Identification of Yeasts From the Suwannee River Florida Estuary

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The yeast flora of the Suwannee River estuary in Florida has been studied. The predominant genera were Candida and Rhodotorula; however, the yeast most frequently isolated was Cryptococcus laurentii. Nine ascosporogenous species were isolated, with Hansenula saturnus predominating. The salinity range of the sediments was 0.4 to 20.6%; in the estuary water, 0.07 to 0.25%; and in the open Gulf of Mexico, 18 to 20%.

The ecological significance of yeasts in fresh, estuarine, and salt water, as well as their adjacent sediments, is currently being studied throughout the world. Enumeration and identification of the yeasts in estuarine waters have shown a wide range in number and species but a similarity in genera. The five most frequently isolated genera are Rhodotorula, Candida, Debaryomyces, Cryptococcus, and Kloeckera (12). Some species which are common terrestrial inhabitants appear to have adapted to marine and aquatic environments, whereas others appear to be strictly oceanic (13). Due to the proximity of sewage pollution and terrestrial runoff some species are more prevalent in estuaries than in the open seas (9-11). Uden (12), comparing the yeast speciation study in polluted streams and sewage treatment plants of Cooke (5) with estuarine yeast studies, concluded that most of the species found in sewage and polluted waters were also the dominant species in the estuaries.

Capriotti (3, 4) found that the frequency of yeasts in the Key Biscayne, Fla., area was similar to that in other warm water localities throughout the world, but that it differed from that in soils of cold marine areas. Fell et al. (6) isolated species of Candida, Cryptococcus, Rhodotorula, Trichosporon, Torulopsis, Debaryomyces, Hansenula, and Saccharomyces from Biscayne Bay, Fla., and adjacent benthic areas. These taxa were similar to yeasts isolated from deep sea sediments from the Bahamas, as well as nonmarine localities. In aquatic regions of south Florida, Ahearn et al. (1) isolated species of Candida, Cryptococcus, Rhodotorula, and Debaryomyces. These strictly oxidative species were the most widespread in all the habitats studied and were generally found in water of relatively low organic content.

Because there is a current interest in the ecology of marine waters, our study was undertaken to examine the yeast flora of the Suwannee River estuary. This area is sparsely developed and relatively free of pollution. In addition, this river is currently being considered for classification as a protected river area. This classification would prevent further industrial development, which might endanger this important marine spawning area.

MATERIALS AND METHODS
Water and sediment samples were collected 4 October 1971 from nine stations in the estuarine portion of the Suwannee River and in the Gulf of Mexico. A standard coring apparatus (2.5 cm in diameter) was employed to sample the sediment for a depth of 30 cm. The sediment samples were obtained from areas covered by about 30 cm of water at low tide and divided into 10-cm intervals giving a top, middle, and bottom section of sediment. Each section was then treated as a separate sample. Water samples were collected at a depth of 30 cm from the surface and about 2 m out from the low-water mark. All samples were obtained during the incoming tide. Sterile Wheaton water sample bottles (125 ml, Wheaton Glass, Millville, N.J.) were used for the sediment and water samples.

