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Identification of *Candida glabrata* by a 30-Second Trehalase Test

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Rapid (30-s) trehalase tests done with material from colonies of 482 yeasts suspended in a drop of trehalose solution on a commercially supplied glucose test strip were positive for 225 (99.1%) of 227 *Candida glabrata* isolates grown on either of two differential media, Candida ID medium or CandiSelect medium. The test was positive for only 3 (1.2%) and 12 (4.7%) of 255 isolates of other medically important yeast species grown on the same two media, respectively. A rapid maltase test done with a subset of 255 yeast isolates was negative for all but 1 of 64 trehalase-positive *C. glabrata* isolates, raising the specificity of the rapid testing for *C. glabrata* to 98.4 to 100%, depending on the isolation medium used. Rapid trehalase and maltase tests done independently in two laboratories with 217 yeast isolates showed sensitivities of 96.0 to 98.0% and specificities of 98.2 to 99.4% for identification of *C. glabrata* from colonies grown on Candida ID medium. The specificity was much lower because of frequent false-positive trehalose test results when the source of colonies was Sabouraud agar formulated with 4% glucose. We conclude that direct recognition of *C. albicans* as blue colonies on Candida ID isolation medium coupled with the performance of the 30-s trehalase and maltase tests for *C. glabrata* among the white colonies on this medium will allow the rapid presumptive identification of the two yeast species most commonly encountered in clinical samples.

While *Candida albicans* remains the principal cause of opportunistic *Candida* infections in immunocompromised patients, other species such as *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* are also implicated. *C. glabrata* is now the second most common yeast species isolated from clinical specimens, constituting up to 26% of yeasts isolated from blood cultures (1, 3, 5, 14, 20–24). To facilitate the yeast identification process for clinical laboratories, several chromogenic isolation media have been introduced that allow immediate identification of *C. albicans* (2, 8, 13, 18, 19, 25) or *C. albicans*, *C. tropicalis*, and *C. krusei* (7, 15) by the colors of their colonies. However, no chromogenic medium allows the specific identification of *C. glabrata*.

C. glabrata rapidly metabolizes trehalose to glucose by means of a potent trehalase enzyme, a property shared by relatively few other yeasts of clinical importance. Stockman and Roberts (L. Stockman and G. Roberts, Abstr. 85th Annu. Meet. Am. Soc. Microbiol. 1985, abstr. 377, 1985) devised a 1-h screening test for *C. glabrata* based on its trehalase activity, but many isolates of *C. tropicalis* and several other *Candida* species gave false-positive results under the conditions used for that test. Various subsequent attempts to improve on the sensitivities and specificities of trehalose utilization tests for identification of *C. glabrata* have been described, but they necessitate preparation of heavy inoculum suspensions (suspensions of 2 to 4 on the McFarland turbidity scale) and incubation times from 1 to 24 h (12, 17). To reduce or eliminate false-positive results from other *Candida* spp., the use of a second assimilation test (12, 16) or incubation of the trehalase test at 42°C (11) has been recommended.

We recently described a trehalase assay in which commercially supplied glucose oxidase test strips are moistened with trehalose solution and smeared with material taken from a yeast colony with an inoculating loop (16). These conditions led to color development within 30 to 60 s for trehalase-positive yeasts. However, in tests with 58 isolates of *C. glabrata* and 86 other yeasts representing 14 other species, we found that the sensitivity and the specificity of this exceptionally rapid trehalase test were influenced by the medium that had been used to isolate the yeasts from clinical specimens.

To evaluate further the utility of rapid trehalase testing for identification of *C. glabrata*, we have extended our investigations to a much larger number of yeast isolates. We have added a systematic investigation of the influence of an additional rapid maltase test on the overall sensitivity and specificity of identification. Finally, we have undertaken an interlaboratory comparison of results obtained with a subset of yeasts.

