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Yeasts from Marine and Estuarine Waters with Different Levels of Pollution in the State of Rio de Janeiro, Brazil

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Yeast counts were made at 24 marine and estuarine sites in the vicinity of Rio de Janeiro, Brazil. Mean salinities of estuarine sites ranged from 14.2 to 27.4‰, and mean temperatures ranged from 25 to 28°C. Total coliform counts varied from 80% above 100,000 colony-forming units (CFU)/100 ml at heavily polluted sites to 100% below 100 CFU/100 ml at unpolluted sites. Total yeast counts above 100 CFU/100 ml were typical of heavily and moderately polluted water but atypical of lightly polluted and unpolluted water. Mean total yeast counts were 2,880 CFU/100 ml for heavily polluted sites, 202 CFU/100 ml for moderately polluted sites, and 3 CFU/100 ml for lightly polluted and unpolluted sites. Total yeast counts had a positive response to increased pollution levels, and *Candida krusei* and phenotypically similar yeasts as a group were prevalent in polluted estuarine water but rare in unpolluted seawater. The 549 strains of yeasts and yeast-like organisms isolated were grouped into 67 species, of which the 21 most prevalent made up 86% of the total yeast population. The prevalent genera in the polluted estuary were *Candida*, *Rhodotorula*, *Torulopsis*, *Hanseniaspora*, *Debaryomyces*, and *Trichosporon*.

Most work on yeasts in estuaries and near shore seawater has been done in Europe and North America (1-3, 5, 11, 12, 15, 18, 19, 25, 27, 28). Work on yeasts in sewage and sewage disposal plant effluents has been restricted to temperate climates, and much of it has been done by enrichment culture which does not necessarily indicate the relative abundance of species (9, 10, 17, 23, 24).

Yeast counts in clean seawater generally range from a few to several hundred per liter, but in the presence of enrichment, such as from pollution or algae blooms, can reach thousands per liter or more. In addition, there is a shift from a prevalence of strictly aerobic yeasts in clean water to a prevalence of fermentative yeasts in polluted water (26). Our work is a quantitative and qualitative evaluation of the yeast population of subtropical marine and estuary sites in Rio de Janeiro, Brazil. We hope to show a similarity of the yeast population in Brazil to those previously reported from other geographic areas and find at what magnitude yeast counts are significantly higher than those typical of clean water, to allow their use as a pollution indicator in marine waters.

MATERIALS AND METHODS

Collection sites. Most samples were taken from six sites along a polluted estuary bordering the Ilha do Fundão, Rio de Janeiro, Brazil (Fig. 1). Sites 1 to 3 were along an enclosed inner channel which received industrial and raw domestic wastes from the Jacaré River and raw domestic sewage from a slum located between sites 1 and 2. Sites 4 to 6 were along another channel (outer channel) which connected at one end with the inner channel and opened into the cleaner water of Guanabara Bay at the other end. Site 7 (Gloria) was along a heavily polluted cove near the central area of the city and within Guanabara Bay. Site 8 (Botafogo) was at the end of Flamengo Park just inside the entrance of Guanabara Bay. Sites 9 (Leme), 10 (Copacabana), 11 (Arpoador), 12 (Leblon), and 13 (Leblon) were all open beach sites in front of heavily populated residential parts of the city. Site 14 was in a heavily polluted freshwater stream which entered Leblon beach 10 meters from site 13 and 100 meters from site 12. Site 15 was on São Conrado beach in front of a moderately populated area of the city. Sites 16 and 17 were on the beach, and sites 18 and 19 were along a lagoon of Barra de Tijuca which was a relatively undeveloped area of the city. Sites 20 to 24 were on an uninhabited island near Arraial do Cabo in the State of Rio de Janeiro. Site 20 was water continuously pumped from 40 meters depth, and sites 23 and 24 were at the shore of the island. Sites 21 and 22 were algae aquaculture tanks with algae populations between 10^7 and 10^8 cells per 100 ml. The sites were grouped according to pollution level as follows: heavy pollution sites 1, 2, 3, 13, and 14 had dark water with strong sewage-like odor; moderate pollution sites 4, 5, 6, and 12 had colorless water with no foul odor but were near heavily polluted areas and heavy algae populations were often evident; light pollution sites 8 to 11 and 15 to 19 had clear water without foul odor