Standard methods agar (Difco) supplemented with 100 mg per liter each of chloramphenicol and chlortetracycline (?) was the isolation medium employed. Undiluted water samples and diluted (1:1) sediment samples were surface inoculated onto the antibiotic medium and incubated at 25 C for 5 days. Isolated
| Species                          | Frequency (%) | Station | Total fungi/g | Yeast/g | Salinity (%) |
|---------------------------------|---------------|---------|---------------|---------|--------------|
| Brettanomyces intermedius       | 0.6           | 2S      | 80            | 14      | 0.43         |
| Candida boidinii                | 0.6           | 8S      | 100           | 12      | 20.64        |
| Candida diversa                 | 0.6           | 4S      | 44            | 21      | 2.43         |
| Candida glaebosa                | 0.6           | 1S      | 165           | 66      | 0.63         |
| Candida guilliermondii          | 0.6           | 1W      | 6            | 2       | 0.07         |
| Candida ingens                  | 0.6           | 1S      | 165           | 66      | 0.63         |
| Candida krusei                  | 3.9           | 1S      | 165           | 66      | 0.63         |
|                               |               | 2S      | 80            | 14      | 0.43         |
|                               |               | 7S      | 72            | 15      | 2.70         |
|                               |               | 9W      | 4             | 1       | 17.95        |
| Candida lambica                 | 6.6           | 1S      | 165           | 66      | 0.63         |
|                               |               | 2S      | 80            | 14      | 0.43         |
|                               |               | 4S      | 44            | 21      | 2.43         |
|                               |               | 7S      | 72            | 15      | 2.70         |
| Candida maritima                | 0.6           | 1S      | 165           | 66      | 0.63         |
| Candida melibiosica             | 0.6           | 1S      | 165           | 66      | 0.63         |
| Candida silvae                  | 2.6           | 1S      | 165           | 66      | 0.63         |
|                               |               | 3S      | 26            | 2       | 0.53         |
|                               |               | 4S      | 44            | 21      | 2.43         |
| Candida solani                  | 2.0           | 1S      | 165           | 66      | 0.63         |
| Candida valida                  | 5.9           | 1S      | 165           | 66      | 0.63         |
|                               |               | 2S      | 80            | 14      | 0.43         |
|                               |               | 3S      | 26            | 2       | 0.53         |
|                               |               | 4W      | 10            | 4       | 0.07         |
| Candida spp.                    | 2.6           | 1S      | 165           | 66      | 0.63         |
| Cryptococcus dimennae           | 0.6           | 1S      | 165           | 66      | 0.63         |
| Cryptococcus laurentii var. laurentii | 10.5 | 1S      | 165           | 66      | 0.63         |
|                               |               | 2S      | 80            | 14      | 0.43         |
|                               |               | 6W      | 12            | 2       | 0.22         |
|                               |               | 7S      | 72            | 15      | 2.70         |
|                               |               | 8S      | 100           | 12      | 20.64        |
| Cryptococcus laurentii var. flavescens | 4.6 | 1S      | 165           | 66      | 0.63         |
|                               |               | 4S      | 44            | 21      | 2.43         |
|                               |               | 5S      | 51            | 9       | 4.50         |
| Debaryomyces cantarellii        | 0.6           | 1S      | 165           | 66      | 0.63         |
|                               |               | 5S      | 51            | 9       | 4.50         |
|                               |               | 7W      | 12            | 2       | 0.25         |
| Debaryomyces phaffii            | 0.6           | 5S      | 51            | 9       | 4.50         |
| Hansenula beijerinckii          | 2.0           | 1S      | 165           | 66      | 0.63         |
| Hansenula saturnus var. saturnus | 4.6 | 1W      | 6             | 2       | 0.07         |
|                               |               | 1S      | 165           | 66      | 0.63         |
|                               |               | 4S      | 44            | 21      | 2.43         |
|                               |               | 7S      | 72            | 15      | 2.70         |
| Hansenula spp.                  | 0.6           | 6S      | 100           | 12      | 20.64        |
| Kluyveromyces polysporus        | 0.6           | 1S      | 165           | 66      | 0.63         |
| Leucosporidium capsuligenum     | 0.6           | 1S      | 165           | 66      | 0.63         |
| Pichia membranefaciens          | 2.0           | 8S      | 100           | 12      | 20.64        |
| Pichia ohmeri                   | 0.6           | 5S      | 51            | 9       | 4.50         |
| Rhodotorula glutinis            | 0.6           | 1S      | 165           | 66      | 0.63         |
| Rhodotorula graminis            | 4.6           | 1S      | 165           | 66      | 0.63         |
|                               |               | 3S      | 26            | 2       | 0.53         |
|                               |               | 5S      | 51            | 9       | 4.50         |
|                               |               | 6S      | 24            | 3       | 6.19         |
Table 1—Continued

