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SHORT COMMUNICATIONS

A Study of the Composition of the Aquatic Bacterial Community of Lake Baikal by the *in situ* Hybridization Method

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Much interest has recently been focused on the biodiversity, composition, and structure of aquatic microbial communities, including that of Lake Baikal. Relevant investigations employ the methods of general microbiology [1, 2] and molecular biology [3, 4], such as the in situ hybridization method, which allows individual microbial cells and particular bacterial groups in water samples to be identified without cultivation [5].

This study aimed to investigate the aquatic microbial community in the southern basin of Lake Baikal by the fluorescence in situ hybridization method with oligonucleotide probes for the main bacterial groups.

Samples of the lake water were taken in the summer of 1999 at the central station on the Listvyanka settlement–Tankhoi settlement transect at various depths (0, 10, 25, 50, 100, 200, 400, and 760 m), as well as from the surface water layer in Peschanaya Bay. The lake water was sampled with a bathometer. The samples were fixed by adding a freshly prepared 4% paraformaldehyde [6] and transported to the laboratory, where they were passed through 0.22-μm pore-size Millipore polycarbonate filters. The filters were washed free of paraformaldehyde with a phosphate buffer and then sterile water and dried in the air. Each filter was cut into sections to be subjected to hybridization with oligonucleotide probes (Table 1) [7] as described by Amann [6]. After hybridization, the total bacterial abundance was determined by staining the filters with the fluorescent dye 4,6-diamidino-2-phenylindole. It should be noted that the loss of the fixed cells from the filters during hybridization was no more than 10%. On each of the filter sections, cells were counted in 10–20 microscope fields. The values obtained were corrected with due consideration for the negative control hybridization.

The results obtained in this study are summarized in Table 2. It can be seen that the total bacterial abundance (TBA) ranges from $(1.6–1.4) \times 10^6$ in the surface water (0–10 m) to $(0.1–0.2) \times 10^6$ in deeper waters of the lake (200–760 m). Eubacteria were found to comprise no more than 78% of the TBA in Lake Baikal. In other bodies of freshwater, the eubacterial probe detects from 43 to 61% of all bacterial cells, while as much as 96% in the oceanic surface waters [7]. It can be seen from Table 2 that the percentage of bacterial cells detected by the negative control hybridization is fairly high in the surface lake water, varying from 13% in Peschanaya Bay to 17% in the surface water at the central station of the southern lake basin and 22% in deeper waters (down to 25 m). This is most likely due to the extensive summer growth of cyanobacteria, whose chlorophyll fluorescence spectrum coincides with the fluorescence spectrum of the tetramethylrhodamine used in the hybridization procedure. This suggestion is in agreement with the fact that the background hybridization in the deeper lake waters does not exceed 3% (Table 2). Noteworthy is the relatively high percentage of cells that are not hybridized with the hybridization probes employed (such cells are referred to as "others" in Table 2). For instance, in the lake water from the depth 10 m,

Table 1. The oligonucleotide probes used in this work [7]

Probe	Probe structure (5'–3')	rRNA nucleotide positions	Detected bacterial group
EUB338	GCTGCCTCCCGTAGGAGT	16S, 338–355	Total eubacteria
ALF968	GGTAAGGTTCTGCGCGTT	16S, 968–986	Alpha-proteobacteria
BET42a	GCCTTCCCACTTCGTTT	23S, 1027–1043	Beta-proteobacteria
GAM42a	GCCTTCCCACATCGTTT	23S, 1027–1043	Gamma-proteobacteria
CF319a	TGGTCCGTGTCTCAGTAC	16S, 319–336	Cytophages–Flavobacteria
NON338	ACTCCTACGGGAGGCAGC	–	Negative control

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Table 2. The number of bacterial cells in water samples detected by fluorescent oligonucleotide probes expressed as a percentage of TBA

Water sampling site	Depth, m	TBA, 10^6 cells/ml	EUB	ALF	BET	GAM	GF	Others	NON
Peschanaya Bay	0	1.4	52	8	7	13	6	18	13
Southern basin of Lake Baikal	0	1.5	64	7	14	11	4	28	17
	10	1.6	70	2	7	4	2	55	18
	25	0.6	78	4	15	13	7	39	22
	50	0.2	71	10	12	15	7	27	3
	100	0.2	60	5	9	14	3	29	2
	200	0.2	52	9	5	10	3	25	2
	400	0.1	67	7	6	4	2	48	3
	700	0.1	64	7	7	10	7	33	<1

only 15% of the 70% of cells determined as eubacterial were able to hybridize with the probes for the main bacterial groups, whereas the other 55% comprised a fraction of the unidentified bacteria. According to data in the literature, such a situation is typical of freshwater ecosystems [7], although the fraction of cells that cannot be detected by the known hybridization probes in other ecosystems is not so high. Our previous studies of nonculturable bacteria in the southern Lake Baikal basin revealed a great diversity of nucleotide sequences with a low degree of homology to the sequences available in the EMBL database [8]. This suggests that a large fraction of the microbial community of Lake Baikal comprise the microorganisms that have not yet been identified by the 16S rRNA gene sequence analysis and are not presented in the EMBL database. If so, these microorganisms cannot be detected by the known oligonucleotide probes, which are based on the available nucleotide sequences.

To conclude, the investigations performed in this study show that in situ hybridization is a feasible method for the analysis of bacterioplankton in Lake Baikal. For a better quantitative analysis of this bacterioplankton, oligonucleotide probes should be designed with allowance made for a great number of nonculturable and endemic microorganisms in the lake.

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