Variable Assimilation of Carbon Compounds by *Candida albicans*

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A total of 215 typical strains of *Candida albicans* were studied for their ability to assimilate 11 carbon compounds. All isolates assimilated lactic acid, ribitol, succinic acid, and methyl α-D-glucopyranoside. None of the isolates assimilated cellobiose and salicin; 1.9% of the isolates assimilated L-arabinose. Citric acid, glycerol, and L-sorbose were assimilated by greater than 97% of the isolates, whereas melezitose was assimilated by 81% of the isolates. Assimilation results depended on duration of incubation, temperature, and methodology.

The taxonomic description of *Candida albicans* includes information on the assimilation of 31 carbon compounds (14). *C. albicans* is listed as giving a variable assimilation result with 11 of the carbon compounds; however, the number of isolates giving either a positive or a negative reaction is not presented. This taxonomic key is based on studies of 22 strains of *C. albicans* isolated in various parts of the world and from a variety of sources. The present study was undertaken to define the variability among typical human isolates of *C. albicans* in the assimilation of these 11 carbon compounds.

**MATERIALS AND METHODS**

*C. albicans* was isolated from 198 specimens submitted to the following clinical microbiology laboratories (number of isolates within parentheses): Emory University Hospital, Atlanta, Ga. (70); Grady Memorial Hospital, Atlanta, Ga. (21); Harborview General Hospital, Seattle, Wash. (7); and Strong Memorial Hospital, Rochester, N.Y. (100). Specimens included respiratory, vaginal, and cervical secretions, urine, rectal or perianal swabs, blood, and other body fluids. No more than one isolate of *C. albicans* per patient was included in this study. An additional 17 strains of *C. albicans* were obtained as follows: 1 strain, *C. albicans* 18804 type culture, from the American Type Culture Collection (ATCC); 3 strains from E. Reiss, National Center for Disease Control (NCDC); 6 strains from H. R. Buckley, Temple University, including ATCC strains 32354 and 38696; 6 strains from B. Cooper, Baylor University; and 1 strain from the Mycology Reference Laboratory, NCDC. The strains were stored at room temperature and at 4°C on modified Sabouraud agar. All isolates were identified as recommended by Haley and Callaway (6) and in accordance with van Uden and Buckley (14). Specifically, most isolates formed germ tubes in fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.), and all produced chlamydospores on rice extract agar or cornmeal-Tween 80 agar (Difco Laboratories, Detroit, Mich.) and assimilated but did not ferment sucrose. Identification of the isolates was confirmed by assimilation and fermentation tests.

Carbon compounds for the assimilation broth tests were obtained as follows: salicin, L-sorbose, ribitol, methyl α-D-glucopyranoside, melezitose, and L-arabinose (all National Research Council standard) (Pfannstiehl Laboratories, Inc., Waukegan, Ill.), and sucrose, succinic acid, lactic acid, anhydrous glycerol, and citric acid monohydrate (Baker Analyzed Reagents, J. T. Baker Chemical Co., Phillipsburg, N.J.).

Differential disks for the auxanographic tests were obtained as follows: sucrose, dextrose, arabinose, and galactose (Difco) and salicin, glycerol, cellobiose, and L-arabinose (BBL Microbiology Systems, Cockeysville, Md.). No commercial differential disks were available for L-sorbose, ribitol, methyl α-D-glucopyranoside, melezitose, succinic acid, or citric acid. To prepare these disks, a 10% solution (wt/vol) of each carbon compound was made in distilled water and filter sterilized, and 25 μl of each solution was dropped onto a sterile 6.5-mm paper blank (Difco) and allowed to air dry at room temperature.

All of the isolates were tested for assimilation of the carbon compounds by the auxanographic method described by Haley and Callaway (6), by using yeast nitrogen base (Difco) with 1.5% Noble agar (Difco) as the base medium. The plates were observed for yeast growth around the disks after incubation at room temperature for 48 and 120 h. Growth was reported as positive, negative, or questionable. All of the isolates of *C. albicans* which showed a questionable reaction around a disk and isolates which showed a reaction different from 95% of the other isolates were retested by the auxanographic technique and tested by a broth assimilation test.

