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Isolation of *Candida albicans* from Freshwater and Sewage

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The isolation and identification of *Candida albicans* from polluted aquatic environments were facilitated by the inclusion of a selective medium and a differential screening medium to detect the reduction of 2,3,5-triphenyltetrazolium chloride. *C. albicans* occurred commonly in low numbers in sewage influents, rivers, and streams.

Candida albicans is found commonly in the alimentary tract and mucocutaneous regions of mammals and birds. This yeast is isolated infrequently from other habitats, including aquatic environments receiving urban sewage effluents (7). Isolations from these sources appear to be due to recent contamination with human or animal excrement (3, 6). It has been shown that *C. albicans* will survive for long periods as a uniculture in sterile water or seawater (4), but it does not compete well in a mixed culture (2). In culture, *C. albicans* has been shown to be inhibited by *Bacillus* sp. isolated from sewage effluents (A. Coleman, W. L. Cook and D. G. Ahearn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, N20, p. 187). Also, it has been suggested that predation by protozoa reduces the population of yeasts in sewage (7). Therefore, *C. albicans* may serve as an indicator of recent fecal pollution when isolated from water.

Buck and Bubucis (5) designed a medium containing bismuth ammonium citrate and sodium sulfite that imparts a brown color to colonies of *C. albicans*. These authors confirmed by germ tube tests that 90% of the brown yeasts isolated from raw sewage, river water, estuaries, and bathing beaches on Long Island Sound were *C. albicans*. Nearly half (47%) of the germ tube-negative isolates were determined by conventional means to be *Candida tropicalis*. Germ tube-negative isolates of *C. albicans* were not reported.

The inability to distinguish the brown colonies of *C. albicans* from those of *C. tropicalis* and other yeasts and the possible existence of germ tube-negative *C. albicans* indicated that additional tests for the rapid identification of *C. albicans* were necessary. This communication describes a two-step isolation procedure which facilitates the selection and identification of *C. albicans* from clean and polluted freshwater.

Water samples were obtained in sterile glass bottles from polluted and clean rivers and streams in the metropolitan Atlanta area, returned on ice, and processed within 1 h. Samples were obtained also from unchlorinated influent and secondarily treated effluent from a sewage treatment facility which receives most of its sewage from domestic sources.

Samples were filtered through sterile 47-mm-diameter membrane filters (Millipore Corp.) with a pore size of 1.2 μm. The volumes filtered varied with sample consistency, but were generally 1.0 ml for influent (diluted with sterile distilled water), 25 ml for effluent, 50 ml for polluted river water, and 500 ml for clear river water. After sample filtration, 25 ml of sterile deionized water was passed through each filter before placing it, organism side up, onto the mCA medium of Buck and Bubucis (5). The medium was prepared by adding 1.0 g of glycine, 3.0 g of maltose, 0.3 g of Na_2SO_3, 0.5 g of bismuth ammonium citrate (City Chemical Corp., New York, N.Y.), 0.05 g of chloramphenicol (Sigma Chemical Co.), and 0.15 g of cycloheximide (Sigma) to 90 ml of deionized water. The solution was warmed to 50°C in a water bath, and the pH was adjusted to 7.1 with 1 N NaOH. Agar (1.5 g) was added, and the solution was heated to melt the agar. Ten milliliters of filter-sterilized 10× yeast nitrogen base (Difco Laboratories) fortified with 5.0 g of glucose was added to the cooled (50°C) medium. The medium was poured into sterile plastic petri plates (12 by 50 mm) and stored in the dark at 4°C until used. The inoculated mCA medium was incubated at 37°C for 3 days. All brown colonies with a diameter of 1.0 mm or larger with a dome shape and a matte surface were transferred by touching each colony with the end of a sterile applicator stick and aseptically depositing the yeast on YM agar (Difco) and YM agar containing 0.1% 2,3,5-triphenyltetrazolium chloride (TTC; 100 μg/ml) and 0.5 g of chloramphenicol per liter. The TTC solution (10×) was sterilized by filtration and added to autoclaved YM agar cooled to 50°C. The plates were incubated at 22°C for 24 h. Colonies which were white to light

pink on the YM-TTC medium were placed in 0.5 ml of calf serum, incubated at 37°C for 2 to 3 h, and examined for germ tube production (1).

