Isolation of a Germ-Tube-Forming Revertant from *Candida albicans* B311V6

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We describe and partially characterize the isolation of a germ-tube-positive revertant from *Candida albicans* B311V6. This revertant has all of the properties of a germ-tube-forming strain of *C. albicans* except that it appears to have a nutrition defect.

A variant of *Candida albicans* was described previously which is unable to produce hyphae at 37°C (2). This variant, designated V6, was nonpathogenic for mice and rabbits and had a reduced ability to infect the vagina of rats maintained in pseudodoe (5). In comparing two derivatives from the original V6 strain, a question arose as to whether the V6 variant strain was actually a derivative of the parental strain (ATCC 32354; B311-Hasenclever). The purpose of this study was to obtain a germ-tube-forming derivative of the V6 variant strain and to compare it with the parental strain. To screen large numbers of colonies, we developed a blood agar plating procedure that helps to distinguish germ-tube-forming colonies from non-germ-tube-forming colonies. The present study describes the method and provides a partial characterization of a germ-tube-forming derivative of *C. albicans* V6.

**MATERIALS AND METHODS**

To obtain revertants from the parent variant strain (B311V6) the cells were maintained in exponential-phase growth in synthetic medium (SMC) (3) at 24.5°C. The B311 (ATCC 32354) strain was grown and treated in the same manner. Cells were diluted in SMC to yield ca. 100 to 200 colonies and were then spread onto sheep blood agar plates (BAP). The plates were incubated at 37°C in anaerobic gas jars. All plates were removed from the gas jars after 4 to 6 days of incubation and examined under a dissecting microscope for colony appearance.

Mutagenesis was attempted by treating 3.5 × 10³ CFU of the V6 strain in the exponential phase of growth with 40 µg of nitrosoquinoline (NTG) per ml for 1 h at 37°C. In other attempts, 4 mg, 400 µg, or 0.4 µg of NTG was placed in 1 drop at the center of plates previously inoculated with approximately 100 CFU of the V6 strain.

After examining the colonies on the BAP plates, the derivatives were transferred to Sabouraud slants and further characterized at 37°C for their ability to produce germ tubes in fetal calf serum, hyphae on cornmeal agar (2), and in selected cases for germ tubes in the N-acetyl-glucosamime induction system (4). These colonies were recloned and tested with the above procedures. The colonies were tested for assimilation patterns on Uniyeast plates (Flow Laboratories, Inc., McLean, Va.). The selected revertant strains were tested for liquid carbohydrate assimilations by the methods of van Uden and Buckley, as described in Lodder’s *The Yeasts* (7).

**Experimental candidal vaginitis.** Pseudodoe was induced in oophorectomized female Sprague-Dawley rats (175 to 200 g) by injection of estradiol valerate in sesame oil (0.5 mg/0.1 ml) (E. R. Squibb & Sons, Princeton, N.J.). Thereafter, weekly injections were given to maintain pseudodoe. At 72 h after estrogen injection, 10 rats were inoculated with 10⁴ blastospores of each of the three strains under study, suspended in 0.1 ml of sterile phosphate-buffered saline (pH 7.2). *C. albicans* intended for injection was grown by inoculation of 10 ml of sterile 1% Phytoene-peptone glucose broth (BBL Microbiology Systems, Cockeysville, Md.) with 100 µl of the stock blastospore suspension prepared for each strain. The cultures were incubated for 22 h at 25°C in a shaking water bath and centrifuged for 10 min at 570 × g at 4°C and counted in a hemacytometer. Yeast suspension (0.1 ml) was administered via a short segment of butterfly tubing fixed to a tuberculin syringe (5).

Vaginal lavage was performed 72 h and 1 week after initial vaginal inoculation, and quantitation of vaginal candidal infection was made by serial dilution of the retrieved lavage fluid and plating on Sabouraud dextrose agar plates. All plates were incubated at 30°C for 48 h for each series of dilutions, and the colony count was used to calculate the log of CFU per milliliter. Vaginal lavage was used to establish presence or absence of infection (6).

