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Evaluation of the Baxter-MicroScan 4-Hour Enzyme-Based Yeast Identification System

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A new 4-h Yeast Identification Panel (YIP; Baxter-MicroScan, W. Sacramento, Calif.) was compared with the API 20C Yeast Identification System (Analytab Products, Inc., Plainview, N.Y.) in the identification of recent clinical yeast isolates. The YIP had a 94% correlation (288 of 306) in identifying 22 species within the genera *Candida*, *Hansenula*, *Pichia*, *Rhodotorula*, *Saccharomyces*, and *Torulopsis*. Correlation dropped to 65% for those species within the genera of slower growing yeasts, i.e., *Blastoschizomyces* spp., *Crpytococcus* spp., *Geotrichum* spp., *Hyphopichia* spp., *Phaeococcomyces* spp., *Prototheca* spp., and *Trichosporon* spp. Overall correlation with the API 20C was 92% (365 of 401) for those taxa included in the data base and 85% (373 of 437) for all yeasts encountered in the study. There were 36 (8.2%) discrepant identifications, which were due in part to the limited data base. Expansion of the data base plus the easy inoculation, reading, and rapid results of the YIP should make it an excellent method for yeast identification.

Opportunistic fungal infections are not uncommon sequelae in the management of immunocompromised patients (10, 13, 24, 25). For this reason, a great deal of effort has been made in the development of rapid methods for the diagnosis of these mycoses and the identification of their etiologic agents (14, 15). Although both moulds and yeasts may cause infections, commercial yeast identification systems have proceeded at a much faster pace than those for mould identification. Reasons for this disparity are severalfold. (i) The single-cell nature of the majority of yeasts lends itself to organized and miniaturized systems similar to those used in clinical bacteriology. (ii) Yeasts, in general, grow at a much faster rate than moulds, which facilitates rapid analysis of their growth patterns on a variety of substrates. (iii) Yeasts isolated from these infections have demonstrated various degrees of in vitro resistance to common antifungal agents, making their correct identity an important aspect of therapeutic planning (1, 9, 17, 26).

The implication that there may be a causal relationship between identity and therapy can best be illustrated in the recent emergence of two yeasts as opportunistic human pathogens. *Candida lusitaniae* is biochemically very similar to several other medically important *Candida* spp., including *Candida parapsilosis* (17). However, unlike *C. parapsilosis*, *C. lusitaniae* commonly demonstrates constitutive resistance to amphotericin B (6, 17, 19). Sucrose-negative variants of *Candida tropicalis* (designated *C. tropicalis*-SN or *Candida paratropicalis*) appear to exist only in human beings (1, 22). Both *C. tropicalis* and its sucrose-negative variants have been associated with severe infections in immunosuppressed patients (1, 26). Hence, the identification of either type of *C. tropicalis* from normally sterile tissue suggests that an aggressive approach to therapy should be used.

To assist in the rapid identification of medically important yeasts that have only subtle metabolic differences compared with less virulent taxa, a number of commercial yeast identification systems have been developed (11, 14). These identification systems may be divided into two groups: growth-based systems and enzyme-based systems. The former approach requires 1 to several days growth to determine a yeast's ability to assimilate a variety of substrates. Assimilation data are then analyzed and used in conjunction with morphology data to determine the identity of an organism. Some systems use carbohydrate assimilation patterns to develop profile numbers (biocodes). Identifications are subsequently made by comparing an unknown's biocode with those in a reference data base.

A recent advancement is the development of direct enzyme detection-based identification systems. These systems regard a yeast as a source of preformed enzymes, and physiologically optimal substrates are provided to determine the yeast's enzymatic profile. By using a heavy inoculum and chromogens to detect enzymatic reactions, growth is no longer needed as an endpoint. Inoculated panels are usually incubated at 37°C for approximately 4 h, and results are read as chromophore production or shifts in pH indicators. Enzyme data are interpreted in much the same manner as for growth-based systems, and either technology (growth or enzyme based) is amenable to automation.

In the study reported herein, the most recent enzyme-based system, the Baxter-MicroScan Yeast Identification Panel (YIP), is compared with the most widely used commercial growth-based system, the Analytab Products, Inc. (Plainview, N.Y.), Yeast Identification System (API 20C).

