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Characterization of Atypical *Candida tropicalis* and Other Uncommon Clinical Yeast Isolates

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Clinical yeast isolates representing α-glucoside-deficient variants of *Candida tropicalis*, *C. lusitaniae*, atypical *C. albicans*, and *Saccharomyces cerevisiae* were characterized. Additional physiological tests, including cellobiose fermentation, rhamnose assimilation, and triphenyl tetrazolium chloride reduction, are recommended for the detection and presumptive identification of uncommon *Candida* spp. in the clinical laboratory.

Certain disease syndromes and associated therapies, particularly those involving cancer or organ transplants, predispose patients to colonization or infection by yeasts. Advances in therapy and surgical procedures that have sustained life in situations where previously it would have been improbable have been accompanied by the increased threat of secondary infection by yeasts. Moreover, the spectrum of yeasts involved in infections of debiliated patients has increased (1, 2, 21).

These developments have resulted in the routine need for the presumptive identification of yeasts in clinical specimens and subsequently in the development of commercial identification systems. These beneficial systems have provided relatively rapid and accurate identifications for the more common, medically important isolates (8, 15, 17, 25). However, the limited test capacities of these commercial systems necessarily result in their being based on selective schemes for the identification of the most common strains of a species. The broad use of these commercial systems has brought about an awareness that atypical strains and less common species occur in clinical specimens. This report examines clinical yeast isolates (see Table 1) that have required other than the routine selective identification regimen to confirm their identification and it compares the diagnostic characteristics of these yeasts with those of authenticated cultures.

MATERIALS AND METHODS

Yeast isolates were obtained from the Mycology Division of the Centers for Disease Control, Atlanta, Ga., from cultures submitted to Georgia State University, and from the Centraalbureau voor Schimmelcultures, Delft, the Netherlands (Table 1). All cultures were subcultured on Mycological Agar (Difco) to assure purity. Yeasts were identified upon their acccession to the collection by procedures described by Wickerham (28), Lodder (18), and Ahearn and Schlitzer (3).

All physiological properties were redetermined for comparative studies. Cells from several colonies growing on corn meal-Tween 80 agar were suspended in appropriate basal medium (yeast nitrogen base, yeast carbon base, vitamin free base; Difco) to a concentration of approximately 10^6/ml. These cell suspensions were incubated at 25°C with intervals of agitation for 3 to 5 days to deplete endogenous nutrients. The depleted cells were diluted in basal medium, and 0.05 ml, about 10^4 cells, was used for inoculum. Broth assimilatory tests were incubated at 30°C on a shaker (150 rpm) and were read at 5, 12, and 21 days. For each culture, growth in the assimilatory tests was recorded and graded on a scale from zero to a maximal turbidity of 4, which was established as equal to or greater than that of the glucose growth control. Tests that gave a reading of no more than 2 by 21 days were repeated by subculturing to a second tube of the respective medium and by inoculation of an entirely new assimilation test; 2 was arbitrarily selected as minimal growth for a positive recording. Fermentation tests were incubated in standing culture at 25°C and recorded as positive at 5 or 12 days when the medium in the Durham tube was displaced with air or when venting of the test tube cap resulted in the release of gas from the broth.

Growth at 37°C was determined on Mycological Agar plates (Difco) and slants. The plates were incubated in a humidified incubator and the slants in a water bath. All plates and slants were examined daily for 7 days for growth.

Resistance to cycloheximide was determined on Mycosel Agar (BBL Microbiology Systems) plates incubated at 25°C for 7 days.

A modified Pagano-Levin medium was prepared by adding an ultra-sterilized solution of 2,3,5-triphenyl tetrazolium chloride (Sigma) (TTC) to a cooled volume of Mycological Agar (50°C) to give a final concentration of 100 μg/ml (8). The medium was dispensed into petri plates and allowed to equilibrate at 25°C for 1 day before use. Yeast cultures were spot-inoculated onto the surface of the medium from a 2 to 3-day-old culture, and the plates were incubated at 25°C for 24 h.

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TABLE 1. Yeast isolates examined

Species	No. of isolates from source:			
	Blood	Sputum	Urine	Other
C. tropicalis	16	2	0	3
C. lusitaniae	1	3	0	3
C. albicans	0	0	0	3
S. cerevisiae	0	4	1	0

Reduction of the TTC to a dark red-maroon formazan was recorded as a positive reaction.

