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Survival of *Candida albicans* in Tropical Marine and Fresh Waters

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A survey of *Candida albicans* indicated that the organism was present at all sites sampled in a rain forest stream and in near-shore coastal waters of Puerto Rico. In the rain forest watershed no relationship existed between densities of fecal coliforms and densities of *C. albicans*. At two pristine sites in the rain forest watershed both *C. albicans* and *Escherichia coli* survived in diffusion chambers for extended periods of time. In near-shore coastal waters *C. albicans* and *E. coli* survival times in diffusion chambers were enhanced by effluent from a rum distillery. The rum distillery effluent had a greater effect on *E. coli* than on *C. albicans* survival in the diffusion chambers. These studies show that neither *E. coli* nor *C. albicans* organisms are good indicators of recent fecal contamination in tropical waters. It further demonstrates that pristine freshwater environments and marine waters receiving organic loading in the tropics can support densities of *C. albicans* which may be a health hazard.

Candida albicans is one of the most studied opportunistic pathogenic yeasts frequently associated with human disease. It can cause superficial infections of the skin, nails, and mucosae, gastroenteritis, vaginitis, and urethritis and is frequently the cause of nosocomial infections in compromised hosts (5, 19, 31). Bonde (7) believes that the members of the genus *Candida* should be considered waterborne pathogens.

Enumeration and characterization of yeasts have been reported for marine coastal waters (1–3, 7, 9, 16, 17), estuaries (1, 3, 20, 26), and fresh waters (1, 3, 13); however, only one of these dealt with yeasts in subtropical (2) or tropical (20) waters. Ahearn et al. (3) suggested in 1968 that *C. albicans* might be used as an indicator of recent fecal contamination of water. Other researchers have supported their hypothesis by showing that high densities of *C. albicans* are associated with sewage or recent human fecal contamination or both in temperate fresh waters (9, 12, 13, 30).

Tropical countries are plagued by a large number of viral, bacterial, protozoan, and helminthic waterborne diseases. As populations in these countries increase, it becomes essential to increase the efforts to control waterborne diseases. Industrial development has only exacerbated contamination of drinking-water supplies. Accurate assessment of fecal contamination is critical to proper treatment and control. Studies by our laboratory have shown that the standard indicator assays, i.e., fecal coliform densities, are not appropriate in Puerto Rico (6, 11, 21, 23). *Escherichia coli* can survive indefinitely in pristine tropical fresh waters and is probably naturally occurring in these waters. Others (15, 18, 22, 25, 28) have also shown that other tropical waters harbor high densities of fecal coliforms in the absence of fecal contamination. Pristine marine waters in Puerto Rico seem to be extremely bactericidal to *E. coli*. This bactericidal effect, however, disappears when these waters are contaminated by domestic and industrial sewage effluents (6, 23). In the present study, we examined the survival of *C. albicans* in situ by using diffusion chambers in both marine and freshwater environments in Puerto Rico. This was done as part of our continuing search to find better indicators and establish appropriate maximum contaminant levels of microbial contamination for tropical waters.

MATERIALS AND METHODS

Study site. The Mameyes River watershed is on the northeast corner of the island of Puerto Rico, 18° 15′ N and 65° 45′ W (Fig. 1). This watershed has a drainage area of 27.3 km^2 and a total length of 17.1 km (11, 20). Annual average precipitation in the upper third of the watershed is 395 cm; this area is classified as a cloud rain forest and is protected as part of the Caribbean National Forest/Luquillo Experimental Forest. The middle third of the watershed is dominated by agricultural land and several housing projects that dump their sewage into the Mameyes River. The lower third of the watershed is dominated by two small towns which contribute municipal, domestic, and light-industry wastes to the river. The Mameyes River empties into the Atlantic Ocean near Puerto Rico's largest public beach, Luquillo. Previous studies have shown that this watershed exhibits no seasonal differences in water quality (21).

Ensenada de Boca Vieja, 18° 27′ 48″ N, 66° 08′ 42″ W, is a protected cove immediately adjacent to San Juan Bay (Fig. 2). This cove has a tidal range of 0.5 m, shoreline length of 855 m, mean depth of 4 m, and surface area of 94,643 m^2 and received 1.4×10^6 liters of untreated effluent per day from a single rum distillery (6, 14). Shore currents moved the effluent plume in a westerly direction, parallel with the shoreline. On rare occasions the wind was from the northwest and created a current that moved the waste plume in a easterly direction, so that it tended to accumulate in the apex of the cove. For a more complete description see Biamón and Hazen (6).

