Evaluation of the Uni-Yeast-Tek Kit for the Identification of Medically Important Yeasts

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Received for publication 6 June 1975

The Uni-Yeast-Tek system, a commercially prepared kit and scheme for the rapid identification of medically important yeasts (Corning Medical), was evaluated in comparison with a conventional procedure in the identification of 623 yeasts. The system permitted the presumptive identification of 99.8% of 436 isolates representing 16 common species commonly isolated in the clinical laboratory. Correct biochemical and morphological analyses were obtained with 48 other species, but their specific identification required additional data.

Detailed procedures for the identification of yeasts have been described by Wickerham (15) and Lodder (8). Unfortunately, since these methods require a broad spectrum of tests, skilled interpretations, and time, they are employed in few clinical laboratories. Adequate schemes for the selective identification of common clinical isolates are available (1, 2, 11), but due to lack of technical help and time even these simplified schemes rarely are used. In hospitals, yeast diagnostic efforts consist mostly of tests for the detection of pseudomycelia and chlamydospores in *Candida albicans* and tests for capsule formation and urease activity in *Cryptococcus neoformans*. These circumstances together with the increased incidence and importance of yeast infections have motivated several companies to prepare yeast diagnostic kits. This paper evaluates the Uni-Yeast-Tek system developed by Corning Medical for the routine identification of clinically important yeasts.

MATERIALS AND METHODS

Six hundred and twenty-three isolates, mainly from clinical specimens, were selected from cultures received at the Mycology Division of the Center for Disease Control (CDC), Atlanta, and from the culture collection at Georgia State University. All isolates were identified by conventional procedures (3) conducted mostly at CDC, and the data were made available for comparison with results of the Uni-Yeast-Tek system. The conventional procedures included testing for formation of germ tubes on bovine serum and tissue culture medium 199 (BBL).

The Uni-Yeast-Tek kit, received from Corning Medical, Roslyn, N.Y., is composed of a multisectioned dish containing seven carbon assimilation agars (pH adjusted to 7.0), urea agar, nitrate assimilation agar (pH adjusted to 5.0), a central well containing corn meal with Tween 80 for mycelium and chlamydospore production, a broth containing 0.05%

glucose and 2.6% beef extract (Wilson Laboratories) for the production of germ tubes, instructions, and a wheeled classification key for the identification of 16 common clinical yeast isolates.

Commercially prepared Sabouraud dextrose agar (Difco), as recommended, was used to obtain colony isolates of yeasts. Cells from a single colony were suspended in 5 ml of sterile distilled water to give a cell suspension of 10^6 to 10^7 /ml (a 1+ reading on a Wickerham card); 1 drop was used to inoculate each assimilation agar via a Pasteur pipette through a small portal on the side of each well. A second Sabouraud plate was used to determine growth at 37 C.

To test for germ tubes, the glucose-beef extract broth was inoculated by touching the tip of a pipette lightly to the surface of a colony and inserting the pipette into the broth and incubating at 37 C for 3 h. Germ tubes and all morphology were determined microscopically. The central corn meal well was inoculated with a standard Dalmau cut, and the plate was incubated at 22 to 26 C for 2 to 10 days. The Dalmau cut was examined microscopically on days 3 and 5 after inoculation for observation of blastospore, pseudomycelium, chlamydospore, or arthrospore production.

The reaction media for the Uni-Yeast-Tek system are listed in Table 1. The carbohydrates vary in concentration from 1 to 4%, and the basal media are prepared in accordance with the formulas of Wickerham (15). Positive reactions on the assimilation agars are denoted by growth accompanied by color changes in the indicators, bromocresol purple for the carbon assimilations and bromothymol blue in the nitrate medium. Agar reactions, i.e., color changes and growth, were read from 2 to 5 days.

RESULTS

In preliminary studies of varied inocula, including cells starved on yeast nitrogen base and yeast carbon base for 48 h, cells starved for 1 week in distilled water, and cells grown on Sabouraud dextrose broth and washed twice in sterile saline and resuspended to 10^8 cells/ml,

similar reactions were produced by representative clinical isolates. Thereafter, all subsequent testing employed the recommended inoculum preparation.

All isolates of *C. albicans* and *C. stellatoidea* were readily distinguished from other yeasts by their germ tubes and assimilation spectra. The glucose-beef extract broth provided with the kit proved comparable to tissue culture media 199 and bovine serum in the production of germ tubes. All isolates of *C. albicans* except three produced germ tubes within 3 h on the glucose-beef extract broth. Two cultures of *C. albicans* from patients who had received polyene therapy gave only sparse numbers of germ tubes in all preparations. The third isolate, *Syngospóra albicans* (14), was somewhat atypical in all its morphological properties. A typical germ tube produced by *C. albicans* is shown in Fig. 1a and b. Unlike pseudohyphal cells, germ tubes are not constricted at their point of origin (3, 9). Pseudohyphal elements, which appear grossly similar to germ tubes, may be produced by some isolates of *C. tropicalis* in the germ tube test. Unlike germ tubes, these elongated buds are constricted at their point of origin (Fig. 1c and d). It should be noted that some *C. albicans* may produce both germ tubes and pseudohyphal cells, but only the germ tubes are diagnostic.

