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EXPERIMENTAL ARTICLES

The Biodiversity of Actinomycetes in Lake Baikal

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Abstract—The taxonomic analysis of 107 actinomycete strains isolated from the bottom sediments and water of Lake Baikal showed that most of the water isolates belong to the genus *Streptomyces* and most of the sediment isolates belong to the genus *Micromonospora.* In the sediments, the number of actinomycetes increased with depth (down to 200 m). Eight *Streptomyces* isolates were identified to a species level.

Key words: Lake Baikal, biodiversity of actinomycetes, water, sediments.

Actinomycetes are widely spread in various bodies of water, where they play a great part in the carbon cycle due to their ability to grow at low concentrations of carbonaceous substances and to degrade recalcitrant organic compounds [1]. In spite of the fact that the bacterial community of Lake Baikal has long been the subject of investigation by various researchers, actinomycetes in this lake are as yet poorly studied. The phylogenetic analysis of microbial 16S rRNA genes showed that actinomycetes comprise up to 30% of the microorganisms present in the lake water [2], the most numerous culturable actinomycetes being micromonosporas and streptomycetes [3–6].

The aim of the present work was to study the biodiversity of actinomycetes in the bottom sediments and water of Lake Baikal with due consideration for the specific characteristics of the lake (such as its oligotrophy, low temperature, and specific mineral composition).

MATERIALS AND METHODS

Water samples were obtained over a period from December 1999 to September 2000. In September 2000, 12 standard transects embracing the entire water area of Lake Baikal were investigated, namely, the Solzan–Maritui, the Listvyanka–Tankhoy, the Kharauz–Krasnyi Yar, the Anga River–Sukhaya River, the Boldakovo–Olkhon Vorota, the Cape Ukhan–Turka, the Cape Khoboy–Cape Krestovyi, the underwater Akademichesky Ridge, the Elokhin–Davsha, the Cape Pokoiniki–Ushkany Islands, the Baikalskoe–Turoli, and the Tyya River–Cape Nemnyanko transects (Fig. 1). In addition, samples were taken in Barguzin and Chivyrkuysky Bays, the Maloye More strait, the Selenga River, and in 12 other tributary rivers, as well as near settlements. Water samples were taken aseptically using a Ruttner bottle.

Actinomycetes were isolated using different techniques, such as surface and submerged cultivation, the membrane filtration technique [7] with the use of Vladipor MFA-MA no. 2 filters, and the plating of preliminarily concentrated samples [8] on selective media, including starch–ammonium agar, starch agar, nutrient agar, CP1 agar, MA1 agar, fish meal–peptone agar, starch–casein–KNO_3 agar [9], nocardia agar [10], non-nutrient agar [11], and Czapek agar with nystatin. Taking into account the fact that Lake Baikal is oligotrophic, the content of the respective carbon sources in the media used was reduced to 10–30% of that in the complete media.

Inoculated agar plates were incubated either at 25°C in a thermostated incubator, or at room temperature (18–20°C), or at 4–8°C in a refrigerator. Depending on the cultivation medium and temperature used, the agar plates were incubated for 2 to 8 weeks.

Table 1. The number of different actinomycete colonies grown on various selective media

Medium	Strain number
Starch–ammonium agar	56
Starch–casein–KNO_3 agar	38
Czapek agar with nystatin	10
Starch agar	9
Fish meal–peptone agar	8
Nocardia agar	4
Nutrient agar	3
CP1 agar	0
Non-nutrient agar	0
MA1 agar	0

