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Identification of Clinical Yeast Isolates by Using the Microring YT

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The Microring YT (MYT; Medical Wire & Equipment Co., Victory Gardens, N.J.) is a system for the rapid (24 to 48 h) identification of yeasts. The MYT system was evaluated and compared with the API20C (Analytab Products, Plainview, N.Y.) system for its ability to identify 677 clinical yeast isolates. Only 458 isolates (68%) were correctly identified by the MYT system, and the accuracy of the system varied considerably (0 to 96%), depending on the species. While MYT was less expensive and convenient to use and results were available 24 h sooner, it is inadequate for identification of many commonly isolated yeasts and is not designed for the identification of *Cryptococcus* species.

In recent years, the incidence of serious infections caused by yeasts has increased significantly, particularly in immunocompromised patients (1, 3, 7). This has placed tremendous pressure on clinical laboratories to accurately identify to the species level a wide variety of medically important yeasts. Because of this, much effort has been devoted to developing rapid and accurate methods designed for use by diagnostic laboratories for the identification of yeasts. Recently, the Microring YT system (MYT; Medical Wire & Equipment Co., Victory Gardens, N.J.) has been introduced as a less expensive and more rapid method for identifying common yeast isolates other than *Cryptococcus* species. Here we report results of a comparison of the MYT system with the API 20C yeast identification system (Analytab Products, Plainview, N.Y.) performed with a large number of clinical isolates representing a wide variety of species. The API 20C system was chosen as the reference standard because it is the commercial system most widely used by clinical laboratories to identify yeasts and because it has achieved a 97% correlation with conventional identification methods (2, 4).

(This study was presented in part at the 92nd General Meeting of the American Society for Microbiology, New Orleans, La., 26 to 30 May 1992.)

MATERIALS AND METHODS

Organisms. A total of 677 yeast isolates were obtained from clinical specimens submitted to the clinical laboratories of Children's Hospital of Philadelphia, St. Christopher's Hospital for Children, and SmithKline Beecham. These represented 21 species classified in the following seven genera: *Candida*, *Geotrichum*, *Hansenula*, *Rhodotorula*, *Saccharomyces*, *Torulopsis*, and *Trichosporon*. All isolates were initially identified by the API 20C system, which was used as the reference method. Isolates were then independently coded and distributed to one of the authors for testing. The identities of the isolates were unknown until the final data were examined. Isolates were maintained at 25°C on Trypticase soy agar slants (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) for the duration of the study and were subcultured onto Sabouraud dextrose agar (SDA) plates (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.) to confirm their purity before being tested by the two identification systems. Quality control organisms recommended by each manufacturer were tested to ensure adequate system performance. These included *Candida tropicalis* ATCC 750, *Pseudomonas aeruginosa* ATCC 27853, *Cryptococcus laurentii* ATCC 18803, and *Blastoschizomyces capitatus* ATCC 10663.

API 20C system. Tests with the API 20C system were performed as directed by the manufacturer (Analytab Products), and results were recorded after 72 h of incubation at 30°C. Kits were stored at 4°C and were brought to room temperature prior to use. A profile number was generated for each isolate, and an identification was made by comparison of the unknown profile number with those listed in the manufacturer's analytical profile index. Appropriate supplemental tests were performed if needed to achieve an "excellent" or "very good" identification rating with the system's data base. Supplemental tests included microscopic morphology on cornmeal-Tween 80 agar, temperature tolerance, carbohydrate fermentations, urease production, capsule detection by India ink, germ tube formation, and nitrate reduction. The Analytab Products computer service was consulted for those profile numbers not listed in the profile index.

MYT system. Test isolates were initially subcultured onto an SDA plate and were incubated for 48 h at 37°C. A suspension equivalent to a no. 2 McFarland standard was made using 3 ml of sterile water and spread with a swab over the surface of an SDA plate. The suspension was examined microscopically to check for purity and microscopic morphology. Once the plate surface was dry, a hexagonal Microring was centered on the plate and was pressed securely onto the surface. Microring tips numbered 1 to 6 were impregnated with the following chemicals: Janus green, ethidium bromide, triphenyl tetrazolium chloride, brilliant green, cycloheximide, and rhodamine 6G. Plates were incubated at 37°C. After 24 h of incubation, the zones of growth inhibition around each tip were measured. Plates were reincubated for an additional 24 h, and pigment formation, regrowth within original inhibition zones, rough colony morphology, and the formation of color around tip 3 (tri-