Of the 623 isolates examined, 436 were common clinical species whose identification pattern was provided in the Uni-Yeast-Tek classification scheme. The accuracy of identification of 16 common clinical isolates as compared to conventional procedures is given in Table 2. Of these isolates, 99.8% were correctly identified. The one isolate of *C. tropicalis* that would not grow on the Uni-Yeast-Tek media was CBS 6418. In initial kits, isolates of this species were distinguished on the basis of cellobiose assimilation. Cellobiose assimilation by *C. tropicalis*, however, is frequently latent to negative on auxanographic procedures. In our preliminary screening of 47 isolates, all of which assimilated cellobiose with prolonged incubation in liquid medium, only 17 gave positive reactions with the kit by 8 days. Thereafter the company modified the kit to include soluble starch (Difco). All isolates of *C. tropicalis*, including the type strain deposited at the Centraalbureau voor Schimmelcultures (CBS), Delft, Holland (D. Yarrow, personal communication), and over 100 cultures in our collection assimilated soluble starch, whereas isolates of *C. parapsilosis* in these same collections failed to utilize starch. The positive assimilation reactions for isolates of *C. tropicalis* on soluble starch were rapid,

TABLE 1. Media and reactions of the Uni-Yeast-Tek system

Reaction media	Reaction color	
	Positive	Negative
Urea	Pink	Straw
Sucrose	Yellow	Purple
Lactose	Yellow	Purple
Maltose	Yellow	Purple
Raffinose	Yellow	Purple
Cellobiose	Yellow	Purple
Soluble starch	Yellow	Purple
Trehalose	Yellow	Purple
Sugar control	Yellow	Purple
Nitrate	Green-blue	Orange
Nitrate control		Orange
Corn meal with Tween 80		

with 79% showing positive assimilation in 2 days and 97.5% in 3 days. The single isolate of *C. tropicalis* that did not react was CBS 6418, which reportedly undergoes bud meiosis (12). This strain has an unknown requirement, as it did not grow on any of the defined media.

One hundred and eighty-seven isolates representing species less frequently associated with humans were also examined with the Uni-Yeast-Tek kit. One hundred percent correlation with conventional procedures was obtained; this correlation included 21 genera (including *Brettanomyces*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Endomycopsis*, *Geotrichum*, *Hansenula*, *Prototheca*, *Saccharomyces*, *Torulopsis*, *Trichosporon*, *Rhodotorula*) and 48 species. Correct biochemical reactions were obtained, but insufficient data were provided with the Uni-Yeast-Tek system for classification. For certain isolates, the use of the kit provided sufficient information for presumptive identification and, in the case of *Prototheca*, definitive identification was possible. The emerging pathogens *Prototheca zopfii* and *P. wicherhamii* grew much better on the Uni-Yeast-Tek medium than in the broth. *Geotrichum candidum* could be distinguished from *Trichosporon capitatum* and *T. penicillatum* with experience in recognizing their distinctive morphologies on the Uni-Yeast-Tek media.

DISCUSSION

The selection of characteristics in the Uni-Yeast-Tek kit were good choices for the identification of the common medically important yeasts. The kit permitted the specific identification of *C. albicans* and *C. stellatoidea* mainly on the basis of their production of germ tubes. The glucose-beef extract broth proved acceptable for germ tube production as compared with bovine serum or tissue culture medium 199.

FIG. 1. Germ tube of *C. albicans* without constriction at origin on yeast cell: (a) high-dry light microscopy, $\times 750$ (b) scanning electron microscopy, $\times 6,500$; elongated pseudohyphal cell of *C. tropicalis* with constriction at origin; (c) high-dry light microscopy, $\times 750$ (d) scanning electron microscopy, $\times 5,285$.

TABLE 2. Accuracy of the identification of common clinical yeast isolates by the Uni-Yeast-Tek system

Organism	No. correct/no. tested	% Correlation with conventional identification
<i>Candida albicans</i>	85/85	100
<i>C. stellatoidea</i>	9/9	100
<i>C. parapsilosis</i>	46/46	100
<i>C. tropicalis</i>	55/56	98.2
<i>C. pseudotropicalis</i>	7/7	100
<i>C. guilliermondii</i>	31/31	100
<i>C. krusei</i>	29/29	100
<i>Cryptococcus albidus</i>	22/22	100
<i>Cryptococcus laurentii</i>	10/10	100
<i>Cryptococcus neoformans</i>	35/35	100
<i>Cryptococcus terreus</i>	3/3	100
<i>Rhodotorula rubra</i>	29/29	100
<i>R. glutinis</i>	4/4	100
<i>Saccharomyces cerevisiae</i>	19/19	100
<i>Torulopsis glabrata</i>	20/20	100
<i>Trichosporon cutaneum</i>	31/31	100

