

DO GERM-TUBE POSITIVE CANDIDA TROPICALIS OCCUR?

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Summary

53 isolates of yeasts thought to produce germ tubes were further examined for basal constrictions to distinguish between pseudohyphae and true germ tubes. 11 isolates produced pseudohyphae with basal constrictions and all were identified to be *Candida tropicalis* based on fermentative and growth characteristics. 21 isolates produced only true germ tubes without basal constriction, while another 21 produced both pseudohyphae and true germ tubes. Both these groups were further identified to be *C. albicans*. No strain of *Candida tropicalis* was shown to produce germ tubes.

INTRODUCTION

Ever since *Candida albicans* was reported to form germ tubes on incubation in serum for 3 hours at 37°C¹, the germ tube test has been widely used to identify and differentiate *C. albicans* from other species of *Candida*. However, recent reports of the isolation of strains of *Candida tropicalis* that form germ tubes under the same conditions have started a controversy over the use of this test as the sole criterion for the identification of *C. albicans*. While isolation of germ-tube positive *C. tropicalis* from oral and pulmonary sources have been reported,^{2,3,4} there have also been suggestions that the filamentous outgrowths of *C. tropicalis* reported to be germ tubes were not germ tubes but pseudohyphae⁵. Germ tubes are cylindrical outgrowths that arise from blastospores and grow continuously by extension (Fig. 1) while pseudohyphae are budding cells that remain attached to the blastospores and may elongate (fig. 2).⁴ When pseudohyphae elongate, they may resemble germ tubes. Differentiation between the two depends on the presence of a constriction at the juncture between the filamentous outgrowth and the mother blastospore in a pseudohypha and the absence of such a constriction in a germ tube⁴

In view of this controversy, we decided to investigate the incidence of germ tube positive *C. tropicalis* among our clinical isolates and how their occurrence might effect our identification of *C. albicans* based on the germ tube test.

MATERIALS AND METHODS

Ninety-five yeast isolates from clinical specimens during a period of 1½ years were

examined for the production of germ tubes. The germ tube test was performed by making a light suspension of each yeast in 0.5 ml of human serum and incubating at 37°C for 3 hours. A loopful was then examined under the light microscope. Where germ tubes appear to be present, careful examination was made to detect constrictions at the juncture of the filaments and blastospores. The presence of constrictions was taken to indicate that the filamentous outgrowths were pseudohyphae and the absence, a true germ tube. Regardless of the presence or absence of constrictions, all such strains were identified further by their fermentation patterns on glucose, maltose, sucrose, lactose and trehalose in Wickerham's Fermentation media, their growth pattern in Sabouraud dextrose broth and on cornmeal agar incorporated with tween 80.

RESULTS

Fifty-three isolates of yeasts produced filamentous outgrowths in the germ tube test. On examination at higher magnification, 21 isolates were found to produce true germ tubes without any constriction; 11 isolates produced only outgrowths with basal constriction and thus are pseudohyphae; and another 21 isolates produced both true germ tubes and pseudohyphae. In all except 4 of the isolates that produced both germ tubes and pseudohyphae, there were more germ tubes than pseudohyphae. In the exceptional 4 isolates, the proportion of both were approximately equal.

On further identification, all 21 isolates that formed germ tubes proved to be *C. albicans*. Similarly, all 21 isolates that formed both germ

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tubes and pseudohyphae were *C. albicans*. The 11 isolates that produced pseudohyphae only turned out to be *C. tropicalis*.

Seven of these strains of *C. tropicalis* were isolated from sputum while the other 4 isolates were from specimens of a vaginal swab, nail clippings, pus and biopsy tissue. The strains of *C. albicans* were isolated from sputum (33), pus (1), vaginal swab (1), stool (2), throat swabs (3) and skin (2).

DISCUSSION

Should *C. tropicalis* form germ tubes, then the use of the germ tube test for the identification of *C. albicans* would not be valid. A search among our clinical isolates for 1% years failed to reveal any true germ tube forming *C. tropicalis*. All 11 isolates of *C. tropicalis* that were initially thought to form germ tubes had basal constrictions, thus indicating that they were pseudohyphae and not true germ tubes. Thus had closer examination not been done, these 11 isolates could have been reported as germ tube positive.

With experience, we found we could detect the constrictions easily and accurately predict on the outcome of further identification methods. Thus, an isolate that produced only pseudohyphae would be *C. tropicalis*; one that produced only true germ tubes would be *C. albicans*; another that produced both germ tubes and pseudohyphae would still be *C. albicans*.

Although we could produce no evidence for the production of germ tubes by *C. tropi-*

calis, it does not mean that it cannot occur. As Martin and White indicate? these strains are rare. In such a case then, the interference by germ tube positive *C. tropicalis* in the germ tube test would be relatively insignificant, and thus the germ tube test can still be regarded as a useful rapid test for the identification of *C. albicans*.

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Fig. 1 : True germ tube in *Candida albicans* (X 900)



Fig. 2 : Pseudohypha in *Candida tropicalis* (X 900)