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Evaluation of a New System for the Rapid Identification of Clinically Important Yeasts

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The rapid system developed by Huppert et al. (1975) for the identification of yeasts based on assimilation and fermentation patterns and on germ tube and pseudohyphal production was evaluated in a comparative study with conventional procedures. The 95 test cultures were members of the genera *Candida*, *Cryptococcus*, *Rhodotorula*, *Saccharomyces*, *Torulopsis*, and *Trichosporon*. The study revealed that approximately 94% of the isolates were correctly identified by the rapid method in comparison with the standard method. With the rapid method identification was accomplished in 72 h, and with the conventional procedures identification was completed in 2 weeks. Although it was difficult with some isolates to obtain definitive speciation by the rapid method, this method promises to be especially useful in clinical laboratories for the identification of yeasts of medical importance. Modifications were made in the procedure of Huppert et al. (1975) to improve the reading of reactions. Commercial media and a disk dispenser to make the method more useful were also investigated.

In recent years a marked increase in the prevalence and incidence of opportunistic yeast infections has occurred among compromised patients throughout the world (3, 6, 7). This phenomenon has made it imperative for diagnostic laboratories to isolate and identify the yeasts found in clinical materials. However, conventional procedures based on the methods of Wickerham (8) for assimilation and fermentation are sophisticated and time consuming (7). The pressing need for simplicity and rapidity in identification procedures has resulted in attempts to develop new tests (P. Bowman and D. G. Ahearn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, F42, p. 92; R. G. Ferrigno, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, F56, p. 94; J. P. Gayrod, R. Gillot, and K. M. Tomfohrde, Abtr. Annu. Meet. Am. Soc. Microbiol. 1975, F54, p. 94; J. G. Newby, S. S. Shadomy, and M. Motley, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, F55, p. 94; 2, 4, 5). Among these, the rapid test of Huppert et al. (4) appeared to be highly suitable for adoption by diagnostic laboratories with limitations in staff and resources that prevent them from undertaking the identification of yeasts by conventional procedures.

Since Huppert et al. (4) had used a single isolate only of each species in a group of previously identified yeasts from several culture collections, it was felt advisable to determine the effectiveness of the procedure in comparison with a standard procedure. Multiple cultures of yeast species recently isolated from clinical material were used in the evaluation. The results are presented here.

MATERIALS AND METHODS

Test cultures. Two groups of yeast cultures were used in the evaluation (Table 1). One group, which served as a control, was made up of 58 previously identified isolates from our culture collection. These represented 16 species classified in six genera. The second group consisted of a series of 37 isolates received as unknowns by the Fungus Diagnostic Branch, Mycology Division, Center for Disease Control.

Parallel tests were conducted on the isolates in both groups by the rapid system and the standard procedures routinely used in the Fungus Diagnostic Branch (1, 8).

Carbohydrate and nitrogen assimilation tests. The rapid tests were performed as described by Huppert et al. (4). This involved the use of a basal agar medium (containing phosphate buffer, bromothymol blue, and agar) and of filter paper disks impregnated in the laboratory with 12 reagent-grade carbohydrates (glucose, maltose, sucrose, inositol, lactose, cellobiose, raffinose, melibiose, erythritol, xylose, trehalose, and dulcitol), KNO_3, and $(NH_4)_2SO_4$. Solidified basal agar plates were streaked with a cotton swab that had been saturated with cell suspensions of the test yeasts. Carbohydrate and nitrogen disks were then positioned on the plates, and the plates were incubated at 25°C for 24 to 72 h.

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TABLE 1. *Identification of 95 yeast cultures (19 species) by a newly described rapid system (≤ 72 hr)*

Identity by conventional methods (10 to 14 days)	No. of isolates[a]		Total no. of isolates
	A	B	
Candida albicans	11 (0)	11 (0)	22 (0)
C. guilliermondii	8 (1)[b]	2 (0)	10 (1)
C. krusei	4 (0)	1 (0)	5 (0)
C. norvegensis	1 (0)[b]		1 (0)
C. parapsilosis	5 (0)	5 (0)	10 (0)
C. pseudotropicalis	1 (0)		1 (0)
C. rugosa	1 (1)[b]		1 (1)
C. stellatoidea		1 (0)	1 (0)
C. tropicalis	13 (0)	3 (1)[b]	16 (1)
Cryptococcus albidus		2 (0)	2 (0)
C. laurentii	1 (0)		1 (0)
C. neoformans	2 (0)	6 (0)	8 (0)
Rhodotorula rubra	1 (0)		1 (0)
R. species	1 (0)	2 (0)	3 (0)
Saccharomyces species		2 (0)	2 (0)
Torulopsis candida	2 (0)	1 (1)[b]	3 (1)
T. glabrata	5 (0)	1 (0)	6 (0)
Trichosporon capitatium	1 (1)		1 (1)
T. cutaneum	1 (0)	1 (0)	2 (0)
Total	58 (3)	38 (2)	96 (5)

[a] (A) Conventional procedures performed concurrently but independently by the Fungus Reference Branch. (B) Previously identified by conventional methods; results unknown to laboratory performing rapid system. Values in parentheses are the number of isolates identified incorrectly by the rapid system.

[b] Identified as to genera by morphology; biochemical properties, as determined by the rapid procedure, did not match any of the species.

For comparative purposes the Wickerham liquid medium assimilation procedure with mechanical agitation and incubation at 25°C for 7 to 12 days was used with the same battery of carbohydrate and inorganic nitrogen compounds (1, 8).

