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EXPERIMENTAL ARTICLES

Distribution of Heterotrophic Bacteria in Lake Shira

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Abstract—A study of the horizontal and vertical distribution of heterotrophic bacteria in brackish Lake Shira in summer periods showed that mesophilic bacteria dominated in all areas of the lake, whereas psychrotolerant bacteria dominated in the metalimnion and hypolimnion of its central part. Nonhalophilic bacteria were mostly mesophilic and dominated in coastal waters. Most psychrotolerant bacteria were able to grow in the presence of 5–10% NaCl. Heterotrophic bacteria isolated in different regions of the lake were identified to a generic level. The isolates were classified into autochthonous and allochthonous microorganisms on the bases of their distribution pattern in the lake water, halotolerance, and ability to grow at low temperatures.

Key words: saline lakes, halotolerance, mesophilic and psychrotolerant heterotrophic bacteria, autochthonous and allochthonous microorganisms.

Saline lakes attract great attention due to their curative waters. The sulfate–sodium–chloride water of Lake Shira, Khakassia Republic, is of great curative value, which poses the problem of protecting it from the anthropogenic impact coming from the resort situated on the lake's shore. The domestic wastewaters of this resort, which are contaminated by human and animal microflora, are discharged into the lake in an amount of up to 1 million m^3 per year, causing adverse changes in the chemical and bacteriological parameters of the lake water [1]. This problem is aggravated by the facts that the only tributary, Son River, carries organic and mineral substances and allochthonous microflora from the surrounding cattle farms [2] and that Lake Shira does not have outlets.

The most vulnerable element of aquatic ecosystems is bacterioplankton. For this reason, its investigation may provide data on the degree of pollution of impacted ecosystems [3–5]. In studies of such type, the proportion between autochthonous and allochthonous microorganisms may serve as one of the parameters characterizing the degree of pollution.

The aim of this work to study the horizontal and vertical distribution of autochthonous and allochthonous heterotrophic bacteria in Lake Shira.

MATERIALS AND METHODS

Water sampling. Brackish Lake Shira is situated in the south of Khakassia Republic, Russia, at 90°014' E and 54°030' N. The lake has no outlets. The only freshwater tributary (the Son River) in its eastern part provides for 42% of the lake water volume. The lake has a length of 9.35 km and a width of 5.3 km. The lake water was sampled, using the Molchanov bottle, at four stations. Station 1 was located in the southeastern part of the lake, near the Son River mouth, at a distance of 4000 m from the resort, where the lake depth is 1.2 m. Station 3 was located in the central part of the lake, at a distance of 2500 m from the resort, where the lake depth is 21 m. Station 7 was located in the southwestern part of the lake, at a distance of 80 m from the resort, where the lake depth is 2.3 m. Station 8 was located in the northeastern part of the lake, near agricultural fields, at a distance of 3500 m from the resort, where the lake depth is 3 m.

The content of Na^+ in the lake water sampled at station 7 (near the resort) was 2.7 g/l; at station 3 (the center of the lake), 2.8 g/l at a depth of 0.5 m, 3.7–4.0 g/l at a depth of 10 m, and 4–5 g/l at a depth of 20 m; at station 1, 0.6 g/l; and at station 8, 2.8 g/l. The salinity of the lake water changes with the season and reaches 18 g/l in the surface layer and 30 g/l in the bottom layer. The lake water is stratified and has the epilimnion with a water temperature of 15–25°C, the thermocline at a depth of 6–8 m with a water temperature of 4–17°C, and the hypolimnion with a water temperature of 1.4–3°C.

The lake water was sampled throughout the summer periods (June, July, and August) in 1999 and 2000 at 7- to 10-day intervals (experimental data were summarized for each month). At the central station (3), the lake water was sampled at three depths: 0.5 m (epilimnion), 10 m (metalimnion), and 20 m (hypolimnion).

Water samples were analyzed in the stationary field laboratory Lake Shira of the Institute of Biophysics.

