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EXPERIMENTAL ARTICLES

Ecophysiological Characteristics of the Mat-forming Bacterium *Thioploca* in Bottom Sediments of the Frolikha Bay, Northern Baikal

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Abstract—A colorless sulfur bacterium of the genus *Thioploca*, which forms bacterial mats, was studied in the region of underwater thermal vents (Frolikha Bay, northern Baikal). The organism occurs under microaerobic conditions in top sediment layers, and its biomass can amount to 65 mg of wet weight per 1 kg of silt. Individual filaments of the bacterium penetrate the anaerobic zone to the depth of 19 cm. *Thioploca* is distributed in a mosaic pattern over the bottom of the bay. *Thioploca* mats are typically found near vents that discharge low-temperature underground water. In the form of separate filaments, this bacterium is more widely distributed in the top sediment layer, particularly in sediments with a more active sulfate reduction. The bacteria from the deep-water and coastal areas of the bay have different morphology. Cells of *Thioploca* are able to accumulate nitrate, and the coefficient of nitrate accumulation in wet bacterial mass in relation to the near-bottom water is 1.3×10^4, suggesting a similarity of metabolism with seawater species. A more lightweight isotopic composition of nitrogen in cell mass as compared to that of representatives of zoobenthos also indicates an active metabolism of nitrogen, apparently, in the process of nitrogen respiration. Comparison of the composition of stable isotopes of carbon in the biomass of representatives of different trophic levels, including *Thioploca*, found at a depth of 105 m indicates its planktonic origin, whereas, in the deeper bay region, the biomass of *Thioploca* incorporates more of the light carbon originating from biogenic methane.

Key words: underwater vent, *Thioploca*, physiology, stable isotopes of carbon and nitrogen.

The exploration of the region of low-temperature hydrothermal vents, discovered in Frolikha Bay in the northern section of Lake Baikal with the help of automatic and man-operated submersibles, revealed the presence of a rich biological community [1, 2]. The predominant organism in this community is the colorless sulfur bacterium *Thioploca* [3].

Representatives of this genus occur in sediments of seawater and freshwater reservoirs and are known to have a peculiar ecophysiology [4]. *Thioploca* forms massive accumulations in bottom sediments along the Pacific coast of South America. The near-bottom water in these regions is deficient in oxygen and rich in organic matter and nitrate. The metabolism of *Thioploca* was studied using its natural samples by measuring the concentrations of different compounds in cells and in the environment, and the rates of their production by the bacterium. In these experiments, a suspension of washed filaments collected from mat samples was used, because *Thioploca* has not been obtained in pure culture to date. Based on these measurements, *Thioploca* was shown to be able to capture nitrate from near-bottom water, accumulating it in vacuoles, and to utilize nitrate oxygen to oxidize intracellular sulfur to sulfates under anaerobic conditions as the bacterium migrates into underlying sediment layers to the depth of 5–15 cm. Studies carried out with the use of carbon radioisotopes showed that marine species of *Thioploca* could grow both autotrophically and mixotrophically by utilizing, in the latter case, acetate and other organic compounds as the source of carbon [5].

The overall purpose of this work was to study the structure of the *Thioploca* sulfur mats and the conditions for their development in bottom sediments in the northern part of Lake Baikal. The issues we were specifically concerned with were (i) the morphology of the filamentous sulfur bacterium occurring at various depths in the Frolikha Bay in the region of underwater hydrothermal vents; (ii) the distribution of this organ-

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Table 1. Characteristics of bottom sediments in Frolikha Bay (October 1997)

