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Screening Protocol for *Torulopsis* (*Candida*) *glabrata*

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A screening test has been developed for the presumptive identification of *Torulopsis* (*Candida*) *glabrata* from other common clinical isolates of yeast-like fungi. An interlaboratory comparison of a protocol consisting of morphology on cornmeal Tween 80 agar and trehalose fermentation at 42°C was successful in differentiating *T. glabrata* from other taxa that are frequent or possible clinical isolates. The screening results for 517 clinical yeast isolates, 241 of which were *T. glabrata*, were compared with their final identification via commercial systems (API 20C Yeast Identification System [bioMERIEUX, Hazelwood, Mo.] and Rapid Yeast Identification Panel [Dade Microscan, Sacramento, Calif.]). The trehalose screening test has a sensitivity and a specificity of 97.8 and 95.8%, respectively, and a positive predictive value of 97.4% and a negative predictive value of 96.5%. Overall, the trehalose screen had an efficiency rating of 93.9% for ruling in or out *T. glabrata*. Since *T. glabrata* represents a substantial part of the workload in a clinical laboratory, a significant reduction in direct and indirect costs should be realized.

Torulopsis glabrata, a member of the family *Cryptococcaceae*, is an anamorphic, nonfilamentous yeast inhabiting the gastrointestinal and genitourinary tracts of humans and animals (20, 23). Although its possible association with bronchopneumonia was reported in 1937, *T. glabrata* has long been considered a saprophyte because it is most often isolated from stools, skin, mouth, vagina, and improperly collected urine specimens (16, 20, 21). Recently, *T. glabrata* has emerged as an opportunistic pathogen in patients with severe underlying primary diseases and/or undergoing complex therapeutic regimens. It has been the suspected etiologic agent of a variety of infections including meningitis (1), endocarditis (4, 8), cholecystitis (20), osteomyelitis (5, 15), spondylitis (3), hyperplasia and infection of the oral pharynx (6, 18), and fungemia (8, 16, 21). Amphotericin B and/or flucytosine have been the standard therapy for these infections, augmented by surgery when necessary (10, 21). However, a potential therapeutic problem is that some clinical strains of *T. glabrata* readily show in vitro resistance to the synthetic imidazoles, suggesting that imidazole resistance could be an in vivo problem as well (7, 10, 11, 24).

As a result of its frequent association with humans, *T. glabrata* is usually one of the three most common yeasts isolated in the clinical laboratory. Because of its ubiquity, possible clinical relevance, and potential therapeutic problems, it has become necessary to identify *T. glabrata* in a timely and cost-effective fashion. Stockman and Roberts developed a screening test based upon *T. glabrata*'s ability to assimilate trehalose in the presence of cycloheximide within 1 h, when incubated at 37°C (22). The test was in a microtiter format, and by equating bromcresol green reduction with trehalose metabolism and observing the plate coordinates, large numbers of isolates could be surveyed at one time. Although this was an excellent approach, the medium has not been made commercially available, nor have published studies been forthcoming as to its efficacy.

Our goal was to develop a screening test for *T. glabrata* that used commercially available reagents, was cost-effective, and offered a relatively rapid turnaround time. In reviewing two yeast compendia and comparing a number of tests, it became apparent that no one test would work with the effectiveness of the germ tube test (2, 12). However, we felt we could capitalize on a combination of tests consisting of morphology on cornmeal agar and two unique characteristics, namely, fermentation of the nonreducing disaccharide trehalose and growth at 42°C (Table 1) (2, 12). Of 33 yeast taxa considered frequent or possible clinical isolates (23), 5 grow well at 42°C and 10 exhibit strain-dependent or variable growth (Table 1). The concomitant ability of these same yeasts to ferment trehalose in a standard Wickerham broth is variable among seven species (*Candida albicans*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida tropicalis*, *Candida viswanathii*, *Hansenula polymorpha*, and *Saccharomyces cerevisiae*), whereas only *T. glabrata* consistently does both. Since cornmeal morphology or its equivalent was required by the commercial systems already in place in our respective laboratories (API 20C and Dade Microscan's Rapid Yeast Identification Panel), only media for the occasional growth plate, media for trehalose fermentation, and a means to attain 42°C had to be supplied.

