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Diagnostic Characters of an Atypical *Candida*

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The morphological and physiological characters of an atypical *Candida* isolated from diverse clinical specimens are described. The colony and microscopic morphologies of the atypical *Candida* most closely resemble those of *Candida tropicalis* or of reported sucrose-negative variants of *C. tropicalis*. However, the atypical isolates differ from *C. tropicalis* by their inability to ferment sucrose or melezitose and from the sucrose-negative variants by their inability to assimilate inulin and their varied utilization of other carbon substrates.

Beginning in 1978 our laboratories have received numerous isolates of an atypical *Candida*. The frequency of its recovery from diverse clinical specimens in laboratories throughout the United States and Canada, as well as the similarity of its diagnostic characters to those of other clinically significant *Candida* species, suggested that this atypical *Candida* could cause diagnostic problems in clinical laboratories.

This report describes the morphological and physiological characters of 29 isolates of the atypical *Candida* as determined by conventional identification techniques. Since the atypical isolates had, in several instances, been misidentified as *Candida stellatoidea* when submitted to our laboratories, we decided to conduct parallel diagnostic studies with authentic strains of the latter species.

MATERIALS AND METHODS

Test organisms. A total of 29 isolates of the atypical *Candida* and 14 of *C. stellatoidea* were used in our study. The atypical *Candida* isolates were originally recovered from blood, sputa, urine, bronchial washings, throat swabs, lung biopsy, and decubitus ulcer. A portion of growth from a stock culture of each test organism was streaked with a sterile transfer loop over the surface of modified Sabouraud dextrose agar (MSDA: 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 the source of inocula for all morphological and physiological studies.

Biochemical tests. Assimilation of 31 carbon and 2 nitrogen sources and fermentation of 11 carbohydrates were determined by the Wickerham procedures outlined by van der Walt (3). Test cultures were incubated at 30°C for 6 weeks and were read once each week. Substrates used are listed in Tables 1 and 2.

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TABLE 1. *Fermentation patterns of 29 atypical Candida and 14 C. stellatoidea isolates*[a]

Substrate	Isolates positive (%)			
	Atypical *Candida*		*C. stellatoidea*	
	3 Weeks	6 Weeks	3 Weeks	6 Weeks
Cellobiose	0	0	0	0
Dextrose	100	100	100	100
Galactose	100	100	0	0
Inulin	0	0	0	0
Lactose	0	0	0	0
Maltose	100	100	93(7)[b]	100
Melezitose	0	0	0	0
Melibiose	0	0	0	0
Raffinose	0	0	0	0
Sucrose	0	0(3)	0	0
Trehalose	100	100	0	50

[a] Fermentation = acid and gas production in the Wickerham broth procedure.

[b] Numbers within parentheses indicate the percentage of weak-positive reactions.

Morphology. Colony morphology of each isolate was examined on MSDA plates after a 72-h incubation at 30°C. Microscopic morphology was evaluated by the Dalmau plate technique, using cornmeal plus 1% Tween 80 agar and incubating at 25°C for 7 days. A known chlamydospore-positive isolate of *Candida albicans* was the positive control for this microscopic character in each test.

Ascospore induction. A portion of a single colony of each isolate was removed from a 72-h MSDA plate with a sterile transfer loop and streaked over the surface of V-8 juice and malt extract agar slants. The cultures were incubated at 25°C for 6 weeks and checked each week for ascospore formation through the use of Schaeffer-Fulton-modified Wirtz stain (3).

Urea hydrolysis test. Several colonies of each isolate were removed from 72-h MSDA plates with a sterile applicator stick, streaked onto a Christensen urea agar slant (BBL Microbiology Systems), incu-

TABLE 2. *Assimilation patterns of 29 atypical Candida and 14 C. stellatoidea isolates*[a]

Substrate	Isolates positive (%)			
	Atypical *Candida*		*C. stellatoidea*	
	3 Weeks	6 Weeks	3 Weeks	6 Weeks
D-Arabinose	0	7(3)[b]	0(7)	21(7)
L-Arabinose	3(3)	55(3)	14(7)	29(7)
Cellobiose	69(3)	72	0	0
Citric acid	100	100	36	57
Dextrose	100	100	100	100
Dulcitol	0	0	0	0
Erythritol	0	0	0	0
Ethanol	100	100	71(14)	79(7)
Ethylamine hydrochloride	100	100	21(21)	57(7)
Galactose	100	100	100	100
Glucitol	100	100	64	79
Glycerol	14	55(3)	43	64
Inositol	0	0	0	0
Inulin	0	3	0	0
2-Ketogluconate	100	100	100	100
DL-Lactic acid	86(7)	97	100	100
Lactose	0	0	0	0
Maltose	100	100	100	100
Mannitol	100	100	100	100
Melezitose	52	86(3)	86	86
Melibiose	0	0	0	0
α-Methyl-D-glucoside	24	31(7)	43(7)	86(14)
Potassium nitrate	0	0	0	0
Raffinose	0	0	0	0
Ribitol	100	100	64(7)	86
Ribose	10	41(10)	14	43(7)
Rhamnose	0	0	0	0
Salicin	24(24)	31(17)	0	0
Sorbose	90(7)	100	0	14
Succinic acid	100	100	100	100
Sucrose	59	93	0	14(7)
Trehalose	100	100	100	100
Xylose	100	100	100	100

[a] Assimilation results obtained with the Wickerham broth technique.