MATERIALS AND METHODS

Yeast isolates and growth conditions. In total, 482 yeasts were tested for rapid trehalase activity. These comprised 5 reference strains (*C. glabrata* ATCC 90030, *C. tropicalis* ATCC 66029, *C. albicans* ATCC 60193, and *Cryptococcus neoformans* ATCC 66031 from the American Type Culture Collection, Manassas, Va., and *Candida dubliniensis* 9709002 from bioMérieux, Marcy l'Etoile, France) plus 477 strains from our stock collections of clinical isolates. The panel included a total of 227 isolates of *C. glabrata* and 255 isolates of 17 other *Candida* spp. (Table 1). The isolates had previously been identified by conventional yeast morphology and assimilation tests. All yeasts were maintained on peptone-glucose agar media.

The yeasts were subcultured by streaking single colonies and were incubated for 24 to 48 h at 30°C on Candida ID agar (bioMérieux, Marcy l'Etoile, France), CandiSelect agar (Bio-Rad, Marnes la Coquette, France), and Sabouraud agar containing chloramphenicol and gentamicin (bioMérieux [referred to as Sab-

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TABLE 1. Positive reactions in rapid trehalase tests with 482 yeast isolates

Species	No. of isolates	No. (%) of positive reactions obtained with yeast colonies grown on the following agars:		
		SAB bio[a]	Candida ID	CandiSelect
C. glabrata	227	223 (98.2)	225 (99.1)	225 (99.1)
Non-*C. glabrata*				
C. tropicalis	66	4	3[b]	9
C. parapsilosis	71	3	0	1
Candida guilliermondii	18	2	0	1
Candida famata	15	0	0	1
Candida lusitaniae	15	1	0	0
Other[c]	70	0	0	0
	255	10 (3.9)	3 (1.2)	12 (4.7)

[a] SAB bio, Sabouraud bioMérieux agar.
[b] Only 13 isolates forming white colonies on Candida ID medium were tested since pink or blue colonies on this medium could not be regarded as possible isolates of *C. glabrata.*
[c] *C. krusei* ($n = 23$), *C. albicans* ($n = 10$), *Candida inconspicua* ($n = 7$), *Candida kefyr* ($n = 7$), *Candida sphaerica* ($n = 5$), *Saccharomyces cerevisiae* ($n = 5$), *Candida norvegensis* ($n = 3$), *Candida lipolytica* ($n = 2$), *Candida pelliculosa* ($n = 2$), *Candida utilis* ($n = 2$), *Candida catenulata* ($n = 1$), *C. dubliniensis* ($n = 1$), *Candida rugosa* ($n = 1$), *C. neoformans* ($n = 1$).

ouraud bioMérieux; this medium contains 2% glucose]). Candida ID agar and CandiSelect agar are chromogenic media on which *C. albicans* colonies develop a characteristic blue color; in addition, Candida ID agar contains a second substrate which allows the classification of several species (but not *C. glabrata*) in a group with pink colonies (5, 9, 26). Thus, for yeasts grown on this medium, only white colonies needed to be tested for trehalase and maltase activities to identify a *C. glabrata* isolate.

Test procedure. For each trehalase test, two glucose reagent strips (Clinistix; Bayer Diagnostics, Puteaux, France) were used. A drop of trehalose solution (1.25 g/liter) was placed on one strip and a drop of water was placed on the other to provide a negative control. In one laboratory, trehalose and maltose were purchased in the form of 2.5-mg tablets (Rosco; Eurobio Laboratory, Ulis, France) and dissolved in 2 ml of distilled water; in the other, trehalose and maltose powders were purchased from Difco Laboratories, Detroit, Mich. Material from one or two identical-looking colonies from the test plates was picked up with a sterile inoculating loop and mixed with the drop of fluid on each test strip. The development of a blue coloration on the trehalose test strip 30 s after mixing and no coloration on the negative control strip was read as a positive result. The development of no coloration or a blue coloration later than 30 s was read as a negative result.