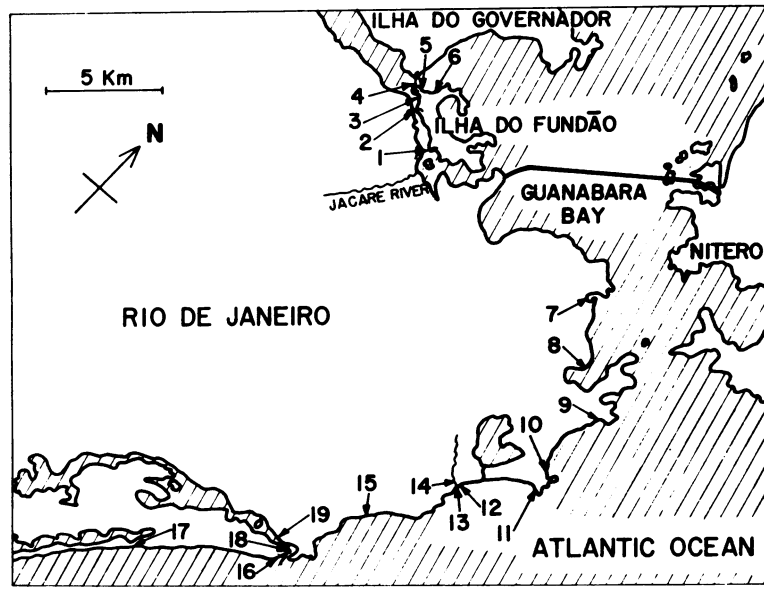

FIG. 1. *Map of collection sites in the city of Rio de Janeiro.*

and no obvious direct entry of sewage but were located in an urban area and received heavy recreational use; unpolluted sites were far from urban areas and received no recreational use.

All samples were taken at the shore line except those from sites 1 and 2 which were taken from bridges at mid-channel, those from site 10 taken from a pier 10 meters from shore, and those from sites 20 through 24 as indicated above. The collections were made between 20 February 1974 and 22 October 1975, and the number of samples taken is indicated in Table 1. With the exception of site 20, all samples were taken at the surface with sterile wide-mouth bottles which were lowered into the water on sterile strings. About 1 m of string was coiled around the mouth of each bottle, which was then wrapped in paper and sterilized by autoclaving. This allowed samples to be taken without directly handling the bottle, which could have contaminated the sample. Samples were transported to the lab on ice within 1 to 3 h.

Water temperature was measured in the field, and the pH was measured with a Beckman SS-3 pH meter immediately upon arrival of the sample in the laboratory. Salinity was determined by titration of chloride in 5-ml aliquots of the samples with 0.1 M silver nitrate (the Mohr method; 22). Total coliforms were enumerated with Levine EMB agar at 37°C for 48 h by using spread plates or membrane filters (Millipore Corp., HA 0.45 μm). The total aerobic heterotropic bacteria were enumerated on spread plates by using marine agar 2216E (Difco Laboratories) at 25°C for 2 and 7 days. Yeast counts were made on modified Sabouraud dextrose agar (2% dextrose–1% peptone–0.5% yeast extract–1.5% agar–50% aged filtered seawater–20 mg of chloramphenicol per 100 ml, and adjusted to pH 3.7 with HCl) by using spread plates and membrane filters, and the agar plates were incubated at 15°C for 2 weeks.

TABLE 1. *Arithmetic means and standard deviations of salinity, temperature, and pH of the collection sites*

Sites	No. of samples	Salinity (‰)	Temp (°C)	pH
1	13	14.2 ± 5.6	25.0 ± 1.4	7.4 ± 0.3
2	32	23.3 ± 5.0	25.6 ± 3.0	7.7 ± 0.4
3	44	23.8 ± 5.5	26.3 ± 3.1	7.7 ± 0.4
4	18	25.4 ± 4.8	27.8 ± 3.0	8.1 ± 0.4
5	16	26.4 ± 3.4	28.0 ± 3.4	8.3 ± 0.2
6	31	27.4 ± 5.2	27.9 ± 2.7	8.2 ± 0.3
7–13; 15–19	15	32.4 ± 2.6	21.9 ± 0.6	7.9 ± 0.2
14	1	0.0	21.0	7.2
20	4	35.2 ± 0.5	15.5 ± 2.6	7.8 ± 0.2
21–24	5	34.4 ± 1.4	19.3 ± 0.1	7.8 ± 0.2

Counts of individual yeast species were determined by making counts of the different colony types in 12 collections at polluted estuary sites and in 1 collection at four recreational ocean beaches and isolating representatives of each colony type for identification. Isolates were maintained on Sabouraud dextrose agar slants grown at 28°C and stored at 4°C under sterile mineral oil.

