species isolated, and as such, it is the second most frequent yeast cultured from clinical samples. Due to the commonly occurring innate or acquired resistance to fluconazole, rapid identification of *C. glabrata* is essential to guide antifungal therapy (2, 3).

Although morphological features of the colonies in cultures, such as glossy, smooth, and dome-shaped colonies, and the presence of small spherical yeasts upon microscopic examination may be an aid for the presumptive identification of *C. glabrata*, definitive identification requires additional tests. However, commercially available methods are time-consuming and expensive. Different methods for rapid screening and identification of *C. glabrata* have thus been developed. They are based on the ability of *C. glabrata* to rapidly use trehalose. By these techniques, false-positive results have been reported, especially with *Candida tropicalis* (1, 7, 8). To overcome these difficulties, we developed a new and simple method, using Rosco diagnostic tablets (purchased from EUROBIO, Les Ulis, France), based on trehalose and sucrose assimilation. Indeed, among yeast species commonly isolated in a clinical mycology laboratory, *C. glabrata* is the only one which utilizes trehalose but not sucrose (6).

Altogether, 440 clinical isolates were tested to characterize the present method (see Table 1). These included *C. glabrata* ($n = 180$), *C. albicans* ($n = 84$), *C. tropicalis* ($n = 62$), *Candida kefyr* ($n = 38$), *Candida krusei* ($n = 22$), *Candida parapsilosis* ($n = 19$), *Candida sake* ($n = 9$), *Candida famata* ($n = 7$), *Candida lusitaniae* ($n = 4$), *Candida guilliermondii* ($n = 2$), *Candida sphaerica* ($n = 1$), *Saccharomyces cerevisiae* ($n = 11$), and *Trichosporon cutaneum* ($n = 1$). These isolates originated from a variety of clinical samples: stool ($n = 149$), throat swab ($n = 82$), urine ($n = 87$), tracheal secretion and sputum ($n = 57$), skin ($n = 23$), blood and catheter ($n = 12$), vaginal ($n = 10$), nose ($n = 8$), and other ($n = 12$) samples. The reference strains *C. albicans* ATCC 26278, *C. parapsilosis* ATCC 22019, *C. glabrata* ATCC 66032, *C. krusei* ATCC 6258, *Candida zeylanoides* CBS 619 and 947, *Candida conglobata* CBS 2018 and 2019, *Pichia farinosa* CBS 185 and 2001, and *Prototheca wick-*

TABLE 1. Analysis of clinical isolates by trehalose-sucrose assimilation test based on Rosco diagnostic tablets

Organism	No. of isolates	No. of isolates testing positive			
		Trehalose		Sucrose	
		4 h	24 h	4 h	24 h
C. glabrata	180	180	180	0	0
C. albicans	84	0	0	21	25
C. tropicalis	62	8	21	53	53
C. kefyr	38	0	0	33	37
C. krusei	22	0	0	0	0
C. parapsilosis	19	0	0	0	1
S. cerevisiae	11	0	1	10	10
C. sake	9	0	1	1	3
C. famata	7	0	0	0	1
C. lusitaniae	4	0	0	2	3
C. guilliermondii	2	0	0	1	1
C. sphaerica	1	0	0	1	1
T. cutaneum	1	0	0	0	0

TABLE 2. Analysis of reference strains by trehalose-sucrose assimilation test based on Rosco diagnostic tablets

Organism	Test result			
	Trehalose		Sucrose	
	4 h	24 h	4 h	24 h
C. albicans ATCC 26278	−	−	−	−
C. parapsilosis ATCC 22019	−	−	−	−
C. glabrata ATCC 66032	+	+	−	−
C. krusei ATCC 6258	−	−	−	−
C. conglobata CBS 2018	−	−	−	−
C. conglobata CBS 2019	−	−	−	−
C. zeylanoides CBS 619	−	−	−	−
C. zeylanoides CBS 947	−	−	−	−
P. farinosa CBS 185	−	−	−	−
P. farinosa CBS 2001	−	−	−	−
P. wickerhamii IP 1202-79	−	−	−	−

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TABLE 3. Use of trehalose-sucrose assimilation test to identify yeast isolates obtained from patients receiving antifungal treatment at time of sampling

Organism	No. of isolates	No. of isolates testing positive			
		Trehalose		Sucrose	
		4 h	24 h	4 h	24 h
C. albicans	9	0	0	0	0
C. glabrata	10	10	10	0	0
C. kefyr	2	0	0	2	2
C. tropicalis	1	0	0	1	1
C. krusei	1	0	0	0	0
S. cerevisiae	1	0	0	1	1

erhamii IP 1202-79 were also included in the study (see Table 2). In addition, a series of clinical isolates obtained from patients receiving antifungal therapy at the time of sampling was tested (see Table 3). *C. albicans* was identified by germ tube formation at 37°C for 4 h in human serum. Other *Candida* species were identified by their carbohydrate assimilation profiles with the API ID 32C kit (bioMerieux sa, Marcy l'Étoile, France). In order to avoid any error possibly resulting from the presence of multiple yeast species on primary cultures of the clinical samples, yeasts were recovered from the API ID 32C or germ tube test samples and subcultured on Sabouraud dextrose agar for the trehalose-sucrose assimilation tests.

