JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1981, p. 652-654
0095-1137/81/040652-03$02.00/0

Vol. 13, No. 4

Use of Rapid Auxanographic Procedures for Recognition of an Atypical *Candida*

JOHN G. BAKER,[1]† IRA F. SALKIN,[2]* DAVID H. PINCUS,[1] AND RICHARD F. D'AMATO[3]

Analytab Products, Division of Ayerst Laboratories, Plainview, New York 11803[1]; Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201[2]; and Division of Microbiology, Department of Pathology, Catholic Medical Center of Brooklyn and Queens, Jamaica, New York 11432[3]

Received 18 September 1980/Accepted 29 December 1980

An atypical *Candida* which can cause diagnostic problems in clinical laboratories has recently been characterized. Assimilation patterns of 29 clinical isolates of an atypical *Candida* were obtained by the API 20C (Analytab Products, Plainview, N.Y.), Uni-Yeast-Tek (Flow Laboratories, Inc., Rockville, Md.), and dye pour-plate auxanographic methods. The low frequency of assimilation of cellobiose, sucrose, and melezitose noted in all of these procedures permitted the early recognition of the atypical character of the isolates.

Baker et al. (2) recently reported the identification, by conventional techniques, of an atypical *Candida*. Atypical isolates were recovered with relatively high frequency from diverse clinical specimens in laboratories in the United States and Canada. They might easily be misidentified as other *Candida* species, such as *C. stellatoidea*, because of their frequent inability to utilize sucrose in auxanographic procedures (2). Morphologically they closely resemble *Candida tropicalis* and its sucrose-negative variants (1), but physiologically they differ from the typical *C. tropicalis* in their inability to ferment sucrose and melezitose, and from the sucrose-negative variants in their inability to assimilate inulin and their varied ability to utilize other carbon substrates (2).

Several auxanographic procedures are currently used to identify clinically important yeasts. The most common commercial methods are Uni-Yeast-Tek (UYT; Flow Laboratories, Inc., Rockville, Md.) and the API 20C microsystem (API 20C; Analytab Products, Plainview, N.Y.). A noncommercial technique, the dye pour-plate auxanograph (DPPA) was recently described by Land et al. (7). All of these newer procedures are accurate as compared with conventional techniques (3–6) and can be read at 3 to 6 days, as opposed to the 2 to 3 weeks required by the conventional Wickerham method.

Since many laboratories now use the rapid auxanographic techniques rather than the conventional procedures, we investigated the efficiency of the UYT, API 20C, and DPPA tests for early recognition of the atypical *Candida*.

† Present address: Microbiology Department, Lahey Clinic, Burlington, MA 01803.

MATERIALS AND METHODS

Test organisms. Twenty-nine isolates of the atypical *Candida*, originally recovered from blood, sputum, urine, bronchial washings, throat swabs, a lung biopsy, and a decubitus ulcer, were used in this investigation. A portion of growth from a stock culture of each test organism was streaked over the surface of modified Sabouraud dextrose agar (2% dextrose, 1% neopeptone, and 2% agar) in 100-mm plastic petri dishes and incubated for 72 h at 30°C. These 72-h cultures were used as the inoculum source for all physiological studies.

Wickerham method. Assimilation of 23 carbon and 2 nitrogen substrates (see Table 1) was determined by the Wickerham procedure as outlined by Van der Walt (8). Tests were incubated at 30°C for 3 weeks and read at the end of each week.

DPPA. Assimilation of 13 carbohydrates was assessed through the use of the DPPA method of Land et al. (7). The sterile basal agar medium with a pH indicator was melted, cooled to 45 to 50°C, inoculated with several colonies from 72-h modified Sabouraud dextrose agar cultures, gently swirled to distribute the yeast cells in the molten agar, and poured into 150-mm plastic petri dishes. Carbohydrate-impregnated disks were then added to the solidified agar surface. Plates were incubated at 27°C for 3 days and read each day for color change and growth, i.e., turbidity around the disks.

API 20C. The API 20C, consisting of 19 dehydrated substrates and a negative control, was performed according to the manufacturer's instructions. In brief, a sterile basal agar medium was melted, cooled to 50°C, inoculated with a portion of a single isolated colony from a 72-h modified Sabouraud dextrose agar culture, swirled to mix the yeast cells, and pipetted into each of the microcupules containing the dehydrated sub-

strates. The test was incubated at 30°C for 3 days and read each day for growth, i.e., turbidity within the agar.

UYT. The UYT plate, composed of 11 separately sealed wells containing nine substrates and two controls in a basal solid medium, was used according to the manufacturer's instructions. A portion of growth from a 72-h modified Sabouraud dextrose agar culture was transferred to sterile water to prepare a 1+ Wickerham suspension; 1 drop of suspension was added to each well. The plate was incubated at 27°C for 6 days and assessed each day for color change of the pH indicator incorporated in the agar medium and for growth, i.e., colony formation on the agar surface.

