Occurrence and Population Densities of Yeast Species in a Fresh-Water Lake

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VAN UDEN, N. and AHEARN, D. C. 1963. Occurrence and population densities of yeast species in a fresh-water lake. Antonie van Leeuwenhoek **29**: 308–312.

Quantitative studies of yeasts present in surface and deep water samples from a fresh water body (Douglas Lake, Michigan) revealed 12 species (*Candida parapsilosis*, *C. pulcherrima*, *Cryptococcus albidus*, *Cr. diffluens*, *Cr. gastricus*, *Cr. laurentii*, *Rhodotorula glutinis*, *R. pilimanae*, *R. rubra*, *Trichosporon cutaneum*, *Debaryomyces* sp., "black yeasts"). In two regions of surface sampling the population densities averaged 39.6 and 5.5 cells per 100 ml respectively, whereas the average deep water count was 40.3 cells per 100 ml. Yeasts of the genus *Rhodotorula* predominated.

INTRODUCTION

The presence of yeasts in marine environments is well documented (Johnson and Sparrow, 1961), and with certain exceptions the species reported are common to terrestrial habitats (van Uden and ZoBell, 1962).

The yeast speciation of fresh water bodies has received only scant attention. Although references exist as to the occurrence of yeasts in lakes (ZoBell, 1946), an examination designed for the specific detection of yeasts in a lake has not been undertaken. Douglas Lake, Cheboygan County, Michigan (45°37′ N, 84°38′ W) was selected as the site for study. The morphometric, physicochemical and photosynthetic aspects of this lake have been examined previously (Welch, 1927, 1945; Welch and Eggleton, 1931, 1935; Eggleton, 1952; Saunders, Trama and Bachmann, 1962).

METHODS

Samples. Surface water samples were collected during July and August, 1962, in sterile 1-liter Erlenmeyer flasks at 21 stations in Douglas Lake (Fig. 1).

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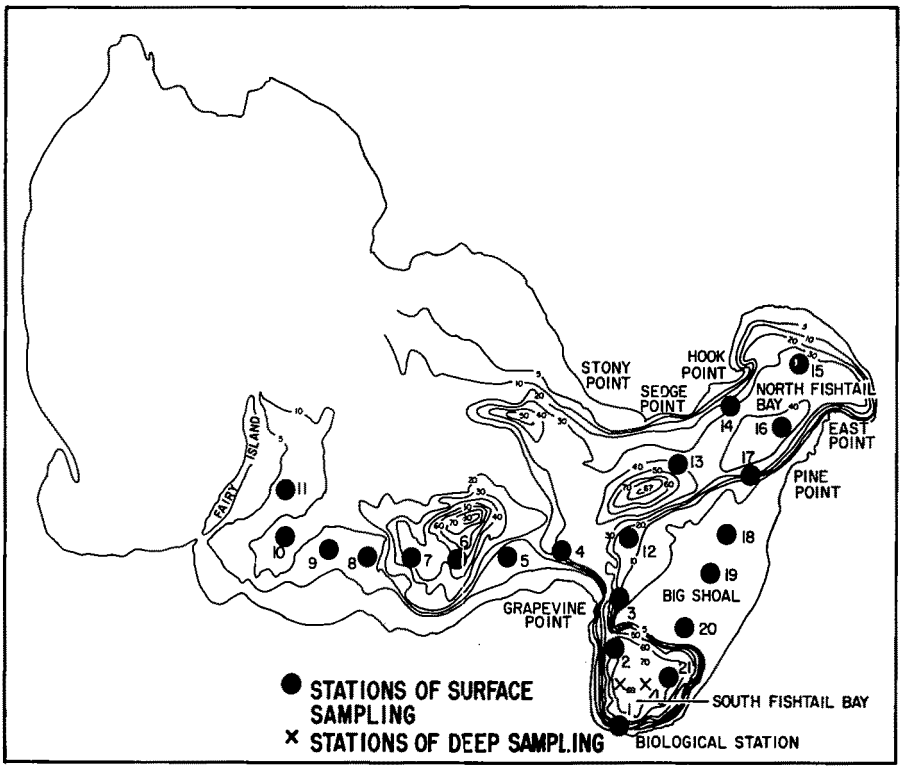

Fig. 1. Area of sampling in Douglas Lake, Michigan.

At two additional stations in South Fishtail Bay deep samples were obtained with a 3-liter Kemmerer bottle at two meter intervals from a depth of 3 to 21 meters. All samples were processed within an hour after collection.

Isolation medium. The isolation medium had the following composition: glucose, 2%; peptone (Difco), 1%; yeast extract (Difco), 0.5%; agar, 2%; distilled water. To limit bacterial growth the medium was adjusted to pH 4.5 with lactic acid.

Isolation procedure. Water samples of 100 ml each were filtered through HA membrane filters of 0.45 μ porosity (Millipore Filter Corp., Bedford, Mass.). The filters were transferred to petri dishes containing the isolation medium and incubated at 18–20 C. After 3 to 5 days, yeast colonies developing on the filter were subcultured on the periphery of the plate. The numbers of each type of colony, distinguished by macro- and microscopic morphological examination of the subcultures, were recorded and representative colonies were subcultured on slants of isolation medium for later identification.

