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Evaluation of the Updated Vitek Yeast Identification Data Base

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Using 398 isolates of yeasts and yeastlike fungi comprising 9 genera and 26 species, as well as the hyphomycete *Geotrichum candidum* and the achlorophyllous alga *Prototheca wickerhamii*, we compared the API 20C yeast identification system with the modified Vitek yeast identification system with an expanded data base. We found 11 discrepancies between the two systems: five (1.3%) of the isolates (*Blastoschizomyces capitatus*, 1; *Candida albicans*, 1; *Hansenula anomala*, 1; *Rhodotorula minuta*, 2) had biocodes not included in the expanded Vitek data base, and six (1.5%) of the isolates (*Candida lusitaniae*, 1; *Candida parapsilosis*, 1; *Cryptococcus uniguttulatus*, 1; *H. anomala*, 1; *Torulopsis candida*, 2) were misidentified by the Vitek system. Overall, the efficacy of the Vitek system compares favorably with that of the API 20C in the identification of clinically important yeasts.

The incidence of mycoses caused by yeasts has increased in the last several years (4). Diagnosis and therapy of these infections, especially in immunocompromised and severely debilitated patients, are necessities (1, 3, 6, 7, 21). Because of the diversity of organisms causing these infections, it is important for the clinical laboratory to have the capacity to rapidly and accurately identify both common and uncommon yeast isolates (13, 15, 20). In response to this increased need, several commercial systems have been developed for the rapid identification of medically important yeasts (2, 5, 12, 16–19). These systems have many advantages over conventional methods; these advantages include prepared substrates, a minimal need for supplementary tests, and a reduced incubation period to achieve a final identification.

The API 20C yeast identification system (Analytab Products, Plainview, N.Y.) has been proven to provide reliable and accurate results within 3 days (2, 12). As a result of its reliability and accuracy in comparison with the Wickerham method, many clinical laboratories now use the system as a standard reference method when dealing with clinical yeast isolates. Even though the system is faster than the traditional (12, 22) assimilation and fermentation methods, the API 20C still requires technical time for preparing and reading the identification panels. While automated yeast identification systems have been available for several years, their accuracy has been less than that achieved with the API 20C (11, 17, 19). For one of these, the AutoMicrobic System (AMS; Vitek Systems, Inc., Hazelwood, Mo.), an updated expanded data base for its Yeast Biochemical Card (YBC) has recently been introduced to provide a more accurate identification of emerging opportunistic yeast pathogens. To determine its usefulness in the clinical microbiology laboratory, we evaluated the accuracy of the revised and expanded data base (R 4.01, 1989) of the Vitek system and compared the system with the API 20C.

MATERIALS AND METHODS

Organisms. A total of 398 yeasts and yeastlike fungi comprising 9 genera and 26 species, as well as the hyphomycete *Geotrichum candidum* and the achlorophyllous alga *Prototheca wickerhamii*, were tested in this study. All organisms were recent clinical isolates obtained from patients hospitalized at the University of Texas Medical Branch at Galveston or the Methodist Medical Center in Dallas, Tex.; other isolates, maintained for less than 2 years in the culture collection of the Mycology Laboratories, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, were also used. The test isolates were either stored as suspensions in sterile distilled water and maintained at 25°C or stored as mature cultures on potato dextrose agar slants at −70°C. Isolates to be studied were subcultured at least twice onto Sabouraud dextrose agar in plates that were incubated at 30°C for at least 18 but not exceeding 48 h before being tested by the two identification systems.

All isolates were identified by the API 20C system, which was considered the reference standard. A correct identification by the API 20C system, to include appropriate supplementary tests, was used as the reference identification of each isolate evaluated in this study. Supplemental tests included cornmeal Tween 80 agar in plates inoculated by the Dalmau method for microscopic determination of morphology, temperature tolerance, and carbohydrate fermentations. Discrepant results between the API 20C and AutoMicrobic systems were resolved by reconfirmation of the purity of the isolate, repeat testing by both systems, morphological examination, and traditional identification by the Wickerham carbohydrate fermentation and assimilation procedures (14). The quality control organisms recommended by each manufacturer were used to ensure that both systems were performing to the specifications of the manufacturer. These included *Blastoschizomyces capitatus* ATCC 10663 and 28576, *Candida albicans* ATCC 14053, *Cryptococcus albidus* ATCC 34140, and *Cryptococcus laurentii* ATCC 18803.

API 20C yeast identification system. All yeast identification procedures were conducted according to the instructions of the manufacturer. A portion of a yeast colony that was 24 to 48 h old and was maintained at 30°C on Sabouraud dextrose agar was removed aseptically with a sterile wooden applicator stick and then adjusted to a Wickerham 1+ suspension in an API 20C ampoule containing agar base medium. Each of

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the 20 cupules of the plastic strip was filled with the yeast suspension to 1 drop above the level point with a sterile Pasteur pipette. Strips were placed in incubation trays, incubated at 30°C, and then read for the presence of growth (turbidity) at 24, 48, and 72 h. A final identification was made when the first-choice biocode number listed was excellent, very good, or acceptable. For isolates having low selectivity, identifications were confirmed by supplemental tests, as recommended by the manufacturer.