Station no.	Depth, m	Presence of *Thioploca* in sample	Horizon, cm	Temperature, °C	pH	Eh, mV	HCO_3^-	Cl^-	SO_4^{2-}	$H_2PO_4^-$
							mg/l			
1	415	+	0–3	6.1	7.00	10	174.4	2.46	41.28	–
2	415	+	–	–	–	–	–	–	–	–
3	405	+	0–5	4.8	6.99	–35	105.1	2.92	1.24	0.79
			1–5	–	–	–	127.2	6.23	4.49	0.95
4	400	–	–	–	7.51	–70	55.3	13.6	7.82	–
5	400	+	0–1	5.0	6.85	33	76.2	2.89	1.96	–
6	215	+	Near-bottom water	–	–	–	59.7	2.08	4.17	–
			0–2	6.2	7.34	5	105.4	1.03	2.35	–
			5–10	–	–	–	106.7	0.47	n. f.	1.05
7	105	+	Near-bottom water	–	–	–	41.1	3.57	5.67	1.24
			0–1	6.0	7.32	6	103.1	0.66	3.68	–
			10–15	–	–	–	90.9	0.70	n. f.	1.1
8	380	–	0–1	–	–	–	53.4	1.77	5.57	1.30
9	690	–	0–1	–	7.10	–	83.6	0.80	5.90	–
10	23	+	0–5	–	7.26	–	72.7	0.63	3.81	–
11	42	+	0–3	–	7.24	–	71.3	0.57	6.58	–
12	32	+	0–1	–	6.71	–	59.6	3.12	5.06	–
13	105	+	Near-bottom water	–	–	–	63.5	0.77	5.73	–

Note: Symbol “–” means that no data is available; n.f. stands for “not found.”

ism across the bay area in the discharge region of low-temperature hydrothermal vents; and (iii) the particular features of metabolism in a freshwater ecosystem that is characterized by low environmental concentrations of oxygen and nitrate.

MATERIALS AND METHODS

Samples were obtained in October, 1997, using a grab and a dredger (*Ocean*) operated from the ship *G. Vereshagin*. Samples for microbiological and chemical analyses were collected in the deep-water and shallow parts of the bay. The presence of filaments of the colorless sulfur bacterium in samples was determined by eye. The temperature, pH, and Eh of the sediments were determined with portable PH-82 and SC-82 meters (Yokogama, Japan). Silt solutions were obtained by centrifuging or by squeezing liquid from the entire core sample. These solutions were analyzed immediately after sampling for the presence of the anions HCO_3^-, Cl^-, SO_4^{2-}, NO_3^-, $H_2PO_4^-$, and Br^- using an EnviroChrom liquid chromatograph that was aboard the ship.

The concentrations of nitrate and sulfate ions in crushed bacterial mass were determined by chromatography. Bacterial filaments were picked out from bottom sediments with a preparation needle and washed with twice distilled water. After a 5-hour drying at 70°C, cells were disrupted by mechanical grinding in a mortar with the addition of twice distilled water. The concentrations of nitrate and sulfate in the obtained homogenate and in the water used to wash filaments were determined on a liquid chromatograph. The water content of the wet biomass was 84%.

To establish whether or not nitrate accumulates inside cells or in the filament sheath, we used the qualitative reaction of diphenyl amine oxidation, which gives a strong blue color in concentrated sulfuric acid in the presence of NO_3^- [6]. Filaments of *Thioploca* were placed on a slide, partly dried with filter paper, and put into a droplet of diphenyl amine in concentrated sulfuric acid. The preparation was then examined under a microscope and its color photograph was taken.

The presence of *Thioploca* biomass in a 20-cm core sample of sediment was determined in 1-cm steps. Each sample consisted of 1 cm^3 of sediment taken in three replicates. The entire volume of these samples

