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NOTES

An Aberrant Variant of *Histoplasma capsulatum* var. *capsulatum*

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We studied an aberrant culture of *Histoplasma capsulatum* var. *capsulatum* isolated from synovial fluid collected from the right elbow of a patient from Kansas. Colonies on Sabouraud glucose agar and other routine mycological media were glabrous to soft, moist, heaped, deeply folded or convoluted, and orange-brown with a white, irregular margin. Microscopically, hyphae were hyaline, septate, and branched and remained totally devoid of conidiation over a period of 2 years on all mycological media. Conversion to the yeast form was achieved on Pine's medium at 37°C. Colonies at early stages of growth were smooth, moist, pasty, shiny, and orange-brown but soon became wrinkled and slightly raised and produced oval, thin-walled cells measuring 2 to 3 by 3 to 4.5 μm which multiplied by polar budding. The identity of the isolate was further confirmed by utilizing the Accuprobe DNA probe and the exoantigen test for *H. capsulatum* var. *capsulatum*.

Histoplasma capsulatum var. *capsulatum* produces moderately growing, white to buff brown, velvety colonies on most mycologic media at 25 to 30°C (1, 2). Typically, two types of holoblastic, solitary conidia, namely, large (8 to 15 μm in diameter), spherical, oblong, pyriform, tuberculate macroconidia and small (2 to 4 μm in diameter), oval microconidia with smooth to finely roughened walls, are produced by the majority of isolates. Variants isolated from soil producing glabrous, wine-red, as well as pink, cottony colonies have been described by Morris et al. (9). Some strains of *H. capsulatum* lack microconidia, while others do not produce tuberculate macroconidia, and macroconidia often remain smooth and devoid of tubercles (4, 8). Variants include pleomorphic colonies (lacking conidiation) and glabrous, leathery to waxy colonies with topography varying from flat to radially furrowed to concentrically ringed to crateriform. Most of these variants remain sterile and fail to produce conidia on routine mycologic media. Many such sterile colonies, however, when grown on nutritionally deficient media, such as soil extract, hay infusion, or yeast starch (YpSs) agar (5, 7, 10), do produce a few characteristic tuberculate macroconidia. Isolates which do not ordinarily convert from the mycelial to the yeast form have also been documented (7, 14).

We recently studied an aberrant culture of *H. capsulatum* isolated from right elbow synovial fluid from a patient from Kansas which manifested very atypical colony morphology and persistently remained devoid of conidiation. Because of the clinical diagnosis of histoplasmosis, the isolate was first tested by DNA probe (Accuprobe; Gen Probe Inc., San Diego, Calif.) and found to be positive for *H. capsulatum*. Its atypical morphology prompted further studies; thus, it was sent to the Centers for Disease Control and Prevention (CDC) to be tested by the exoantigen test and conventional methods. At the CDC, the isolate was retested with the DNA probe and the exoantigen test for *H. capsulatum* was done at the same time. Both tests confirmed the identity of UTHSC 93-310 as *H. capsulatum*. Because of its atypical colonial morphology and lack of conidiation, conversion to the yeast form and growth on nutritionally deficient media to induce micro- and macroconidia were undertaken.

Colony morphology. Colonies on Sabouraud glucose agar (Sab; Difco Laboratories, Detroit, Mich.), potato dextrose agar

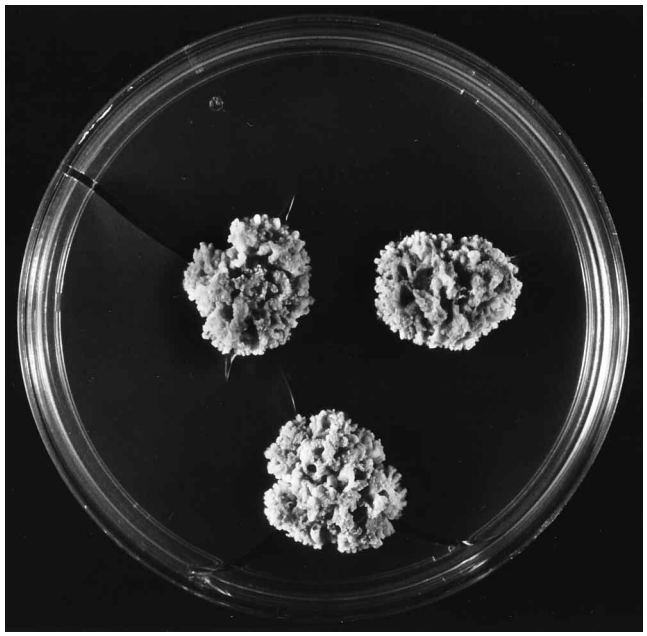

FIG. 1. Four-week-old colony of *H. capsulatum* (ATCC 90723) on Sab at 25°C.