RESULTS

The physiological reactions for the isolates of *Candida tropicalis* and *Candida lusitaniae* are given in Table 2. The isolates of *C. tropicalis* failed to assimilate α-glucosides and showed strain variation in the assimilation of β-glucosides. When incubation periods were extended beyond 21 days and additional subculturings were performed, a few of the isolates assimilated cellobiose, sucrose, or melezitose. None of the isolates fermented sucrose, nor did they produce germ tubes in fetal calf serum, although all formed numerous elongate pseudophyphal cells, some grossly resembling germ tubes, but all with constrictions at their point of origin. All isolates reduced TTC to a dark red formazan within 24 h. This type of reaction is also observed for typical isolates of *C. tropicalis* (8, 9). The isolates included three strains (GSU 59, GSU 372, GSU 446) initially reported as sucrose-negative variants of *C. tropicalis* (2). Previously these strains were reported to assimilate inulin; however, by our procedures and criteria, only one isolate (GSU 372) gave weak growth which approached our minimal criterion for a positive reaction. All isolates developed *Candida* to *Mycotoruloides* type pseudomycelium on corn meal agar with Tween 80. Unlike typical isolates of *C. tropicalis*, several sucrose-negative isolates produced on initial testing only sparse strands of pseudohyphae; however, with extended incubation, subculturing, on both, more typical morphology developed. All isolates were resistant to cycloheximide, a variable characteristic for more typical strains of *C. tropicalis*, and all isolates grew well at 37°C.

The assimilation and fermentation reactions of the seven isolates of *C. lusitaniae* varied in their degrees of fermentation with α-glucosides and in several assimilatory reactions, but, in contrast to *C. tropicalis*, assimilated rhamnose, fermented cellobiose, and utilized taurine for growth. All cultures of *C. lusitaniae* were sensitive to cycloheximide and gave only a weak pink reaction with TTC. All isolates grew well at 37°C and only occasionally produced elongated pseudohyphal cells in bovine serum.

Salient physiological characteristics distinguishing *C. tropicalis*, its sucrose-negative vari-

TABLE 2. Assimilation and fermentation patterns of *C. lusitaniae* and of sucrose-negative variants of *C. tropicalis*

Test	Pattern[a]	
	C. tropicalis (21 isolates)	*C. lusitaniae* (7 isolates)
Assimilation of:		
D-Arabinose	−	−
L-Arabinose	−	−
Cellobiose	v(9)	+
Citrate	−	−
Erythritol	−	−
Ethanol	+	v(6)
Galactose	+	+
Galactitol	−	−
Glucitol	+	+
Glucose	+	+
Glycerol	v(1)	+
Inositol	−	−
Inulin	−	−
2-Keto-gluconate	+	+
5-Keto-gluconate	+	−
Lactose	−	−
Maltose	+	+
Mannitol	+	+
Melezitose	v(2)	+
Melibiose	−	−
α-Methyl-D-glucoside	v(2)	v(4)
N-Acetyl-D-glucosamine	+	+
Raffinose	−	−
Ribitol	+	+
Ribose	v(1)	+
Rhamnose	−	+
Salicin	v(1)	+
Sorbose	−	+
Starch	+	−
Succinate	+	+
Sucrose	−	+
Trehalose	+	+
Turanose	v(2)	+
Xylitol	v(15)	+
Xylose	+	+
KNO_3	−	−
Taurine	−	+
Vitamin-free substrate	−	−
Fermentation of:		
Cellobiose	−	+
Galactose	+	+
Glucose	+	+
Inulin	−	−
Lactose	−	−
Maltose	+	v(3)
Melezitose	−	v(2)
Melibiose	−	−
Raffinose	−	−
Sucrose	−	v(1)
Trehalose	+	v(5)

[a] +, All isolates positive; −, all isolates negative; v, isolates varied. Figures in parentheses are number of isolates giving positive reactions.

TABLE 3. Characteristics of selected yeasts phenotypically similar to *C. tropicalis*[a]

Yeast (no. of isolates)	Assimilation of:									Fermentation of:			TTC reaction	Growth on Mycosel	Growth at 37°C
	Glycerol	Sucrose	Melezitose	α-Methyl-D-glucoside	Rhamnose	Cellobiose	Salicin	Sorbose	Starch	Cellobiose	Sucrose	Maltose			
C. albicans (110)	v	(+)	v	v	−	−	−	v	v	−	(−)	+	−	+	+
C. lusitaniae (7)	+	+	+	(+)	+	+	+	+	−	+	v	v	−	−	+
C. tropicalis (105)	(−)	+	+	+	−	(+)	(+)	(−)	+	−	+	+	(+)	v	+
C. tropicalis, sn[b] (21)	(−)	(−)	(−)	(−)	−	v	(−)	−	+	−	−	+	+	+	+
C. viswanathii (1)	+	+	+	+	−	+	−	−	+	−	−	+	−	+	+
C. zeylanoides (3)	+	−	−	−	−	v	v	+	−	−	−	−	−	−	−
B. naardenensis (13)	−	−	(−)	(−)	(−)	+	v	(−)	(−)	−	−	−	−	−	−