Because a few, rare isolates of species other than *C. glabrata* were found to yield a positive trehalase test result, an additional rapid test with a drop of maltose solution (1.25 mg/liter) in place of trehalose solution was done to distinguish isolates with false-positive results (*C. glabrata* does not react in the maltase test) from other species. This additional test was evaluated with a subset of 255 of the yeasts. The results for isolates of *C. glabrata* that gave positive results by the trehalase test and negative results by the maltase test were regarded as true positives for the presumptive identification of *C. glabrata*. Similar results for other species were regarded as false positive. To compare the results obtained in different laboratories by the rapid trehalase and maltase tests, a subset of 217 of the yeast isolates, including 50 isolates of *C. glabrata*, was retested in two laboratories. In both laboratories the yeasts were grown on Sabouraud bioMérieux agar. In laboratory A the yeasts were grown in parallel on Candida ID and CandiSelect agars; in laboratory B they were grown in parallel on two further Sabouraud agar formulations, namely, Sabouraud Oxoid agar, comprising 1% Mycological Peptone L40 (Oxoid, Basingstoke, United Kingdom), 4% glucose, and 2% agar, and Sabouraud Difco agar, comprising 1% Neopeptone (Difco), 4% glucose, and 2% agar. The operator in both laboratories was blinded to the identity of the yeast being tested. Isolates that gave a positive result by the rapid trehalase tests were subjected to rapid maltase tests as described above.

Finally, 58 isolates of *C. glabrata* were evaluated for their activities in the rapid trehalase test after initial growth on CHROMagar Candida from two different suppliers: CHROMagar Microbiology (Paris, France) and Becton Dickinson (Le Pont de Claix France).

RESULTS

Rapid trehalase testing with full yeast panel. Table 1 summarizes the results of all the trehalase tests done to date with 482 yeast isolates. The distribution of species included in the tests was weighted toward those, apart from *C. glabrata*, that were known to potentially possess rapid trehalase activity. The results in Table 1 show true-positive test results for almost all isolates of *C. glabrata* and a small number of false-positive results for other species. The data indicate sensitivities and specificities for the rapid trehalase test of 98.2 and 96.1%, respectively, for colonies from Sabouraud bioMérieux agar, 99.1 and 99.8%, respectively, for colonies from Candida ID agar, and 99.1 and 95.3%, respectively, for colonies from CandiSelect agar.

Rapid trehalase and maltase testing. The trehalase and maltase tests were both performed with 255 yeast isolates (Table 2). One *C. glabrata* isolate was negative by the trehalase test, and a further isolate gave a false-positive reaction by the maltase test. Among the other species tested, isolates giving a false-positive result by the trehalase test alone seldom gave negative maltase reactions, reducing the overall level of false positivity by the trehalase test. Thus, addition of the maltase test to the trehalase test slightly reduced the sensitivities of the rapid tests, but it increased their specificities for presumptive identification of *C. glabrata* (Table 2).

Rapid trehalase and maltase testing in two laboratories. The results of the tests from two laboratories, both of which used Candida ID agar as a common medium plus two other agar media unique to each laboratory, are shown in Table 3. The results obtained in both laboratories were comparable for

TABLE 2. Results of rapid trehalase and maltase testing with 255 yeast isolates

Species	No. of isolates	No. of positive reactions obtained with yeast colonies grown on the following agars[a]:					
		SAB bio		Candida ID		CandiSelect	
		Tre+	Tre+, Mal−	Tre+	Tre+, Mal−	Tre+	Tre+, Mal−
C. glabrata	65	64[b]	63[c]	64[b]	63[c]	64[b]	63[c]
C. tropicalis	43	3	0	1[c]	0[d]	7	1
C. guilliermondii	18	2	1	0	0	1	1
C. parapsilosis	68	3	2	0	0	1	1
C. famata	15	0	0	0	0	1	0
C. lusitaniae	10	1	0	0	0	0	0
Other[e]	36	0	0	0	0	0	0
Test sensitivity (%)		98.5	96.9	98.5	96.9	98.5	96.9
Test specificity (%)		95.3	98.4	99.5	100	94.7	98.4

[a] Tre+, trehalase test positive; Tre+, Mal−, trehalase test positive, maltase test negative; SAB bio, Sabouraud bioMérieux agar.
[b] One *C. glabrata* isolate gave a negative trehalase test.
[c] One *C. glabrata* strain gave a positive maltase test.
[d] Only 14 isolates forming white colonies on Candida ID medium were tested since pink or blue isolates could not be regarded as possible isolates of *C. glabrata.*
[e] *C. krusei* ($n = 19$); *C. inconspicua* ($n = 7$), *C. norvegensis* ($n = 3$), *C. kefyr* ($n = 2$), *S. cerevisiae* ($n = 2$), *C. lipolytica* ($n = 1$), *C. pelliculosa* ($n = 2$).