Enumeration and isolation of heterotrophic bacteria. Water samples were plated onto solid M9 medium containing (g/l) Na_2HPO_4, 6; KH_2PO_4, 3; NaCl, 0.5; NH_4Cl, 1; peptone, 5; and agar, 20. After

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Table 1. Dominant heterotrophic bacteria in Lake Shira and their characteristics relevant to environmental temperature and NaCl concentration in the medium

Bacteria	Temperature-related characteristic	Maximum tolerable NaCl concentration, %
Pseudomonas sp. 1	Psychrotolerant	10
Pseudomonas sp. 2	Mesophilic	5
Pseudomonas sp. 3	Psychrotolerant	5
Flavobacterium sp.	Mesophilic	0.05
Acinetobacter sp. 1	Mesophilic	5
Acinetobacter sp. 2	Mesophilic	10
Halococcus sp.	Psychrotolerant	10
Escherichia coli	Mesophilic	0.05
Bacillus sp. 1	Mesophilic	0.05
Bacillus sp. 2	Mesophilic	0.05

autoclaving, the medium was supplemented with Mg and Ca by adding 1 ml of each 20% $MgSO_4$ and 0.5% $CaCl_2$ [6]. To isolate halotolerant heterotrophic bacteria, the concentration of NaCl in the medium was increased to 50 and 100 g/l. Colonies were enumerated after incubating the plates for 7 to 14 days. The halotolerance of microorganisms was determined by plating them on medium containing different concentrations of NaCl. To isolate psychrotolerant and mesophilic bacteria, the plates were incubated at 5 and 25°C, respectively [7].

Identification of heterotrophic bacteria. Bacterial isolates were identified on the basis of their morphological [8] and biochemical characteristics (including tests for cytochrome oxidase and glucose fermentation) using the identification criteria of *Bergey's Manual* [9].

RESULTS AND DISCUSSION

Table 1 summarizes data on the generic diversity of heterotrophic bacteria isolated from the lake water and their characteristics relevant to environmental temperature and NaCl concentration in the medium. Mesophilic and psychrotolerant isolates were found to belong to the genera *Pseudomonas*, *Flavobacterium*, *Acinetobacter*, *Halococcus*, *Bacillus*, and *Escherichia*. Most of the isolates (*Pseudomonas* sp. 2, *Flavobacterium* sp., *Acinetobacter* sp. 1, *Acinetobacter* sp. 2, *Bacillus* sp. 1, *Bacillus* sp. 2, and *Escherichia coli*) were mesophilic. Three isolates (*Pseudomonas* sp. 1, *Pseudomonas* sp. 3, and *Halococcus* sp.) were psychrotolerant.

Mesophilic heterotrophic bacteria dominated in all of the water samples investigated (Table 2). The population of psychrotolerant bacteria was most dense in the central part of the lake (station 3) and at station 8, whereas the number of these bacteria at stations 7 (the resort area) and 1 (the Son River mouth) was minimal (Table 2). The distribution of mesophilic and psychrotolerant bacteria followed the distribution of the average water temperature. For instance, the surface water at station 3 had a temperature of 18–22°C and was dominated by mesophilic bacteria (Table 3). At the same station, the metalimnion water (10 m) and hypolimnion water (20 m) had temperatures of 2.8 and 1.5–1.7°C, respectively [10], and were dominated by psychrotolerant bacteria. At station 8, the lake water had a temperature of 10–11°C and was dominated by psychrotolerant bacteria, especially in June (Table 2). At stations 7 and 1 located near the resort and the Son River mouth, the lake water warmed to 23–25°C and was dominated by mesophilic bacteria. It should, however, be noted that the prevalence of mesophilic bacteria at the two last stations could be due to their receiving wastewaters from the resort (station 7) and the cattle farm runoff carried by the riverine water (station 1).