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TABLE 1. Yeast identification results with MYT

Isolate	No. (%) of isolates				
	Total	Correct	Incorrect	Choice[a]	No code
Blastoschizomyces capitatus[b]	1	0	1	0	0
Candida albicans	195	187	3	0	5
Candida famata	4	3	0	0	1
Candida guilliermondii	7	0	6	1	0
Candida krusei	6	5	0	1	0
Candida lipolytica	2	0	2	0	0
Candida lusitaniae	17	5	6	4	2
Candida parapsilosis	123	44	47	28	4
Candida paratropicalis	3	0	3	0	0
Candida pseudotropicalis	3	2	1	0	0
Candida rugosa	4	0	4	0	0
Candida tropicalis	151	112	12	23	4
Candida zeylanoides	2	0	1	0	1
Geotrichum sp.	1	0	1	0	0
Hansenula sp.	1	0	1	0	0
Rhodotorula apili	1	0	1	0	0
Rhodotorula glutinis	2	0	2	0	0
Rhodotorula rubra	9	2	7	0	0
Saccharomyces cerevisiae	13	2	10	1	0
Torulopsis glabrata	104	95	8	0	1
Trichosporon beigelii	28	1	22	0	5
Total	677	458 (67.6)	138 (20.4)	58 (8.6)	23 (3.4)

[a] Two or more possible choices, one of which was the correct identification.
[b] Formerly *Trichosporon capitatum*.

phenyl tetrazolium chloride) were noted. If an isolate was noted to grow poorly or failed to grow at 37°C, no recommendation was suggested by the manufacturer. In such cases, the test was repeated at 30°C. Reactions were scored for each tip, and a six-digit code number was generated for each isolate. Code numbers were then compared with a data base supplied by the manufacturer. In some cases, the code number generated matched with multiple organisms. In these cases, the exact inhibition zone size for specific tips, the presence of regrowth at 48 h within previous zones of inhibition, and coloration of the growth were used to discriminate between different species, as directed by the manufacturer.

RESULTS

Table 1 shows the total number of each of the yeast species tested and the number correctly or incorrectly identified, the number where a choice of two or more organisms was listed in the manufacturer's analytical profile index but with no differentiating test, and the number that yielded a profile number not present in the data base. MYT gave correct identifications for 458 of 677 (68%) of the isolates. The species most likely to be identified correctly by this system were *Candida albicans* (187 of 195; 96%), *Torulopsis glabrata* (95 of 104; 91%), *Candida krusei* (5 of 6; 83%), and *Candida famata* (3 of 4; 75%). The MYT system incorrectly identified 138 of 677 (20.4%) of the isolates. The MYT system had the most difficulty correctly identifying *Candida guilliermondii* (0 of 7; 0% correct), *Candida lusitaniae* (5 of 17; 29%), *Candida parapsilosis* (44 of 123; 36%), *Rhodotorula* spp. (2 of 12; 18%), *Saccharomyces cerevisiae* (2 of 13; 15%), and *Trichosporon beigelii* (1 of 28; 3.6%). Of the yeast isolates which were incorrectly identified, the organisms most commonly mistaken with each other were *C. parapsilosis* with various other *Candida* species, *T. beigelii* with *Geotrichum* or *Candida* spp., and *S. cerevisiae* with *Candida* spp. The most common misidentifications are listed in Table 2.

With 58 of 677 (8.6%) of the isolates, the profile number generated by MYT yielded two or more possible answers, one of which was correct. This occurred most often with *C. parapsilosis* (28 of 123; 23%) and *C. tropicalis* (23 of 151; 13%). In all such cases, the differentiating characteristic offered by the data base was either not present or inconclusive, making a correct identification impossible. Isolates which yielded profile numbers not present in the data base included *C. albicans* (n = 5 isolates), *C. famata* (n = 1), *C. lusitaniae* (n = 2), *C. parapsilosis* (n = 4), *C. tropicalis* (n = 4), *Candida zeylanoides* (n = 1), *T. glabrata* (n = 1), and *T. beigelii* (n = 5). All 12 *Rhodotorula* isolates and 30 of 104 (29%) *T. glabrata* isolates failed to grow within 24 h on test plates incubated at 37°C. They did, however, yield a readable profile when incubated at 30°C.