TABLE 3. Trehalase and maltase test results for 217 yeast isolates tested in two laboratories[a]

Species	Total	No. of positive reactions obtained with yeast colonies grown on the indicated agar[b]											
		Laboratory A						Laboratory B					
		CandiSelect		Sabouraud bioMérieux		Candida ID		Candida ID		Sabouraud Oxoid		Sabouraud Difco	
		T+M−	T+M+	T+M−	T+M+	T+M−	T+M+	T+M−	T+M+	T+M−	T+M+	T+M−	T+M+
C. glabrata	50	48	1	48	1	48	1	49	1	49	1	49	1
C. famata	15	0	0	0	4	0	0	0	1	0	12	2	13
C. guilliermondii	19	1	2	0	1	1	0	0	4	1	17	1	17
C. inconspicua	7	0	0	0	3	0	0	0	2	0	5	1	5
C. kefyr	2	0	0	0	0	0	0	0	0	1	0	1	0
C. krusei	18	0	0	0	0	0	0	0	0	1	3	10	5
C. lipolytica	1	0	0	0	0	0	0	0	0	0	1	0	1
C. lusitaniae	10	0	0	0	1	0	0	1	1	3	5	5	5
C. norvegensis	3	0	0	0	0	0	0	0	0	0	3	0	3
C. parapsilosis	68	1	2	2	2	0	0	1	2	1	32	5	57
C. tropicalis	24	0	5	0	1	0	5	1	0	11	2	6	6
Test sensitivity (%)		96.0		96.0		96.0		98.0		98.0		98.0	
Test specificity (%)		98.8		98.8		99.4		98.2		89.2		81.4	

[a] Only *C. glabrata* isolates should give positive trehalase test results and negative maltase test results.
[b] T+M−, trehalase positive, maltase negative; T+M+, trehalase positive, maltase positive.

tests done with colonies picked from Candida ID agar and indicated a very high level of sensitivity and specificity for the trehalase and maltase test combination. For colonies from Sabouraud Oxoid and Sabouraud Difco agars, however, the specificity of the test was notably lower; in particular, isolates of *C. tropicalis* grown on both media gave false-positive results, and high frequencies of false-positive results were also seen with isolates of *C. krusei* and *C. parapsilosis* grown on Sabouraud Difco agar.

Rapid trehalase results for yeasts grown on two formulations of CHROMagar Candida. Since previous studies showed that yeast colonies grown on the chromogenic medium CHROMagar Candida were unsuitable for testing by the rapid trehalase test (16), we conducted a pilot evaluation of a novel formulation of this medium from Becton Dickinson for its suitability. In comparative tests with 16 *C. glabrata* isolates grown on the two formulations of this chromogenic medium for 24 h, 4 (25%) grown on the original formulation from CHROMagar Microbiology were positive and 11 (68%) grown on the new formulation from Becton Dickinson were positive. Among 42 isolates grown on CHROMagar Candida for 48 h, 29 (69%) grown on the CHROMagar Microbiology formulation were trehalase positive and 39 (92%) grown on the Becton Dickinson formulation were trehalase positive.

DISCUSSION

This study confirms that the 30-s trehalase test is capable of identifying *C. glabrata* isolates with levels of sensitivity and specificity in excess of 95%. This result is as good as those that Land et al. (11) achieved by longer-duration trehalase tests. Widely used commercial yeast identification systems such as the API 20CAUX and Vitek YBC systems often perform less well than our trehalase test-maltase test combination (8). Multiplex PCR methods offer 100% sensitivity and specificity for identification of *C. glabrata* (4), but they are less rapid and far more expensive than the trehalase test that we have presented here.