**RESULTS**

In a photograph of a BAP inoculated with the V6 strain and incubated at 37°C in an anaerobic gas jar, the plate shows smooth, pinpoint colonies (Fig. 1A). In a photograph of the B311, the ATCC strain that forms germ tubes, the majority of the colonies (stars) are tiny, with myceliumlike spikes growing into the agar (Fig. 1B). The stars were isolated from both NTG-mutagenized and nonmutagenized cultures with approximately equal frequencies (Table 1). None of the stars isolated from mutagenized cultures formed germ tubes or hyphae. Of the seven star colonies from nonmutagenized plates, four formed germ tubes and hyphae. One of these colonies was designated V6R (for V6 revertant) and was used in further studies. This strain when tested in extended carbohydrate assimilations

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TABLE 1. Screening of V6 derivatives for germ-tube-forming cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of colonies screened</th>
<th>No. of stars</th>
<th>Star formation frequency (10^3)</th>
<th>Germ tubes in serum (colonies/total stars)</th>
<th>Hyphae on cornmeal (colonies/total stars)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTG</td>
<td>2,161</td>
<td>3</td>
<td>1.39</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>None</td>
<td>3,263</td>
<td>7</td>
<td>2.15</td>
<td>4/7</td>
<td>4/7</td>
</tr>
</tbody>
</table>

V6R taken from Sabouraud plates and grown for one transfer in SMC, 90% produced germ tubes, whereas the V6 parent produced none (Table 2). Addition of 1% yeast extract to the SMC during growth at 25°C yielded 87% germ-tube-forming cells from the V6R strain (Table 2). The revertant strain produced germ tubes in serum at 37°C and on cornmeal agar at 25°C when tested anaerobically.

These data suggest that the V6R derivative isolated in this study has a nutrition defect and is not a chance contaminant from a germ-tube-forming C. albicans such as B311. In additional support of this view is a detectable frequency of reversion of the germ-tube-positive V6R strain to the V6 germ-tube-negative phenotype, with about 0.1% stars producing smooth colonies on sequential transfers. These colonies failed to produce germ tubes in serum. The occasional smooth colonies derived from the B311 germ-tube-forming strain always produced germ tubes in serum (see above).

The V6 strain and its revertant were examined in the rat vaginal model. The V6 strain showed significant reduction in infectivity as compared with the B311 (5). However, the V6R was as effective as the original germ-tube-producing strain (B311) in establishing vaginitis in the rat model (Table 3). The V6 strain infected only 20% of the rats inoculated after 1 week, whereas 80% of the rats inoculated with the V6R were still infected.

DISCUSSION

The V6 strain described in our previous studies has unique characteristics. First, it is temperature sensitive for hyphal formation on solid media, such as cornmeal agar (2). Second, it does not form germ tubes in fetal calf serum or in cultures shifted from 24.5 to 37°C in SMC. Third, it is not pathogenic for animals.

In addition to the temperature-sensitive defect discussed above, the V6 strain is also unable to produce mycelia on SMC agar (2), suggesting a nutritional defect. The V6 revertant which we have isolated here possesses all the properties of a germ-tube-forming C. albicans with one
exception. After repeated transfers in SMC it appears to require a yeast extract supplement to form germ tubes in SMC. The nature of the nutritional defect is under investigation.

In the rat model for experimental candidal vaginitis the revertant of the V6 strain was equally as efficient as the B311 strain in eliciting vaginal infection. By 72 h the V6 strain had infected only 50% of the animals tested, which showed lower quantitative vaginal titers; and after 1 week it had infected only 20% of the animals, which showed even lower quantitative titers.

Since the V6R described here was obtained spontaneously from the V6 strain, the two strains should prove useful in future studies of the requirements for germ tube formation.

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LITERATURE CITED