Yeast isolates were characterized by using techniques described in Lodder (16) and employing the velvet pad replica plate technique (20) to inoculate carbon assimilation and temperature growth tests. Identifications were made by using the keys in Lodder (16) and Barnett and Pankhurst (3).

RESULTS

The salinities of sites 1 through 6 reflect their position along the estuary by the decrease in salinity with decreased distance from the fresh-

water source (Table 1). Sites of the outer channel (sites 4, 5, and 6) averaged about 26‰ salinity. Inner channel sites 2 and 3 averaged about 23‰ salinity, and site 1 averaged about 14‰ salinity. The salinities of the outer sites except site 14 were close to the 35‰ expected for seawater.

With the exception of the subsurface site 20, water temperatures were warm, especially the polluted estuary sites which ranged up to 31°C (Table 1). The pH of all samples was slightly alkaline, ranging from 7.2 to 8.7. Lower pH readings were from the inner channel, and higher readings were from the outer channel of the polluted estuary. Variation in pH was lowest in the open ocean sites and highest in the polluted estuary sites (Table 1).

Total coliform counts listed in Table 2 ranged from less than 10^2 per colony-forming units (CFU) per 100 ml in seawater from unpopulated areas (sites 20, 21, and 22) to more than 10^5 CFU/100 ml in heavily polluted sites (sites 1, 2, 3, 7, 13, and 14). Total aerobic heterotropic bacteria counts (Table 2) were generally 2 to 4 orders of magnitude higher than the total coliforms and were highest in polluted water but were also elevated when high algae populations existed as at sites 21 and 22. The geometric means of total yeast counts and pink yeast counts are given in Table 2. The frequency table of total yeast counts shows greater incidence of high yeast counts at sample sites with highest pollution levels (Table 3). More than one-half of the samples from inner channel sites had total yeast counts in the range of 1,000 to 5,000 CFU/100 ml, and more than half the outer channel samples had total yeast counts in the range of 50 to 500 CFU/100 ml. Total yeast counts from city recreational beach sites (sites 8–11 and 15–19) were 79% within the range of 10 to 100 CFU/100 ml, and more than half of the total yeast counts from unpopulated beach sites (sites 20–24) were less than 10 CFU/100 ml. The only count above 100 CFU/100 ml at unpolluted sites was from an aquaculture tank with a high algae population.

Pink yeast counts generally paralleled those of total yeast counts, but averaged about 10% of the total yeast counts in the inner channel, 25% in the outer channel, and about 50% at recreational and clean beach sites (Table 2).

The 21 species which occurred in three or more of the 12 collections in which yeast were identified at sites 1 through 6 represented 86% of the total yeast counts. The species isolated and their frequency in 12 collections were: *Aureobasidium pullulans*, 5; *Candida boidinii*, 2; *C. brumptii*, 3; *C. cifferrii*?, 1; *C. diddensii*, 1; *C. guilliermondii* var. *guilliermondii*, 4; *C. humicola*, 1; *C. ingens*?, 1; *C. intermedia*, 4; *C. krusei*, 12; *C. lusitaniae*, 1; *C. lipolytica*, 4; *C. membranaefaciens*, 2; *C. mogii*?, 1; *C. norvegensis*, 1; *C. parapsilosis*, 3; *C. sake*?, 1; *C. shehatae*?, 1; *C. sorbosa*, 11; *C. sorboxylosa*, 7; *C. steatolytica*?, 1; *C. tenuis*?, 1; *C. tropicalis*, 7; *C. utilis*, 1; *C. valida*, 1; *C. zeylanoides*, 1; *Candida* sp. A 4; *Candida* sp. B, 1; *Candida* sp. C, 1; *Candida* sp. D, 1; *Candida* sp. E, 1; *Candida* sp. F, 1; *Cryptococcus albidus* var. *albidus*, 2; *Debaryomyces hansenii* + *Torulopsis candida*, 9; *Hansenula anomala* var. *schneggii*?, 2; *H. anomala*?, 4; *Kloeckera apiculata* + *Hanseniaspora uvarum*, 9; *Metschnikowia*? sp., 2; *Pichia membranaefaciens*, 3; *P. ohmeri*, 1; *P. terricola*, 7; *Prototheca zopfii*, 1; *Rhodotorula glutinis* var. *dairenensis*, 1; *R. pilimanae*, 1; *R. rubra*, 12; *Saccharomyces cerevisiae*, 1; *S. exiguus* + *Torulopsis holmii*, 8; *Torulopsis ernobii*?, 1; *T. glabrata*, 2; *T. gropengiesseri*?, 1; *T. maris*?, 2; *Torulopsis* sp. A, 1; *Trichosporon cutaneum*, 4; *Torulopsis penicillatum*, 9; *Torulopsis variable*?, 1.