RESULTS

While the three auxanographic procedures gave generally similar assimilation patterns (Table 1), they showed apparent differences in the ability of the atypical isolates to utilize selected carbohydrates. The relatively low frequency of assimilation of maltose in the API 20C test (55%) contrasts with the greater than 90% utilization in both the DPPA and UYT. Whereas approximately a third of the isolates assimilated sucrose in the UYT, fewer than 10% did so in the API 20C or DPPA. Finally, fewer than 7% of the isolates assimilated cellobiose in the API 20C, but 22% did so in the UYT and 31% did in the DPPA.

TABLE 1. *Results obtained with 29 atyypical Candida isolates in four assimilation procedures*

Substrate	% Positive			
	Wickerham	DPPA	UYT	API 20C
L-Arabinose	3(3)[a]	0	—[b]	0
N-Acetyl glucosamine	—	—	—	100
Cellobiose	69(3)	31(15)	22	7
Dextrose	100	100	—	100
Erythritol	0	—	—	—
Galactitol (dulcitol)	0	0	—	—
Galactose	100	100	—	100
D-Glucitol (sorbitol)	100	—	—	100
Glycerol	14	—	—	2
Inositol	0	0	—	0
2-Keto-gluconate	100	—	—	100
Lactose	0	0	0	0
Maltose	100	92(8)	94	55
Melezitose	52	0(4)	—	1
Melibiose	0	0	—	—
α-Methyl-D-glucoside	24	—	—	0
Nitrate	0	—	0	—
Raffinose	0	0	0	0
Ribitol (adonitol)	100	100	—	100
Soluble starch	—	—	39(6)	—
Sucrose	59(3)	4(4)	33(6)	7
Trehalose	100	100	100	100
Urea	—	—	0	—
Xylitol	—	—	—	0
Xylose	100	100	—	100

[a] Parentheses indicate percent weak-positive reactions.
[b] —, Not part of procedure.

Utilization of the carbon and nitrogen substrates by the atypical isolates in the three auxanographic tests was in general agreement with Wickerham results (Table 1). However, significantly more isolates assimilated cellobiose (69%), sucrose (59%), and melezitose (52%) in the Wickerham procedure.

DISCUSSION

The differences in assimilation patterns between the Wickerham and the rapid auxanographic procedures, as well as differences among the auxanographic techniques, may be due to differences in methods of inoculation, substrate concentrations, duration of incubation, physical phase of the medium (i.e., agar or broth), or pH indicators. Therefore, detailed direct comparison of these systems is not valid.

The variable frequencies of assimilation of sucrose, α-methyl-D-glucoside, cellobiose, and melezitose found with the Wickerham technique should alert the laboratorian to the atypical character of these *Candida* isolates. Alternatively, the limited frequency of assimilation of sucrose, especially in the DPPA (4%) and API 20C (7%), the inability to utilize melezitose in these same two procedures, and the low utilization of cellobiose in all three methods should permit the laboratorian to initially recognize the atypical *Candida*.

The results obtained with either the Wickerham procedure or the commonly used rapid auxanographic techniques are not sufficient to completely identify the atypical *Candida*. Only when these procedures are used in association with other physiological and morphological tests, such as the germ tube test, fermentation of sugars, and morphology on corn meal agar plus 1% Tween 80, can one identify the atypical *Candida* isolates (2).

ACKNOWLEDGMENTS

We thank G. A. Land and G. D. Roberts for supplying cultures used in this study, and M. McGinnis and M. A. Gordon for their critical review of the manuscript.

LITERATURE CITED

1. **Ahearn, D. G., S. A. Meyer, G. Mitchell, M. A. Nicholson, and A. I. Ibrahim.** 1977. Sucrose-negative variants of *Candida tropicalis*. J. Clin. Microbiol. **5**: 494-496.
2. **Baker, J. G., I. F. Salkin, D. H. Pincus, and R. F. D'Amato.** 1981. Diagnostic characters of an atypical *Candida*. J. Clin. Microbiol. **13**:199-203.
3. **Bowman, P. I., and D. G. Ahearn.** 1976. Evaluation of commercial systems for the identification of clinical yeast isolates. J. Clin. Microbiol. **4**:49-53.
4. **Buesching, W. J., K. Kurek, and G. D. Roberts.** 1979. Evaluation of the modified API 20C system for identification of clinically important yeasts. J. Clin. Microbiol.

9:565-569.

5. **Cooper, B. H., J. B. Johnson, and E. S. Thaxton.** 1978. Clinical evaluation of the Uni-Yeast-Tek system for rapid presumptive identification of medically important yeasts. J. Clin. Microbiol. **7**:349-355.
6. **Land, G. A., B. A. Harrison, K. L. Hulme, B. H. Cooper, and J. C. Byrd.** 1979. Evaluation of the new API 20C strip for yeast identification against a conventional method. J. Clin. Microbiol. **10**:357-364.
7. **Land, G. A., E. C. Vinton, G. B. Adcock, and J. M. Hopkins.** 1975. Improved auxanographic method for yeast assimilations: a comparison with other approaches. J. Clin. Microbiol. **2**:206-217.
8. **Van der Walt, J. P.** 1970. Criteria and methods used in classification, p. 36-113. *In* J. Lodder (ed.), The yeasts: a taxonomic study, 2nd ed. North-Holland Publishing Co., Amsterdam.