Water quality. Seven water quality parameters were measured simultaneously with water collection for bacteria density. Measurements were taken in situ for conductivity, salinity, pH, dissolved oxygen, light intensity, and temperature. The pH was measured with a digital pH meter, model 201 (Orion Research, Inc., Cambridge, Mass.) and dissolved

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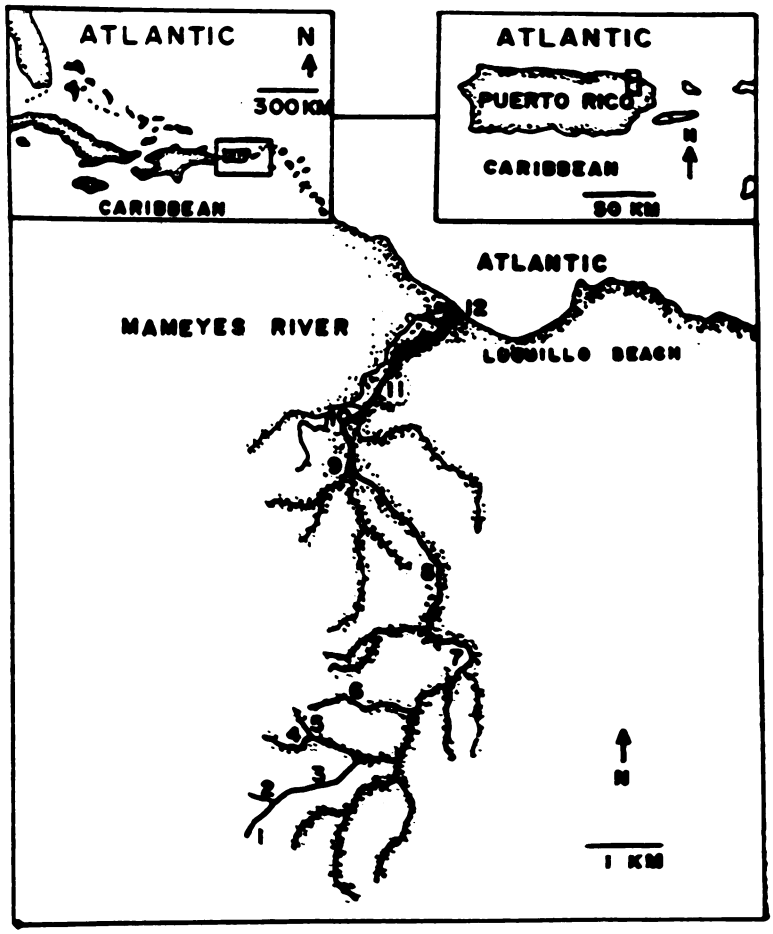

FIG. 1. Map of sites in the Mameyes River watershed.

oxygen was measured with a model 57 DO meter (Yellow Springs Instrument Co., Yellow Springs, Ohio). A model 33 S-C-T meter (Yellow Springs Instrument Co.) was used to measure conductivity and salinity. Turbidity, alkalinity, hardness, and ammonia measurements were done in the field, using a Mini Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). Three liters of water was collected and divided into various bottles; small amounts of the following preservatives were added: sulfuric acid, zinc acetate, and mercuric chloride. Amber bottles were used for samples to be analyzed for chlorophyll. All samples were then placed on ice for transport to the laboratory. The preserved samples were analyzed for the following parameters: nitrates plus nitrites, sulfates, inorganic phosphates, total phosphorus, and chlorophyll *a*, using standard methods (4).

Sampling protocols. Several monthly grab samples were taken at Ensenada de Boca Vieja and the Mameyes River, using Whirl-Pak bags (Nasco, Ft. Wilkinson, Wis.). Aliquots were filtered through 0.45-μm-pore size membrane filters (Millipore Corp., Bedford, Mass.). Filters were placed on sterile pads soaked with Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.) supplemented with chloramphenicol (0.5 g/liter) and incubated at 25°C for 48 h (10). Yeast colonies selected at random were identified by germ tube test, carbohydrate assimilation and fermentation, nitrate, urea, caffeic acid reaction, chlamydospores, pseudohyphae, temperature, cycloheximide resistance, and other tests according to Lodder (27) with the use of rapid tests and alternative methods (24).

