

Formation of Aerial Hyphae in *Candida albicans*

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Each of 22 isolates of *Candida albicans* was induced to produce aerial hyphae by culturing on a solid medium containing a peptone, acid-hydrolyzed casein, soluble starch, and agar in an atmosphere of 10% CO₂ at 37 C and room temperatures. Production of aerial hyphae is not diagnostic for *C. albicans*. Some of the other species of *Candida* may also produce such structures.

Candida albicans, one of the fungi most frequently isolated from certain parts of the human body, may grow as a typical yeast or as a filamentous fungus, depending upon the medium and the condition of incubation. On Sabouraud dextrose agar or blood-agar at both 37 C and room temperature, young colonies are wet, pasty, and cream-colored. Microscopically yeast cells and blastospores predominate. In older cultures, pseudohyphae are found which tend to grow into the substratum. Factors which control the dimorphism of this fungus are at present not precisely understood (4, 5). The importance of certain carbohydrates, particularly polysaccharides, certain amino acids, and carbon dioxide, have been studied. Langeron and Guerra (2) reported that CO₂ stimulated mycelium production of *C. albicans*. Weld's method (6) of identification of the organism is based on this property.

Invariably this fungus is described as a surface clinging organism, not at all considered as a mold with hyphae protruding into the air. An exception is the membranous or filamentous strain of *C. albicans* (582) which MacKinnon (3) described. On potato-agar slide cultures he observed aerial hyphae. In the method to be described, all *C. albicans* isolates tested have been found to produce aerial hyphae, making them behave somewhat like molds.

The medium consisted of 10 g of tryptone, 10 g of Casamino Acids, 10 g of soluble starch, and 20 g of agar (all Difco) made up to 1 liter with deionized water. After autoclaving for 15 min at 121 C, it was cooled and about 15 ml was poured into 90-mm petri dishes. The pH of the medium after autoclaving was 6.3. The somewhat dry agar surface needed for the experiments was obtained by permitting the water of condensation to evaporate while the petri dish lids were left ajar for about 0.5 hr.

Twenty-two *C. albicans* strains were isolated from clinical specimens and identified by three standard methods, namely, chlamyospore production, carbohydrate fermentation, and germ tube formation in serum (1). The fungi were maintained on Sabouraud dextrose agar at room temperature and were transferred weekly; seven of the isolates had been maintained for about 1 year, and the remaining strains were freshly isolated.

Cultures were made by streaking very thinly the yeast forms over the special agar medium

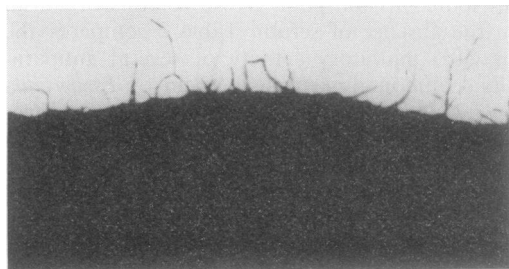


FIG. 1. Aerial hyphae of *Candida albicans* growing out on the edge of a cube of special agar medium incubated at 37 C in 10% CO₂ for 2 days.

with a bacteriological loop. Four to six isolates were inoculated onto each petri plate, placed in a jar charged with 10% CO₂, and incubated at 37 C.

As early as 16 hr, heavy filamentous growth occurred in about half of the isolates. The remaining strains produced good hyphae in 24 to 48 hr. Aerial hyphae, which are easily observed at 100× magnification, were found in all 22 isolates tested: plentiful in some and scanty in others. The aerial heights attained in one isolate ranged from 0.1 mm in 1 day to 0.7 mm in 3 days. Figure 1 shows growth at the edge of an

agar cube cultured in an atmosphere of 10% CO₂. Although the majority of the isolates produced filaments and aerial hyphae rapidly at 37 C, a few produced these structures better at room temperature. Aerial hyphae were produced by some isolates in the presence of 5% CO₂.

Sixteen additional yeast strains were tested on this medium with and without 10% CO₂. Of three *C. tropicalis* and two *C. stellatoidea* isolates tested, two of the former and one of the latter produced short aerial hyphae on this medium even without the 10% CO₂. In 10% CO₂, however, aerial hyphae attained greater heights, similar to those given by *C. albicans* described above. Two *C. krusei* strains tested under 10% CO₂ gave very short or rudimentary aerial hyphae. One *C. guilliermondii* isolate failed to produce aerial hyphae. Two strains each of *Cryptococcus*

neoformans, *Torulopsis glabrata*, *Rhodotorula* sp., and *Saccharomyces* sp. did not produce aerial hyphae by this method.

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