MATERIALS AND METHODS

Organisms. Four hundred and thirty-seven recent clinical isolates of yeasts and yeastlike fungi were used in this study. Isolates were obtained from the laboratories at Methodist Medical Center, Dallas, Tex., the New York State Department of Health, Albany, and the University of Texas Medical Branch, Galveston. Quality control isolates included American Type Culture Collection type cultures and quality

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control organisms supplied by the manufacturer (cultures designated AmMS). They are as follows: *Blastoschizomyces capitatus* ATCC 28576, *Candida albicans* ATCC 14053, *C. albicans* AmMS 225 B1013-30, *Candida pseudotropicalis* AmMS 226 B1013-21, *C. tropicalis* AmMS 227 B1013-22, *Cryptococcus albidus* AmMS 228 B1013-23, *Cryptococcus laurentii* ATCC 18803, *Cryptococcus neoformans* AmMS 229 B1013-24, *Cryptococcus uniguttulatus* AmMS 234 B1013-26, and *Torulopsis glabrata* AmMS 231 B1013-27. Yeasts were maintained on Sabouraud dextrose agar plates or slants (Emmon's modification) at room temperature until testing was completed. Experiments were conducted as per manufacturer's directions using 18- to 48-h-old isolates obtained from primary Sabouraud cultures following confirmation of their purity.

API 20C yeast system. Kits were stored at 4°C prior to use, and all tests, including microscopic morphology, were performed according to manufacturer's directions (Analytab Products) and as previously described (12, 15). Briefly, ampoules of the API 20C basal medium were melted and allowed to cool to approximately 45°C before inoculation. A 1+ Wickerham suspension of the unidentified isolate was made by touching several well-isolated colonies with the tip of a sterile applicator stick and emulsifying the yeast in the cooled basal medium. Each cupule of the dehydrated API 20C strip was filled with the agar suspension. Care was taken not to overfill the wells, to permit agar from several wells to become confluent, or to cross contaminate the substrates by pipetting back and forth between wells. Positive reactions were recorded at 72 h by comparison to both a positive and negative control, and an analytical profile number (biocode) was derived from the results. Identifications were made by comparing biocodes of the unknowns with those listed in the manufacturer's analytical profile index. The API computer service was consulted for those biocodes not listed in the profile index. An identification was accepted if listed by either of the above as excellent, good, or acceptable. Isolates which had a low probability or selectivity were confirmed by additional conventional tests, which included carbohydrate fermentation profiles, assimilation of carbohydrates not on the strip, and testing for the presence of urease and nitrate reductase.

YIP. Swab sweeps of an unidentified yeast grown on Sabouraud dextrose agar were suspended to a density of a McFarland standard of 5 in sterile water and vortex mixed to homogeneity. Filamentous or mucilaginous isolates were disrupted and mixed via a sterile, disposable tissue homogenizer (no. 3500; Sage Products Inc., Cary, Ill.). Aliquots (50 μl) were inoculated by an automatic pipette outfitted with a sterile tip into each of 27 dehydrated substrates contained in a microtiter tray. Panels were stacked in groups of four or five, covered, and incubated at 35 to 37°C for 4 h in a non-CO_2 incubator.

The appearance of chromophores released by enzymatic hydrolysis of amino acid β-naphthylamides or nitrophenyl-linked carbohydrates was detected by the addition of either *p*-dimethylaminocinnamaldehyde or 0.05 N NaOH, respectively, to the appropriate wells (Table 1). The assimilation of sucrose and trehalose was determined by a visible acidic shift in the chlorophenol pH indicator. An insoluble blue to blue-gray precipitate indicated the presence of indoxyl phosphatase, and urease was detected by an alkaline shift in a pH indicator as urea was split to form ammonia and CO_2. Test results were converted to a nine-digit number (biotype) and compared with those listed in the manufacturer's printed database. Codes not in the biotype index were evaluated by the manufacturer's computer reference service.

Discrepant identifications. All yeasts with conflicting identifications, i.e., YIP identifications with less than an 85% confidence level or whose YIP code was not in the data base, were retested in both systems. After being retested, those yeasts having discrepant results were identified by conventional assimiliation and fermentation tests and by Dalmau mount to determine microscopic morphology on cornmeal agar (19). Those isolates whose identities remained in doubt when compared with the reference methods were considered misidentified.

***C. neoformans* serotyping.** To determine the correlation between YIP identification and serotype, 15 isolates from each of the four serogroups of *C. neoformans* were identified by both systems. Serotyping of each strain was confirmed by reaction with serogroup-specific *C. neoformans* latex antibody conjugates as previously described (2).

