Biochemical identification of clinically important yeasts

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SUMMARY Four commercially available kits for the speciation of yeasts were tested against 50 clinical isolates that had been identified by conventional methods. On biochemical grounds alone, the four systems varied from 71% to 100% in their efficiency in identifying *Candida albicans*. Yeasts other than *C. albicans* were identified with an efficiency varying from 24% to 83%. Conclusions are drawn on the value of these systems to the routine laboratory.

Although *Candida albicans* is the principal pathogen of the genus, a number of other *Candida* species cause disease in man (Hurley, 1967; van Uden and Buckley, 1970) as do yeasts of other genera (Gentles and La Touche, 1969). Since antifungal agents with a selective or narrow range of activity are available, it is important that speciation of yeasts in routine clinical microbiological practice should be effected rapidly and reliably. Conventional methods of identification (van der Walt, 1970) are cumbersome and often beyond the range of expertise available in non-specialist laboratories. Commercially available kits have been marketed to aid identification of pathogenic yeasts, and it is the purpose of this study to report results obtained with four of these, using the criteria of identification of Lodder (1970) as the reference system and basis of comparison.

Material and methods

STRAINS STUDIED

Fifty isolates from man were studied, 25 originating from patients of Queen Charlotte's Maternity Hospital and 25 kindly supplied by the Mycology Reference Laboratory.

The organisms were identified using auxanograms, zymograms, and other tests (Lodder, 1970).

TEST KITS

The kits tested were:

(a) API 20C, which consists of 19 fermentation and assimilation tests and a control presented as a strip

of cupules and accompanied by an ampoule of suspending medium.

(b) API 20C (Auxanogramme), an improved version of (a), in which all 19 reagent cupules contain substrates for assimilation.

(c) Mycotube (Roche), based on the principle of the Enterotube, an inoculated needle being drawn through eight agar-filled compartments. The first two compartments contain urea and citrate, followed by five carbohydrate substrates. There is the facility to observe gas production from glucose and sucrose.

(d) Auxodisk (Sobiota), which consists of an eight-pronged blotting paper disc containing carbon sources and actidione with glucose, which is applied to a seeded base medium free from utilisable carbon source. The tests contained in the four systems are shown in Table 1.

Procedure

Fresh cultures of the 50 isolates studied were made on Sabouraud's dextrose agar using a numerical system of identification, so that subsequent investigations were carried out 'blind'. Selected colonies were used to inoculate all four kits according to the manufacturers' instructions. The recommended times for incubation were: Auxodisk, 18-24 hours; API 20C, 48 hours; Mycotube and API 20C (Auxanogramme), 72 hours at 37°C. After incubation the results were read from the identification tables accompanying each kit. API 20C, Mycotube, and Auxodisk had simple tables of reactions for the recognition of 27, 13, and 9 species/groups of fungi respectively. The API 20C (Auxogramme) System is computer-based and contains information for the identification of 26 taxa. The reactions obtained are converted into

Table 1 Reagents contained in the four systems tested

	API 20C	API 20C Auxano- gramme	Auxodisk	Mycotube
Dextrose	+/+*	-/+	+/-	+/-
Xylose				+/-
Sucrose	+/+	-/+	+/-	+/-
Raffinose	+/+	-/+	+/-	+/-
Lactose	+/+	-/+	+/-	+/-
Trehalose	+/+	-/+		+/-
Citrate				+
Urea				+
Maltose	+/+	-/+	+/-	
Galactose	+/+	-/+	+/-	
Cellobiose	-/+	-/+	+/-	
Actidione	+		+	
Melibiose	+/+			
Inositol	-/+	-/+		
2-keto-D-gluconate		-/+		
Arabinose		-/+		
Xylose		-/+		
Adonitol		-/+		
Xylitol		-/+		
Glycine		-/+		
Sorbitol		-/+		
Methyl-D-glucoside		-/+		
N-acetyl-D-glucosamine		-/+		
Melzitose		-/+		

*Fermentation/Assimilation

a numerical profile, which is read from a profile index. All four systems had a shelf-life at 4°C of 12 months or longer.