[a] +, All isolates examined gave a positive reaction; −, all isolates examined gave a negative reaction; v, reactions commonly varied among isolates; (−), reaction usually negative; (+), reaction usually positive.
[b] sn, Sucrose negative.

ants, and *C. lusitaniae* from phenotypically similar yeasts are presented in Table 3. There is considerable overlapping of these various physiological properties among the strains of a species, and some of the uncommon reactions have been observed only under certain conditions. For example, sucrose fermentation by isolates of *Candida albicans* was noted as a delayed reaction with incubation at 37°C (18). Growth of *Candida zeylanoides* at near 37°C was achieved for initial clinical isolations, but subsequent testing of the cultures gave negative results. Similarly, an isolate of *Brettanomyces naardenensis* from a soft drink and another from sputum initially grew slowly at 37°C, but on subsequent retesting did not grow at 37°C (16). The reactions with α-glucosides and β-glucosides are the most difficult to categorize since these reactions appear to be more directly influenced by the composition of the agar medium from which the inocula for the various tests are prepared. We have not presented variation for our results with *Candida viswanathii* because we accepted only the type strain CBS 4024 as representing the species. Two other cultures that we have examined under this binomial failed to grow at 37°C and reduced TTC. These two cultures appeared to be related to *Candida saki*.

Five clinical isolates were identified, with some difficulty, as *Saccharomyces cerevisiae*. Three of these failed to produce ascospores. Of these, culture 80036, which gave a typical fermentation spectrum, failed to grow in the defined assimilation broths. When the pH of these broths was adjusted to 7.0, assimilation reactions representative of the species were obtained. Two isolates gave unusual morphology: one produced a highly mucoid colony, and the other produced extensive candida-type pseudohyphae. Two cultures eventually produced asci and ascospores on dimalt agar. One isolate (CDC 161), from a kidney autopsy, failed to ferment or assimilate maltose; the second isolate, from a cancer patient, grew poorly, gave variable assimilation reactions in repeated tests, and produced amoeba-like cells (Fig. 1).

One clinical isolate of *C. albicans* (GSU 435) produced typical germ tubes, chlamydospores, and fermentation reactions, but failed to grow in defined media. A number of isolates of *C. albicans* failed to produce chlamydospores and produced only rudimentary pseudohyphae or aberrant germ tubes, particularly on initial testing. These isolates, typified by GSU Ca 9 (Fig. 2), occasionally produced revertants to a typical morphological form with subculturing and maintenance.

DISCUSSION

Variants of *C. tropicalis* and *C. albicans* deficient in α-glucoside activity have been previously described (2, 20). Ahearn et al. (2) chose not to elevate four sucrose-negative strains to a separate anamorph taxon since serological, physiological, morphological, and DNA homology data supported their inclusion within the anamorph taxon *C. tropicalis*. These sucrose-negative variants differed from several authenticated strains mainly in their assimilation of inulin. This property, not considered sufficient to affect the initial classification, could not be verified in the reexamination of these strains in this study. Inulin and starch assimilation reactions often are suspect since these carbohydrates usually are sterilized by autoclaving and may be hydrolyzed. Moreover, different lots and