All of the isolates were tested for assimilation of glycerol, L-sorbose, melezitose, and methyl α-D-glucopyranoside by the Wickerham broth method for carbon assimilation as described by Haley and Callaway (6) with the following modifications: tubes (16 by 125 mm) were incubated at 35°C with constant shaking for 21 days. Growth in the broth was measured by a reading on a Bausch and Lomb Spectronic 20 spectrophotometer. Results were recorded as positive (60% T or
RESULTS

The agar-disk diffusion test results were as follows. All of the isolates assimilated lactic acid, ribitol, and succinic acid. Citric acid was assimilated by 97.7% of the strains, including the three ATCC reference strains. L-Arabinose was assimilated by 5 out of 215 strains (2.3%), including ATCC strains 18804 and 32354. None of the strains assimilated cellubiose or salicin by the agar-disk diffusion or broth techniques. Agar-disk assimilation tests of mezalitose, glycerol, and methyl D-glucopyranoside gave equivocal results; therefore, assimilation of these carbon compounds and L-sorbose was assessed by the tube-broth technique.

The results of the 21-day tube assimilation tests were as follows. Methyl a-D-glucopyranoside was assimilated by all isolates. Glycerol was assimilated by 98.6% of the isolates, L-sorbose was assimilated by 97.7% of the isolates, and mezalitose was assimilated by 80.9% of the isolates. Glycerol and L-sorbose were assimilated by the three reference strains. Mezalitose was assimilated by ATCC strains 18804 and 32354 but not 38996.

Differences in the rate of assimilation of sorbose, glycerol, and mezalitose were apparent among isolates of C. albicans (Fig. 1). Sorbose was assimilated by 70.8% of the isolates within 7 days and by 94.2% of the isolates within 14 days. Glycerol was assimilated by 80.3% of the isolates within 7 days and by 94.3% of the strains within 14 days, whereas mezalitose was assimilated by 61.0% of the strains within 7 days and by 70.4% of the isolates within 14 days.

DISCUSSION

Although C. albicans is the fungus most frequently isolated from clinical specimens, it is not possible to readily distinguish between isolates. Antigenic analysis has demonstrated only two serogroups (7, 11). Differences in the pathogenicity of strains of C. albicans for laboratory animals have been observed, but these differences have not been correlated with colonization rates of humans or with serological or biochemical differences between strains (8, 10). Variation in assimilation of carbon compounds has been reported (4, 14) but not quantitated. This study expands and defines the data on carbon assimilation tests for typical strains of C. albicans.

Four criteria were used for the presumptive identification of clinical isolates. Identification of the isolates was confirmed by assimilation and fermentation tests. The formation of germ tubes in fetal bovine serum is characteristic of approximately 96% of the clinical isolates of C. albicans (2, 3, 9, 12); 96.7% of the isolates tested in these experiments formed germ tubes. Chlamydosores are also formed by most, but not all, strains of C. albicans (3, 5). All of the isolates formed chlamydosores on rice extract or cornmeal-Tween 80 agar; most, but not all, of the isolates formed chlamydosores on both media. All of the isolates assimilated, but did not ferment, sucrose. The latter two criteria, in conjunction with the first two requirements, should exclude isolates of C. stellatoidea and germ-tube or chlamydosporo-producing strains of C. tropicalis, respectively. Some atypical clinical isolates of C. albicans may have been excluded by the above criteria.

