Rapid Fluorogenic Assay for Differentiation of the Candida parapsilosis Group from Other Candida spp.

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A 30-min fluorogenic test was developed for differentiation of members of the Candida parapsilosis group from other Candida species commonly encountered in clinical material. The fluorogenic substrate, 4-methylumbelliferyl-β-D-glucoside, was utilized to assay β-glucosidase activity. A total of 50 C. parapsilosis isolates and 135 isolates of four other Candida species were tested. Assay sensitivity and specificity were 100 and 99.3%, respectively. The procedure was adapted for use with a spectrofluorometer.

Candidiasis has been increasingly implicated as a complication in both immunocompromised and immunocompetent individuals. Even though Candida parapsilosis has been estimated to make up only 2 to 5% of the commensal yeast flora, this organism shows affinity for traumatized endocardial tissue and has been reported to be responsible for 25% of candidal endocarditis cases (8). Similarly, the yeasts demonstrate a propensity to cause infection in patients receiving total parenteral nutrition (5). In a 1985 review, Dyess et al. (2) documented that patients with candidemia caused by C. parapsilosis had better survival rates than patients infected with other Candida species and pointed to the uniqueness of this yeast group in human medicine.

Confirmation of infections caused by yeasts presently requires laboratory isolation and subsequent identification of the infectious agent. Traditionally, taxonomic assessment of a yeast isolate has been determined according to the morphological characteristics, coupled with biochemical testing (6). Although this biochemical testing, whether with commercial kits or by traditional biochemical tests, has been based predominantly on the expression of the inherent enzymatic capacities of each yeast isolate, results are determined by growth of the agent. Direct assay of enzymatic activity could considerably shorten the identification process.

In recent years, the utilization of fluorophores for the detection of specific enzymatic activities in yeasts has been described (1, 7, 9). These substrates (such as 4-methylumbelliferyl-β-D-glucoside [4-MUB]) are nonfluorescent conjugates of umbellif erone which upon specific enzymatic cleavage liberate the fluorescent umbelliferone. 4-Methylumbelliferone is considered one of the strongest fluorescent compounds, being detectable at concentrations of 10⁻¹⁵ M (14). By comparison, few substances can be estimated colorimetrically below 10⁻⁷ g/ml (13). Umbelliferone possesses characteristic electric blue fluorescence when viewed under long-wavelength UV light (3), a characteristic which is readily detectable and also indicative of specific types of enzymatic activity with the appropriate substrates (4).

The following is a description of the use of 4-MUB formulated for the rapid differentiation of the C. parapsilosis group (10, 12) from other yeasts morphologically characterized as Candida species.

Yeast cultures. Fifty C. parapsilosis isolates originally isolated from clinical material in the Laboratory Services Branch were identified by traditional morphological and biochemical testing (development on corn meal, oxgall bile, yeast malt, peptone-dextrose, and cycloheximide agar; production of urease; pellicle or sediment formation; growth at 37 and 41°C; fermentation of glucose, lactose, sucrose, maltose, galactose, and trehalose; and assimilation of D-glucose, D-galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, D-xylose, L-rhamnose, erythritol, D-mannitol, inositol, salicin, L-arabinose, and potassium nitrate). These isolates were cultured on peptone dextrose agar and incubated at 28°C for 24 to 48 h. A total of 100 Candida albicans, 20 Candida tropicalis, 10 Candida krusei, and 5 Candida stellatoidea strains originally stored at −20°C were similarly prepared.

Substrate. 4-MUB (Sigma Chemical Co., St. Louis, Mo.) was prepared at 1.0 and 1.5 mM as described previously (1). Broths were prepared in 0.1 M sodium acetate-hydrochloric acid, phosphate, and boric acid-borate buffers at pH values ranging from 4.5 to 8.0, filter sterilized, and refrigerated until use.

Enzyme. β-Glucosidase (EC 3.2.1.21) (Sigma) was prepared fresh in sodium acetate (pH 5.1) and phosphate (pH 7.0) buffers at concentrations for final use of 5 × 10⁻³, 1.25 × 10⁻², and 2.5 × 10⁻² U/ml.

Visual UV test. A bench-model UV box (model cc-10; Ultra-Violet Products, Inc., San Gabriel, Calif.), was used at the long-wavelength UV setting for detection of fluorescence. Tubes with broth substrates were preincubated at room temperature or 37°C and then inoculated with a heavy, milky suspension of Candida species. Uninoculated broth was used as a control. The development of the characteristic electric blue fluorescence was observed at 10-min intervals over 1 h and recorded as a range from − to +++.