Results

The original identity of the cultures was confirmed using the procedures described by Lodder (1970). The number of isolates studied and the number of correct identities obtained with each of the identification schemes are shown in Table 2. The three isolates grouped as *Candida* species (Table 2) were *C. pulcherrima*, *C. claussenii*, and *C. pelliculosa*, and the three species of *Geotrichum* were *G. asteroides*, *G. matalense*, and *G. candidum*. All four systems

Table 2 Number of yeasts correctly identified by the four schemes

Yeast	No.	API 20C	API 20C Auxano- gramme	Auxodisk	Mycotube
<i>C. albicans</i>	21	16	19	21	15
<i>C. parapsilosis</i>	2	2	2	1	0
<i>C. tropicalis</i>	2	0	2	2	2
<i>C. pseudotropicalis</i>	3	3	2	3	3
<i>C. stellatoidea</i>	2	0	2	0	0
<i>Candida</i> species	3	0	0	0	0
<i>T. glabrata</i>	7	7	6	0†	7
<i>S. cerevisiae</i>	3	3	3	0†	3
<i>C. neoformans</i>	3	2*	3	0	3*
<i>Geotrichum</i> species	3	0	3	0	0
<i>Trichosporon cutaneum</i>	1	1*	1	0	0

*Identity of best fit

†Species not listed

were reasonably efficient at identifying *C. albicans*, API 20C (Auxanogramme) and Auxodisk being marginally better than the other two. The Auxodisk scheme does not include reactions for *Torulopsis glabrata* and, as a result, the seven isolates of this species would have been identified as *C. krusei* using biochemical results alone. The majority of *Torulopsis* were correctly identified by the other schemes. Only API 20C (Auxanogramme) identified satisfactorily the three isolates of *Cryptococcus neoformans*. Using Mycotube they would probably have been identified correctly by their positive urea reactions, although they were all citrate negative. Using Auxodisk, they were identified as *C. tropicalis* because of the absence of a positive reaction with cellobiose. Using API 20C, Auxodisk, and Mycotube, we were unable to identify species of *Candida* or *Geotrichum* infrequently isolated from man. Two of the *Candida* species were identified correctly to genus level using API 20C (Auxanogramme), as were three isolates of *Geotrichum*. For the remaining yeasts tested, API 20C (Auxanogramme) was consistently more reliable than the other systems.

The auxanogram reactions of *T. glabrata* and *C. krusei*, and those for *C. parakrusei* and some strains of *C. tropicalis*, are the same for the seven carbohydrates contained in the Auxodisk. Isolates of *T. glabrata* and *S. cerevisiae* studied could not be correctly identified since neither species is included in the Auxodisk table of reactions.

In addition to the isolates that gave reactions uncharacteristic for any of the species listed by the manufacturers there were 36 occasions when the reactions obtained gave a false-positive identification (Table 3). This was due largely to the failure of one or more reagents to give the expected positive result. Five of the discrepant results obtained with API 20C were due to the failure of *C. albicans* (3 isolates) and *C. stellatoidea* (1 isolate) to grow in the actidione substrate. The yeasts, therefore, appeared to give the reactions of *C. tropicalis*.

Correct identification of three isolates of *C.*

Table 3 Number of yeast strains wrongly identified on the basis of biochemical reaction

Yeast	No.	API 20C	API 20C Auxano- gramme	Auxodisk	Mycotube
<i>C. albicans</i>	21	3	0	0	6
<i>C. parapsilosis</i>	2				2
<i>C. pseudotropicalis</i>	3		1		
<i>C. stellatoidea</i>	2	1		2	1
<i>Candida</i> species	3	2†	2†	2†	2*
<i>T. glabrata</i>	7			7	
<i>C. neoformans</i>	3			3	
<i>Geotrichum</i> species	3	1			1

*1 Isolate identified as different *Candida* species†2 Isolates identified as different *Candida* species

neoformans was impossible with Auxodisk because of failure to grow around the cellibiose or raffinose disks. Thus the yeasts gave the reactions of *C. tropicalis*. Two isolates of *C. stellatoidea* gave a positive reaction with sucrose using Auxodisk and therefore gave the reactions of *C. albicans*. Mycotube gave unreliable results for citrate utilisation with *C. neoformans* and *C. parapsilosis*, the latter giving the reactions of *C. stellatoidea* in consequence. Gas production from glucose and sucrose was difficult to determine with Mycotube. As a result, six isolates of *C. albicans* produced the biochemical reactions of *C. stellatoidea*, *C. krusei*, or *T. glabrata*. The overall results obtained with these four systems are shown in Table 4.

