Atypical Isolate of *Cryptococcus neoformans* Cultured from Sputum of a Patient with Pulmonary Cancer and Blastomycosis

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Cryptococcus neoformans was isolated repeatedly from a patient with epidermoid carcinoma and pulmonary blastomycosis. The isolate was atypical in that it had only a minute capsule, caused persistent infection but no perceptible disease in mice, and initially appeared not to assimilate trehalose. Only after an incubation of 2 to 3 weeks did utilization of this substrate become apparent.

During the past few years a rash of publications and commercially available products have appeared intended to simplify isolation and identification of medically important yeasts (1, 3-7, 10, 11, 14, 16; Randolph Multi-Test mycology plate, Randolph Biologicals, Houston, Tex.; Uni-Yeast-Tek, Corning Medical Diagnostic, Roslyn, N.Y.; API 20C, Analytab Products, Inc., Plainview, N.Y.; Micro-Drop, Clinical Sciences, Inc., Whippany, N.J.). The sheer numbers of reports and products attest to the magnitude of the yeast identification problem, one in no small part attributable to the occasional isolate that does not conform to the set pattern. It is the purpose of the present report to describe such an isolate.

A 65-year-old white male from southern Mississippi was evaluated in clinic on 1 March 1977, after 3 months of bronchitis associated with hemoptysis and increasing shortness of breath. For many years he had been a clerical worker in an appliance store. He denied any history of exposure to pigeons or other birds. On April 19 he was admitted to the hospital because of a 15-pound (ca. 2.269-kg) weight loss and recurrence of hemoptysis. Results of his physical examination were still unremarkable. His chest X-ray film in March had shown only hyperlucent areas in the apices consistent with pulmonary function tests that demonstrated decreased air flow; film revealed a mass in the base of the left lower lobe with probable enlargement of the left hilum. Another film 1 week later showed lucent areas within the left lower lobe density. At bronchoscopy a bloody and friable lesion was seen that extended along the medial wall of the left main bronchus and obstructed 60% of the lumen. Biopsies of this area demonstrated poorly differentiated large cell carcinoma. On April 27 the patient underwent left pneumonectomy. Poorly

differentiated epidermoid carcinoma was seen on the pathological sections of the left main bronchus. Tissue was not submitted for culture, but many yeasts morphologically compatible with *Blastomyces dermatitidis* were seen in the histological sections from a localized area of granulomatous pneumonitis at the base of the left lower lobe. This was the zone of consolidation noted on the last two preoperative chest X-ray films. No forms resembling *Cryptococcus neoformans* were found, even in mucicarmine-stained sections, and neither were any seen in sections sent for fluorescent-antibody studies to Leo Kaufman and William Kaplan, Center for Disease Control, Atlanta, Ga., although the presence of *B. dermatitidis* was confirmed.

Nothing suggested central nervous system involvement. (The patient returned to his home and developed complications including cardiac arrest; it has not been possible to determine whether he did, in fact, have cryptococcosis or was merely colonized.)

After the surgical resection, sputum specimens were submitted daily for 6 days. Each was inoculated onto Littman agar containing 0.03% chloramphenicol, Sabouraud dextrose agar, and Sabouraud dextrose agar containing 0.03% chloramphenicol and 0.05% cycloheximide. Incubation was at ambient temperature (22 to 25°C). Within 1 week, each had a heavy growth of yeast, which eventually proved to be *Candida albicans*. On cycloheximide-containing medium, the growth appeared to be that of a single species, but on Littman agar at least two different colony types were recognizable by virtue of differences in pigmentation; each plate had 3 to 10 colonies that failed to absorb the crystal violet stain—a signal to the possibility of cryptococcal identity. Grossly, these colonies were nonmucoid, but microscopically some of the yeasts had

a small yet distinctive capsule. No hyphae or pseudohyphae were seen, and none formed during growth on a cornmeal agar. These yeasts produced urease and grew at 37°C. One colony was screened by inoculation on urea, nitrate, sucrose, lactose, melibiose, raffinose, cellobiose, trehalose, and soluble starch from a kit sold for the purpose of identifying clinically significant yeasts (Uni-Yeast-Tek). Incubation was at room temperature, with daily observation through 6 days as prescribed by the manufacturer. At that time strongly positive reactions were apparent in the wells containing urea, sucrose, maltose, raffinose, and soluble starch; the cellobiose had just started to convert to positive; but trehalose was negative. Failure to have utilized trehalose by this time eliminated the possibility of identification as *C. neoformans*, according to interpretation of the instructions provided. Even after incubation for 14 days, there was no indication of trehalose utilization, although by day 23 a shift occurred toward a positive reaction. Reactions with three additional isolates from this patient, using media from three different lots, were identical. A known *C. neoformans* behaved appropriately.