Joshi et al. (6) previously showed that *C. albicans* produced germ tubes in various peptone broths at 37 C. Of the three isolates of *C. albicans* that gave only sparse germ tube production in all preparations, two were from patients with a history of chronic vaginitis and extensive polyene therapy. In general, fermentation of sugars and mycelial production by these two isolates were weak. It has been observed that exposure to polyene antibiotics alters the physiological and morphological characteristics of *C. albicans* (5). Our unpublished observations on *C. albicans* from recalcitrant infections support this observation. The third isolate of *C. albicans* was obtained from the CBS as *Syringospora albicans*, the reported perfect stage of *C. albicans*. van der Walt (13) initially considered this strain a heterobasidiomycete but recently amended his description to include it in the ascomycetes (14). The somewhat atypical morphology and physiology of this strain are reflected in the confusion concerning its life cycle and taxonomy.

Among the yeasts producing germ tubes was the type culture of *C. novellii* CBS 6552. Saëz (10) isolated this species from animals in a Paris zoo. *C. novellii* does not assimilate xylose and thus differs from the standard description of *C. albicans*. However, Ahearn (2) reported that rare strains of *C. albicans* from humans did not assimilate xylose. Therefore, we consider *C. novellii* a synonym of *C. albicans*.

The identifications of most of the other clinical yeast isolates by the Uni-Yeast-Tek kit, although proven correct in this study by a conventional procedure, would have to be regarded

as presumptive. As with any limited system, verification of certain identifications requires a more complete assessment of morphological and physiological properties. Additional sugar assimilations (melibiose, dulcitol, inositol) and florescent antibody tests or mouse pathogenicity tests are required to verify the identification of *Cryptococcus neoformans*. *C. parapsilosis* could not be distinguished from *C. maltosa* (7) due to the false-negative cellobiose reactions by this latter species. Modification of the cellobiose medium formulation to overcome the difficulty of interpretation of latent reactions would enhance distinction of species of the *C. tropicalis* group. Fortunately, *C. maltosa* has not been reported from clinical specimens and seems to occur in nature mainly in oil-soaked soils. The identification of *C. pseudotropicalis* and *C. guilliermondii* also must be classified as presumptive. For example, to differentiate between *C. pseudotropicalis* and *C. kefir*, the ability to assimilate xylose must be determined. Interest in identifying the above yeasts in clinical laboratories may increase in the future due to their potential as food yeasts. Barnett and Pankhurst (4) list *C. guilliermondii*, *Debaryomyces hansenii*, *Pichia guilliermondii*, and *Torulopsis candida* in a group with similar fermentation and assimilation characteristics. Combinations of morphological properties, including ascospore characteristics, are necessary for their distinction. Species of *C. rugosa*, *C. valida*, *C. lipolytica*, and the *C. krusei* complex also require further biochemical tests, such as xylose, erythritol, D-mannitol, and sorbitol assimilation media, for definitive identification. Verification of *Saccharomyces cerevisiae* would require the observation of ascospores. Only three isolates of *Cryptococcus uniguttulatus* and two isolates of *Cryptococcus luteolus* were examined. The isolates of *Cryptococcus uniguttulatus*, except for failure to utilize dulcitol, had the same assimilation pattern as most of the isolates of *Cryptococcus neoformans* (erythritol-negative strains). *Cryptococcus luteolus*, which assimilates melibiose in contrast to *Cryptococcus neoformans*, and *Cryptococcus uniguttulatus* grow on most routine media at temperatures up to 33 to 34 C, whereas rare strains of *Cryptococcus neoformans* fail to grow in vitro at 37 C but do grow at 35 C. Although not found in this study, the temperature ranges for these species rarely may overlap if strict temperature control is not maintained. Therefore, expanded sugar assimilation patterns should be used for definitive identifications of the cryptococci.

A major advantage of using the Uni-Yeast-Tek system in clinical laboratories is that a

single technician can inoculate 50 to 75 plates in 1 day. Most species can be identified presumptively in 3 to 4 days; however, some may take 6 days for complete results. The Uni-Yeast-Tek plates are easy to store and maintain during growth, since they are incubated at room temperature. One possible drawback of the kit is the need to keep it refrigerated prior to use. When the Uni-Yeast-Tek kit is assembled for commercial use, each plate will be sealed with a plastic cover, making it air tight. This modification should increase the shelf life beyond the 2 to 3 months experienced in this study.

In general, the Uni-Yeast-Tek kit should greatly increase the capabilities of yeast identification in routine clinical laboratories.

ACKNOWLEDGMENTS

This research was supported in part by the Microbiology Research Fund of Georgia State University.

We thank Corning Medical for their gift of materials used in this study.

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