[b] Numbers within parentheses indicate the percentage of weak-positive reactions.

bated at 30°C, and read after 7 days for growth and for color change of the pH indicator. An isolate of *Cryptococcus albidus* and an isolate of *C. albicans* were used as positive and negative controls, respectively, in each test.

Germ tube test. The ability of each isolate to form germ tubes was evaluated by the technique of Silva-Hutner and Cooper (2). *C. albicans* and *Candida tropicalis* were the positive and negative controls, respectively, in each test.

Temperature tolerance test. A portion of a single colony of each isolate was removed from a 72-h MSDA plate with a sterile transfer loop and streaked over the surfaces of two MSDA plates. One plate was then incubated at 37°C, and the other was incubated at 44°C; both were read for growth after 7 days.

Growth on Mycosel agar. A portion of a single colony of each isolate was removed from a 72-h MSDA plate with a sterile transfer loop, streaked over the surface of a Mycosel (BBL Microbiology Systems) slant, incubated at 30°C, and read for growth after 7 days.

RESULTS

All of the atypical *Candida* isolates formed ovoid blastoconidia (2.5 to 6.5 by 3.5 to 14.0 μm), singly or in a few small clusters, at the nodes of branched hyphae and pseudohyphae (Fig. 1). There was no evidence of germ tube, chlamydospore, or ascospore formation. Colonies were cream colored, smooth to wrinkled, glossy to dull glossy, and convex with spreading filamentous margins. In contrast, *C. stellatoidea* isolates produced somewhat smaller (2.0 to 4.0 by 4.0 to 12.0 μm) ovoid blastoconidia that were found in abundant, large clusters at the nodes of branched hyphae and pseudohyphae (Fig. 2). Furthermore, 11 of 14 isolates produced typical spherical chlamydospores, and all but 1 formed germ tubes. The one germ tube-negative isolate did form chlamydospores. The colonies, like those of the atypical *Candida*, were cream colored, smooth to wrinkled, slightly glossy to dull glossy, and convex and had entire to slightly filamentous spreading margins.

All of the atypical isolates fermented dextrose, galactose, maltose, and trehalose at 3 weeks (Table 1). Although none had fermented sucrose after 3 weeks, one isolate showed a weak sucrose fermentation after 6 weeks. Isolates of *C. stellatoidea* fermented only dextrose and maltose in 3 weeks, but at 6 weeks, approximately half of these isolates fermented trehalose.

At 3 weeks, eight of the carbon sources had been assimilated by all isolates of both types, nine were not utilized by either, and five were assimilated variably by the two organisms (Table 2). However, differences were found in the assimilation of seven carbon substrates: ribitol, glucitol, cellobiose, sucrose, sorbose, citric acid, and salicin. In addition, *C. stellatoidea* isolates showed a latent ability at 6 weeks to assimilate D-arabinose, α-methyl-D-glucoside, sucrose, sorbose, and ribose, whereas isolates of the atypical *Candida* latently utilized glycerol, L-arabinose, sucrose, melezitose, and ribose. Neither organism utilized potassium nitrate as the sole nitrogen source, but all atypical isolates had assimilated ethylamine hydrochloride at 3 weeks (Table 2). In contrast, only about 20% of the *C. stellatoidea* isolates had utilized this nitrogen source at 3 weeks, and only a total of 57% had utilized it at 6 weeks.

These morphological and physiological characters of the *C. stellatoidea* isolates agree closely with van Uden and Buckley's description of this species (4). The sole difference is in the frequency of assimilation of four carbon substrates.