Table 4 Biochemical results obtained from 50 yeast isolates (as a percentage)

	API 20C	API 20C Auxano- gramme	Auxodisk	Mycotube
Correct identity— overall	68	86	54	66
Correct identity— <i>C. albicans</i>	76	90	100	71
Correct identity— other yeasts	65	83	24	65
Unidentified yeasts	16	10	18	10
Wrong identity	16	4	28	24

The four systems varied in their ease of application. Preparation of Auxodisk was uncomplicated provided that care was taken to standardise the inoculum to an absorption of 0.5–0.6 at 450 nm and a pathwidth of 1 cm. Mycotube required no preliminary preparation, but with many of the drier colonial types the viable inoculum was completely removed from the inoculating wire before it reached the chamber containing glucose. Repeat examination usually produced the same effect. The API 20C systems were more difficult to use. The density of the yeast suspension and the filling of the cupules were critical if accurate and reproducible results were to be obtained. Although final identification using the API 20C (Auxanogramme) system requires 72 hours' incubation, it was found that many of the more common pathogenic yeasts could be reliably identified after 24 or 48 hours' incubation.

Discussion

The identification of many yeasts is facilitated by their colonial or cellular morphology and characteristics. However, in many laboratories such characterisation is limited to the demonstration of germ tubes and/or chlamydospores by *C. albicans*. Demonstration of biochemical reactions is required

to establish or to confirm identity, and this comparative study has been confined to the biochemical reactions of yeasts. If it is to be of value, the information obtained from commercially available kits should be accurate, reliable, reproducible, and unambiguous and should, ideally, provide identification more quickly than the conventional methods.

On biochemical grounds alone the four schemes varied in their efficiency in identifying *C. albicans* from 71% with Mycotube to 100% with Auxodisk.

Most laboratories identify *C. albicans* by germ tube production, reserving biochemical investigation for germ tube negative yeasts. The efficiency with which the latter were identified varied from 24% with Auxodisk to 83% with API 20C (Auxanogramme). The reliability of Auxodisk with eight tests and Mycotube with 10 tests was not high, due mainly to particular substrates giving false-negative results consistently. The API 20C systems were better in this respect, and the adverse effect of a single discrepant result was minimised because of the larger number of tests. The omission of *T. glabrata* and *S. cerevisiae* from the Auxodisk table of reactions, coupled with failure of the system to differentiate biochemically *C. parakrusei* from some strains of *C. tropicalis*, is a serious drawback. The API 20C system is capable of identifying many more species than either Auxodisk or Mycotube. The size of the identification tables, however, makes it difficult to use. This problem was solved by the numerical profile of API 20C (Auxanogramme).

The failure of the systems tested to identify the less common *Candida* species and, to a lesser extent, the *Geotrichum* species tested is less disturbing than the poor results obtained with *C. neoformans* and the large number of yeasts wrongly identified.

Conclusions

AUXODISK

Unsatisfactory substrates, overlap between species listed in the identification table, and omission of pathogenic yeasts from it severely limit the value of the system which can be relied upon to identify only *C. albicans*.

MYCOTUBE

This is more reliable than Auxodisk, but the consistent failure of the citrate substrate, the difficulties associated with the determination of gas production and xylose fermentation, and repeated failure of the inoculating wire to seed all the agar compartments are drawbacks.

API 20C

This is significantly better than Auxodisk or Myco-

tube, although it is more difficult to put up. Cost would probably confine its routine use to yeasts other than *C. albicans*. The complexity of the tables of reactions makes interpretation difficult. The unreliability of the actidione sensitivity test also limits its usefulness.

API 20C (AUXANOGRAMME)

Many of the problems attendant on the other systems have been resolved by replacing fermentation tests with an increased range of assimilation reactions, although accurate standardisation of the suspension and filling of the cupules are essential. The high percentage of yeasts identified solely on biochemical grounds by this system, and the ease with which the results can be interpreted, commend it for use in the routine laboratory.

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