Bacteriological analyses. Water samples for fecal coliform analysis were placed in sterile Whirl-Pak bags and kept on ice until assayed. Samples were analyzed by membrane filtration as indicated in *Standard Methods for the Examination of Water and Wastewater* (4), with m-FC media (Difco). Random isolates were further characterized with API-20E strips (Analytab Products, Plainview, N.Y.).

Diffusion chamber studies. Pure cultures of *C. albicans* obtained from Bill Copper (Baylor University Dallas, Tex.) were grown in 3% Sabouraud dextrose broth at 35°C for 24 h, transferred in 5% Sabouraud dextrose broth, and incubated for another 24 h at 45°C with shaking. Pure cultures of *E. coli* (ATCC 11775) were grown in nutrient broth at 37°C for 24 h. Cells of each organism were harvested by centrifugation and washed twice in sterile phosphate-buffered saline. The number of cells per milliliter was determined with a model ZF Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) and adjusted to 10^7 cells per ml. Bacterial and yeast suspensions, 100 ml each, were individually placed into sterile diffusion chambers. The chambers used were a modification of those of McFeters and Stuart (29) as described by Hazen and Esch (22).

Four chambers each for *E. coli* and *C. albicans* were suspended 0.5 m below the surface at sites 1 and 4 of the Mameyes River (Fig. 1) and at sites A and B at Ensenada de Boca Vieja (Fig. 2). Samples (1 ml) were taken with a sterile

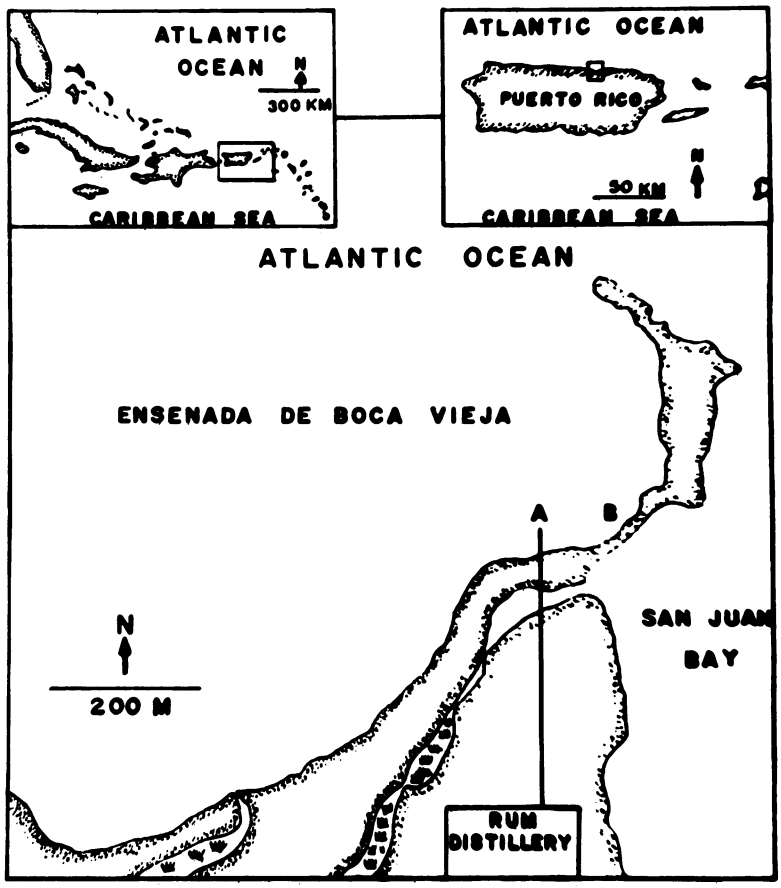

FIG. 2. Map of sites in Ensenada de Boca Vieja.

syringe from each chamber at regular intervals for 96 h. Each sample was immediately fixed in 10% phosphate-buffered Formalin (pH 7) and refrigerated for later reading with the Coulter Counter as described by Hazen and Esch (22).

Data analysis. Programs developed on Apple II and IBM 370-148 computers were used for all statistical tests. Two-factor analysis of variance was used to test differences between sites and times. Heteroscedastic data as determined by skew and kurtosis were made more homoscedastic by transformation with log(× 4 1). Any statistical probability ≤0.05 was considered significant (32).