RESULTS

The Baxter-MicroScan YIP had an 85.4% (373 of 437) correlation with the API 20C for all yeasts tested (Tables 2 and 3). Excluding uncommon taxa or biocodes that were not in the data base (33 of 437), correlation improved to 92%. There were 36 (8.2%) YIP misidentifications and 1 (0.2%) discrepant API result. The latter was a white yeast, identified as *Rhodotorula glutinis* by the API 20C, whose actual identity was confirmed as *C. lusitaniae* by both the YIP and reference procedures.

In order to determine whether there was a correlation between yeast metabolism and misidentifications by YIP, organisms were separated into two groups on the basis of their growth rates on Sabouraud dextrose agar. There was a 94% correlation between both systems for rapid growers (group 1) and 98% (300 of 306) when organisms and biocodes not in the data base were excluded (Table 2). *Candida guilliermondii* was the most inconsistently identified group 1 organism. Two strains were misidentified, and the biocodes of two others were not in the data base. Although the YIP had an 87% correlation with API for strains of *C. lusitaniae*, there were some problem isolates. Two isolates had the same biocode as *C. albicans*, and one other was identified with less than an 85% confidence level.

Slower growing yeasts (group 2) posed the greatest challenge to the YIP system (Table 3). There was a 65% (85 of 131) correlation with API 20C, but it was only 68% when unrecognized biocodes and taxa were excluded. The YIP had the greatest difficulty identifying the highly filamentous yeastlike fungi and *C. neoformans*. Only four of nine *Trichosporon beigelii* biocodes appeared in the published compendium. The computer service provided additional information which improved identification to seven of nine isolates. Similar difficulties were noted in identifying *Blastoschizomyces capitatus* and *Geotrichum* spp.

The YIP correctly identified 55 of 80 (69%) random clinical isolates of *C. neoformans* (Table 3) and 40 of 60 (67%) known serotypes (Table 4). There was one incorrect identification (one strain of serotype A), and 19 codes were not in the data base. Serogroup D biocodes had the fewest correct biocodes in the data base (4 of 15), whereas serogroups B and C, *C. neoformans* var. *gattii*, had the most correct biotypes, 25 of 30 (83%).

TABLE 1. Substrates, reagents, and interpretation of reactions of the Baxter-MicroScan YIP

Substrate	Abbreviation	Reagent or directions	Color reaction	
			Positive	Negative
Hydroxyproline β-naphthylamide	HPR	Add 1 drop of peptidase reagent. Allow color reaction to develop for at least 30 s, but no longer than 3 min. Compare with β-naphthylamide control well.	Any shade of pink to red to magenta in the solution	Yellow to orange
L-Isoleucine β-naphthylamide	ILE			
L-Proline β-naphthylamide	PRO			
L-Tyrosine β-naphthylamide	TYR			
Glycine β-naphthylamide	GLY			
Glycylglycine β-naphthylamide	GGLY			
Glycyl-L-arginine 4-methoxy-β-naphthylamide	GLAR			
Glycyl-L-proline 4-methoxy-β-naphthylamide	GLPR			
L-Arginyl-L-arginine β-naphthylamide	AARG			
L-Lysyl-L-alanine 4-methoxy-β-naphthylamide	LYAL			
L-Alanine 4-methoxy-β-naphthylamide	ALA			
L-Seryl-L-tyrosine β-naphthylamide	STY			
L-Histidine β-naphthylamide	HIS			
Sucrose[a]	SUC1 or SUC2		Any brown or yellow	Purple
Trehalose	TRE			
p-Nitrophenyl-α-D-glucopyranoside[a]	AGL1	Compare with nitrophenyl control well	Any shade of yellow	Clear
p-Nitrophenyl-β-D-glucopyranoside	BGL			
o-Nitrophenyl-β-D-galactopyranoside	BGAL			
Urea	URE		Orange to pink to magenta	Yellow
3-Indoxyl phosphate	IDX		Any shade of blue	Clear
p-Nitrophenyl-α-D-glucopyranoside[a]	AGL2	Add 1 drop of 0.05 N NaOH. Wait at least 5 s but no longer than 5 min before recording result. Compare with nitrophenyl control well.	Any shade of yellow	Clear
p-Nitrophenyl-β-D-fucopyranoside	BDF			
p-Nitrophenyl-α-D-galactopyranoside	AGAL			
p-Nitrophenyl-*N*-acetyl-β-D-glucosamine	NAG			
p-Nitrophenyl-β-D-cellobiose	CELL			
p-Nitrophenyl-*N*-acetyl-β-D-galactosaminide	NGAL			

[a] Different formulations of the same substrate are used.