TABLE 3. *Frequency table showing the number of samples from different levels of pollution with total yeast counts within 5 logarithmic ranges*

CFU/100 ml	No. of samples		
	Heavy pollution, sites 1–3	Moderate pollution, sites 4–6	Light and no pollution, sites 8–11; 15–24
<10	0	5	13
10–<100	1	20	12
100–<1,000	12	40	1
1,000–10,000	63	12	0
>10,000	15	2	0

TABLE 2. *Geometric means of selected microbial populations in CFU per 100 ml at four levels of pollution*

Pollution level	Total coliforms	Total aerobic heterotrophs	Total yeasts	Pink yeasts	*C. krusei* group	*C. tropicalis* group
Heavy	3.4×10^5 (15)[a]	3.4×10^7 (15)	2.8×10^3 (95)	2.8×10^2 (95)	5.0×10^2 (29)	4.3×10^2 (29)
Moderate	9.6×10^3 (11)	2.3×10^6 (11)	2.0×10^2 (79)	5.3×10^1 (79)	4.5×10^1 (10)	1.1×10^2 (10)
Light	2.3×10^2 (8)	4.9×10^4 (14)	9.9×10^0 (14)	1.5×10^0 (12)	8.8×10^{-1} (4)	9.8×10^0 (4)
None	7.3×10^1 (3)	6.3×10^5 (17[b])	5.8×10^{-1} (12)	5.8×10^{-1} (12)	(0)	(0)

[a] Numbers in parentheses are the number of samples.
[b] High density of algae present in 14 samples.

The genus *Rhodotorula* was almost exclusively represented by the species *R. rubra*, which had considerable variation among the 67 strains isolated (14). *H. uvarum* and its imperfect form were present as a dominant species, after rain storms. The *C. krusei-P. membranefaciens* group (which included *C. krusei*, *C. sorbosa*, *C. sorboxylosa*, *C. valida*, *C. norvegensis*, *P. membranefaciens*, *P. kluyveri*, *P. terricola*, *Candida* sp. A), and the *C. tropicalis* group (which included *C. tropicalis*, *C. guilliermondii*, *C. parapsilosis*, *C. intermedia*, *C. shehatae*, and *C. sake*) were each collectively responsible for large portions of the total yeast counts and included several individually important species. Each made up about 15 to 20% of the total yeasts in the inner channel sites. In the outer channel the *C. kursei-P. membranaefaciens* group made up about 20% of the total yeasts, but the *tropicalis* group made up about 50%. In sites 8 through 11 the *C. krusei-P. membranaefaciens* group made up less than 5%, but the *C. tropicalis* group made up about 50% of the total yeast counts (Table 2).

The dominate species at recreational beach sites 9 to 11 on 3 July 1975 were *C. guilliermondii*, *C. parapsilosis*, *Candida* sp. A, *R. rubra*, and *T. candida*, which were each found at three sites. *C. lipolytica*, *Candida* sp. I, *Candida* sp. J, and *T. holmii* were each found at two sites, and *A. pullulans*, *Candida* sp. G, *H. anomala*, and *K. apiculata* were found at one site each. With the exception of the four unidentified *Candida* species, all were among the most common species of the polluted estuary. However, the populations of the individual yeast species were much lower in the recreational beach water than in water of the polluted estuary.

DISCUSSION

The extremely polluted sites of the inner channel had mean total coliform counts several orders of magnitude above the corresponding fecal coliform level suggested by Geldrich (13), as a standard for recreational waters (200 fecal coliforms per 100 ml), and all total yeast counts from these sites were above 100 CFU/100 ml. Yeast counts from polluted sites with total coliform counts about 10 times the suggested recreational water standard were above 100 CFU/100 ml in 70% of the samples. All but one of the 26 samples from low-pollution and nonpolluted sites had less than 100 yeasts per 100 ml. Although total yeast counts above 100 CFU/100 ml can be obtained from unpolluted water, they are unusual, and counts above this level are characteristic of polluted water. Relationships between yeast counts and pollution indicators in polluted water have been reported by Buck (5) for the Miami River and Viviani and Tortorano (29) for the Po River.