AMS-YBC. The YBC consists of a 30-well disposable plastic card containing 26 conventional biochemical tests and 4 negative controls. The YBC is used in conjunction with an automated system (AMS), which includes a programmed computer, reader incubator unit, filling module, sealer module, and printer. The automated portion and data management system of the AMS have been previously described (5, 11, 16).

All AMS-YBC procedures were conducted as recommended by the manufacturer. A McFarland no. 2 suspension was made by picking several well-isolated yeast colonies with a sterile wooden applicator stick and then emulsifying them in 1.8 ml of a sterile 0.45% NaCl solution. For this study, each yeast inoculum was standardized with a Vitek colorimeter, which is a single-beam filter photometer with a 450-nm filter and 46 to 56% transmittance that corresponds to a McFarland no. 2 standard. Inocula made from encapsulated yeast isolates or isolates with extensive mycelial or pseudomycelial growth were prepared by taking the unsettled supernatant of a suspension prepared in 3 ml of a 0.45% NaCl solution after mixing it and letting it stand for several minutes. Suspensions were inoculated into the cards via the filling module, and the cards were then sealed by the sealer module and incubated at 30°C for either 24 or 48 h, depending on readings provided by the instrument. After being inspected for the absence of air bubbles, the cards were read in a single reading in the reader incubator module, and the biochemical patterns were analyzed by the Vitek program computer. Identifications consisted of converting biochemical test results into nine-digit biocodes and matching the biocodes with codes in the in situ data base of the computer. Responses were expressed as the one or two most likely possibilities; only those matches whose first choice had an 85% or greater probability of being correct were considered the most likely identification for the yeast.

RESULTS

A total of 398 clinical yeast isolates, including 245 isolates of commonly encountered taxa and 153 isolates of uncommon taxa, were tested by both the API 20C and AMS-YBC yeast identification systems (Table 1). The AMS-YBC system correctly identified 387 (97.2%) isolates; 282 (70.8%) were identified after 24 h of incubation, 105 (26.4%) required 48 h of incubation, 67 (17.8%) required supplemental morphological studies, and 15 (3.8%) required both supplemental morphological and biochemical tests before an identification could be reached. Of the 245 common clinical isolates studied (*Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Cryptococcus neoformans*, and *Torulopsis glabrata*), 243 (99.2%) were correctly identified by the AMS-YBC. In contrast, 144 (94.1%) of the 153 uncommon isolates tested were correctly identified by the AMS-YBC. There were some discrepancies between the two systems: five isolates (1.3%) had biocodes that were not in the Vitek data base, and six isolates (1.5%) were misidentified by the AMS-YBC.

TABLE 1. Yeast identification results with AMS-YBC

Species	No. of isolates				
	Total	Correctly identified at:		Discrepant	
		24 h	48 h	Not identified	Incorrectly identified
Blastoschizomyces capitatus	23	0	22	1	0
Candida albicans	61	52	8	1	0
C. guilliermondii	4	2	2	0	0
C. krusei	36	0	36	0	0
C. lipolytica	4	4	0	0	0
C. lusitaniae	18	10	7	0	1
C. parapsilosis	58	48	9	0	1
C. paratropicalis	2	1	1	0	0
C. pseudotropicalis	10	8	2	0	0
C. rugosa	2	2	0	0	0
C. stellatoidea[a]	3	2	1	0	0
C. tropicalis	28	25	3	0	0
Cryptococcus albidus	2	2	0	0	0
C. laurentii	1	1	0	0	0
C. neoformans[b]	65	63	2	0	0
C. terreus	2	2	0	0	0
C. uniguttulatus	3	2	0	0	1
Geotrichum candidum	3	2	1	0	0
Hansenula anomala	5	3	0	1	1
Pichia ohmeri[c]	1	1	0	0	0
Prototheca wickerhamii	2	2	0	0	0
Rhodotorula glutinis	1	1	0	0	0
R. minuta	2	0	0	2	0
R. rubra	1	1	0	0	0
Saccharomyces cerevisiae	13	13	0	0	0
Torulopsis candida	8	2	4	0	2
T. glabrata	33	27	6	0	0
Trichosporon beigelii	7	6	1	0	0

[a] Reported as *C. albicans* (sucrose negative).
[b] Isolates consist of all serotypes.
[c] Not included in the API 20C data base.