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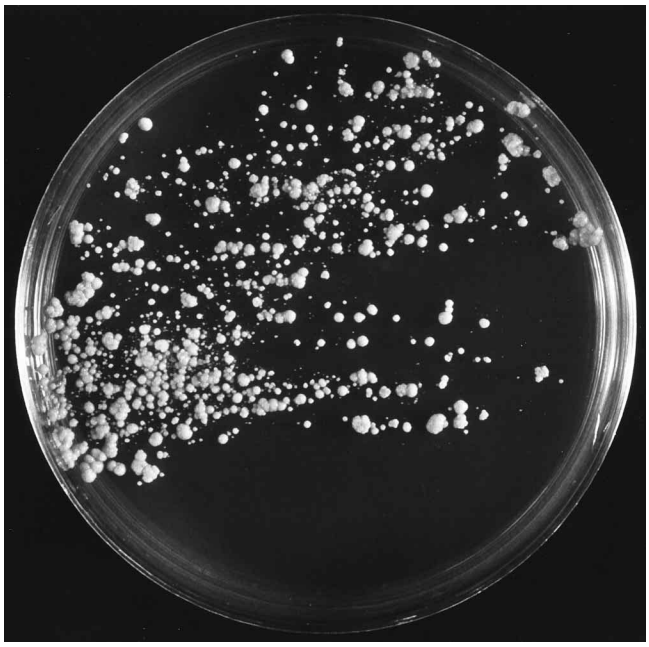

FIG. 2. Two-week-old yeast form colonies of *H. capsulatum* (ATCC 90723) on Pine's agar at 37°C.

(Difco), potato flakes agar (13), malt extract agar, phytone yeast extract agar (Becton Dickinson & Company, Cockeysville, Md.), Mycosel agar (Becton Dickinson), cornmeal agar (Difco), pablum cereal agar (10), and lactritmel agar (3) were glabrous, soft, moist, heaped, deeply folded or convoluted, and orange-brown with a white, irregular margin reaching diameters of 15 to 18 mm after 3 weeks at 25°C (Fig. 1). Morphologically, colonies grown on Sab at 37°C were very similar to those grown at 25 to 30°C, except that growth was slower. Growth was not inhibited by cycloheximide. To induce conidiation, the isolate was subcultured on the above media in two laboratories at the CDC approximately every 30 days in duplicate cultures. These serial subcultures were incubated at 25°C in the dark and examined microscopically at the end of the incubation period for production of conidia. If examination revealed no conidia, the subsequent subculture was done on a different medium.

Microscopically, hyphae were hyaline, septate, and branched and developed a few intercalary chlamydoconidia in 3-week-old cultures on potato flakes agar. The isolate did not produce either microconidia or tuberculate macroconidia on any of the above-mentioned mycologic routine and sporulation media over a period of more than 2 years and has remained sterile.

Conversion to the yeast form was achieved on Pine's medium (12) at 37°C. Initial young colonies were smooth, shiny, orange, and moist but soon became wrinkled and slightly raised (Fig. 2) and produced oval, thin-walled hyaline cells measuring 2 to 3 by 3 to 4.5 μm which multiplied by polar budding.

The Accuprobe DNA probe used to confirm the identity of the isolate employed a single-stranded rDNA sequence probe specific for *H. capsulatum* linked to an acridinium ester. Pinhead-sized portions of both mycelial and yeast form growths were tested. The inocula were prepared from a 10-day-old mycelial growth on Sab at 25°C and yeast form growth on Pine's medium at 37°C. The inocula were suspended in a tube with a lysing reagent with 0.04% sodium azide and a buffered solution of probe diluent and glass beads. The mixture was vortexed briefly, and the resulting cell lysate was heated to 95°C for 15 min in a sonicator water bath to inactivate viable cells. One-hundred-microliter samples of the two lysed specimens were pipetted into tubes containing labeled DNA and incubated at 60°C in a water bath for 15 min. This hybridization step allowed the labeled probe to combine with rDNA to form a stable DNA-RNA hybrid. The tubes were removed from the water bath, and 300 μl of a selection reagent was added to each tube. The selection reagent preferentially inactivated the label on any nonhybridized single-stranded probe, whereby any chemiluminescence would be that retained by the probe hybridized with the rDNA target. Chemiluminescence was measured by a luminometer (provided by Gen Probe Inc.) as probe light units (PLU's), and results were compared with those of a positive control culture of *H. capsulatum* (ATCC 38904) and a negative control culture of *Blastomyces dermatitidis* (ATCC 60916). A reading of 1,500 or more PLUs indicated a positive reading for *H. capsulatum* (11). Both specimens gave readings of 9,300 PLUs and thus confirmed the identity as *H. capsulatum.*

Exoantigen test. A 10-day-old mycelial colony on Sab was tested for *H. capsulatum* according to procedures of Kaufman and Standard (6). The results were read after 48 and 72 h. The exoantigens from the mycelial culture, when reacted with the reference *H. capsulatum* in a microimmunodiffusion test, produced two precipitin bands (H and M) of identity with the reference system.

Because of its atypical morphologic characteristics, isolate UTHSC 93-310 (CDC B-5392) has been deposited in the American Type Culture Collection (ATCC 90723).

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