Use of Autobac 1 for Rapid Assimilation Testing of *Candida* and *Torulopsis* species

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We devised a system of presumptive identification of some yeasts that uses the Autobac 1 (Pfizer Inc.) instrument to detect carbon assimilation by 218 strains of *Candida* and *Torulopsis*. This system compared favorably with a conventional system of yeast identification and also with the Uni-Yeast-Tek (Corning Medical) and API (Analytab Products Inc.) methods.

The Autobac 1 is a semiautomated instrument used for rapid determination of antimicrobial susceptibility of bacteria. This instrument functions by detecting small changes in bacterial population with a light-scattering photometer. It was decided to combine the technology of this instrument with carbon assimilation studies to devise a system of yeast identification. Two microsystems API-20C, Uni-Yeast-Tek (UYT), and a conventional system of yeast identification were used in comparison with the new Autobac 1 method in terms of accuracy, practicality, speed, and economy.

MATERIALS AND METHODS

A total of 218 yeast isolates were tested. Most of these were from clinical specimens received in the Mount Sinai Hospital Microbiology Laboratory and some were generously contributed by colleagues in other hospitals. American Type Culture Collection strains (18804, 11006, 750, 4135, 28474, 6260, 16528, and 15126) were used as controls for each system. All yeasts were first coded and subcultured to Sabouraud dextrose agar (Difco), and fresh 24-h cultures of yeast were used in all tests. Identification by any method was recorded independently of the results of tests by other methods. Results were compared only on conclusion of all tests.

Germ tube production. This was an essential preliminary to all four testing procedures. For the UYT system, a glucose-beef extract broth supplied with the kit was used, whereas production of germ tubes in other systems was detected by the method of Weld (17), using an eosin-methylene blue medium. A light inoculum of yeast was streaked on eosin-methylene blue agar, incubated in a humidified 5% CO₂ atmosphere at 37°C for 3 h, and examined microscopically for germ tube production. A cover glass was placed over each isolate, and, after overnight incubation under the same conditions, plates were examined

microscopically for clusters of blastospores typical of *Candida albicans* (16, 17).

Autobac 1 method: (i) assimilation media. This consisted of a fivefold concentration of yeast-nitrogen base (Difco) made by dissolving 33.5 g in 1 liter of distilled water. This solution was sterilized by filtration through a membrane filter (0.22 µm) and dispensed in 15-ml fractions in test tubes (20 by 125 mm).

(ii) Carbohydrate disks. The following carbohydrates were used: glucose, galactose, maltose, sucrose, lactose, raffinose, trehalose, melibiose, cellobiose, soluble starch, and inositol. Carbohydrate disks were prepared by using special elution paper disks, supplied by Pfizer Diagnostic Division, and were sterilized by autoclaving. These disks were saturated with a 10% carbohydrate solution, except for raffinose (20%) and sucrose (5%). They were dried in a 56°C incubator and left overnight in a desiccator before storing at 4°C.

(iii) Standardization solution. Six milliliters of phosphate-buffered saline, pH 7.2 (Pfizer product no. 201M), containing 0.01% of Tween 80 was distributed in special optically clear standardization tubes (Pfizer product no. 203). For assimilation studies, yeast isolates were inoculated into standardization solution, mixed on a vortex mixer, and standardized in the upper blue region of the Autobac 1 light-scattering photometer. This concentration of yeast represented approximately 3×10^6 colony-forming units per ml. Three milliliters of the inoculated solution was added to 15 ml of assimilation medium; this solution was mixed by inversion and dispensed in a cuvette as described by the manufacturers for antibiotic sensitivity testing (14).

