JOURNAL OF CLINICAL MICROBIOLOGY, May 1991, p. 1081–1082
0095-1137/91/051081-02$02.00/0

Vol. 29, No. 5

Candida albicans Colony Identification in 5 Minutes in a General Microbiology Laboratory

STEPHEN F. DEALLER

Microbiology Department, University of Leeds, 1 Thoresby Place, Leeds LS1 9JT, United Kingdom

Received 22 October 1990/Accepted 7 February 1991

A total of 381 fully identified yeast isolates were tested by the germ tube (GT) and Albistrip (Lab M Ltd., Bury, United Kingdom) methods, and the results were compared. As a test system for the identification of *Candida albicans*, the Albistrip showed two false-positive and two false-negative results, whereas the GT showed seven false-negative and no false-positive results. With the same methods, 736 yeast isolates from clinical samples were tested in a laboratory that did not specialize in mycology. In this second experiment, when the results of the tests disagreed, further identification was carried out with the API 20C Yeast Identification System (API-Biomerieux Ltd., Vercieu, France). When the statistics of the first experiment were used to justify the results, this second experiment showed the Albistrip to be 98% sensitive and 98% specific, whereas the GT was 98% sensitive and 95% specific.

Candida albicans is the most common yeast isolated in the clinical laboratory. With the rise in the numbers of AIDS patients and the extended use of chemotherapy for neoplastic conditions, the increase in the numbers of immunocompromised patients has made the diagnosis of yeast conditions more important. The current methods of clinical laboratory yeast identification depend on the germ tube (GT) test, the production by the yeast of chlamydospores, and the ability of the yeast to utilize sugars. Full yeast identification by the last two tests takes 2 to 3 days, so the GT test, in which a germ tube (mycelium) is produced after 2 h in serum at 37°C, is popular. However GT-negative strains of *C. albicans* represent 1 to 2% of the species (3, 4), and other mycelium-like forms, such as pseudohyphae, may be misinterpreted as GTs in non-*C. albicans* species. These two problems may mean that laboratory staff that have not had full mycology training may find the GT test more inaccurate than is expected from the clinical literature. A cost-effective test that would permit early yeast identification and would help staff with less than full mycology training to identify *C. albicans* is obviously desirable. The GT test is relatively inexpensive, well known, and available.

A 90-min test for *N*-acetyl-β-galactosaminase coupled with a test for prolyl aminopeptidase has been shown to be as accurate as the GT test (5). An assessment of a 5-min form of these rapid enzyme tests in a busy general microbiology laboratory was thought to be of value.

I describe here a method of using these two enzyme tests in a format (Albistrip; Lab M Ltd., Bury, United Kingdom) that takes advantage of the fact that these enzymes are produced in adequate amounts without enzyme induction. The Albistrip was compared with the GT test for the identification of yeast isolates of known identity as well as clinical yeast isolates. Neither experiment was carried out in a mycology laboratory. The Albistrip consisted of a small sheet of plastic holding two filter strips: one of these was impregnated with proline-*p*-nitroanilide (PRO), and the other was impregnated with 4-methylumbelliferyl-*N*-acetyl-β-galactosamine (NAG). The yeast isolate to be tested was grown on selective agar overnight (or for 48 h, if required for adequate growth) and inoculated onto each of the strips. An adequate inoculum was considered present if it was visible to the naked eye. The filters were dampened with water and incubated at 37°C for 5 min. One drop of 0.01 M phosphate buffer (pH 10) was added to the NAG strip, and 1 drop of 0.1% (wt/vol) *p*-dimethylaminocinnamaldehyde in 1 M HCl was added to the PRO strip. The production of an immediate bright blue fluorescence on the NAG strip and a purple color on the PRO strip at the inoculum site under a hand-held long-wavelength UV light (Lab M Ltd.) within 30 s was considered positive. If both strips were positive, the organism was considered to be *C. albicans*.