DISCUSSION

The development of differential media and dichotomous keys on the basis of growth of an unknown yeast on those media made yeast identification feasible for any laboratory which could prepare them (23). This led to the production of growth-based commercial kits with extensive data bases that enabled any laboratory to identify yeastlike fungi in 3 to 7 days (4, 7, 12). Recent commercial improvements have included media to support faster growth of the yeasts, semiautomation of systems, and computer-enhanced data bases (AMS-YBC, Vitek Systems Inc., Hazelwood, Mo.; and Quantum II, Abbott Laboratories, Irving, Tex.) (8, 15, 18). Increased expertise in mycology and the availability of these systems and data bases have led to timely and accurate identification of uncommon yeasts. These organisms may vary only slightly in their metabolic profiles from more common clinical isolates but pose a greater health threat to humans (1, 6, 9, 24).

The clinical relevance of a yeast identified by these growth-based systems is limited by the yeast's generation time. Consequently, yeasts identified later than 48 h post-isolation diminish the chances for a physician to administer a less toxic form of therapy. Considering the single-cell nature and metabolism of the majority of yeastlike fungi, it seemed logical that they could be identified as rapidly as bacteria (3, 5). Attempts to develop rapid methods for determining carbohydrate (16), urea (20, 27), and nitrate metabolism (11) were the first steps toward detecting existing enzymes rather than growth for yeast identifications. These methods were based on a large inoculum (McFarland standard of 5 or greater), metabolically balanced substrates, and color indicators rather than growth to detect substrate utilization.

The first enzyme-based detection system to be commercially introduced was the API Yeast Ident (API-YI). Published evaluations reported a 55 to 60% correlation between API-YI and the API 20C in identifying routine clinical isolates (18, 21). This reduced accuracy was due to several problems. The major difficulty was in interpreting the API-YI aminopeptidase results, which required considerable experience in discriminating between subtly different shades of pink and red. Other factors that contributed to discordant results were a limited number of substrates used to define taxa and a database that was too small.

The Baxter-MicroScan YIP containing substrates for detecting preexisting enzymes appears to have overcome some of the API-YI's shortcomings. The YIP has 27 substrates, which increases by 7 the number of datum points used to define a taxon. Panels are easy to inoculate, and positive reactions are readily determined without prior experience. The indoxyl phosphatases and the pH indicators for sucrose 1 and 2 and trehalose are the exceptions, giving somewhat inconsistent results. Since YIP results correlated better with the API 20C than the API-YI (92% for the more common

TABLE 2. Comparison of identifications of rapidly metabolizing yeastlike fungi

Organism[a]	No. of strains tested	No. (%) BMS correct[b]
Candida albicans	61	61[c] (100)
C. ciferrii	1	NIDB
C. guilliermondii	7	3[c] (43)
C. intermedia	1	NIDB
C. krusei	30	29[c] (97)
C. lambica	1	0 (0)
C. lipolytica	7	7[c] (100)
C. lusitaniae	23	20[c] (87)
C. parapsilosis	57	55[c] (96)
C. paratropicalis	3	3[c] (100)
C. pseudotropicalis	11	11[c] (100)
C. rugosa	2	2 (100)
C. salmanticensis	1	NIDB
C. stellatoidea	3	3 (100)
C. tropicalis	31	31[c] (100)
Hansenula anomala	5	3[c] (60)
Pichia ohmeri	4	3 (75)
Rhodotorula minuta	1	1 (100)
R. rubra	3	3[c] (100)
Saccharomyces cerevisiae	14	13[c] (93)
Torulopsis glabrata	34	34[c] (100)
T. candida	6	6[c] (100)
Total	306	288 (94[d])

[a] Taxa were established by the API 20C 72-h growth-based system.
[b] BMS, Baxter-MicroScan YIP enzyme-based 4-h system. YIP identifications were listed in the manufacturer's code compendium as having a >85% probability of being correct. Discrepant observations were resolved by conventional identification methods. NIDB, Isolate not in data base.
[c] Common clinical isolates of yeastlike fungi found in the United States. Of the 292 isolates of this sort tested, 279 (96%) were correctly identified by the YIP.
[d] The percentage of isolates correctly identified rises to 95% if the three NIDB isolates are discounted.