RESULTS AND DISCUSSION

Over a 2-year period, all random sampling at the rum distillery effluent were positive for *C. albicans*; however, because of the large number of *Saccharomyces cerevisiae* from the effluent, it was impossible to obtain accurate density estimates of either *C. albicans* or total yeasts. Rum distillery effluent (slops) in Ensenada de Boca Vieja has been shown to have serious effects upon the marine life not only at the point source of discharge but also at adjacent areas (14). The distillery effluent is a viscous, odorous effluent with a complex organic fraction containing simple sugars, polysaccharides, free amino acids, and aromatic compounds. This study showed, as have others (6), that slops significantly increase the concentration of nitrates, phosphates, total phosphorus, and sulfates and the temperature (Table 1). The amounts of nitrogen, phosphorus, and other inorganic compounds at the effluent point source are comparable to levels found in raw sewage. The 5-day biochemical oxygen demand for this rum distillery effluent is the highest ever

TABLE 1. Water quality of Ensenada de Boca Vieja (EBV) and Río Mameyes (MR), Puerto Rico[a]

Site	Air Temp (°C)	Water Temp (°C)	Dissolved oxygen (mg/liter)	pH	Salinity (ppt)	NO_{2-3} (mg/liter)	PO_4 (μg/liter)	Total phosphorus (μg/liter)	Chlorophyll[a] (mg/liter)
EBV-A	27.0 ± 2.0	33.4 ± 1.5	4.2 ± 1.3	5.1 ± 0.3	34.3 ± 4.0	4.8 ± 0.3	20 ± 15	26.8 ± 9.8	1,342 ± 5,495
EBV-B	27.0 ± 2.0	27.3 ± 0.6	5.3 ± 1.7	7.6 ± 0.3	32.2 ± 3.0	0.2 ± 0.1	<0.1	<0.1	133 ± 116
MR-1	23.1 ± 1.0	21.1 ± 0.5	7.9 ± 0.2	6.2 ± 0.1	0	0.4 ± 0.1	2.4 ± 0.9	3.5 ± 1.2	148 ± 71
MR-4	23.6 ± 0.9	21.6 ± 0.9	8.1 ± 0.2	6.8 ± 0.1	0	0.7 ± 0.2	1.0 ± 0.7	5.7 ± 2.8	47 ± 19
MR-5	23.7 ± 0.9	21.7 ± 0.4	8.2 ± 0.2	7.2 ± 0.1	0	0.5 ± 0.1	1.4 ± 0.9	3.0 ± 1.2	77 ± 29
MR-9	28.2 ± 1.4	27.1 ± 0.9	6.9 ± 0.5	7.0 ± 0.1	0	1.4 ± 0.7	6.8 ± 2.3	9.0 ± 2.6	106 ± 63
MR-12	30.1 ± 2.0	25.0 ± 0.3	6.2 ± 1.0	7.4 ± 0.2	5.0 ± 3.0	0.2 ± 0.1	3.6 ± 1.9	8.6 ± 3.9	54 ± 53

[a] All values are mean ± 1 standard error of six samples. NO_{2-3}, Nitrites plus nitrates.

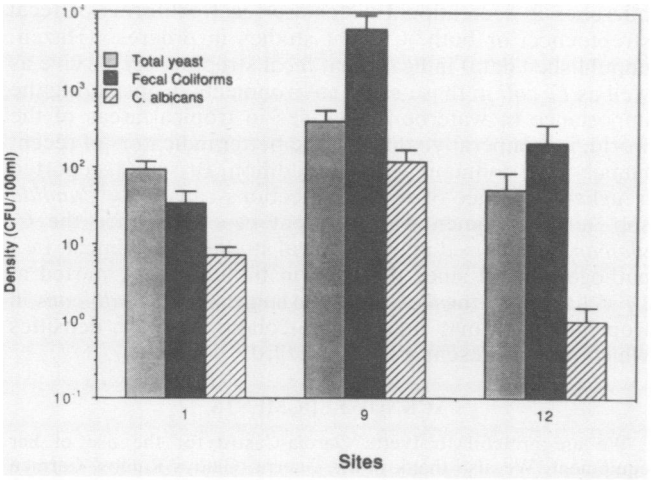

FIG. 3. Densities of *C. albicans* and total yeasts and fecal coliforms by site at the Mameyes River (mean ± 1 standard error; n = 3).

reported for any effluent, >32,000 mg of O_2 per liter (14). Generally, site A at Ensenada de Boca Vieja is a strongly polluted site and site B is a relatively pristine site. Site B at Ensenada de Boca Vieja was lower than any of the sites sampled for all inorganic nutrients, although chlorophyll *a* levels were the highest at this site. Further proof that the effluent is the source of these between-site differences was seen when the effluent was shut off after 48 h. Neither site at Ensenada de Boca Vieja was different for all water quality parameters measured within 3 h after the discharge was stopped. Biamón and Hazen (6) reported similar findings and that this effluent increased the survival of *Aeromonas hydrophila*.