In the first experiment, 369 yeast isolates, supplied fully identified by the Mycology Department, University of Leeds, were subcultured on Sabouraud agar (Oxoid Ltd., Basingstoke, United Kingdom) containing 40 mg of chloramphenicol per liter and incubated for up to 48 h at 30°C. Cultures unable to grow at this temperature were subcultured at 27°C or room temperature. Subcultures were tested by the GT test and the Albistrip. In the second experiment, 736 unidentified yeast isolates from clinical samples which had been cultured for 48 h as described above were tested with the GT test and the Albistrip. When these two tests yielded results which were not compatible, the yeast isolate was further tested with the API 20C system (API-Biomerieux Ltd., Vercieu, France), which required 3 days of incubation at 30°C.

Results from the first experiment are shown in Table 1. The sensitivity and specificity of the Albistrip were 98%, and the major fault of the GT test was the lack of GT production by 3% of the *C. albicans* strains. The sensitivity and specificity of the Albistrip (98%) were not significantly different from those of the GT test (χ^2). Probably of most importance is that in no instance were both the Albistrip and the GT test incorrect, justifying the protocol and results of the second experiment (Table 2), in which only the yeast isolates that had incompatible Albistrip and GT test results were further tested. However, the finding of NAG-positive and PRO-positive results for separate strains of *C. guilliermondii*, *C. glabrata*, *C. parapsilosis*, and *Geotrichium* spp. indicated that strains could be positive for both enzymes. The second experiment involved unidentified yeast isolates from clinical samples and was done by laboratory technicians who were not specialists in mycology. Hence, the results represent

TABLE 1. Comparison of the Albistrip tests NAG and PRO with the GT test for the identification of 381 yeast isolates previously identified by a mycology reference laboratory

Organism	No. of isolates with the following:					
	Combination of possible Albistrip results:				GT test result	
	NAG +, PRO +	NAG +, PRO −	NAG −, PRO +	NAG −, PRO −	+	−
Candida albicans	191	0	1	1	186	7
C. famata	0	0	8	1	0	9
C. guilliermondii	0	1	12	0	0	13
C. glabrata	0	1	5	27	0	33
C. kefyr	0	0	1	2	0	3
C. krusei	0	0	0	8	0	8
C. lipolytica	0	0	2	0	0	2
C. lusitaniae	1	0	6	1	0	8
C. norvegensis	0	0	1	0	0	1
C. parapsilosis	0	1	48	8	0	57
C. tropicalis	0	17	0	7	0	24
Cryptococcus albidus var. *diffluens*	0	0	0	1	0	1
C. neoformans	0	0	0	2	0	2
C. uniguttulatus	0	0	1	0	0	1
Geotrichum sp.	0	1	1	0	0	2
Saccharomyces cerevisiae	0	0	2	10	0	12
Sporobolomyces salmonicolor	0	0	1	0	0	1
Trichosporon beigelii	1	1	6	0	0	8
T. capitatum	0	0	0	3	0	3

those expected in a general microbiology laboratory. In this experiment, the Albistrip was 98% sensitive and specific, the GT test was 98% sensitive, but GT test specificity, at 95%, was significantly lower (χ^2, $P < 0.05$) than that of the Albistrip. This work was carried out by six technicians, and none was apparently better than another at GT interpretation (not statistically assessed). The problems with the GT test were GT-negative *C. albicans*, accounting for the sensitivity of 98%, and the misinterpretation of results, for example, the production of pseudomycelia, even in *C. glabrata*, which has no mycelial form, accounting for the relatively low specificity of 95%.

