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Sucrose-Negative Variants of *Candida tropicalis*

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Four cultures of a *Candida* sp. that lacked alpha-glucosidase activity were isolated from clinical specimens. Physiological, morphological, and serological characterizations of the yeasts and deoxyribonucleic acid reassociation studies supported their classification as a variant of *C. tropicalis*.

Between 1971 and 1975, four yeast isolates with an apparent unique spectrum of morphological and physiological properties were isolated from three different patients. Two patients were diagnosed as having candidiasis prior to their death. One was a 53-year-old male with third degree burns over approximately 35% of his body. This patient had received intravenous fluids and had been treated topically with daily Phisohex scrubs and applications of Sulfamylon (4-homosulfanilamide). Antibiotic therapy during his first 22 days of hospitalization had consisted of penicillin, streptomycin, gentamicin, and cephalosporin. On day 6 of hospitalization, the patient showed symptoms of fungemia with a temperature of 40°C. At that time, yeasts were isolated from burned areas of the lip, abdomen, and right axilla, and an isolate (GSU 372) was obtained from the blood. The patient ran a high fever and steadily deteriorated, with low urinary output and symptoms of a blocked intestine. On the day 23, amphotericin B therapy was begun and continued for 2 days until urinary output ceased. Culture GSU 446 was isolated from the blood at that time, just a few hours prior to death. Culture GSU 59 was isolated from the blood of a 72-year-old male patient who later died of a pulmonary infection thought to be due to a *Candida* species. The patient had received extensive broad spectrum antibiotic therapy. Culture GSU 79 was obtained from cerebral spinal fluid of a terminal cancer patient who had been suspected of developing cryptococcosis. The four yeast cultures were studied extensively to determine their taxonomic status.

The physiological and morphological characterization of the isolates was according to the methods of Lodder (4) and Ahearn (1). Serological groupings of the yeasts were accomplished with the slide agglutination test, using absorbed rabbit antisera (Sweet and Kaufman; 4), and with soluble antigen, using a microimmunodiffusion technique of double diffusion in two dimensions (3) with adaptations according to Meyer et al. (5). Procedures for determining deoxyribonucleic acid (DNA) relatedness were those of Meyer et al. (5).

Selected sugar fermentation and assimilation reactions of the four clinical isolates are contrasted with those established for similar yeasts in Table 1. The isolates were intermediate in these physiological properties between *C. albicans*, *C. stellatoidea*, and *C. tropicalis*. All but one of the four, GSU 372, failed to utilize alpha-glucosides as a sole carbon source for growth, and this strain assimilated sucrose latently. All four isolates had essential requirements for biotin, and thiamine was stimulative for growth. The maximum temperature for growth ranged from 42 to 43°C. Initially, GSU 372 formed only sparse strands of pseudomycelium with grape-like clusters of blastospores at regular intervals. This mycotorula type filamentation is typical of *C. albicans*. In subsequent culture, consistent with the other isolates, GSU 372 produced profuse pseudohyphae with irregular length lateral branches interspersed among restricted clusters of blastospores. This mycotoruloides type pseudohyphae is found both in *C. albicans* and *C. tropicalis*, but it is more characteristic of the latter species. Single or mixed cultures of the four isolates were observed not to produce ascospores. None of the four produced germ tubes in appropriate media, but all produced elongated pseudohyphal cells. Minimal inhibitory concentrations of amphotericin B for the four strains ranged from 0.1 to 0.3 μg/ml. Strain GSU 446, isolated from the burned patient after amphotericin B treatment, showed the highest minimal inhibitory concentrations.