Preliminary studies (data not shown) revealed that we could combine growth at elevated temperatures with trehalose fermentation and that either a heating block or an incubator could be used for incubation. Additionally, we found that we could usually use yeasts from primary cultures on chocolate agar, blood agar, or Columbia CNA. Other modifications which may be used include substituting a deep mineral oil overlay for Vaspar and bromthymol blue dye instead of bromcresol purple as an indicator (yeast fermentation broth with bromthymol blue with trehalose with Durham tube and yeast fermentation broth with bromcresol purple with trehalose with Durham tube, respectively [Remel Laboratories, Lenexa, Kans.]).

From this departure point, we developed the following protocol based upon standard procedures (20, 23). Upon isolation of a yeast from a primary clinical specimen, a germ tube test was performed, morphology medium (cornmeal Tween 80 or its equivalent) was inoculated, and when necessary, a plate was streaked for isolation of pure colonies. Growth from pure

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TABLE 1. The ability to grow at 42°C and ferment trehalose for those yeasts considered to be possible clinical isolates and their morphology on cornmeal Tween 80 agar or its equivalent (2, 12, 23) [a]

Organism	Morphology on CMA	42°C growth	Trehalose fermentation
Torulopsis glabrata	MLB, NF	+	+
Blastoschizomyces capitatus	FSN, TH, Ar	+	−
Candida albicans	MLB, S/E PSH, TH	+	V
Candida catenulata	MLB, S/E PSH	−	−
Candida guilliermondii	MLB, F, N to S/E PSH	V	−
Candida kefyr	MLB, F, N to S/E PSH	V	−
Candida krusei	MLB, S/E PSH	V	−
Candida lambica	MLB, S/E PSH	−	−
Candida lipolytica	MLB, F, N to E PSH	−	−
Candida lusitaniae	MLB, F, N to E PSH	V	V
Candida parapsilosis	MLB, S/E PSH	V	V
Candida rugosa	MLB, S/E PSH	−	−
Candida tropicalis	MLB, S/E PSH, TH	+	V
Candida viswanathii	MLB, E PSH, TH	V	V
Candida zeylanoides	MLB, F, N to S/E PSH	−	D
Cryptococcus neoformans	MLB, NF	−	−
Cryptococcus albidus	MLB, NF	−	−
Cryptococcus laurentii	MLB, NF	−	−
Cryptococcus luteolus	MLB, NF	−	−
Cryptococcus terreus	MLB, NF	−	−
Cryptococcus uniguttulatus	MLB, NF	−	−
Debaryomyces spp.	PB, F	−	−
Geotrichum candidum	FSN, TH, Ar	−	−
Hansenula anomala	MLB, F, N to S/E PSH	−	−
Hansenula polymorpha	MLB, NF	+	V
Pichia ohmeri	MLB, E PSH	V	−
Prototheca wickerhamii	EC	−	−
Rhodotorula glutinis	MLB, NF	−	−
Rhodotorula minuta	MLB, NF	−	−
Rhodotorula pilimanae	PB, NF	−	−
Rhodotorula rubra	MLB, NF	−	−
Saccharomyces cerevisiae	MLB, F, N to S PSH	V	V
Torulopsis candida	MLB, F, N or S PSH	−	D
Torulopsis pintolopesii	MLB, F, N or S PSH	V	−
Trichosporon beigelii	FSN, PB, TH, S/E PSH, Ar	V	−
Trichosporon pullulans	FSN, PB, TH, S/E PSH, Ar	−	−

[a] CMA, cornmeal agar; MLB, multiple lateral budding; N, no/none; F, filaments; S, simple; E, elaborate; PSH, pseudohyphae; TH, true or septate hyphae; PB, polar budding; Ar, arthroconidia; EC, endocytoplasmic cleavage; FSN, vegetative reproduction by splitting. Example: MLB, F, N to S/E PSH = multiple lateral budding; filaments, none to simple or elaborate pseudohyphae. V, strain variable; D, delayed fermentation (≥36 h).

cultures of small (3 to 5 μm in diameter), nonfilamentous yeasts were transferred to 7.0-ml Durham yeast fermentation tubes containing bromcresol purple (0.0016%) and 1% trehalose to the density of a 3 or 4 McFarland standard (Remel), overlaid with melted paraffin or Vaspar, and incubated at 42°C for 24 h. For those isolates demonstrating sparse primary growth, the trehalose tube was inoculated from a growth plate on the following day. A positive result was the production of both acid and gas in trehalose broth at the elevated temperature. All yeasts were completely identified via the API 20C or Dade Microscan Yeast Identification Panel commercial system, according to their respective manufacturer's directions, and microscopic morphology was determined at 24 to 48 h.