Identification of yeast isolates. The methods of Lodder and Kreger-van Rij (1952), Wickerham (1951), van Uden and Farinha (1958) and Ahearn et al., (1962) were employed in the identification studies.

RESULTS AND DISCUSSION

Eighteen out of 21 surface samples contained viable yeasts in numbers ranging from 1–59 cells per 100 ml sample (Table 1). The highest densities of yeasts were found at the first five stations (Fig. 1). These water samples

TABLE 1

Yeast species and colony counts from surface water samples

Station	Species	Number/ 100 ml	Total	Station	Species	Number/ 100 ml	Total
1	*Candida pulcherrima*	5		10	*R. pilimanae*	8	8
	Cryptococcus laurentii	3		11	*R. glutinis*	3	3
	Rhodotorula pilimanae	5		12	negative		
	Trichosporon cutaneum	6		13	*R. glutinis*	2	
	unidentified yeast	7	26		*Cr. albidus*	3	5
2	*Debaryomyces* sp.	14		14	negative		
	R. glutinis	7	21	15	*R. pilimanae*	5	5
3	*Cr. diffluens*	14		16	*R. glutinis*	7	
	R. pilimanae	45	59		*C. pulcherrima*	3	10
4	*R. pilimanae*	55	55	17	*R. pilimanae*	1	
5	*Cr. diffluens*	5			*T. cutaneum*	1	2
	R. pilimanae	32	37	18	*R. pilimanae*	1	
6	*Cr. diffluens*	3			*C. pulcherrima*	3	4
	R. rubra	12	15	19	*Cr. gastricus*	1	1
7	*R. pilimanae*	13	13	20	*R. pilimanae*	3	3
8	*R. rubra*	3	3	21	negative		
	Debaryomyces sp.	5					
	R. glutinis	11	16				

averaged 39.6 cells per 100 ml while samples from all other surface stations averaged less than 10 cells per 100 ml. The genus *Rhodotorula* was the most widespread with representatives occurring in 15 out of the 21 samples. *R. pilimanae* was the most common species. All samples from the two deep stations demonstrated viable yeasts in number ranging from 19 to 110 cells with an average count of 40.3 cells per 100 ml (Table 2). The speciation of the deep samples was similar to that of the surface waters.

The yeasts isolated from Douglas Lake belonged to only 12 different species, occurring with relative incidences ranging from 1 to 20%: *Rhodotorula pili-*

TABLE 2

Yeast species and colony counts from deep water samples

Station I				Station II		
Depth	Yeast species	Number/ 100 ml	Total	Yeast species	Number/ 100 ml	Total
3 m	*Cryptococcus albidus*	17		*Cr. albidus*	13	
	Candida parapsilosis	3	20	*R. glutinis*	24	37
5 m	*Rhodotorula pilimanae*	27		*Cr. albidus*	8	
	Black yeasts	11	38	*R. rubra*	34	42
7 m	*Cr. albidus*	13		*C. parapsilosis*	8	
	R. rubra	9	22	*R. glutinis*	19	27
9 m	*Cr. albidus*	3		*R. glutinis*	23	
	R. rubra	5		unidentified	10	33
	Black yeasts	39	47			
11 m	*Cr. albidus*	12		*C. parapsilosis*	31	
	R. pilimanae	8	20	*R. glutinis*	79	110
13 m	*R. rubra*	17		*C. parapsilosis*	48	
	R. pilimanae	15	32	*Cr. albidus*	9	57
15 m	*Cr. albidus*	13		*R. glutinis*	37	
	R. rubra	15	28	*C. parapsilosis*	7	44
17 m	*Cr. albidus*	21		*R. glutinis*	31	
	R. rubra	29	50	*Cr. albidus*	11	42
19 m	*Cr. albidus*	13		*C. parapsilosis*	54	
	R. rubra	17	30	*R. rubra*	49	103
21 m	*Cr. albidus*	4		*R. glutinis*	32	32
	R. pilimanae	12				
	Black yeasts	3	19			

manae, 20%; *Cryptococcus albidus*, 19%; *R. glutinis*, 16%; *R. rubra*, 14%; *Candida parapsilosis*, 9%; *C. pulcherrima*, 4%; *Cr. diffluens*, 4%; *Trichosporon cutaneum*, 3%; *Debaryomyces* sp., 3%; black yeasts, 3%; *Cr. laurentii*, 1%; *Cr. gastricus*, 1%, unidentified, 3%. With the possible exception of *Cr. gastricus*, unreported from marine substrates, the species collected are common in the seas and occur in terrestrial substrates. The yeast *Metschnikowia zobellii*, which has not been isolated from terrestrial habitats, was not found during this survey. However, this species was repeatedly isolated on other occasions from trematodes infesting snails in Douglas Lake (Dr. K. Hussey, personal communication).

The results of the present study suggest that fresh water lakes may constitute a natural environment for a number of yeast species that also occur in marine and terrestrial habitats.

This investigation was supported by the Gulbenkian Foundation, Lisbon, and grants E-2587 and E-1546 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

Author N. van Uden is indebted to the following individuals of the University of Michigan Biological Station, Pellston, Michigan: Dr. A. H. Stockard, Dr. R. E. Beer, Dr. F. K. Sparrow, Dr. G. W. Saunders and Mr. R. Eisenberg.

Received 26 *June* 1963

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