Densities of *C. albicans* in the diffusion chambers were significantly higher at the effluent site after only 18 h of exposure (Fig. 3). However, these differences were not significant after 72 h. Densities of *E. coli* in adjacent diffusion chambers were significantly higher after only 12 h of exposure (Fig. 3). The differences between the two sites for *E. coli* were not significant after 48 h. Discharge of the effluent from the distillery ceased at 48 h. Thus, the effluent increased the survival of both *C. albicans* and *E. coli*. Densities of *E. coli* were much more affected by the presence of the effluent since they responded faster to both the presence and the absence of discharge. The survival of *C. albicans* and *E. coli* in tropical coastal waters can be enhanced by high organic-containing effluents. Many tropical waters receiving effluents may therefore support high densities of both *C. albicans* and *E. coli* in the complete absence of recent fecal contamination.

Water quality at the three sites along the Mameyes River was quite variable (Table 1). Site 1, the highest point in the watershed, was quite low in nutrients compared to the other sites and yet had densities of fecal coliforms (30 CFU per 100 ml) (Fig. 4) that would indicate some fecal contamination, even though none was apparent. High densities of *C. albicans* were also observed at site 1. Site 9, which receives primarily treated sewage, had higher densities of both fecal coliforms and *C. albicans*, yet the fecal coliforms showed a much greater increase at this site. Site 12, an estuary, had very low densities of *C. albicans* even though fecal coliform densities were >300 CFU per 100 ml. Thus, *C. albicans* densities were not significantly correlated to densities of fecal coliforms in the watershed.

Other studies (11) have shown that *E. coli* can survive indefinitely even in pristine areas of this watershed (Fig. 5). No differences in survival of *E. coli* were observed between the two sites. *C. albicans* also survived well at these sites, and again no between-site differences were observed (Fig. 5). With 24 h densities of *C. albicans* had increased by 80% of its orginal densities; however, after 60 h densities declined until they were close to the initial concentration at 108 h. This increase was not cryptic growth since cells were well washed before putting them in the chambers. Microscopic examination of samples revealed that this increase was an actual increase in cell density since the number of budding cells had increased proportionally. Studies with bacteria in this same watershed have shown that they also undergo cyclic fluctuations in this environment (11, 21).

Further evidence of the prolonged survival or indigenous nature of *C. albicans* in tropical freshwater is provided by examining the species composition on yeast selective media (Table 2). Even at site 1, a pristine area with no known source of fecal contamination, *C. albicans* comprised 15% of the yeasts present. Site 9, which was known to receive sewage effluent, had the highest proportion of *C. albicans* (46%), yet site 12, which was equally contaminated with sewage, had no indentifiable *C. albicans* at the times sampled.

Ahearn et al. (3) examined the survival of *C. albicans* in situ and found that it survived better in marine than in fresh waters. Buck (9) reported similar results. The present study shows that *C. albicans* survives equally well in both fresh and marine tropical waters, although high organic effluents