In a survey of 517 clinical yeast isolates, isolates of *T. glabrata* and *C. tropicalis* were the only taxa that fermented trehalose at 42°C (Table 2). *C. tropicalis*, however, was an inconsistent fermenter as only 5% (4 of 87) strains produced both acid and gas in trehalose at 42°C, while 96% (231 of 241) of *T. glabrata* isolates were positive (Table 2, $P = <0.0001$, chi square with Yates' correction). *T. glabrata* was readily differentiated from *C. tropicalis* by the rapid fermentation of trehalose (8 to 24 h) and the lack of pseudohyphae or filaments on morphology medium. None of the other frequently encountered yeasts with the potential to ferment trehalose appeared capable of doing so at the restrictive temperature. Several taxa demonstrated delayed acid production in trehalose broth at 42°C (≥36 h) but did not produce both acid and gas (Table 2). Some isolates of *S. cerevisiae* bear a slight morphological resemblance to *T. glabrata* and are said to survive elevated temperatures and ferment trehalose (12, 21) and thus have the potential to be confused with *T. glabrata*. However, the 12 isolates that we tested did not appear capable of fermenting trehalose above 37°C. Should a rare strain of *S. cerevisiae* be found capable of fermenting trehalose at the restrictive temperature, it can be differentiated from *T. glabrata* on the basis of size and the inability of the latter to form rudimentary pseudohyphae composed of short chains of elongated budding cells.

The overall sensitivity and specificity for the trehalose screen were 97.8 and 95.8%, respectively. This translated to a positive predictive test value of 97.4% and a negative predictive value of 96.5%, indicating that 93.9% of the time the screen would effectively rule in or rule out *T. glabrata*. These values are well within the range considered acceptable for the *C. albicans* germ tube test (14). In using the trehalose screen for 6 months in a routine clinical microbiology laboratory (Methodist Medical Center), there were three instances (333 total isolates, 0.9%) in which a yeast failing to ferment trehalose at 42°C

TABLE 2. Determination of the ability of clinical yeast isolates to ferment trehalose at 42°C and their concomitant morphology on cornmeal Tween 80 agar[a]

Organism	No. of strains	Morphology on CMA	42°C trehalose[b]	Time positive (h)
Torulopsis (*Candida*) *glabrata*	241	MLB, NF	(231) A/G	8–18
Blastoschizomyces capitatus	5	FSN, TH, Ar	–	–
Candida albicans	31[c]	MLB, S/E PSH, TH	–	–
Candida catenulata	1	MLB, S/E PSH	–	–
Candida guilliermondii	5	MLB, F, N to S/E PSH	–	–
Candida kefyr	1	MLB, F, N to S/E PSH	–	–
Candida krusei	8	MLB, S/E PSH	–	–
Candida lambica	6	MLB, S/E PSH	–	–
Candida lusitaniae	2	MLB, F, N to E PSH	–	–
Candida mogii	2	MLB, PSH/clusters of B	–	–
Candida parapsilosis	24	MLB, S/E PSH	–	–
Candida philyla	2	MLB, NF, NPSH	–	–
Candida rugosa	2	MLB, S/E PSH	(1) A	≥36
Candida stellatoidea	2	MLB, F, N to E PSH	–	–
Candida tropicalis	87	MLB, S/E PSH, TH	(4) A/G	≥36
Candida viswanathii	4	MLB, E PSH, TH	–	–
Candida zeylanoides	3	MLB, F, N to S/E PSH	–	–
Candida spp. not otherwise identified	5	MLB, PSH		
Cryptococcus neoformans	4	MLB, NF	–	–
Cryptococcus albidus	1	MLB, NF	–	–
Cryptococcus laurentii	1	MLB, NF	–	–
Cryptococcus luteolus	2	MLB, NF	–	–
Cryptococcus terreus	1	MLB, NF	–	–
Cryptococcus uniguttulatus	1	MLB, NF	–	–
Debaryomyces spp.	1	PB, F	–	–
Geotrichum candidum	7	FSN, TH, Ar	–	–
Hansenula anomala	6	MLB, F, N to S/E PSH	–	–
Hansenula polymorpha	11	MLB, NF	–	–
Pichia ohmeri	4	MLB, E PSH	–	–
Prototheca wickerhamii	3	EC	–	–
Rhodotorula glutinis	15	MLB, NF	–	–
Rhodotorula minuta	3	MLB, NF	–	–
Rhodotorula pilimanae	1	PB, NF	–	–
Rhodotorula rubra	2	MLB, NF	–	–
Saccharomyces cerevisiae	12	MLB, F, N to S PSH	–	–
Torulopsis candida	2	MLB, F, N or S PSH	(1) A	≥36
Torulopsis pintolopesii	1	MLB, F, N or S PSH	–	–
Trichosporon beigelii	6	FSN, PB, TH, S/E PSH, Ar	–	–
Trichosporon pullulans	1	FSN, PB, TH, S/E PSH, Ar	–	–