Table 2. Proportions between different groups of heterotrophic bacteria isolated from the water of Lake Shira in the summer periods 1999–2000

Station	M/P ratio (%) in 1999			M/P ratios (%) in 2000		
	June	July	August	June	July	August
1	86/14	93/7	93/7	95/5	97/3	90/10
3	55/45	77/23	67/43	67/33	67/33	21/79
7	99/1	93/7	86/14	95/5	85/15	83/17
8	43/57	83/17	22/78	8/92	83/17	65/35
	N/I/H ratios (%)			N/I/H ratios (%)		
1	85/15/0	90/10/0	77/23/0	92/8/0	95/5/0	74/26/0
3	65/35/0	56/31/13	58/38/4	57/6/37	57/12/31	52/24/24
7	95/5/0	90/10/0	84/16/0	89/11/0	84/16/0	77/23/0
8	35/75/0	10/19/71	38/60/2	32/72/0	55/20/25	47/51/2

Note: M, P, N, I, and H stand for mesophilic, psychrotolerant, nonhalophilic, intermediately halophilic, and moderately halophilic bacteria, respectively.

Table 3. Vertical distribution of different groups of heterotrophic bacteria isolated from the water in the central part of Lake Shira (station 3) in the summer periods 1999–2000

Depth, m	M/P ratio (%) in 1999			M/P ratios (%) in 2000		
	June	July	August	June	July	August
0.5	67/37	82/18	77/23	75/25	58/15	57/43
10	77/33	18/82	38/62	42/58	30/70	24/76
20	25/75	15/85	24/76	20/80	24/76	10/90
	N/I/H ratios (%)			N/I/H ratios (%)		
0.5	65/35/0	85/15/0	75/25/0	74/0/26	95/0/5	61/25/14
10	30/70	19/68/13	33/58/9	43/0/57	30/44/26	24/44/32
20	25/75	13/48/39	24/53/23	18/77/5	25/52/23	7/32/61

Note: M, P, N, I, and H stand for mesophilic, psychrotolerant, nonhalophilic, intermediately halophilic, and moderately halophilic bacteria, respectively.

The presence of psychrotolerant bacteria in these water areas can be explained by the migration of these bacteria from other areas of the lake.

The horizontal distribution of heterotrophic bacteria was characterized by the occurrence of bacteria of some genera (as *Pseudomonas* and *Flavobacterium*) throughout the lake area (Fig. 1), indicating that these bacteria are autochthonous. In contrast, other bacteria (as *Escherichia*) were encountered only in the lake regions subject to anthropogenic impact, namely, at stations 1 and 7 (Figs. 1b, 1d). In the resort area (st. 7), the relative content of *Escherichia* bacteria increased from 5% in June to 30% in July (Fig. 1b) likely due to the fact that bathing in July is in full swing. As a result, the content of allochthonous microorganisms in the lake water increased in July to 1300 CFU/ml. In August, when bathing is not intense, *Escherichia* bacteria are almost absent from the water (Fig. 1b). Wastewaters from the resort may also serve as a source of *Escherichia* bacteria in the lake water. In addition to these bacteria, the