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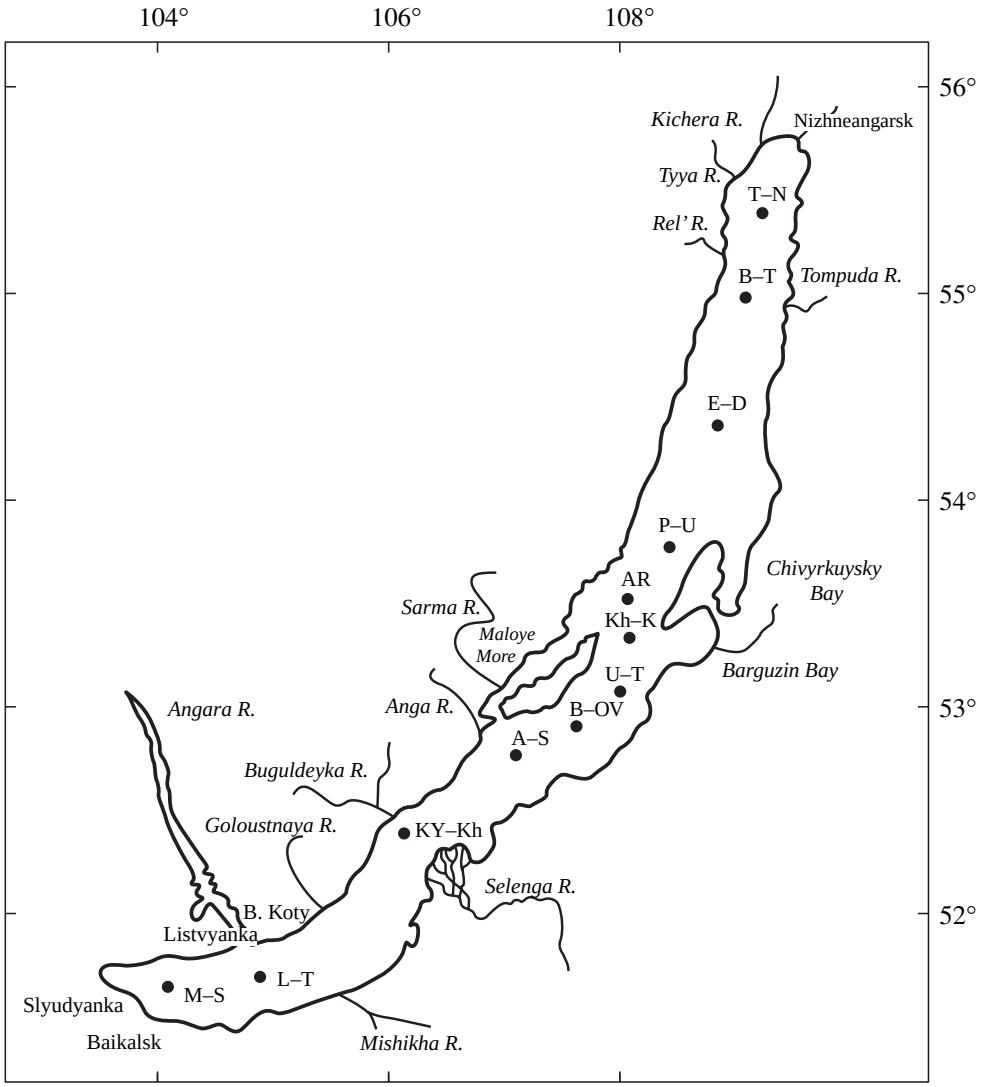

Fig. 1. Map showing the sampling of water in Lake Baikal with the following stations at the midpoints of transects: T–N, Tyya River–Cape Nemnyanko; B–T, Baikalskoe–Turoli; E–D, Elokhin–Davsha; P–U, Cape Pokoiniki–Ushkany Islands; AR, Akademichesky Ridge; Kh–K, Cape Khoboy–Cape Krestovyi; U–T, Cape Ukhan–Turka; B–OV, Boldakovo–Olkhon Vorota; A–S, Anga River–Sukhaya River; KY–Kh, Krasnyi Yar–Kharauz; L–T, Listvyanka–Tankhoy; and M–S, Maritui–Solzan.

Bottom sediment samples were obtained in June 2000 in the southern region of Lake Baikal (near a paper and pulp mill on Baikal's southern shore) from depths of 20, 22, 100, 200, and 314 m using a grab bottom sampler. The sediment samples were stored at 4°C and analyzed by plating their serial dilutions [12] onto the selective agar media described above. The number of actinomycetes found in 1 g of sediments was calculated by the formula presented in the textbook [12]. The isolated actinomycetes were analyzed microscopically and identified to the genus level using the identification criteria of Bergey's Manual [13]. Some of the isolated streptomycetes were identified to a species level according to the classification scheme presented in the Gauze Manual [14].

RESULTS AND DISCUSSION

Using the selective media and cultivation conditions described in the previous section, we succeeded in isolating 107 actinomycete strains (Table 1). Most of the actinomycetes belonging to the genera *Streptomyces* and *Micromonospora* were obtained using starch-containing agar media. All of the strains isolated through incubation on Czapek agar with nystatin belonged to the genus *Micromonospora*. It should be noted that the isolation procedures employing nocardia agar did not yield nocardia species.

The direct inoculation of water and sediment samples onto agar and into liquid media allowed us to isolate only a small number of actinomycetes (Table 2). The isolation procedure with membrane filters did not give good results either: all of the colonies grown on the

Table 2. The number of actinomycete strains isolated from the lake water by various methods

Sampling location and date	Strain number			
	Direct inoculation		Germination on filters	Sample concentration
	surface cultivation	submerged cultivation		
Southern Baikal, December 1999	0	–	–	–
Southern Baikal, March 2000	5	–	–	–
Baikal, June 2000	5	10	–	–
Baikal, September 2000	–	–	0	27
Baikal affluents, September 2000	–	–	0	12

Note: "–" stands for "not determined."

Table 3. The taxonomic composition of the actinomycetes present in the lake water and sediments

Morphological characteristics	CFU number		Number of isolates		Genus
	water	sediment	water	sediment	
Developed branched substrate mycelium and aerial mycelium with straight or spiral (monopodial or tufted) sporophores with nonmotile spores	39	22	31	18	*Streptomyces*
Undeveloped aerial mycelium; developed substrate mycelium with short or long sporophores with one or two nonmotile spores	12	29	12	22	*Micromonospora*

filters were fungal or bacterial. This was likely because of the inappropriate membrane pore size. At the same time, the use of concentrated samples for inoculation allowed 39 actinomycete strains to be isolated.