[a] CMA, cornmeal agar; MLB, multiple lateral budding; N, no/none; F, filaments; S, simple; E, elaborate; PSH, pseudohyphae; TH, true or septate hyphae; PB, polar budding; Ar, arthroconidia; EC, endocytoplasmic cleavage; FSN, vegetative reproduction by splitting. Example: MLB, F, N to S/E PSH = multiple lateral budding; filaments, none to simple or elaborate pseudohyphae. A, acid production; G, gas production.

[b] Numbers in parentheses reflect the number of isolates positive for trehalose fermentation at 42°C. *T. glabrata* was within 24 h; other yeasts were ≥36 h.

[c] Germ tube-negative *C. albicans* clinical isolates that were referred for further identification.

went on to be identified as *T. glabrata* by API. Each of these strains grew poorly on Sabouraud dextrose agar, the trehalose screening medium, and the API 20C basal medium supplemented with trehalose, 5 to 7 days being taken to identify them as *T. glabrata* in the last. These patients were on cytotoxic therapy for their primary diseases or had finished a course of antifungal therapy for another organism.

Although excellent commercial systems now exist for the relatively rapid identification of yeasts, the cost per identification is high for small- to modest-volume laboratories (≥\$4.00 to ≤\$8.00). The total hands-on time involved in inoculating and reading the commercial panels is also significant. Thus, the major problem of yeast identification in this era of cost containment is not the lack of technical expertise experienced 10 years ago, but rather the cost of reagents and personnel. This challenge has been met by devising screening strategies for the more common yeasts. Precedence for the screening approach was the germ tube test developed by Reynolds and Braude (19), a mainstay for over 40 years in the clinical laboratory. It permits the laboratory to rapidly dismiss approximately 70% of its work at less than \$2.00 per test, and its efficacy is not diluted by the fact that *Candida stellatoidea* (a sucrose-negative variant of *C. albicans*) is also germ tube positive and not all *C. albicans* strains are germ tube positive. A biochemical alternative to the germ tube test appears to be even more sensitive at about the same cost (17). Similar screening tests are available for *Cryptococcus neoformans* (birdseed agar, caffeic acid) and *C. parapsilosis* (β-glucosidase), and rapid urease and nitrate tests have been developed for screening large numbers of respiratory specimens for possible pathogenic yeasts (9, 13, 25).

In summary, *T. glabrata* and *C. tropicalis* were the only yeasts among our clinical isolates that fermented trehalose at 42°C. The nonfilamentous morphology of *T. glabrata* combined with high-temperature trehalose fermentation within 24 h readily differentiated it from *C. tropicalis* and the few other filamentous yeasts that had delayed acid production only. The trehalose screen for *T. glabrata* offers a more rapid and cost-effective alternative to commercial identification systems and takes about one-fifth of the setting-up and reading time of the more expensive panels. An experienced laboratorian can look at the colonial morphology of an unknown yeast on a Columbia CNA agar or blood agar plate, inoculate a trehalose tube and morphology medium, and have the results by the next morning (for some strains, within the same day). This is in contrast to the 3-day incubation period for the API 20C, and while the Dade Microscan Yeast Identification Panel provides an answer in 4 to 6 h, panels are more costly than the tubes (approximately $1.50) and a 24- to 48-h growth plate is always required for the inoculum. Savings in media and time are diluted slightly if a growth plate has to be inoculated, but the overall savings compared with commercial identification systems are still significant. Another advantage of 42°C trehalose fermentation is that it is not used to discriminate between organisms having good likelihood but low selectivity identification or rare biotypes in commercial databases. These are yeast-like fungi that metabolize very few substrates with much strain variability such as *Blastoschizomyces* (*Trichosporon*) *capitatus*, *Candida krusei*, *Candida lambica*, *Hanseniaspora valbyensis*, and *S. cerevisiae* and are incapable of growing at 42°C, fermenting trehalose, or both (Table 1). Considering the data presented herein, we feel that a test consisting of appropriate morphology and fermentation of trehalose at 42°C effectively differentiates *T. glabrata* from other yeasts and has the potential not only to significantly reduce laboratory workload, but also to generate substantial savings in direct and indirect costs to the laboratory.

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