Isolates GSU 59, 372, and 446 were each inoculated intravenously (10^6 viable cells) into groups of 12 Swiss white mice (20 to 25 g). Two

TABLE 1. *Fermentation and assimilation characteristics of selected species of Candida*[a]

Species	Dextrose	Galactose	L-Sorbose[b]	Maltose	Sucrose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Soluble starch[b]	Xylose[b]	Rhamnose[b]	α-Methyl D-glucoside[b]	Salicin[b]	Mannitol[b]
Candida albicans	F	F	V	F	+	–	VF	–	–	–	V	–	+	+	–	V	–	+
C. stellatoidea	F	+	–	F	–	–	+	–	–	–	–	–	+	+	–	V	–	+
C. tropicalis	F	F	V	F	F	V	F	–	–	–	VF	–	+	+	–	+	V	+
GSU 59, 79, 372, 446	F	F	–	F	V	+	F	–	–	–	–	+	+	+	–	–	+	+

[a] F is fermented and assimilated; V is strain variation in assimilation; + is assimilated; VF is assimilated with variable fermentation; – is not fermented or assimilated.

[b] Tested for assimilation only.

to three mice were killed by each isolate within 25 days. The dead mice showed typical signs of candidiasis, primarily with lesions of the kidneys.

Whole cells of the four unknown isolates shared common antigens with *C. albicans* serotype A and *C. tropicalis*. The isolates did not react with the species-specific antisera to *C. guilliermondii*, *C. krusei*, *C. parapsilosis*, and *C. pseudotropicalis*, nor to the antiserum of the *C. albicans* B–*C. stellatoidea* complex. Antiserum to soluble antigens of isolate GSU 446 formed a line of identity with soluble antigen of *C. albicans* A and *C. tropicalis*. With one of the three isolates of *C. tropicalis* tested (culture AJ5338, see Nakase et al., 6), two lines of identity were formed. When unadsorbed antiserum to GSU 446 was reacted with *C. albicans* A, *C. albicans* B, *C. stellatoidea*, and its homologous antigen, it showed no reaction with *C. albicans* B, two lines with the homologous antigen, and a single precipitin band with *C. stellatoidea*. Adsorption with *C. albicans* B antigens did not eliminate any reactions, although their intensity was reduced. The precipitin line with *C. stellatoidea* and one of the lines with GSU 446 did not appear when the antiserum was adsorbed with the antigen of *C. stellatoidea*. Studies with the soluble antigens and whole cells support a closer affinity between GSU 446 and certain isolates of *C. tropicalis* than to the *C. albicans* B–*C. stellatoidea* complex. The data also support the findings of Nakase et al. (6) that differing serotypes exist in *C. tropicalis*. Similarly, *C. stellatoidea* may be regarded as an additional serotype of *C. albicans*.

The guanine plus cytosine content of the DNA of isolates GSU 59, 372, and 446 ranged from 33.2 to 34.6% (median value, 34.6%), whereas the guanine plus cytosine content of nine isolates of *C. tropicalis* ranged from 33.9 to 36.1% (median value, 35.1%). DNA reassociation studies conducted with GSU 372, GSU 46, and the type strain of *C. tropicalis* (ATCC 750) gave 96% relative binding (homologous system, ATCC 750, set at 100%) between GSU 372 and ATCC 750 and 90% between GSU 446 and ATCC 750. The DNA of GSU 372 showed only 14% and that of GSU 446 only 17% reassociation with the DNA of *C. stellatoidea* (CBS 1905, type strain). These data further support the classification of the four unknown clinical isolates as *C. tropicalis*.

There has been frequent question of the validity of distinguishing yeast species by a single difference in physiology or morphology. In certain instances, e.g., the separation of *C. tropicalis* from *C. maltosa* by inability of the latter

to assimilate soluble starch, species distinction was supported by DNA reassociation studies (5). Herein, comparative evaluation of physiological, morphological, and serological properties and DNA reassociation studies did not support the creation of a new species on the basis of differences in alpha-glucosidase activity. The distinction of *Candida* species by the presence or lack of alpha-glucosidase activity has been questioned previously. For example, *C. stellatoidea* may be considered an alpha-glucosidase-negative variant of *C. albicans* (2). This relationship is further supported by the capacity of both yeasts to produce germ tubes. The alpha-glucosidase-negative variants of *C. tropicalis* are readily distinguished from *C. stellatoidea* by their failure to produce germ tubes. The variants did produce elongated pseudohyphal cells in germ-tube induction media, and all were pathogenic for mice and implicated as pathogens for humans.

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