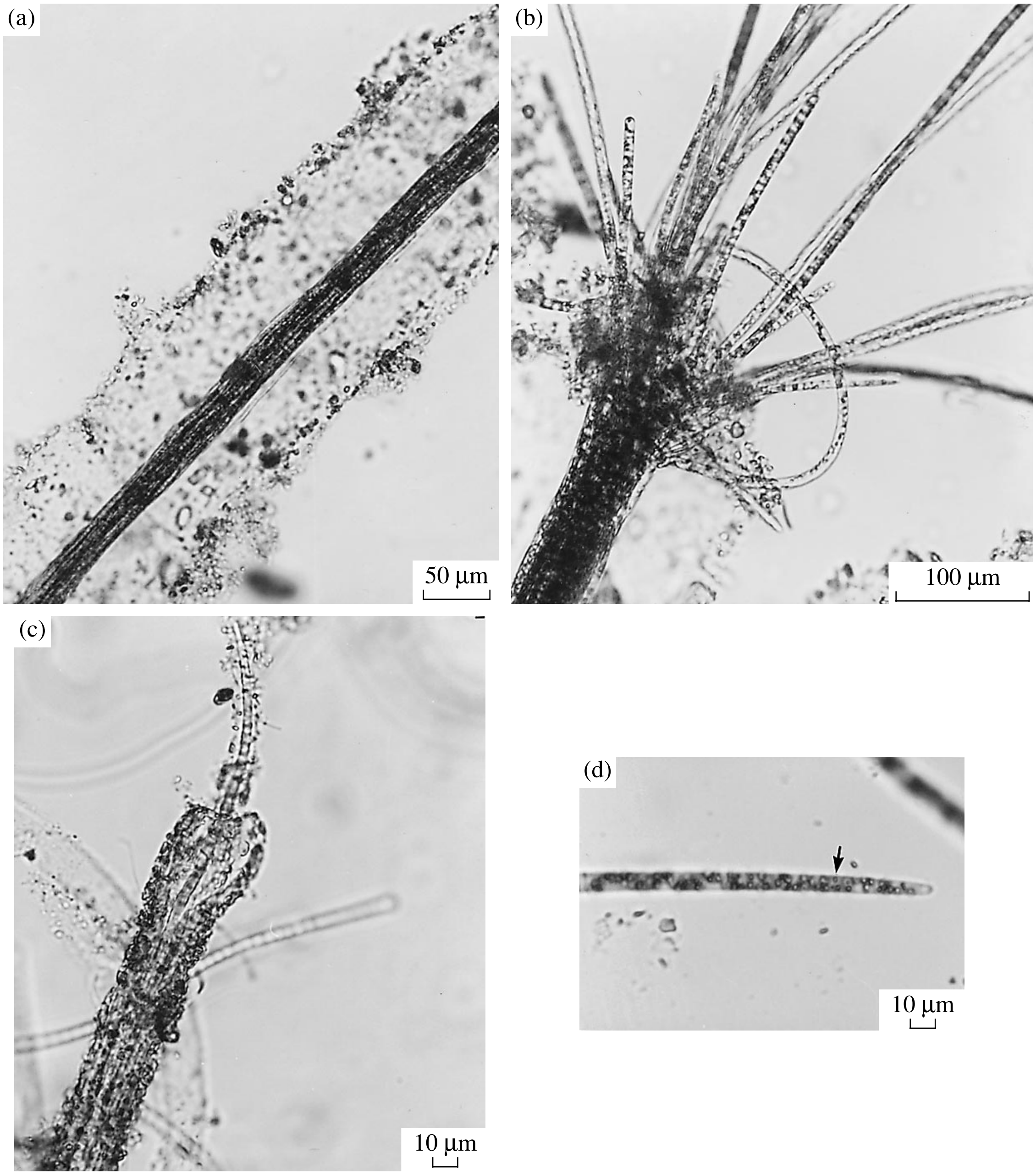

Fig. 1. Baikalian *Thioploca* from Frolikha Bay, northern Baikal: (a) general view of a bacterium found on sediment surface in a deep-water region at a depth of 415 m; (b) separate filaments of *Thioploca* emerging from the sheath; (c) general view of a bacterium found on sediment surface in a littoral zone at a depth of 105 m; and (d) an individual filament of *Thioploca* with inclusions of sulfur.

was examined in a binocular microscope and the number of *Thioploca* filaments and their lengths were determined. The cell volume was calculated based on a cylinder model, and the dry biomass weight was determined using a coefficient of 1.6 [7].