(iv) Cuvette. This is an optically clear, molded polystyrene disposable receptacle that provides a single row of 13 separate chambers (Pfizer product no. 200M). In a laminar flow cabinet, the first chamber was pierced with a hot hypodermic needle, and 20 µl of 10% glucose was inoculated to make a growth control chamber. The opening was then sealed with adhesive tape. Eleven carbohydrates were added to the next 11 chambers, and a sugar-free control disk was added to the last chamber. The cuvette was placed in an incubator shaker at 35°C for 12 h and read by using an antibiotic sensitivity card, substituting positive assimilation for "resistant" and "intermediate"

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readings; "sensitive" denoted negative assimilation. A chart showing characteristic assimilation reactions with the Autobac 1 system is shown in Table 1.

Commercial microsystems. Both the API and UYT systems were used as described by their manufacturers, and yeasts were identified according to their respective identification charts (5, 8).

Conventional system. This system consisted of tests and criteria used in identification of medically important yeasts (2, 9, 12, 13). Microscopic morphology was studied on cornmeal agar with Tween 80 (9), and chlamydospore formation was tested on Oxgall agar by the method of Fisher and Kane (10). Urea hydrolysis was tested on Christensen urea agar, and nitrate assimilation was established by a method described by Ajello et. al. (4). Fermentation of glucose, galactose, maltose, sucrose, and lactose was tested by the method of Dolan (9), and assimilation of glucose, galactose, maltose, sucrose, lactose, raffinose, trehalose, cellobiose, melibiose, soluble starch, and inositol was tested by using a modified Wickerman medium described by Adams and Cooper (1).

RESULTS

Table 2 shows that the API correctly identified 93, the UYT 95, and the Autobac 1 system 97% of the yeast isolates tested.

Three isolates of *C. albicans* failed to grow in the API test strip despite prolonging incubation to 7 days, and two were misidentified by API due to false positive sucrose fermentation. Three

isolates of *C. albicans* failed to produce germ tubes and were negative for chlamydospores on the medium provided with the UYT kit. All of the remaining *Candida* species were identified correctly by the three systems, except *C. tropicalis* and *C. parapsilosis*. These two species presented some difficulty in identification with the three systems. In the case of the API system, four isolates of *C. tropicalis* were misidentified as *C. parapsilosis* due to false negative fermentation of maltose and sucrose with negative cellobiose assimilation, whereas five isolates of *C. parapsilosis* were identified as *C. tropicalis* because of false positive maltose and sucrose fermentation tests. None of these five isolates assimilated cellobiose. In the UYT system, there were five false negative assimilations of soluble starch by *C. tropicalis*, and two isolates of *C. parapsilosis* were misidentified as a result of false negative assimilation of sucrose. Repeat tests of these seven isolates showed that assimilation of carbohydrates was detected when incubation was extended to 7 days. Delayed assimilation of starch was also noted in the Autobac 1 system with four of the five isolates of *C. tropicalis* that proved troublesome to UYT. Two isolates of *C. parapsilosis* were misidentified in Autobac 1 because of false negative assimilation of maltose. All of the isolates of *Torulop-*

TABLE 1. *Autobac 1 carbon assimilation reactions*

Organism	Carbohydrates										
	Glucose	Galactose	Maltose	Sucrose	Lactose	Raffinose	Trehalose	Melibiose	Cellobiose	Soluble starch	Inositol
<i>Candida albicans</i>	+	+	+	+	—	—	+	—	—	+	—
<i>C. stellatoidea</i>	+	+	+	—	—	—	+	—	—	+	—
<i>C. tropicalis</i>	+	+	+	+	—	—	+	—	V ^a	+	—
<i>C. pseudotropicalis</i>	+	+	—	+	+	+	—	—	V	—	—
<i>C. parapsilosis</i>	+	+	+	+	—	—	+	—	—	—	—
<i>C. guilliermondii</i>	+	+	+	+	—	+	+	V	+	V	—
<i>C. krusei</i>	+	—	—	—	—	—	—	—	—	—	—
<i>Torulopsis glabrata</i>	+	—	—	—	—	—	+	—	—	—	—

^a V, Variable results expected. The spellings of specific epithets in this and all tables are from reference 11.