Counts of individual species or groups of species of yeasts should allow better correlation to environmental factors than total yeast counts, but enumeration of most individual yeast species or groups is generally too tedious for routine application. Some yeasts or groups of yeasts do form distinctive colonies on spread plates and membrane filters which allow them to be enumerated individually, and Buck and Bubucis (7) have developed a medium to count *C. albicans*. The most obvious are the pink colonies which usually are formed by *Rhodotorula* species and are easily to enumerate if incubation time is sufficient for their carotenoid pigments to develop. The use of pink yeasts as a pollution indicator was encouraged by Simard and Blackwood (21). However, pink yeasts do not appear to represent a consistent proportion of the yeast population in other studies, including ours, nor have they been correlated with a more specific factor of pollution. Furthermore, pink yeast counts made up a higher proportion of the total yeast population in clean water than in polluted water, indicating that total counts are more responsive to pollution levels. *C. krusei*, *P. membranaefaciens*, and similar species were a phenotypically similar group forming typical rugose colonies with radiating ridges on the acid Sabouraud agar used for isolation which allowed enumeration of the group. Since representatives of this group did not grow in the alkaline conditions or low phosphate levels typical of seawater and had relatively low survival in seawater (Hagler, unpublished data), they should make good indicators of recent contamination from terrestrial sources. Xylose-assimilating forms of the *C. krusei-P. membranaefaciens* group have previously been found associated with pollution from pulp mills and are a possible indicator of this type of pollution (17). Our counts of this group increased with the increased pollution levels (Table 2).

We found *Candida*, *Rhodotorula*, *Torulopsis*, *Hanseniaspora* (+ *Kloeckera*), *Debaryomyces*, and *Trichosporon* to be the most frequently isolated genera in polluted estuary water, which is in basic agreement with existing literature. *Crytococcus* was of minor importance in the polluted estuary, which is in accord with the results of Woollett and Hedrick (26), who indicated that this genus is more important in unpolluted than in polluted water.

C. krusei was present in all of 12 collections from which yeasts were isolated for taxonomic studies, and the phenotypically similar species *C. sorbosa*, *C. sorboxylosa*, *P. terricola*, and *P. membranaefaciens* were also frequently iso-

lated, making this group especially important. *C. tropicalis*, *C. parapsilosis*, and *C. guilliermondii* appeared to replace each other in the yeast population, and when one of these species was present it usually made up a major proportion of the total yeast count. *D. hansenii* and its imperfect form *T. candida* were isolated in 75% of the collections from the polluted estuary and also in 75% of the samples from recreational beaches, which suggests that these are normal inhabitants of seawater which were able to grow in the presence of pollution. *S. exiguus* and its imperfect form *T. holmii* were found in 8 of 12 collections, but *S. cerevisiae* was encountered only once in the polluted estuary. *H. uvarum* and *K. apiculata* were in 75% of the collection and were especially numerous after rain storms, indicating their entrance into the estuary with runoff. *T. penicillatum* was frequently isolated in our collections, but is not a common isolate from aquatic environments, although it and the similar species *T. capitatum* have been reported as a prevalent yeasts in polluted water (15, 17, 23, 24). *H. uvarum* and *T. penicillatum* were reported by Meyers et al. (17) to occur in increased densities in an area polluted by pulp mill wastes compared with other areas of Lake Champlain.

Our failure to isolate *C. albicans*, which is often associated with polluted water (6), may be explained by the 15°C incubation temperature used since Buck (4) isolated *C. albicans* at 37°C but not at 20°C. However, we isolated *T. glabrata*, *T. holmii*, *C. parapsilosis*, *C. guilliermondii* var. *guilliermondii* and *C. krusei* at 15°C incubation temperature, which Buck (4) isolated at 37°C but not at 20°C. Work in progress here shows that with a 40°C incubation temperature, few *C. albicans* were detected in heavily polluted water. Even if low incubation temperature interfered with isolation of *C. albicans* in this study, the species appears to have a low incidence in these waters.

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