Discrepancies involving the identification of common yeast taxa included (i) a germ tube-positive isolate of *Candida albicans* which did not assimilate palatinose and was susceptible to cycloheximide and thus resulted in no identification and (ii) one isolate of *Candida parapsilosis* which did not assimilate xylose, arabinose, trehalose, or adonitol and was misidentified as *Torulopsis candida* or *Cryptococcus neoformans*, with good confidence and marginal separation. Among the uncommon yeast taxa evaluated in this study, an isolate of *B. capitatus* had a biocode not included in the data base, and one isolate of *Cryptococcus uniguttulatus* was misidentified as *Candida tropicalis*. Additionally, two isolates of *Rhodotorula minuta* could not be identified because this taxon is not included in the data base, and two isolates of *T. candida* were misidentified as *Pichia ohmeri*, which is a species newly added to the data base. Among new taxa included in the expanded data base, an isolate of *Candida lusitaniae* was misidentified as either *T. candida* or *P. ohmeri*, with good confidence and marginal separation. Of the two isolates of *Hansenula anomala*, one had a biocode not included in the data base, and the other was misidentified as either *Candida lusitaniae* or *Cryptococcus neoformans*, with good confidence and marginal separation.

DISCUSSION

The Vitek system is designed to provide the clinical laboratory with the capability for rapid, accurate, reference

level yeast identification. The major concerns experienced by previous investigators have included the facts that the data base was too limited and that certain key biochemical characteristics were improperly weighted when the taxonomic keys were designed (5, 11, 16). In previous studies, the YBC of the Vitek system was reported to have an overall agreement with the API 20C, ranging from 83 to 98.8% (5, 11, 16, 17). The present evaluation of the new data base (R 4.01) showed an overall agreement of 97.2% (99.2% with common isolates and 94.1% with less common isolates) with the API 20C, which represents significant improvement in the ability of the YBC to identify clinically important yeasts.

Of the correctly identified yeast isolates, 70.8% were reported after 24 h of incubation. Occasionally, more slowly growing isolates required more than 24 h to assimilate the YBC biochemicals. Isolates of *Candida krusei* and *B. capitatus* cannot be identified by the system until the 48-h reading is completed, since some strains of these species may react similarly in the YBC test by giving positive assimilation reactions only for glucose and glycerol (according to the manufacturer, the same applies to *Prototheca zopfii*, which we did not include in this study). When all the isolates tested are considered, 26.4% of the correctly identified yeast isolates required 48 h of incubation.

Rapid and cost-effective presumptive identification of *Candida albicans* is usually accomplished in the diagnostic laboratory by using the germ tube test (14). Therefore, inoculation of the YBC with germ tube-positive yeasts is normally not necessary. However, it is useful in identifying germ tube-negative variants of *C. albicans*. No isolates of *Candida stellatoidea* included in this study assimilated sucrose in either system. With the new data base, each of these isolates was listed as *Candida albicans* (sucrose negative). Recent studies (8, 10) demonstrated that the isolates identified as *Candida stellatoidea* on the basis of carbon assimilation tests can be either type I or type II, depending on their electrophoretic karyotype. The isolates designated type II are considered sucrose-negative *Candida albicans*, whereas type I isolates are considered *Candida stellatoidea*. The rarity of *Candida stellatoidea*, as discussed in a recent study (10), plus the fact that *Candida stellatoidea* is less virulent than *Candida albicans* in experimental animals (9), may support the reporting of these isolates as *Candida albicans* (sucrose negative).

Our data have shown that the new updated, expanded Vitek software has markedly improved the accuracy of the YBC, especially for the common yeast taxa isolated in the clinical laboratory. The expanded data base accommodates the identification of nine additional species (*Candida humicola*, *Candida lambica*, *Candida lusitaniae*, *Candida paratropicalis*, *H. anomala*, *P. ohmeri*, *P. wickerhamii*, and *Sporobolomyces salmonicolor*) of medical importance. The system has some problems recognizing yeasts included in the expanded data base. These identification problems appear to be related to the fact that the new data base does not contain a large enough pool of biocodes that reflect variations typically associated with a given species. As the data base is expanded and additional unusual isolates are included, the system should become more accurate.

The Vitek AMS-YBC yeast identification system is more rapid than the API 20C and is comparable in accuracy (97.2%) in the identification of clinical yeast isolates. The reading, collecting of data, and final reporting of the YBC are totally automated, with good computer software support that can be easily updated to reflect both biochemical profile changes and the increasing number of fungi that need to be identified. The Vitek system does not appear to force fit an isolate not included in its data base into another taxon.

Only 21.3% of the correctly identified yeast isolates required supplemental morphological or biochemical tests or both to confirm their identification. From morphological features such as germ tubes, pseudohyphae, hyphae, and the ontogeny of conidia, many taxa could be easily distinguished from each other at the generic level without supplemental biochemical tests. Morphological examination was effectively used to help resolve discrepancies that occasionally occurred between the two systems. However, in the future, as the data base is expanded by the incorporation of more biocodes that reflect organism variability, it is anticipated that the system may provide more accurate identifications. Thus, the updated, expanded data base of the AMS-YBC system provides a reliable method for the rapid, automated identification of clinical yeast isolates. The data from this evaluation indicated that the final identifications of yeasts obtained by the composite YBC biochemical profile correlated well with those obtained by using the API 20C system.

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