The rate of sulfate reduction was determined by the radioisotopic method using $Na_2^{35}SO_4$. Near-bottom water and 3 ml of fresh sediment were placed in 20-ml penicillin bottles which, after injection of 0.1 ml (7 µCi) of $Na_2^{35}SO_4$ solution with a specific activity of 100 mCi/mmol (Amersham) were incubated for 24–96 h at 4–9°C. Acid-soluble sulfides were determined using a special apparatus [8]. The radioactivity of the sulfides formed was counted on a RackBeta 1219 scintillation spectrophotometer (LKB, Sweden). Stable carbon and nitrogen isotopes in the biomass of various representatives of zoobenthos, phytoplankton, and *Thioploca* collected from Baikal samples were analyzed on a mass spectrometer (delta-S, Finigan-mat) at the Center for Ecological Studies of the Kyoto University (Japan).

Table 2. Nitrate and sulfate concentrations (μM) in *Thioploca* biomass and their weighted mean values across the water column

Concentrations of ions	NO_3^-		SO_4^{2-}	
	in wet biomass	in dry biomass	in wet biomass	in dry biomass
Cell mass	136000	800000	3600	21100
Weighted mean across the water column	10.2		53.5	
K_a	1.3×10^4	7.5×10^4	65	3.9×10^2

Note: K_a is the accumulation coefficient defined as the ratio between the concentration of ions in the biomass and their weighted mean concentration in the water depth.

RESULTS

Distribution of Thioploca and Physicochemical Characteristics of the Environment

The bottom profile of the bay where *Thioploca* mats were found is quite complex. There is an underwater canyon, which is believed to have been formed during the latest glacial period [9]. In all, 13 stations were surveyed (Table 1), starting from the depth of 690 m and moving up to small depths closer to the shore. In selecting the stations, the morphology of the bay floor and the locations of vents discharging low-temperature abyssal underground waters with low mineral content were taken into account. Samples of pebble material from a depth of 415 m near the vent where underground water was discharged (station 2) had abundant sulfide coating, and individual filaments of *Thioploca* could be readily observed. This sampling area was also inhabited by mollusks. Another sediment sample from the same depth had a different granulometric composition, but its topmost layer was also reduced, and its surface had an abundant white coating formed by *Thioploca* filaments. It should be mentioned that *Thioploca* developed in the form of isolated patches at depths ranging from 415 to 32 m and only on one slope of the underwater canyon, where low-temperature vents discharging water with low mineral content were noted.

The redox potential in the top layer of bottom sediment, where the development of sulfur mats was observed, ranged from –35 to +33 mV, which corresponds to the variation of rH_2 from 12.8 to 15.4 and defines the conditions in the topmost sediment layer as microaerobic.

The sites of *Thioploca* development were typically close to underwater vents, as shown by the enrichment of sediment porous water with ions of hydrocarbonate, sulfate, and chloride as compared to Baikal water and sediments from other regions of the lake (Table 1). Specifically, in sediment pore water from the depth of 400–415 m, where sulfur mats of *Thioploca* occurred on a mass scale, the concentration of sulfate reached 41 mg/l, being, on average, only 3–5 mg/l at other stations. The concentrations of hydrocarbonate and chloride were as high as 174 and 13.6 mg/l, respectively.