TABLE 3. Comparison of identifications of slower-growing yeastlike fungi

Organism[a]	No. of strains tested	No. (%) BMS correct[b]
Blastoschizomyces capitatus	15	11[c] (73)
Cryptococcus albidus	7	5[c] (71)
C. laurentii	4	2[c] (50)
C. neoformans[d]	80	55 (69)
C. terreus	2	2[c] (100)
C. uniguttulatus	4	3[c] (75)
Geotrichum sp.	3	1[c] (33)
Hyphopichia burtonii	4	NIDB
Phaeococcomyces sp.	1	NIDB
Prototheca wickerhamii	2	2 (100)
Trichosporon beigelii	9	4[c] (44)
Total	131	85 (65[e])

[a] Taxa were established by the API 20C 72-h growth-based system.
[b] BMS, Baxter-MicroScan YIP enzyme-based 4-h system. YIP identifications were listed in the manufacturer's code compendium as having a >85% probability of being correct. Discrepant results were resolved by conventional identification methods. NIDB, Isolates not in data base.
[c] Common clinical isolates of yeastlike fungi found in the United States. Of the 109 isolates of this sort, 86 (79%) were correctly identified by the YIP.
[d] If data for *C. neoformans* serotype D isolates are omitted, then 56 of 65 (86%) isolates were correctly identified; totals change to 86 of 116 (74% [77% if the five NIDB isolates are discounted]) isolates correctly identified.
[e] The percentage of isolates correctly identified rises to 67% if the five NIDB isolates are discounted.

clinical isolates [Tables 2 and 3]), these particular reactions appeared to have limited impact on identification. Retesting of 169 isolates resulted in the correct identification of one isolate each of *C. neoformans* and *Saccharomyces cerevisiae*. The remainder reproduced either the same biocode or only a slight modification thereof.

The majority of misidentifications occurred in the slowly growing or metabolizing group of yeasts. This could be because of insufficient enzymatic activity within the 4 h of incubation, too few group 2 biotypes contained in the data base, or a combination of both. Because of the slow growth rate of most of the misidentified yeasts, the first explanation appeared to be the most plausible. However, several observations did not support growth (metabolism) per se as being the reason for the following discrepancies: (i) the data were reproducible, (ii) the increased incubation did not improve identification, and (iii) all classes of group 2 organisms should have had equivalent misidentifications but did not (Table 2 and 3). Almost 40% (25 of 40) of the incorrect identifications listed in Table 3 occurred with strains of *C. neoformans*. This indicated that the group 2 misidentifications might be a data base problem and not a function of metabolism.

C. neoformans is a ubiquitous yeast that has been divided into four serogroups (A, B, C, and D) on the basis of specific polysaccharide capsular antigens. These serogroups have a limited geographical distribution, with serotypes B and C confined to tropical and subtropical regions and the West Coast of the United States. Serotype A strains are common to most of North America, and serotype D strains are common to Europe and other temperate regions. Studies with serotyped isolates of *C. neoformans* revealed that the D serogroup had the fewest correct biotypes (27%) included in the data base (Table 4). Groups B and C, on the other hand, representing serotypes geographically closest to the manufacturer, had the highest number of correct biotypes (83%) in the data base. Serotype A isolates, common throughout the remainder of the United States, occurred at a rate (73%) between the two extremes. These results suggest that, at least for *C. neoformans*, the YIP data base is limited in biotypes geographically removed from the area of the manufacturer. The efficiency of the YIP in identifying all serotypes of *C. neoformans*, as well as other taxa, should improve as the data base is expanded to include geographically representative biotypes.

In summary, there was a 92% correlation between YIP and API 20C in the identification of common clinical isolates of yeasts and an 85% correlation for all strains encountered during the study. The YIP panel is easy to inoculate, and reactions are easy to read. Although the data base is limited

TABLE 4. Identification of serotyped isolates of *C. neoformans* with the YIP

Serotype	No. of identifications			Efficacy (%)[a]
	Correct	Incorrect	Not made[b]	
A	11	1	3	73
B	12	0	3	80
C	13	0	2	87
D	4	0	11	37

[a] Efficacy is the number of identifications correlating with API 20C divided by the number of isolates tested. Overall efficacy was 66.6% (40 correct identifications/60 isolates tested).
[b] Identifications were not made because the isolate was not in the data base.

at present, its expansion and the refinement of the phosphatase- and acid-detected carbohydrate reactions should make the YIP an excellent alternative for the identification of medically important yeasts.

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