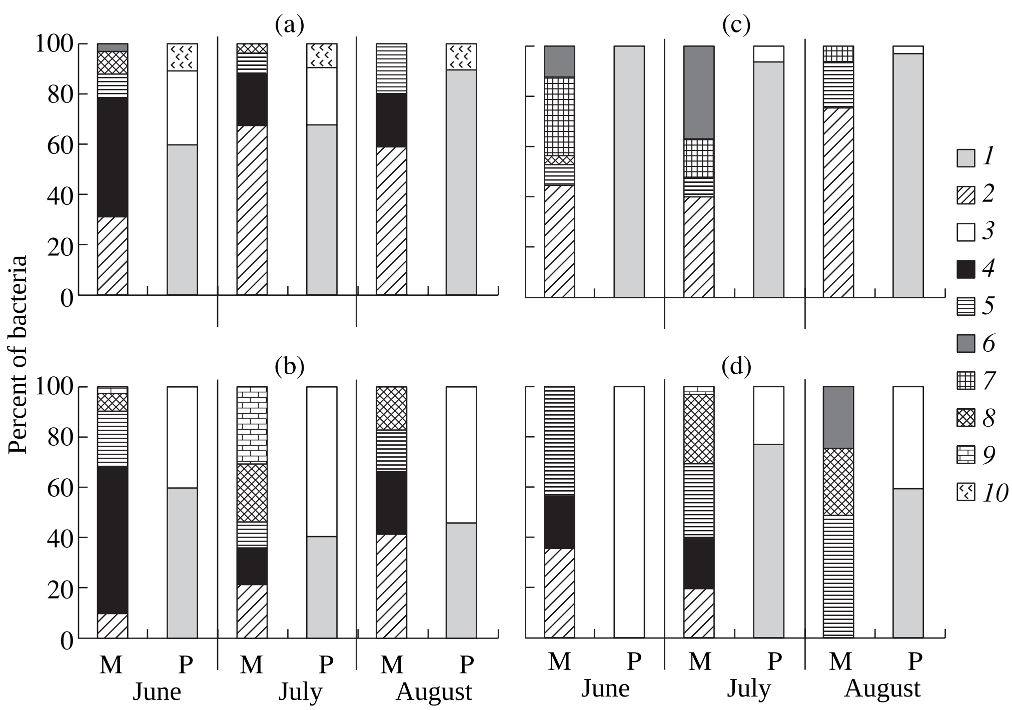

Fig. 1. Proportions of the mesophilic (M) and psychrotolerant (P) heterotrophic bacteria of different taxa isolated from the water of Lake Shira in the summer periods 1999–2000 at (a) station 3 (average data), (b) station 7, (c) station 8, and (d) station 1. *1*, *Pseudomonas* sp. 1; *2*, *Pseudomonas* sp. 2; *3*, *Pseudomonas* sp. 3; *4*, *Acinetobacter* sp. 1; *5*, *Flavobacterium* sp.; *6*, *Acinetobacter* sp. 2; *7*, *Bacillus* sp. 1; *8*, *Bacillus* sp. 2; *9*, *Escherichia coli*; *10*, *Halococcus* sp.