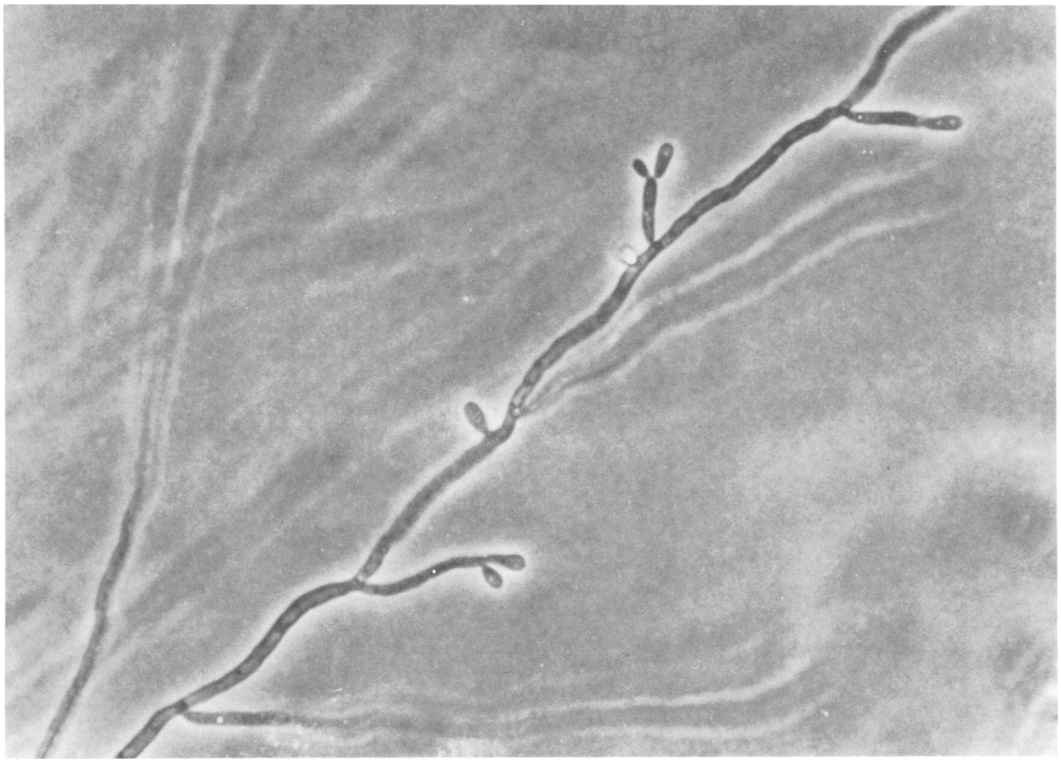

FIG. 1. *Hyphae of atypical Candida in vitro with blastospores formed singly or in small clusters at nodes.* ×*700.*

Our assimilation results are based upon a study of 14 isolates as opposed to the 5 studied by van Uden and Buckley.

Although all isolates grew on Mycosel at 27°C and on MSDA at 37°C, only those of the atypical *Candida* grew on MSDA at 44°C. Both organisms were urease negative.

DISCUSSION

Cursory examination of the morphological characters of the atypical *Candida* could lead to its misidentification as *C. albicans*, *C. stellatoidea*, or *C. tropicalis*. However, its inability to form germ tubes within 3 h at 37°C and the absence of chlamydospores, even after 7 days of incubation on cornmeal agar plus 1% Tween 80, suggest a closer relatedness to *C. tropicalis* than to *C. albicans* or *C. stellatoidea* (4). This suggestion is reinforced by a detailed microscopic examination. The ovoid blastoconidia are consistent in shape and size with those of *C. tropicalis*, but they are slightly larger than those described for *C. stellatoidea* and *C. albicans* (4). Furthermore, the appearance of the blastoconidia singly or in small sparse clusters at the nodes is similar to that noted in *C. tropicalis* but contrasts sharply with the abundant, large clusters characteristic of *C. albicans* and *C. stellatoidea*. Finally, the cream-colored, smooth-to-wrinkled, slightly glossy colonies with spreading, filamentous margins are more suggestive of *C. tropicalis*.

The physiological characters of the atypical *Candida* also differ in a number of respects from those of *C. stellatoidea*. Most significantly, 59% of the atypical isolates assimilated sucrose after 3 weeks of incubation (93% at 6 weeks). The inability of *C. stellatoidea* to utilize this sugar is generally employed to differentiate it from *C. albicans* (2). The two species also differ in the assimilation of cellobiose and sorbose and in their ability to use ethylamine hydrochloride as the sole nitrogen source. In addition, all atypical isolates fermented galactose and trehalose at 3 weeks, whereas no isolate of *C. stellatoidea* fermented either sugar. Thus, although several isolates of the atypical *Candida* had originally been misidentified as *C. stellatoidea*, the present morphological and physiological studies clearly indicate only a superficial similarity.