Spectrofluorometric studies. A Perkin-Elmer 203 fluorescence spectrophotometer with a Perkin-Elmer 150 xenon power supply was used. Samples (1 ml) of pure enzyme dilutions were added to 1 ml of substrate to determine reaction characteristics. Fluorescence of free umbelliferone was scanned for maximum excitation and emission wavelengths from 255 to 550 nm at each pH. Uninoculated substrates were used to provide the baseline readings. In the yeast studies, cultures were adjusted to a McFarland density standard of 10 in sterile distilled water, and 1 ml was inoculated into 1 ml of the substrate to a final concentration.
of approximately 10^6 cells per ml. All testing was conducted at room temperature.

**Semiquantitative assay with a bench-top UV box.** Preliminary studies indicated that a substrate system of 1 mM 4-MUβ in phosphate buffer (pH 7.0) was most suitable for the assay. 4-MUβ broth was prepared by dissolving 0.034 g of 4-MUβ in 0.2 ml of dimethyl sulfoxide (Sigma) and adding 0.1 M phosphate buffer (pH 7.0) to give a final volume of 100 ml. This solution was filter sterilized through a membrane filter (0.22-μm pore size) and then refrigerated as 0.5-ml aliquots. Higher substrate concentration and different buffers, ionic strengths, and pHs produced variable results in different yeast groups and were of nonselective value. Figure 1 illustrates the fluorescence obtained in the 30-min assay.

All 50 *C. parapsilosis* isolates incubated at room temperature and at 37°C demonstrated strong glucosidase activity (+ + + fluorescence) within 30 min. Incubation of the other *Candida* species at 37°C resulted in development of fluorescence in some isolates similar to that of the positive (+ + +) control and therefore lacked differential value. Room-temperature incubation of 100 *C. albicans*, 20 *C. tropicalis*, 9 of 10 *C. krusei*, and 5 *C. stellatoidea* isolates resulted in the absence of or distinguishably less fluorescence than the positive (+ + +) enzyme control within the 30-min assay time, thus permitting differentiation from the *C. parapsilosis* group.

**Spectrofluorometric assay.** The spectrofluorometric assay was standardized with purified β-glucosidase. The same substrate system described above was found to be suitable for this quantitative assay. Figure 2 shows results obtained at three concentrations of β-glucosidase. Estimates of fluorescence with the bench-top UV box scored as +, ++, and +++ were found to correspond approximately to spectrofluorometric readings of 20, 40, and 60, respectively.

Figure 3 shows results obtained spectrofluorometrically with different yeast isolates. *C. albicans*, *C. tropicalis*, and *C. krusei* gave readings equal to or less than 30 within 30 min of incubation at room temperature. All 15 isolates of *C. parapsilosis* demonstrated elevated fluorescence evolution with no overlap with other *Candida* species tested.

The above-described rapid, semiquantitative assay shows potential application in the diagnostic laboratory as a rapid differentiating method for members of the *C. parapsilosis* group. Preliminary results obtained with a substrate-buffer system of low pH supported the findings of Bobey and

![FIG. 1. Negative control (left) and positive test (right) with C. parapsilosis incubated in 1.0 mM 4-MUβ (pH 7.0) at room temperature for 30 min.](image)

![FIG. 2. Fluorescence produced by free umbelliferone liberated by cleavage of 1.0 mM 4-MUβ with different levels of β-glucosidase (pH 7.0) at room temperature. Excitation was at 365 nm; emission was at 465 nm. A 1/10 sensitivity setting was used.](image)
Ederer (1) that β-glucosidase activity was not significant in C. parapsilosis under certain conditions. Hydrolysis of glycosides can be affected by enzyme specificity and by reaction conditions (13). The results of Polacheck et al. (11) with β-glucosidase activity in C. albicans using different substrates and conditions illustrate such variation. In the present work, assay conditions were carefully selected and standardized for detection of the β-glucosidase of the C. parapsilosis group. No overlap was observed with other medically important related yeasts tested.

Use of a fluorogenic substrate permits rapid detection of β-glucosidase activity. Use of a UV box and minimal manipulation and material facilitate incorporation into routine mycological testing. The spectrophotometric assay, while not as advantageous for routine screening of yeast isolates because of the additional labor and instrumentation involved, has the advantage of providing a quantitative result. The latter assay therefore has the potential to provide finer differentiation between yeasts, perhaps even within the C. parapsilosis group under certain conditions.

The fluorogenic assay has the potential to replace up to 20 different tests and the several weeks of incubation time which may be necessary at times for complete identification of C. parapsilosis yeasts commonly encountered in clinical specimens. Perhaps a battery of such specific fluorogenic assays could provide rapid differentiation for other yeast groups. By comparison with traditional methods, the assay is therefore inexpensive and economical in terms of labor and time. When used according to the described criteria, it has the potential to provide rapid differentiation of the C. parapsilosis group from other frequently isolated species morphologically consistent with members of the genus Candida. The sensitivity and specificity are 100 and 99.3%, respectively.

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LITERATURE CITED