TABLE 2. Comparison of the GT test with the Albistrip for the identification of 736 yeast isolates from clinical samples

Result of the following test:			No. of isolates with the indicated combination of results	Identity of the yeast isolates (*n*) in instances in which the GT test result was not consistent with the NAG and PRO test results[a]
GT	NAG	PRO		
+	+	+	480	
−	−	−	111	
−	−	+	77	
−	+	−	32	
−	+	+	15	*C. albicans* (11), *T. beigelii* (1), nonclassical identification (3)
+	+	−	11	*C. albicans* (10), *C. tropicalis* (1)
+	−	−	10	*C. glabrata* (6), *S. cerevisiae* (1), *C. tropicalis* (1), nonclassical identification (2)

[a] Identities were determined with the API 20C system incubated at 27 and 30°C for at least 3 days.

The *Candida albicans* Screen (Carr-Scarborough Microbiologicals, Inc., Stone Mountain, Ga.) takes 90 min but relies on the same enzymes as does the Albistrip for the identification of *C. albicans*. When the *Candida albicans* Screen was compared with the GT test, results similar to those obtained in the Albistrip-GT test comparison were obtained (5). Previous work with NAG also yielded similar results (4). These studies also reported stronger reactions in more mature colonies, as were also found with the Albistrip. The *Candida albicans* Screen and the Albistrip rely on the same enzymes having been formed by the yeast cells before inoculation, and it is unlikely that the Albistrip would be effective if the relevant enzymes were inside the yeast cells.

Previous attempts to produce reliable rapid enzyme tests for *C. albicans* by use of the API ZYM system (API-Biomerieux) have not been successful (1, 8), and the finding of glucosidases in *C. albicans* and *C. parapsilosis* have made them less useful in yeast identification (6, 7). Screening of large numbers of potential enzyme substrates in the API 50CH and ATB 32GN systems (API-Biomerieux) as indicators for *C. albicans* revealed single chemicals to be inadequately specific or sensitive (2). Hence, it is not surprising that chemical substrate galleries have remained the most reliable technique (2).

As a test for *C. albicans*, the Albistrip was as sensitive and as specific as was the GT test, was easily used by technical staff not fully trained in mycology, could be stored for at least 6 months, gave bright colorimetric results with yeast colonies taken directly from clinical isolation, chloramphenicol-containing Sabouraud agar plates (including colonies over 1 week old), and yielded reliable macroscopic results in 5 min. Its value in the identification of *C. albicans* seems worthwhile, although the GT test was more specific. The Albistrip yielded few false-positives in this study, but a small percentage of non-*C. albicans* isolates would be expected to yield false-positives, and cultures mixed with bacteria would not be expected to yield reliable results.

REFERENCES

1. **Casal, M., and M. J. Linares.** 1983. Contribution to the study of the enzymatic profiles of yeast organisms with medical interest. Mycopathologia **81**:155–159.
2. **Hunter, P. R., and C. A. M. Fraser.** 1989. Application of a numerical index of discriminatory power to a comparison of four physiochemical typing methods for *Candida albicans*. J. Clin. Microbiol. **27**:2156–2160.
3. **Odds, F.** 1988. Candida and candidosis. A review and bibiography, p. 63. Bailliere Tindall, London.
4. **Perry, J. L., and G. R. Miller.** 1987. Umbelliferyl-labeled galactosaminide as an aid in identification of *Candida albicans*. J. Clin. Microbiol. **25**:2424–2425.
5. **Perry, J. L., G. R. Miller, and D. L. Carr.** 1990. Rapid, colorimetric identification of *Candida albicans*. J. Clin. Microbiol. **28**:614–615.
6. **Polacheck, I., M. Melamed, H. Bercovier, and I. F. Salkin.** 1987. β-Glucosidase in *Candida albicans* and its application in yeast identification. J. Clin. Microbiol. **25**:907–910.
7. **Smitka, C. M., and S. G. Jackson.** 1989. Rapid fluorogenic assay for differentiation of the *Candida parapsilosis* group from other *Candida* spp. J. Clin. Microbiol. **27**:203–206.
8. **Williamson, M. I., L. P. Samaranayake, and T. W. MacFarlane.** 1987. A new simple method for biotyping *Candida albicans*. Microbios **51**:159–167.