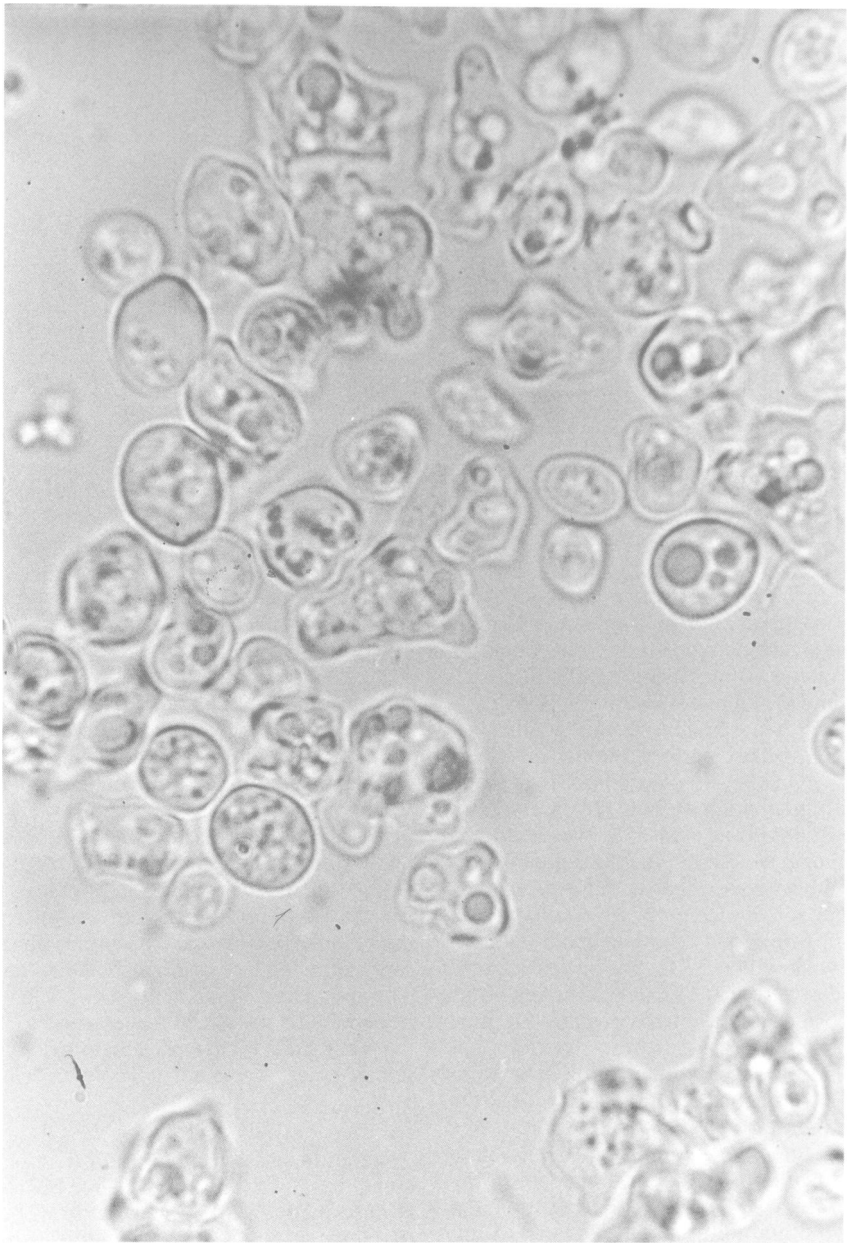

FIG. 1. Irregular shaped cells of *S. cerevisiae* grown on malt agar; ×1,000.

brands of starch and inulin often give variable results because of the presence of impurities. Inulinase also has been shown to be inducible for some species of *Candida* (12). The initially described sucrose-negative variants of *C. tropicalis* and several similar isolates studied herein have been classified elsewhere as *C. tropicalis* on the basis of serology (25) and the near identity of the proton magnetic resonance spectrum of their cell wall mannans to that of *C. tropicalis* (Shinoda, personal communication). In other studies, yeasts with these characteristics have been considered atypical *Candida* (4, 5) and subsequently were proposed as a new anamorph taxon, *Candida paratropicalis* (6).

C. lusitaniae was implicated by Holzschu et al. (14) as an opportunistic pathogen of a cancer patient. We had occasionally obtained this species among isolates submitted to the Centers for Disease Control over the past 10 years. Depend-