The Rosco diagnostic tablets used in this investigation contain 2.5 mg of a specific carbohydrate substrate, together with a weak buffer and a pH indicator (phenol red) that turns from red to yellow upon utilization of the carbohydrate. For each isolate tested, a colony was suspended in 600 μl of sterile 0.85% NaCl, and if necessary the suspension was adjusted to a no. 2 McFarland standard by dilution with 0.85% NaCl or the addition of yeasts from a second colony. A 300-μl aliquot of the resulting suspension was incubated at 37°C with either trehalose or sucrose tablets. Results were read after 4 h and 24 h of incubation. A test was positive if a yellow color was observed in the reaction tube. Tests were considered negative if a pink or red color was observed.

The results of the tests performed with clinical isolates are shown in Table 1. All *C. glabrata* isolates gave a positive reaction with trehalose tablets after 4 or 24 h of incubation. None of the *C. albicans*, *C. kefyr*, *C. krusei*, *C. parapsilosis*, *C. famata*, *C. lusitaniae*, *C. guilliermondii*, *C. sphaerica*, or *T. cutaneum* isolates were able to utilize trehalose. One *C. sake* isolate and one *S. cerevisiae* isolate gave positive reactions with trehalose after 24 h of incubation. Among the 62 *C. tropicalis* strains tested, 8 gave positive reactions after 4 h of incubation and 21 gave positive reactions after 24 h of incubation. All *C. tropicalis*, *C. sake*, and *S. cerevisiae* isolates that gave positive reactions with trehalose at either 4 or 24 h were also positive for sucrose utilization at 4 h. The trehalose-sucrose assimilation assay was also applied to reference strains of *C. conglobata, C. zeylanoides, P. farinosa*, and *P. wickerhamii*, organisms that are occasionally isolated in clinical laboratories and that have the ability to utilize trehalose (6, 9). None of these four species gave a positive result by the trehalose assimilation test (Table 2) in four separate experiments that included *C. glabrata* and *S. cerevisiae* as controls for trehalose or sucrose assimilation. Finally, analysis of clinical isolates from patients receiving antifungal treatment at the time of sampling showed that all *C. glabrata* isolates obtained after antifungal treatment had the trehalose-positive, sucrose-negative profile at 4 h (Table 3). The combination of the trehalose and sucrose assimilation tests thus allowed identification of *C. glabrata* in 4 h with 100% sensitivity and specificity.

Several groups have described trehalose assimilation-based assays for the identification of *C. glabrata* (Table 4). Most of these methods, however, present one or several disadvantages such as the need for homemade reagents, the need for large yeast inocula, false-positive results with *C. tropicalis*, or the need for the use of a microtiter plate format, which implies the simultaneous testing of a large number of isolates for cost-effectiveness. The Rosco system for determination of the carbohydrate metabolism of microorganisms was previously developed in clinical bacteriology laboratories to identify fastidious bacteria (4, 5), and our goal was to adapt this system to identification of a yeast organism. In preliminary experiments, the proportions of *C. glabrata* isolates at 1.5, 1, or 0.5 McFarland standard suspensions that showed trehalose assimilation at 4 h were 100, 60, and 0%, respectively. Suspensions of *C. glabrata* at a no. 3 McFarland standard suspension always produced the trehalose-positive, sucrose-negative profile at 4 h. However, higher densities resulted in cloudy suspensions that made interpretation of colors difficult. Any inoculum from a no. 1.5 to a no. 3 McFarland standard suspension thus appeared adapted to the 4-h detection of trehalose assimilation by *C. glabrata*. We therefore decided to characterize the method with a no. 2 McFarland inoculum, which provided a reasonable margin of error, since a no. 1.5 McFarland inoculum might, due to inaccurate calibration, result in a suspension at no. 1 McFarland standard, which could cause a false-negative result. The approach described herein presents several advantages over previously described techniques: (i) results are obtained in 4 h with a 100% sensitivity and specificity, (ii) the test format is adapted to the work flow of a clinical laboratory, (iii)

TABLE 4. Sensitivity, specificity, and cost of different tests for rapid identification of *C. glabrata*

Method (reference)	Inoculum	Incubation	Sensitivity (%)	Specificity (%)	Cost ($)
Hardy trehalose fermentation (1)	Four to five colonies	24 h, 42°C	96	100	1.10
Mayo Clinic trehalose assimilation (1; Stockman and Roberts[a])	Heavy; not adjusted	1 h, 35°C	96.6	74.1	0.05
Remel rapid trehalose assimilation (1)	Cloudy suspension	3 h, 42°C	91.5	96.3	1.59
Remel yeast fermentation, modified (1, 7)	No. 3–4 McFarland standard	24 h, 42°C	95–97.8	89–95.8	4.15
Rosco diagnostic tablets (current study)	No. 2 McFarland standard	4 h, 37°C	100	100	0.70
Trehalase-generated glucose (8)	One colony/50 μl	3 h, 37°C	98.8	99.1	0.30

[a] L. Stockman and G. Roberts, Abstr. 85th Annu. Meet. Am. Soc. Microbiol. 1985, abstr. F-80, p. 377, 1985.

when the test is performed with a 48-h-old culture, a unique middle-size colony is sufficient, (iv) all reagents are commercially available, and (v) the method is cost-effective. Further evaluation will be necessary to determine the usefulness of the proposed method in a clinical mycology laboratory, with special emphasis on yeast species that were not tested during the current investigation.

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