The Morphological and Physiological Characteristics of Thioploca

Filaments of *Thioploca* were enclosed in a sheath, and their numbers within a sheath varied depending on the habitat of the bacterium. The morphologies of bacteria in the littoral and deep-water bay regions were also to some extent different (Fig. 1). The littoral sulfur bacterium had a thinner sheath that contained 1 to 3 filaments, whereas the number of filaments in the deep-water organism could be as large as 21. The sheath of *Thioploca* from littoral samples of a more heavily oxidized sediment was incrusted with yellow inclusions, which was likely to be the result of the accumulation in the sheath of iron or manganese oxides [10]. Cells of these littoral bacteria had much less inclusions of sulfur than those of deep-water bacteria. In the deep-water bacteria, the diameter of the trichome varied from 2.5 to 5.0 μm, and, together with the individual sheath, measured 15–50 μm; the diameter of the outer sheath was as large 100–200 μm. In the littoral bacterium, the trichome diameter varied from 2 to 4.3 μm, and that of the outer sheath, from 17 to 28 μm. The mean breadth of the *Thioploca* filaments collected in the autumn of 1997 was somewhat smaller than we observed in the summer of 1991 [3]. Some filaments were as long as 2 cm. Bacterial filaments in the bottom sediment had a vertical orientation and could be readily discerned in core samples owing to their large size and deposits of elemental sulfur. The depth of bacterial penetration into the sediment was 19 cm (the core sample length was 20 cm). The maximum biomass (65 mg wet weight per 1 kg) was found in the topmost horizon (0–1 cm), and, at a depth of 11 cm, the biomass amounted to about half of that in the top layer.

The ability of *Thioploca* filaments to move at a maximum velocity of 8.18 μm/s was established when freshly collected material consisting of washed sheaths with filaments was placed on the surface of dense agar medium. The filaments moved inside the slime relative to each other, got out of the sheath, and slid over agar.

Inside *Thioploca* cells, large vacuoles could be readily discerned, which, under the action of diphenyl amine, turned bright blue, revealing the presence of nitrate. The sheaths of the colorless sulfur bacteria remained uncolored. Additional evidence for the ability of *Thioploca* to accumulate nitrate in its cells is furnished by the results of nitrate determinations in the ground cell mass. The concentration of nitrate in wet *Thioploca* biomass exceeded that in the water column in the same region by a factor of 13000 (Table 2). As noted above, no nitrate was found in the pore water of bottom sediments in the Frolikha Bay (as determined by the ion chromatography method). Therefore, we used the weighted mean concentration of the nitrate ion in the water depth as the reference model. It is also worth noting that the sulfate concentration in the *Thioploca* biomass was also very high (a factor of 65 greater than that of the water column).

Rate of Sulfate Reduction

To find out if the distribution of was related to the bacterial production of H_2S, the rate of sulfate reduction was measured by the radioisotopic method employing $Na_2^{35}SO_4$. The results are given in Table 3. Sulfate reduction was established in all samples studied; its rate varied within an order of magnitude. The maximum rate of 11 μg S^{2-} per kg silt per day was observed at a depth of 415 m at a station where the mass development of the colorless sulfur bacterium occurred. In the littoral zone at a depth of 32 m (station 2), the rate of sulfate reduction was lower than in samples with the bacterium obtained near the vents in the open part of the bay; this is in agreement with the corresponding figures of the loss of C_{org} (Table 3).

Table 3. Rate of sulfate reduction (A) and utilization of C_{org} (B) in bottom sediment of Frolikha Bay (October 1997)

Depth, m	Horizon, cm	SO_4^{2-}, mg/l	A, μg S/(kg day)	B, μg C/(kg day)
685	0	5.6	1.2	0.9
690	0	5.9	6.6	4.9
415	0	41.3	10.8	8.0
405	0	1.2	0.63	0.47
	1	4.5	0.72	0.54
400	0	2	1.31	0.98
215	0	2.2	1.22	0.91
	5	10	1.14	0.85
105	0	3.7	4.56	3.4
	10	5	1.85	1.4
42	0	6.6	4.42	3.3
32	0	5.1	3.36	2.5
23	0	3.8	4.61	3.5