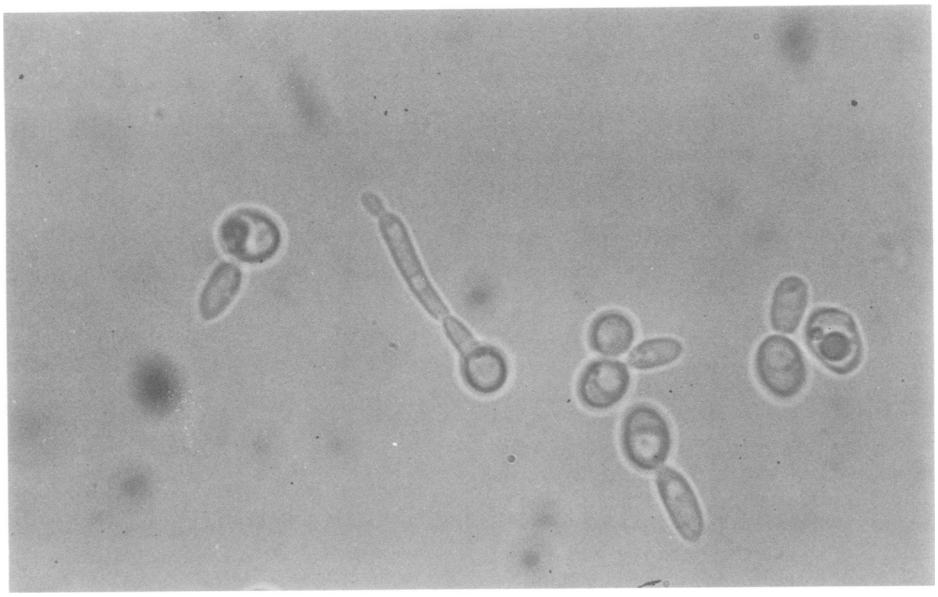

FIG. 2. Short aberrant germ tubes of *C. albicans* produced in bovine serum after 3 h at 37°C; ×700.

ing on the maltose fermentation reaction of the strain and the spectrum of tests, the isolates could be confused with *C. tropicalis* or *Candida parapsilosis*. Serological screening classifies *C. lusitaniae* with *C. albicans* serotype A, whereas analysis with the API 20 system classifies *C. lusitaniae* with *C. tropicalis* (25). *C. lusitaniae*, which is an anamorph of *Claviaspora* (24), possesses numerous distinctive properties, but the sucrose-negative variants of *C. tropicalis* have phenotypic properties that overlap with several taxa (Table 3). Fortunately, the incidence of *B. naardenensis*, *C. zeylanoides*, and *C. viswanathii* in clinical specimens is rare. The guanine plus cytosine content of these latter two is above 40%; that of *B. naardenensis* has not been reported. *C. albicans* and *C. tropicalis* have guanine plus cytosine contents near 35%, but no significant hybridization occurs between their DNAs (20).

In most cases, the simplest effective method for distinguishing sucrose-negative variants of *C. tropicalis* from *C. albicans* is the germ tube test. However, for some strains, a routine germ tube test is insufficient, and varied media and controlled inocula are necessary (22). Several investigators have observed that strains of *C. albicans* may show negligible germ tube production, particularly on routine screening (11, 22). Tierno and Milstoc (26) reported that four strains of *C. tropicalis* recovered from sputum produced germ tubes in serum and fermented sucrose when the fermentation tests were incubated at 37°C. The cultures failed to grow on cycloheximide and on later examination were reported to have lost their ability to form germ tubes (27). Apparently only rare isolates of *C. tropicalis* produce true germ tubes and this capacity is lost with maintenance in culture (19). Hedden and Buck (13) re-emphasized that germ tubes diagnostic for *C. albicans* lack constrictions at their points of origin. Huppert el al. (15) and Bowman and Ahearn (7) found that clinical isolates of *C. tropicalis* produce elongated pseudohyphal cells grossly similar to germ tubes but with constrictions at their points of origin. Most of the isolates of the sucrose-negative variants of *C. tropicalis* produce these elongated pseudohyphal cells, but no true germ tubes have been observed. Careful microscopic examination usually permits the pseudohyphal cells to be distinguished from germ tubes, but the inclusion of a TTC test greatly simplifies the separation process (9, 10). The presence of a deep redmaroon pigment at 24 h is typical of *C. tropicalis*, and rarely is a negative reaction observed.

It is generally stated that a yeast must assimilate a sugar in order to ferment that sugar. Certain strains obtained from clinical specimens, however, have nutritional requirements (usually purines or pyrimidines) that preclude their growth in the defined assimilation media based on the Wickerham formulae. These strains, such as our isolates of *C. albicans* and *S. cerevisiae*, do grow in the complex fermentation media with the production of gas. Occasionally we have seen such fastidious yeasts misidentified as *Malassezia*.

Wickerham (28) discussed the need for recognizing the common occurrence of strain variation among yeasts in morphological and physiological characteristics. He stated, "It is necessary to determine, therefore, the physiological reactions of a large number of strains of each species, in order to ascertain which reactions are common to all strains and which are variable." Price et al. (23) discussed the unsoundness of species discrimination based on one or two sugar reactions, particularly if these reactions have not been weighted by correlation with the genotype. The yeasts examined in this study were assigned to a species on the basis of their overall physiological and morphological properties. The identification of yeasts based on a restricted and unweighted number of characteristics should not be construed to provide other than a presumptive identification. The additional tests suggested for the detection of *C. lusitaniae* and the sucrose-negative variants of *C. tropicalis* are recommended with this philosophy.

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