TABLE 2. Most common misidentifications with MYT

Isolate (no.)	Identification by MYT (no. of isolates)
C. guilliermondii (6)	*C. krusei* (4), *C. famata* (1), *T. glabrata* (1)
C. lusitaniae (6)	*C. krusei* (2), *C. famata* (1), *C. guilliermondii* (1), *C. parapsilosis* (1), *T. glabrata* (1)
C. parapsilosis (47)	*C. guilliermondii* (16), *C. famata* (12), *C. krusei* (7), *C. tropicalis* (4), *C. lusitaniae* (2), *C. rugosa* (2), *S. cerevisiae* (1), *C. albicans* (1)
S. cerevisiae (10)	*T. glabrata* (7), *C. lusitaniae* (1), *C. rugosa* (1), *C. parapsilosis* (1)
T. beigelii (22)	*Geotrichum* sp. (9), *C. famata* (7), *C. pseudotropicalis* (3), *C. krusei* (1), *C. tropicalis* (1), *T. glabrata* (1)

DISCUSSION

Compared with the API 20C system, the MYT system was a rapid and simple method to use for identifying yeasts. With the MYT system, results were available approximately 24 h sooner than they were with the API 20C system and the MYT system required minimal technical proficiency. The procedure was similar to that of the disk diffusion test performed in most clinical laboratories. The faster time that results were obtained, however, did not outweigh the overall poor performance of the system. Interpretation of results was quite subjective, particularly when reading extremely small zones of inhibition or reading for the presence of regrowth within a previous zone of inhibition when the plates were read at 48 h. All results in this study were read by two microbiologists, and even with experience, interpretation of results was often difficult. The cost of the MYT system was $2.70 per isolate ($1.80 per ring plus $0.90 per SDA plate), compared with $3.90 per isolate with the API 20C system.

The present study represents the largest evaluation of the MYT system in terms of number of isolates tested ($n = 677$) and tested the widest range of species thus far examined in a single study. In the present study, 68% of the clinical isolates were correctly identified. In addition, 20.4% were misidentified and 12% could not be identified. Two previously published evaluations of the MYT system used 142 (6) and 355 (5) clinical yeast isolates. Their results were similar to ours in that they found that 52.8 (6) and 72.6% (5) of their isolates were correctly identified.

The MYT system was best at identifying *C. albicans* (96%) and *T. glabrata* (91%) but was unable to accurately identify other very common clinical isolates such as *C. guilliermondii* (0%), *T. beigelii* (3.6%), *S. cerevisiae* (15%), *Rhodotorula* spp. (17%), *C. lusitaniae* (29%), and *C. parapsilosis* (36%). Failure to accurately identify these species was a major problem. Particularly noteworthy was the fact that none of the 7 *C. guilliermondii* and only 1 of 28 *T. beigelii* isolates was correctly identified. Isolates which were incorrectly identified by the MYT system were easily identified by the standard API 20C system, and supplemental tests were not required for any of these isolates. At the time of the study, *C. zeylanoides*, *C. paratropicalis*, *Rhodotorula glutinis*, *Rhodotorula apili*, and *Blastoschizomyces capitatus* were not included in the data base of the MYT system. In addition, the study revealed profile numbers for *C. albicans*, *C. famata*, *C. lusitaniae*, *C. parapsilosis*, *C. tropicalis*, *T. glabrata*, and *T. beigelii* which were also not present in the data base. The present MYT system data base is too limited and needs to be more extensive.

The manufacturer recommends that MYT system test plates be incubated at 37°C. Following these instructions, 42 isolates failed to grow sufficiently for readings to be done at 24 h. All 42 of the isolates grew well when incubated at 30°C, but insufficient data are available to allow us to know whether this should be the preferred incubation temperature for all isolates.

In conclusion, the identification of yeasts soley on the basis of the limited number of tests offered in the MYT system was not reliable. It may be possible to improve the overall sensitivity of the test if it were combined with determination of microscopic morphology features on cornmeal-Tween 80 agar. Many of the organisms which yielded identical profile numbers by the MYT system could undoubtedly be distinguished on the basis of such features. In its present format, MYT is not a complete system for a clinical laboratory. The inability of the system to handle such medically important yeasts as *Cryptococcus neoformans*, *Cryptococcus laurentii*, and *Cryptococcus albidus* is unfortunate. While the MYT system stresses the importance of rapid yeast identification, the need to determine the presence of *C. neoformans* in a clinical specimen cannot be emphasized enough. The inability to identify *Cryptococcus* spp., a limited data base, and inaccurate identification of many commonly isolated yeasts limit the usefulness of the MYT system in clinical settings.

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