Ratio of Stable Isotopes of Carbon and Nitrogen

The composition of stable isotopes of carbon and nitrogen in organisms of different trophic levels in the Frolikha Bay was determined in samples collected at a depth of 105 m (station 7, Table 4). $\delta^{13}C$ of organic carbon of sulfur bacteria was found to be –30.7‰, a figure fairly close to that of phyto- and zooplankton (–27.7‰) and comparable to their isotopic composition in other regions of Lake Baikal [12]. The organic carbon isotopic composition of amphipods and polychaetes collected from the same depth was somewhat heavier (–22.8 and –23.8‰) but still in agreement with the values typical of the pelagic food chain. The isotopic composition of nitrogen determined for the same groups of animals, alga, and bacteria showed some difference of sulfur bacteria from other specimens studied. Colorless sulfur bacteria turned out to be enriched in the light isotope of nitrogen, their value of $\delta^{14}N$ being negative and equal to –5.2‰, which is in contrast to a positive value of $\delta^{14}N$ (from +6 to +7‰) in the representatives of phyto- and zooplankton and zoobenthos of Frolikha Bay of Lake Baikal [11]. Purple bacteria in mats in a hot spring on the shore of Zmeinaya Bay was the only species having the isotopic composition of nitrogen (–3.6‰) close to that of *Thioploca.*

DISCUSSION

Bacterial mats formed by colorless sulfur bacteria of the genus *Thioploca* were noted in Lake Baikal only in Frolikha Bay near the vents discharging low-temperature underground water and had a clear-cut mosaic distribution pattern. In marine environments, colorless sulfur bacteria of the genus *Thioploca* were shown to develop in the microaerobic zone under low concentrations of hydrogen sulfide and high organic and nitrate content of near-bottom water [4, 5, 10]. In Lake Baikal, these bacteria occur in places of seepage of underground waters enriched, in comparison to the Baikal water, in hydrocarbonate, sulfate, and chloride ions. The hydrothermal flow is discharged mainly in the deep-water section of the bay, which is precisely the region with the most active development of *Thioploca*. *Thioploca* also occurs in the littoral bay zone that lacks any obvious manifestations of hydrothermal activity. Littoral sediments at places inhabited by *Thioploca* are covered with vegetative residues of terrigenous origin and have a thin oxidized layer. Cells of bacteria inhabiting this region are smaller and contain few inclusions of elemental sulfur. In all cases, when this bacterium developed away from the outlets of underground waters, this was in the areas characterized by a sufficiently high rate of bacterial production of hydrogen sulfide (1.8–4 μg/kg).

Likewise, in marine ecosystems, filamentous sulfur bacteria in Lake Baikal occupy a relatively vast zone, encompassing water layers most close to the bottom, the microaerobic zone of the topmost sediment layer, and deeper horizons of sediment material. *Thioploca* filaments are clearly visible on the sediment surface, forming a white, delicate, and an easy-to-ruin carpet. The largest biomass is shown to be in the layer of

Table 4. Relative abundances of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{14}N$) in benthic animals and bacterial mats in lake water of the northern part of Baikal and in a terrestrial thermal spring near Zmeinaya Bay

Organism	Sampling site and depth	$\delta^{13}C$, ‰	$\delta^{14}N$, ‰	Source
Purple mats	Zmeinaya Bay	–27.7	–3.6	Our data
Phyto- and zooplankton	Frolikha Bay, layer, 0–50 m	–28.1	7.5	"
Amphipods	" 105 m	–22.8	6.0	"
Polychaetes	" 105 m	–23.8	7.6	"
Thioploca	" 105 m	–30.7	–5.2	"
Bottom sediment, Corg	" 420 m	–49.5	–	[13]
Thioploca mats	" 420 m	–41.6	–	[13]
Sponges	" 420 m	–61.2	–	[13]
Planarians	" 420 m	–66.0	–	"
Phyto- and zooplankton	Near Promontory Zavorotnyi; layer, 0–50 m	–24.8	7.3	Our data

0–1 cm; it decreased with depth and was totally absent in horizons below 19 cm.