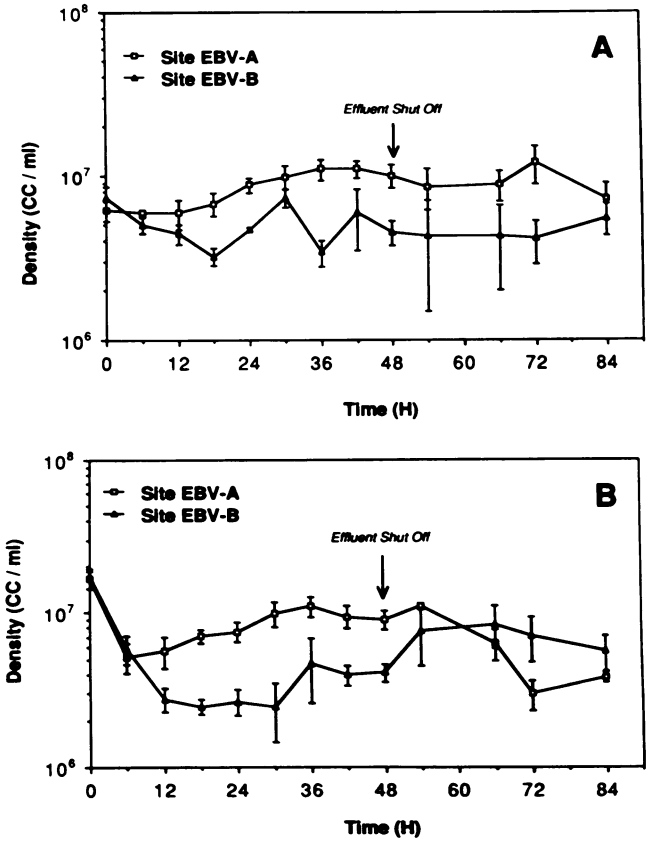

FIG. 4. Survival of *C. albicans* (A) and *E. coli* (B) at Ensenada de Boca Vieja (mean ± 1 standard error; n = 4).

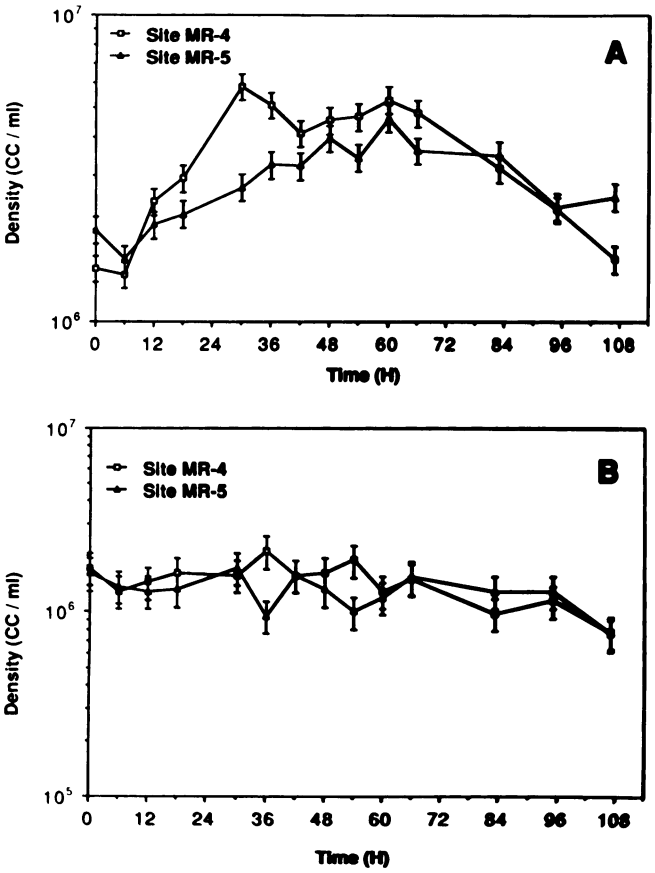

FIG. 5. Survival of *C. albicans* (A) and *E. coli* (B) at Mameyes River (mean ± 1 standard error; $n = 4$).

may allow growth in coastal waters. Our survival times in situ for *C. albicans* were significantly longer than any reported previously (3, 9).

The long survival times observed from both *C. albicans* and *E. coli* in both fresh and marine waters suggest that neither can be accepted as a suitable indicator of recent fecal contamination in tropical waters. High densities of either organism may not necessarily indicate fecal contamination. The current recommended maximum contaminant levels for microbes in recreational water are fecal coliforms or fecal streptococci or both. Current studies in progress (Hazen, unpublished data) indicate that fecal streptococci survive as well as *E. coli* in these same environments. Considering the importance of waterbone diseases in tropical areas of the world, it is imperative that we find better indicators of recent human fecal contamination. In addition, Brisou (8) reported a higher incidence of vaginal infections caused by *Candida* spp. among women who frequent beaches. Since the *C. albicans* strain used in our survival studies was clinical, i.e., pathogenic, and since densities in the chambers started at 10^7 cells per ml, these studies also suggest that *C. albicans* in tropical waters may under some conditions reach densities which may represent a health hazard.

TABLE 2. Yeast isolation from the Mameyes River by site[a]

Site	Species	% of total
1	*Saccharomyces* spp.	15.38
	Candida guillermondii	30.76
	C. parapsilosis	7.69
	C. albicans	15.38
	Cryptococcus dimennae	7.69
	Unidentified	15.38
9	*Candida parapsilosis*	7.69
	C. albicans	46.14
	Cryptococcus albidus	7.69
	Torulopsis candida	7.69
	Unidentified	23.08
12	*Cryptococcus albidus*	33.33
	Torulopsis candida	66.66

[a] There were 30 isolates at each site.

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