The importance of using a variety of assimilation testing techniques was noted. Agar diffusion assimilation tests of methyl a-D-glucopyranoside, glycerol, and mezalitose gave negative or questionable results for the majority of iso-
lates examined despite clearly positive results in broth assimilation tests. Agar-slant assimilation tests, by the method recommended by Adams and Cooper (1), were used in the initial experiments. However, growth was frequently observed on the basal medium without added carbon compounds, apparently due to the carry-over of nutrients. Growth due to the carry-over of nutrients did not interfere with the interpretation of results in the broth assimilation tests. The tube assimilation technique was found to be the most reliable method for all of the carbon compounds evaluated; however, for all of the carbon compounds tested, with the exceptions of methyl α-D-glycopyranoside, glyceral, and melezitose, the agar-disk diffusion technique gave comparable results. The latter test is easier, faster, and more economical when testing the ability of an isolate to assimilate a number of compounds. However, this test has several limitations. It can only be used when carbon compounds readily diffuse into the agar and when the yeast tested can metabolize the compound within a week. Slow assimilation of a compound, such as was observed for some isolates with glyceral, L-sorbose, and melezitose, may be obscured by a number of artifacts, including drying of the plate and slow growth of the yeast due to carry-over of nutrients.

Differences in assimilation techniques may account for the failure to confirm the variable assimilation of lactic acid, ribitol, succinic acid, and methyl α-D-glucopyranoside observed by van Uden and Buckley (14). Their studies utilized the same media, but different incubation conditions. Alternatively, since only clinical isolates of C. albicans were examined in this study, differences in the sources of strains may have accounted for the failure to confirm variable assimilation of these carbon compounds (5). None of the isolates assimilated cellobiose or salicin, two carbohydrates assimilated by C. tropicalis. The taxonomic description lists one purported strain of C. albicans which weakly assimilates these compounds.

The assimilation of melezitose by strains of C. albicans depends on a number of variables including concentration of melezitose, aeration, duration of the test, and the organism itself. The failure to assimilate melezitose is used in one commercial yeast identification kit (Modified API 20C, Analytab Products, Plainview, N.Y.) to distinguish C. albicans from C. tropicalis. Our results suggest that this is an improper criterion for distinguishing between these two species, as 80.9% of our isolates were capable of assimilating melezitose; 7% of these assimilated melizitose within 3 days of inoculation. This conclusion is supported by the report of Buesching et al. (4) that 3% of their strains of C. albicans were misidentified by the Modified API 20C system as a result of this test. It is well known that different techniques can yield different results in the same test (13). As long as users of the Modified API 20C kit adhere to the technical instructions issued with the kit, this problem should be minimal.

Although most carbohydrates were not affected by the initial pH of the yeast nitrogen base (pH 5.7), cellobiose and melezitose may be susceptible to acid hydrolysis after 21 days at 37°C at this pH. Acid hydrolysis could cause the disaccharide cellobiose (two glucose moieties) and the trisaccharide melezitose (one glucose and two fructose moieties) to disassociate into monosaccharide components, which would lead to false-positive results. Hydrolysis did not occur under our experimental conditions, as evidenced by failure of all of the isolates to grow in cellobiose and by the failure of 19% of the isolates to grow in the presence of melezitose. Evaluation of yeast nitrogen base broth with 4% melezitose at 21 days of incubation at 35°C showed that the pH of the broth remained constant; enzymatic analysis showed that the melezitose did not undergo hydrolysis into its glucose and fructose components.

A strain of C. albicans may develop the ability to assimilate a carbon compound through the growth of a mutant clone. Mutants, appearing as isolated colonies in the agar diffusion test, do not confuse interpretation of these tests. However, mutants which develop in broth assimilation tests may cause a delayed assimilation of the test compound. Development of clones of C. albicans able to assimilate L-sorbose, glyceral, or melezitose did not account for the differences in the rate of assimilation of these compounds as evidenced by the results of repeated tests of positive and negative assimilators and by failure to demonstrate mutant populations on selective solid medium.

Assimilation of the carbon compounds was remarkably uniform, with the exception of melezitose. Only 19 strains (8.8%) exhibited an assimilation pattern different from the other isolates. Each of these isolates assimilated or failed to assimilate one carbon compound as compared with the other 215 strains.

Further studies are underway to determine the genetic stability of the observed differences in assimilation of melezitose among strains of C. albicans and to examine the mechanisms involved in this difference.
LITERATURE CITED