As we show elsewhere, the population density of unicellular sulfur bacteria (autotrophic and mixotrophic, aerobic and denitrifying) in *Thioploca* mats does not exceed 10^2–10^4 cells/g [15], and, according to our calculations, their total biomass amounts only to about 0.01% of the *Thioploca* biomass (65 mg/kg silt). Therefore, the major role in the oxidation of sulfur compounds in sulfur mats is apparently played by colorless filamentous sulfur bacteria. The results of determination of nitrate in the biomass of Baikalian *Thioploca* suggests that these organisms, like many marine species of this genus [13], can utilize both oxygen and nitrate as electron acceptors. *Thioploca* filaments, raising over the sediment and looking like white hairs, can utilize nitrate from the water column. It is worth noting that nitrate was not found in the pore water of the sediment in Frolikha Bay during the time period studied but was present in the water column at a concentration as high as 10 μM. At the same time, as shown in the accompanying paper [15], nitrifying bacteria that supply nitrate are widespread in regions inhabited by *Thioploca*. Having the capacity to move fast both inside the sheath and on the surface of dense substrates, *Thioploca* can transport nitrate from the surface into deeper sediment horizons, where the energy substrate—hydrogen sulfide—is available. Likewise in the marine species, the stored nitrate is utilized in the upper part of the anaerobic zone to oxidize sulfide to sulfur and, eventually, to sulfate. This is confirmed by the accumulation in vacuoles of the Baikalian *Thioploca* of up to 21 mM of sulfate (about 3 g SO_4^{2-}/l, as compared to 50 mg/l outside cells).

Having the capacity to replenish its stock of nitrate from near-bottom water and transport it into the sediment depth, where hydrogen sulfide is in constant supply, *Thioploca* becomes less dependent on the environmental conditions. It is probably this advantage that allows *Thioploca* to proliferate in the form of sulfur mats in bottom sediments of Lake Baikal in the region of outflow of underground waters enriched in hydrogen sulfide. However, the obtained data provide only indirect evidence that sulfur mats of the *Thioploca* from Lake Baikal oxidize sulfides in the process of nitrate reduction (as is the case with *Thioploca* species inhabiting marine environments). The definitive conclusion can be made upon studying the metabolism and enzymatic activities of pure cultures of these microorganisms.

The filament diameter of the Baikalian *Thioploca* varied between 2 and 5 μm. This span is wider than the corresponding ranges of other known freshwater morphotypes. The different morphology of *Thioploca* cells in the open and littoral parts of the bay might point to the existence in this region of a mixed population of *Thioploca*, as is the case with filamentous sulfur bacteria occurring near the coast of Chile and Peru [4]. This is a very interesting issue for further studies of these bacteria.

The ratio of stable carbon isotopes in representatives of different trophic levels, including *Thioploca*, varied markedly in the deep-water and shallow bay regions. It was previously shown that the source of carbon for the benthic community (including bacterial mats) developing at the deep-water station (420 m) of Frolikha Bay is organic matter derived from biogenic methane [12, 14]. This fact is evidenced by a lighter isotopic composition of the carbon of bottom sediment, bacterial mats, and benthic animals [12]. The carbon isotopic ratio for organisms of different trophic levels occurring at the depth of 105 m is shifted to heavier values in comparison to the previously published data [12]. The range of carbon isotopic variation for *Thioploca* and benthic animals found at the same depth was similar to that recorded in phyto- and zooplankton. It may be assumed the *Thioploca* inherits the isotopic composition of the carbon of the available organic matter; this may be indicative of its mixotrophic metabolism. The data on the isotopic composition of bacteria and animals in different bay regions attest to a wide spectrum of carbon sources utilized. These carbon sources originate from plankton or can be organic carbon enriched in the light isotope and deriving from biogenic methane.

A lighter isotopic composition of nitrogen in *Thioploca* biomass as compared to that in representatives of plankton and zoobenthos suggests active metabolism of mineral nitrogen, apparently, in the process of nitrogen respiration.

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