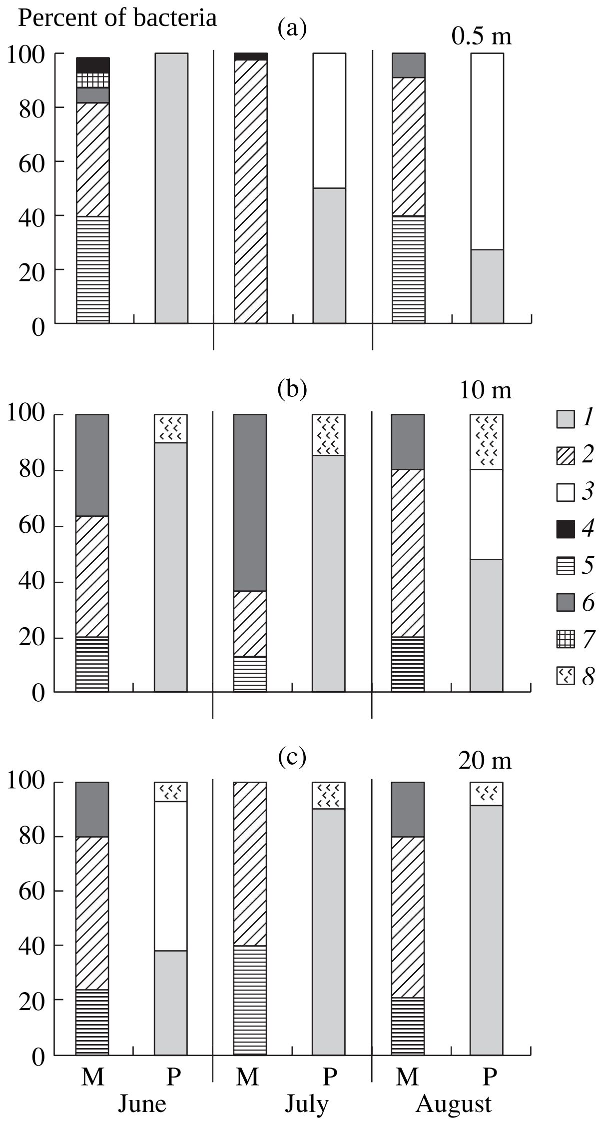

Fig. 2. Vertical distribution of the mesophilic (M) and psychrotolerant (P) heterotrophic bacteria of different taxa isolated from the water in the central part of Lake Shira (station 3) in the summer periods 1999–2000. *1*, *Pseudomonas* sp. 1; *2*, *Pseudomonas* sp. 2; *3*, *Pseudomonas* sp. 3; *4*, *Acinetobacter* sp. 2; *5*, *Acinetobacter* sp. 1; *6*, *Flavobacterium* sp.; *7*, *Bacillus* sp. 1; *8*, *Halococcus* sp.

lake water at stations 1, 7, and 8 contained bacilli (Figs. 1b–1d), which are likely to be allochthonous microorganisms too. The sources of bacilli may be different. At stations 1 and 7, they may come with the resort wastewaters and coastal runoff. At station 8, bacilli may come to the lake water with agricultural runoff. This suggestion is confirmed by the fact that the species composition of bacilli at these stations was different: the lake water at stations 1 and 7 was dominated by *Bacillus* sp. 2, whereas at station 8 by *Bacillus* sp. 1 it was dominated.

The diversity of autochthonous bacteria was studied at station 3 (the central part of the lake), which is most distant from the possible sources of contamination with allochthonous microorganisms. As can be seen from Fig. 2, the lake water at station 3 was dominated by the mesophilic bacteria *Pseudomonas* sp. 2, *Acinetobacter* sp. 1, and *Flavobacteria* and psychrotolerant bacteria of the genera *Pseudomonas* and *Halococcus.* The relative content of *Bacillus* sp. 1 (which is dominant in the contaminated lake waters) at this station was low (a maximum of 5% in the surface lake water in June). The most abundant bacteria of the central part of the lake belonged to the genera *Pseudomonas*, *Acinetobacter, Flavobacterium*, and *Halococcus.*

The water of Lake Shira contains up to 30 g/l salts and hence is brackish [11]. It is evident that the autochthonous microorganisms of this lake must be well adapted to high salt concentrations, i.e., must be halotolerant. Consequently, halotolerance can be used for the differentiation of autochthonous and allochthonous microflora. Analysis showed that nonhalophilic bacteria were present in the lake water sampled at all four stations (Table 2), but were dominant at stations 1 and 7, where the lake water is diluted to 0.6 g/l and 2.4 g/l NaCl, respectively, by fresh water from the Son River and wastewaters from the resort [2, 12]. In contrast, in the central part of the lake (st. 3), nonhalophilic heterotrophic bacteria were scarce in the surface water and were entirely absent at depths of 10 and 20 m, where the lake water had a salinity of 21–22 g/l NaCl and contained intermediately and moderately halotolerant bacteria (Table 3).

On the basis of the distribution pattern in the lake water, halotolerance, and ability to grow at low temperatures (psychrotolerance), the lake bacteria were classified into autochthonous (*Pseudomonas*, *Flavobacterium, Halococcus*, and *Acinetobacter* sp. 2) and allochthonous (*Escherichia*, *Bacillus*, and *Acinetobacter* sp. 1). This work is a continuation of the comprehensive study of bacterial diversity in Lake Shira, the effect of anthropogenic factors on the drug resistance of allochthonous bacteria, and the transmission of this trait to autochthonous bacteria [12].

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