Our experiments represent an ongoing study of a test whose fundamental efficacy has already been established (16). Our data represent the findings of rapid trehalase testing for all 482 yeast isolates that we have evaluated since we conducted our earliest tests. The smaller numbers of yeasts included in the prospectively designed evaluations of interlaboratory reproducibility and of additional maltase testing represent the subsets that were readily available for inclusion in these studies. We consider the numbers of yeasts evaluated in all three parts of the study to be adequate to provide a realistic assessment of the value of the rapid tests.

The main problem with the rapid trehalase test concerns contamination with glucose carried over from the agar medium used to grow yeast colonies for the test. The phenomenon is only partly related to the species being tested. In this study, as in our previous investigation (16), culture media containing 4% glucose were prone to generate false-positive results. Often, yeasts from media containing 4% glucose gave color changes in the control strip (no trehalose), giving uninterpretable results. However, this was by no means always the case, and we therefore speculate that media with high levels of glucose may enhance the intrinsic levels of trehalase activity in some *Candida* species.

Although we used maltase activity to confirm the identifications for *C. glabrata* isolates from media with 4% glucose, we consider that the trehalase test should be done routinely only with yeast colonies grown on media containing no more than 2% glucose. The overall sensitivity of the rapid trehalase test for the identification of *C. glabrata* is very high (at least 95%) for colonies picked from such media. Use of the additional rapid maltase test for trehalase-positive yeasts boosts the test specificity above 98%, which is an excellent specificity for any phenotype-based identification test. For yeast identification purposes, test specificity is more important than test sensitivity.

Occasionally missing a *C. glabrata* isolate in the rapid presumptive test is not a serious problem since the missed isolate will later be identified correctly by more elaborate conventional methods. However, misidentifying a yeast isolate might have serious clinical consequences. We therefore recommend routine addition of the maltase test for trehalase-positive yeasts. Lopez et al. (12) similarly recommended a combination of rapid trehalose and sucrose tests for the ideal specificity of identification of *C. glabrata*. However, their test technology differs from ours; in our hands a rapid test for sucrose hydrolysis done in the same way as our maltose test did not yield useful results with a 30- to 60-s incubation time.

An ideal approach to efficient identification of clinical yeast isolates would make use of preliminary presumptive identifications from a polyvalent chromogenic medium such as CHROMagar Candida agar, which would reduce the need for trehalase testing only to those colonies that did not form the colors characteristic of *C. albicans*, *C. krusei*, or *C. tropicalis* (15). However, among the media that we tested in our previous study (16), that medium performed worst of all as a source of colonies for trehalase testing. CHROMagar Candida agar has recently been reformulated by a new supplier (10). Our preliminary tests with the new formulation indicate that pink colonies from this medium (*C. glabrata* forms only pink colonies on CHROMagar Candida agar) now give higher sensitivity and specificity results in our rapid trehalase test, so long as colonies are picked after 48 h of incubation on the new formulation from Becton Dickinson and not after 24 h. These initial findings encourage us to believe that it may be possible to perform rapid trehalase and maltase tests for identification of *C. glabrata* with colonies from this differential isolation medium, but the new formulation first requires full evaluation.

Meanwhile, our results with yeast colonies from CandiSelect agar suggest that clinical laboratories could expect to combine detection of the specific blue *C. albicans* colony color from this medium with a rapid trehalase and maltase result for *C. glabrata* to provide a pragmatic and effective means of identification of the two most prevalent yeast species isolated from clinical samples. Even more effective would be to combine our 30-s trehalase and maltase tests with Candida ID medium as the isolation medium, since the specificity of *C. glabrata* identification is enhanced on this medium by the appearance of *C. glabrata* only as white colonies, not pink colonies (6, 9).

We conclude that the rapid trehalose test works well for the presumptive identification of *C. glabrata*. When supported with the rapid maltase test to confirm the true positivity of trehalase test results, the test offers a useful adjunct to the work of the routine medical mycology laboratory.

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