Meanwhile, because of reluctance to rely upon a single feature, viz., failure to utilize trehalose, to rule out such an important pathogen, assimilation tests were conducted by another system (9) with galactose, sucrose, melibiose, cellobiose, trehalose, lactose, raffinose, rhamnose, inositol, xylose, melezitose, L-arabinose, galactitol (dulcitol), and glucose. Only lactose and melibiose were not utilized, thus conforming to the pattern described in Lodder (8) for *C. neoformans*, but even this pattern was not evident until week 3 because of the slowness with which trehalose was utilized.

In an effort to determine what may have gone wrong with the commercial system in our hands, determinations were repeated with inocula of four different concentrations: one having the Wickerham value prescribed, and twice, $\frac{1}{2}$, and $\frac{1}{4}$ as concentrated. One set was inoculated at room temperature and a duplicate was inoculated at 30°C. Utilization of trehalose was again slow. (About 6 months later, however, this isolate seemed to have acquired a capacity to utilize trehalose more rapidly.) It was apparent also that the optimal temperature of growth of the isolate was 30°C as compared with either room temperature or 37°C, although after several transfers at 37°C this temperature preference became less pronounced.

Finally, an effort was made to determine virulence for mice. Groups of five male Swiss Webster mice, 25 to 30 g, were inoculated intravenously with 10^2 , 10^4 , or 10^6 viable particles. Dur-

ing observation over 120 days, none died and none showed torticollis or other signs of illness, although mice sacrificed 14, 35, 60, and 120 days after inoculation had *C. neoformans* in the brain when observed microscopically and by culture. One animal, inoculated with 10^6 viable particles and sacrificed 60 days later, had *C. neoformans* that was both observed in situ and cultured from the animal's lungs.

The identity of this isolate was confirmed by B. H. Cooper (Baylor Medical Center, Houston, Tex.) and Leanor Haley (Center for Disease Control, Atlanta, Ga.).

The *B. dermatitidis* did not appear until week 6, at which time a few filamentous colonies were barely perceptible on plates from three of the six specimens, for the most part located deep in the areas having confluent growth of *C. albicans*. In time, aerial hyphae began to emerge, and the organism was isolated and identified.

Our report of this experience serves to emphasize two important points. First, is the necessity of observing plates for a long period of time. Had not the tissue sections shown *Blastomyces*, the original plates in this case probably would have been discarded or at least would not have received the close scrutiny warranted. The second point focuses once again upon isolation of an atypical pathogen, at least the third reported for this species (13, 15). In our case the identification of an important pathogen could have been missed because of aberrant behavior on a single substrate, trehalose. Bowman and Ahearn (1) noted that some strains of *C. neoformans* isolated from pigeon droppings did not utilize trehalose at all or did so only weakly or slowly upon primary isolation, but readily utilized this substrate after maintenance in culture.

Our isolate did form capsules but only minimally, and these easily could have been missed. It should be emphasized, however, that this characteristic is highly variable and in fact, even if present, is not unique to cryptococci. It is perhaps not generally appreciated that at least six noncryptococcal yeast species form capsules (8). Furthermore, one other of the main features of cryptococci, urease production, is not limited to this genus; seven noncryptococcal species form urease. Obviously, there are other characteristics by which such yeasts can be separated but temperature of growth is not always one of them. Although it is usually stated that *C. neoformans* can grow at 37°C, the optimal temperature of growth of some isolates is perhaps closer to 30 than 37°C, especially upon primary isolation (15; Irene Weitzman, Analysis of proficiency testing in mycology, NYCHD, August 1976).

Another widely used test that warrants comment is that of browning *C. neoformans* colonies

on media containing seeds of *Guizottia abyssinica* (12). Our isolate proved to be positive on this medium, but in our experience some isolates are inordinately slow in developing pigment, requiring as long as 2 weeks. In fact, however, pigmentation is not de facto evidence of *C. neoformans*, for Bowman and Ahearn (1) observed both false positive and negative cultures with such media, as well as with a half-dozen modifications that have been developed. In their experience, the synthetic medium of Chaskes and Tyndall (3) proved most efficient, but even this was not without false reactions.

A final comment concerns the use of rapid identification systems, especially those that are commercially available. Each is based upon identification of only clinically important yeasts, but this in turn is based upon the assumption that only 20 or so species will ever be encountered in the clinical laboratory. The potential for error can be well exemplified by citing the relationships between certain *Candida* species; there are eight that have assimilation patterns identical with *Candida guilliermondii*, and nine that are identical with *C. krusei* (8), using the six carbohydrates commonly used (e.g., Uni-Yeast-Tek) in yeast identification, viz., raffinose, cellobiose, sucrose, maltose, lactose, and trehalose. Most of these species are capable of growth at 37°C, and thus there is no reason to believe they are incapable of causing human disease. At best these systems provide only presumptive identification.

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