Fermentation, urease, germ tube, and mycelium-chlamydospore production tests. For the rapid method, fermentation and urease production tests were performed with commercially available fermentation (glucose, maltose, sucrose) and urease tablets (Key Scientific Products, Los Angeles, Calif.) as described by Huppert et al. (4). Liquid fermentation and urease production tests, as performed in the Fungus Diagnostic Branch, were used as controls. The fermentation tests consisted of liquid-sugar fermentation tubes inoculated with yeast cells grown and starved on yeast nitrogen base (Difco Laboratories, Detroit, Mich.) and incubated at 25°C for 7 days, whereas urease production was tested on Christensen urea medium incubated for 7 days at 25°C. Sterile bovine serum was used for germ tube formation. Homemade cornmeal agar supplemented with Tween 80 was used for mycelium and chlamydospore production for the rapid method. The procedure that is performed in the Fungus Diagnostic Branch and that requires the use of oxgall and cornmeal-Tween agar was also used for comparative purposes.

Inoculum. A dense cell suspension that gave a 3+ reaction with a Wickerham card was prepared by harvesting, in 8 ml of sterile water, the growth from cultures on a yeast-malt agar plate. This suspension served as the inoculum for carbohydrate and nitrogen assimilation, fermentation, and urease production tests in the rapid method. The serum for germ tube formation and the cornmeal-Tween 80 agar for mycelium-chlamydospore production were inoculated directly from the yeast-malt agar plates. The inocula used for the standard procedures were identical to those described for the Wickerham method (8).

Dispenser. In an effort to streamline the rapid system, commercially prepared disks, impregnated with the basal medium and the assimilation compounds, were evaluated along with a mechanical disk dispenser.

RESULTS AND DISCUSSION

In tests with 58 yeasts, previously identified by standard procedures (see Table 1), the rapid method demonstrated about 93% accuracy in comparison with the standard procedures. By the rapid method, one isolate of *Candida guilliermondii* and single isolates of *C. norvegensis*, *C. rugosa*, and *Trichosporon capitatum* could be identified only as to genus on the basis of morphology (Table 1), since they failed to assimilate some carbohydrates (cellobiose or xylose) and thus did not fit into the diagnostic pattern of the rapid system. Furthermore, four other isolates of *C. guilliermondii*, which could be identified by using one of the identification charts of the rapid method, failed to assimilate melibiose in the time limits of the method (up to 72 h). This indicated a limitation of the technique due to the long (greater than 72 h) induction period required by some yeasts for the expression of certain enzymes. With the rapid method, 28 of the 58 isolates were identified 24 h after the tests were begun, 12 were identified after 48 h, and 18 others were identified after 72 h. In contrast, the standard procedure required 10 to 14 days for identification. The majority of the isolates (23/28) that were identified within 24 h were *Candida* species (five *C. albicans*, three *C. krusei*, three *C. parapsilosis*, and twelve *C. tropicalis*); the others were *Torulopsis candida* (one isolate), *T. glabrata* (three isolates), and *Trichosporon cutaneum* (one isolate). These preliminary tests demonstrated that the rapid method not only gave quick re-

sults but that it had a high specificity. Limitations, however, were revealed in regard to the speciation of the less common yeasts.

The results obtained with 38 other isolates, which were concurrently undergoing conventional and rapid identification procedures in independent laboratories, are also presented in Table 1 (column B). By the rapid method 36 of the 38 unknowns (94%) were identified, but with the standard procedure all of the 38 unknowns were identified. The two isolates that were not identifiable by the rapid method were a *C. tropicalis* culture and an isolate of *T. candida*. The rapid procedure gave an assimilation pattern compatible for the latter species. However, the sucrose fermentation test was negative instead of positive. The culture thus could not be identified as *C. tropicalis*. By the procedure of Huppert et al., this isolate would have been identified as *C. albicans* despite a negative germ tube test, since some *C. albicans* isolates fail to form germ tubes.

With the rapid procedure 20 of the 38 isolates were identified within 24 h. Of the remaining 18 isolates, 12 were identified in 48 h, and 6 were identified by 72 h. In contrast, by the standard procedure, 14 days were required for identification of the fungi.

During the study we found that some of the growth zones around the carbohydrate disks overlapped and presented interpretation problems. Acting on the assumption that a reduction in the diffusion rate of the carbohydrates would eliminate this problem, we prepared two media with the agar concentrations in the basal medium raised to 2.5% and 3%. These media did reduce the size of the diffusion areas. However, the 3% agar also slowed down the assimilation rates of the yeasts. The 2.5% agar concentration gave zones that were well demarcated and that did not overlap adjacent growth zones. In addition, the assimilation reactions remained within the time limits of the original medium, i.e., 24 to 72 h. The 2.5% agar concentration gave reactions that were easily read, an important consideration for inexperienced personnel.

In addition, the basal medium and assimilation disks were prepared on an experimental basis by Difco Laboratories. Thirty of the isolates were tested with these products. These proved to be just as effective as our laboratory-prepared media and disks. Preliminary tests were also carried out with a mechanical disk dispenser. However, technical problems were encountered that could not be resolved, and the use of the dispenser was discontinued.

In summary, in spite of limitations in the rapid system in regard to definitive speciation of some isolates, the procedure promises to be of value in clinical laboratories. It is simple to use and significantly reduces the time required to identify the yeasts of medical importance. The isolates for which only presumptive speciation is obtained can be retested by the classic methods without delaying the immediate presumptive clinical diagnosis. The modifications that we made, namely, the use of a higher agar concentration and commercially produced assimilation disks, increased the usefulness of the method.

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