C. albicans was isolated, generally in low numbers, from all types of water samples (Table 1). From a total of 51 samples *C. albicans* was isolated 23 times, with the highest incidence in sewage effluents and the lowest in river water. The increased incidence in sewage effluents probably was caused by incomplete treatment of the influent. Although the majority of colonies developing on mCA medium resembled those of *C. albicans*, only 3% of the brown colonies from river water and 8% of the brown colonies from sewage influent proved to be *C. albicans*. The process of differentiating *C. albicans* from other brown yeasts was facilitated by subculturing all dark brown, matte, dome-shaped colonies to YM and YM-TTC agar plates. On YM-TTC medium *C. albicans* and other yeasts unable to reduce TTC produced white to light pink colonies. Yeasts such as *C. tropicalis*, *Candida guilliermondii*, and *Pichia* sp. reduced TTC and produced a pink to deep red colony on the YM-TTC medium after 24 h. The color change is essentially the same as that produced by various yeasts on Pagano-Levin medium (10). Our method provided a simple and rapid procedure for readily distinguishing *C. albicans* from other maltose-assimilating, cycloheximide-resistant yeasts. Inoculating YM-TTC medium with cells from brown colonies is less tedious than testing all of the colonies on mCA medium for germ tube production. The mCA medium inhibited most bacteria and fungi from river and stream samples, but frequently demonstrated only static inhibition of bacteria in sewage. Inocula from mCA medium used for germ tube tests were generally overgrown by the bacteria, and germ tube production was inhibited. In preliminary studies, mixed cultures of bacteria and yeasts occasionally gave a false-positive red color to the growing yeast cells of *C. albicans*. This difficulty was eliminated with the addition of chloramphenicol to the YM-TTC medium. The YM agar served as a control to detect possible inhibition of germ tubes by the antibiotic (9).

In our work about 25% of the environmental isolates on mCA medium were *C. albicans*. The data of Buck and Bubucis (5) indicate that 90% of the dark brown, dome-shaped, matte-textured colonies growing on mCA medium from bathing beaches, sewage, rivers, and estuaries were *C. albicans*. In water samples obtained from beaches on Lake Ontario over 90% of the suspected *C. albicans* colonies growing on mCA were *C. albicans* (11). In our studies brown, dome-shaped, matte-textured colonies grew on all mCA agar plates within 3 days, but only 23 of 51 plates had *C. albicans*. *C. albicans* was present on some sampling days and not on others. This variation in incidence occurred for all types of samples and probably was due not only to variations of the input of animal excrement but also to the complexities of predation, microbial antagonisms, and toxicities in sewage. The differences in incidence of *C. albicans* between our studies and others may be related to geography.

TABLE 1. *Isolation frequency of C. albicans from freshwater and sewage*

Sampling site	Samples[a]	No. of brown colonies	No. of *C. albicans*	Range of no. of *C. albicans*/100 ml
Sewage influent	5/13	159	13	0–400
Sewage effluent	11/14	143	68	0–38
River	1/10	36	1	<1
Stream	6/14	80	24	0–5

[a] Number of samples containing *C. albicans*/total number of samples.

The mCA medium is designed for the rapid detection of *C. albicans* and may be used by quality control personnel who may lack training in the identification of yeasts. The differentiation of *C. albicans* from *C. tropicalis*, one of the most common yeasts in sewage, is dependent on accurate germ tube analysis. Occasionally, pseudohyphal cells of *C. tropicalis* are formed under or on top of a cell and appear to have a base typical of germ tubes when viewed with conventional microscopy. As stated by Hedden and Buck (8), many individuals improperly call pseudohyphae, which are constricted at their point of origin from the mother cell, germ tubes and thus misidentify yeasts as *C. albicans*. In instances of sparse germination or questionable morphology repeat germ tube tests are necessary. These repeat tests may involve isolates of both *C. albicans* and *C. tropicalis* and may require skilled technology (9). The use of the TTC medium markedly reduces the number of needed microscopic observations.

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