| Species                     | Frequency (%) | Station | Total fungi/g | Yeast/g | Salinity (%) |
|-----------------------------|---------------|---------|---------------|---------|--------------|
| *Rhodotorula lactosa*      | 0.6           | 1S      | 165           | 66      | 0.63         |
| *Rhodotorula marina*       | 5.3           | 1S      | 165           | 66      | 0.63         |
| *Rhodotorula minuta var. minuta* | 0.6       | 7W      | 12            | 2       | 0.22         |
| *Rhodotorula rubra*        | 7.9           | 1S      | 165           | 66      | 0.63         |
| *Rhodotorula spp.*         | 3.9           | 1S      | 165           | 66      | 0.63         |
| *Saccharomyces spp.*       | 0.6           | 4S      | 44            | 21      | 2.43         |
| *Torulopsis candida*       | 3.9           | 3S      | 165           | 66      | 0.63         |
| *Torulopsis inconspicua*   | 2.0           | 1S      | 165           | 66      | 0.63         |
| *Torulopsis mogii*         | 0.6           | 7S      | 72            | 15      | 2.70         |
| *Torulopsis spp.*          | 5.9           | 1S      | 165           | 66      | 0.63         |
| *Trichosporon aculeatum*   | 0.6           | 4S      | 44            | 21      | 2.43         |
| *Trichosporon cutaneum*    | 1.3           | 7S      | 165           | 66      | 0.63         |
| *Trichosporon penicillatum*| 0.6           | 1S      | 165           | 66      | 0.63         |

* The number designates station whereas W or S refers to water or sediment, respectively.
* All values for sediments are an average of the top, middle, and bottom 10-cm portion.
* Water sample values are averages of individual determinations.

Colonies were picked and inoculated into YM broth (Difco), incubated for 3 days, and streaked onto YM agar plates to check for purity. Final transfers were then made onto YM slants. After incubation the isolates were stored at 5°C and transferred approximately every 8 weeks. Species identification was performed by using the method outlined by Lodder (8). Salinity determinations were performed as outlined by the American Public Health Association (2).

RESULTS

Table 1 is a summary of the identified yeasts, total fungi, yeast count, and salinity at selected stations of the Suwannee River estuary. Sediments from station 1 (Fig. 1), located at the northwest tip of an uninhabited island in the west pass of the Suwannee River, yielded the highest average number of yeasts (66/gram). Stations 2 and 3 are dredged canals. Station 2 has been recently dredged, whereas 3 is a much older canal. Both of these areas have a small number of houses nearby. Stations 4 and 5 are located in the main channel opening into the Gulf, with station 7 being at the mouth of this channel. Station 6 is located on a segment of

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**Fig. 1.** Map of Suwannee River estuary showing collection stations.
Salt Creek. Station 8 is located on a sandy oyster bar, whereas station 9 is across the bar in approximately 4 fathoms of water. Sediment samples had higher numbers of yeasts than the water samples; however, the majority of yeasts isolated from the sediments were only recovered from the upper 10-cm portion.

A total of 152 isolates representing five ascoporogenous and six asporogenous genera were obtained from the nine stations. The two most commonly isolated genera were Candida and Rhodotorula; these were followed by Cryptococcus and Torulopsis. The predominant species was Cryptococcus laurentii followed by Rhodotorula rubra and Candida lambica. Seventy-five yeasts, which were isolated primarily from the sediment portions, were identified from station 1. This station also yielded the greatest number of species, 31.

**DISCUSSION**

The Suwannee River estuary offers an excellent opportunity to examine the natural yeast flora within a relatively undisturbed area. There has been little commercial development of this area, consequently the environment has not been markedly disrupted.

The species of yeasts that were isolated from this estuary approximate the results of Fell et al. (6), when they determined the yeast flora of Biscayne Bay, Fla. Differences exist in the predominant species and in other species isolated; however, essentially the same genera were isolated. The higher temperatures, salinities, and the degree of water pollution in the Biscayne Bay, Fla, area could contribute to this variation.

Highest yeast densities were recovered from the lower salinity and less inhabited areas; however, no ascoporogenous yeasts were isolated from the two areas that had the lowest salinities. No other distribution patterns were observed.

The limited nutrient availability and selective environmental pressures appear to restrict the amount of active growth as well as the species of yeast in the sediment and water sample. This is indicated by the relatively low number of organisms found and the limited variety of genera isolated.

This is the first attempt to determine the yeast flora of the Suwannee River estuary. Because of Florida's rapidly increasing growth, many of our sparsely developed estuarine and marine areas are becoming sites for homes and industries. Because this results in an alteration of the natural ecological system, this present work can help provide baseline data for comparative studies of this area in the future.

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