Streptomyces colonies reached sufficient sizes after 7 to 10 days of incubation at 25°C and after 2 to 3 weeks of incubation at room temperature, while *Micromonospora* colonies reached sufficient sizes, after 2 weeks of incubation at 25°C and after 3 to 4 weeks of incubation at room temperature. The incubation of agar plates in the refrigerator did not give rise to actinomycete colonies. However, *Streptomyces* colonies did appear on these plates after they had been drawn out of the refrigerator and incubated for 5–7 days.

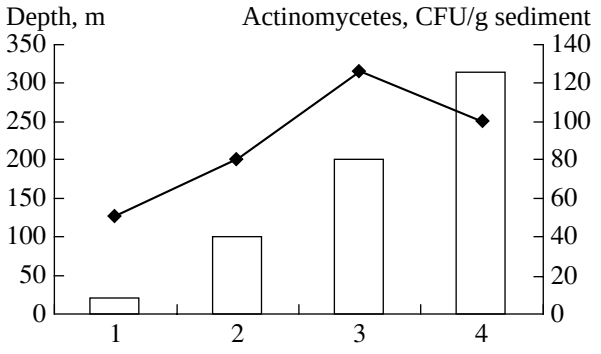

Fig. 2. Number of the actinomycetes (curve) found in 1 g of sediment samples obtained from different depths (bars): (1) 20, (2) 100, (3) 200, and (4) 314 m.

The number of actinomycetes in the bottom sediment samples was maximum in the sample taken from a depth of 200 m and was slightly less in the sample taken from a depth of 314 m (Fig. 2). We believe that the number of actinomycetes in bottom sediments in the environs of the paper and pulp mill correlates with the content of recalcitrant organic substances in these sediments, which are contaminated with wastes from the mill.

The taxonomic analysis of the actinomycetes present in the water and sediment samples on the basis of their morphological characteristics showed that the lake water was dominated by representatives of the genus *Streptomyces* (66% of all water isolates) and that the lake sediments were dominated by representatives of the genus *Micromonospora* (51% of all sediment isolates) (Table 3 and Figs. 3 and 4). We failed to identify seven strains even to the genus level. At the same time, eight isolates of the genus *Streptomyces* were identified to the species level. Three strains were ascribed to one species, *S. globisporus*, and five strains were identified as the species *S. aureofaciens, S. catenalue, S. candidus, S. baarnensis*, and *S. violaceoruber.*

The domination of streptomycetes in the lake water can be explained by two reasons. First, this may be due

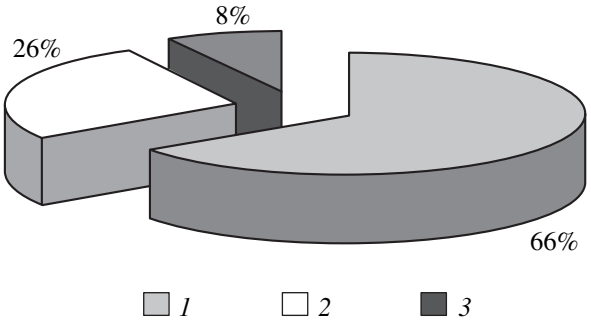

Fig. 3. The percentage of (*1*) *Streptomyces*, (*2*) *Micromonospora*, and (*3*) other actinomycetes in the water of Lake Baikal.

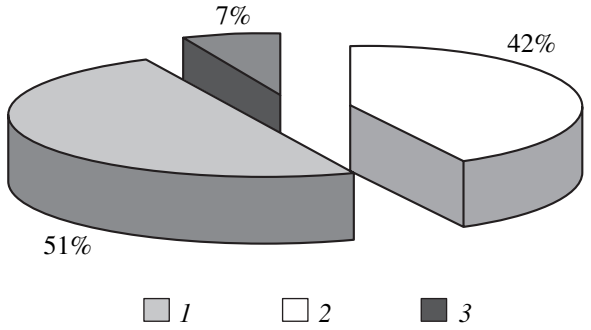

Fig. 4. The percentage of (*1*) *Streptomyces*, (*2*) *Micromonospora*, and (*3*) other actinomycetes in the bottom sediments of Lake Baikal.

to the isolation procedure used, which included the step of sample concentration. Second, most streptomycetes were isolated from the samples taken from depths of 0, 50, and 100 m, i.e., from the upper epilimnion, which undergoes the influence of river waters, air, and runoff from the surrounding soils. It should, however, be noted that some streptomycetes were isolated from the bottom most layer of the lake water and from the bottom sediments, which implies that the isolated streptomycetes may be not only of allochthonous but also of autochthonous origin.

There is controversy in the literature as to the occurrence, growth, and role of streptomycetes in aquatic environments [3, 7, 15]. These questions, as well as the survival, physiological activity, and antagonistic properties of streptomycetes in the cold and oligotrophic water of Lake Baikal, will be the subject of our further studies.

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