TABLE 2. *Number and percentage of identification by three methods, using the conventional method as standard*

Yeast isolates	No. of strains	No. and (%) correctly identified by:		
		API	UYT	Autobac 1
<i>Candida albicans</i>	90	85 (94.4)	87 (96.6)	90 (100)
<i>C. stellatoidea</i>	5	5 (100)	5 (100)	5 (100)
<i>C. tropicalis</i>	50	46 (92)	45 (90)	46 (92)
<i>C. pseudotropicalis</i>	6	6 (100)	6 (100)	6 (100)
<i>C. parapsilosis</i>	8	3 (37.5)	6 (75)	6 (75)
<i>C. guilliermondii</i>	14	14 (100)	14 (100)	14 (100)
<i>C. krusei</i>	14	14 (100)	14 (100)	14 (100)
<i>Torulopsis glabrata</i>	31	30 (96.7)	31 (100)	31 (100)
Total accuracy		203 (93)	208 (95)	212 (97)

sis glabrata were identified correctly by all three systems except for one that failed to ferment or assimilate trehalose and was misidentified by API as *C. krusei*.

Comparison of approximate costs of each system (Table 3), which includes only the cost of reagents, shows that the Autobac 1 method was significantly less expensive than the others based on costs of materials at the time of the study.

DISCUSSION

A precedent was established by Matsen and co-workers (16) when they adapted the Autobac 1 system to the identification of bacteria based on inhibitory profiles.

In designing the Autobac 1 method for yeast identification, we were aware that incubation in a shaker might reduce the time necessary to detect growth of yeasts as was reported by Ahearn et al. (3). It was also hoped that the light-scattering photometer of the Autobac 1 would detect early proliferation of yeasts utilizing certain carbohydrates in tests of assimilation. The only technical problem that had to be surmounted before embarking on this study, apart from preparing carbohydrate-impregnated elution disks, was to gain access to the first chamber in the cuvette for inoculation with a glucose broth control culture. This was readily accomplished by perforating the standard Pfizer cuvette with a hot needle. This procedure presented little technical difficulty.

The Autobac 1 system embodies an excellent method for standardizing the yeast inoculum and, in this respect, is superior to the other three methods. In practice, it was found that the Autobac 1 could detect appreciable changes in concentration of yeast in the cuvettes in 12 h, a marked improvement on the incubation time of the other methods. The selection of carbohydrates was based on recommendations of Wickham and Burton (18) but could be modified in light of future experimentation, particularly in an attempt to reduce the number of misidentifications of *C. parapsilosis* and *C. tropicalis*.

For these initial studies, it was decided to

concentrate on the identification of *Candida* and *Torulopsis* species for two main reasons. First, these account for the vast majority of clinical isolates of yeasts, and second, because the identification of other medically important yeasts such as *Cryptococcus*, *Rhodotorula*, *Trichosporon*, and *Geotrichum* by Autobac 1 requires further technical research.

Although varying in technical complexity and interpretive difficulty, both the API and UYT systems offer good alternatives to the conventional procedure. They have advantages over the conventional system on the basis of media preparation and shortened identification time. The API is inexpensive and gives a faster identification than the UYT system but the former requires greater technical expertise. They both demonstrated a high degree of accuracy, and some of the misidentifications seen in our study were similar to those reported by Bowman and Ahearn (7) and by Roberts and his colleagues (15). The results of the Autobac 1 system are of special importance to those laboratories that possess this instrument, and the method devised is clearly superior to the other methods in terms of speed, simplicity, and economy; there is very little sacrifice in accuracy when compared with the conventional method for identification by species of *Candida* and *Torulopsis*.

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TABLE 3. Cost, evaluation, accuracy, and identification time of systems tested

Method	Approx cost/identification (\$)	% Accuracy	Identification time
API-20C	1.95	93	2-3 days
UYT	4.00	95	4-5 days
Autobac 1	1.25 ^a	97	12 h
Conventional	2.60	100	7-14 days

^a Excluding cost of the instrument.

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