A comparison of the atypical *Candida* with *C. albicans* shows an identical fermentation pat-

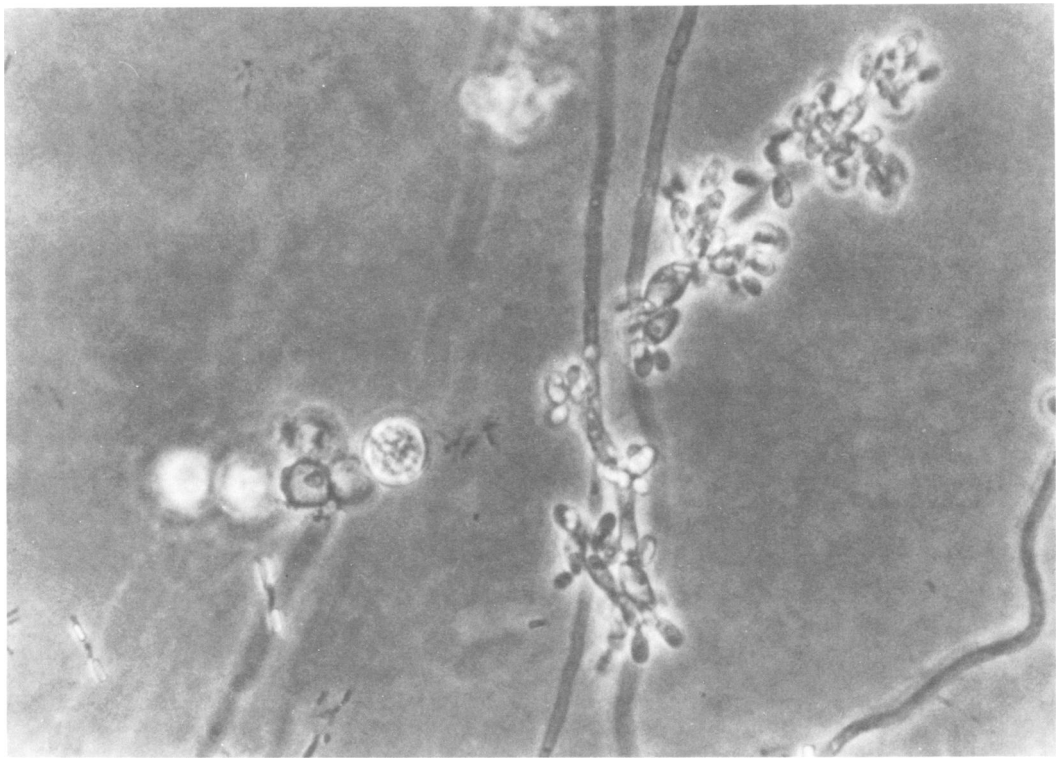

FIG. 2. *Chlamydospores and hyphae of C. stellatoidea in vitro with large clusters of blastospores at hyphal nodes.* ×*700.*

tern. However, the assimilation patterns are strikingly different. Whereas *C. albicans* assimilates sucrose, the atypical isolates were varied (only 59% utilized this carbohydrate). Furthermore, *C. albicans* isolates vary in their assimilation of ribitol, succinic acid, and citric acid (4), whereas all atypical isolates utilized these carbohydrates. Finally, *C. albicans* cannot use cellobiose as a carbon source, but 69% of the atypical *Candida* isolates did so.

Unlike *C. tropicalis*, the atypical *Candida* did not ferment sucrose and melezitose. The atypical isolates also varied in their assimilation of sucrose (59%), melezitose (52%), and α-methyl-D-glucoside (24%); all *C. tropicalis* isolates are described (4) as utilizing these three sugars. The *C. tropicalis* isolates vary in their assimilation of citric acid, whereas all atypical isolates utilized it.

Although the atypical *Candida* and the sucrose-negative variants of *C. tropicalis* described by Ahearn et al. (1) have identical fermentation patterns, they differ in the assimilation of carbon substrates tested. The sucrose-negative variants do not assimilate α-methyl-D-glucoside, melezitose, or sorbose, whereas variable assimilation of these sugars was noted with the atypical isolates. Whereas inulin and cellobiose are assimilated by all of the sucrose-negative *C. tropicalis* strains, none of the atypical *Candida* isolates utilized inulin, and they varied in their assimilation of cellobiose.

The salient morphological and physiological characters of these atypical *Candida* isolates are thus sufficiently clear for clinical laboratorians to readily recognize and identify them. Their colony and microscopic morphologies most closely resemble those of *C. tropicalis* or its sucrose-negative variant described by Ahearn et al. (1): it does not form germ tubes or chlamydospores, and its ovoid blastoconidia develop singly or in small clusters at the nodes of branched hyphae and pseudohyphae. However, the atypical isolates differ physiologically from *C. tropicalis* in their inability to ferment sucrose or melezitose and from the sucrose-negative form of *C. tropicalis* in their inability to assimilate inulin. In addition, whereas none of the sucrose-negative variants utilizes α-methyl-D-glucoside, melezitose, or sorbose, some isolates of the atyp-

ical *Candida* assimilated these three carbohydrates. A complete taxonomic description of the atypical *Candida* is in preparation.

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