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**SHORT COMMUNICATIONS**

# Spatial Distribution of Various Physiological Groups of Bacteria in the Region of Underwater Thermal Vents in Frolikha Bay, Northern Baikal

**T. I. Zemskaya*, [1], B. B. Namsaraev**, N. M. Dul'tseva***, T. A. Khanaeva*, L. P. Golobokova*, and L. E. Dulov*****

**Institute of Limnology, Siberian Division, Russian Academy of Sciences, ul. Lermontova 281, Irkutsk, 664033 Russia*

***Institute of General Biology, Siberian Division, Russian Academy of Sciences, Ulan-Ude*

****Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia*

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In this communication, we present our findings on the structure of bacterial communities dominated by colorless sulfur bacteria of the genus *Thioploca* [1, 2]. In Lake Baikal, such bacterial communities are known to occur in the region of underwater low-temperature vents which discharge water rich in hydrocarbonate, sulfate, and chloride ions [2]. Similar marine communities were shown to have a complex structure [3]. Some physiological characteristics of *Thioploca* and its distribution pattern in the Frolikha Bay are presented in an accompanying paper [2]. The goal of this work was to find out (1) what physiological groups of microorganisms, along with *Thioploca*, oxidize inorganic sulfur compounds in the bacterial communities under study; (2) what is the population size of sulfur-reducing bacteria producing hydrogen sulfide (the energy substrate for *Thioploca*); and (3) whether or not the community includes microorganisms that supply to the medium nitrate, which is required for anaerobic metabolism of *Thioploca*, and ammonium, from which nitrate is formed.

Samples were collected during an expedition to Lake Baikal in October, 1997. The physical and chemical characteristics of the samples and the coordinates of the sampling sites are reported in an accompanying paper [2]. Populations of various physiological groups of bacteria were enumerated in samples from the top layer of the bottom sediments taken from open and littoral bay regions. For two stations, the vertical distributions of bacteria in the top 20-cm layer of the sediment were determined.

Bacteria of various physiological groups were enumerated by the method of serial tenfold dilutions on selective nutrient media. Saprophytic bacteria were enumerated on tenfold diluted fish-peptone agar; ammonifying organisms, on a medium with urea; denitrifiers, on Giltay medium without asparagine; sulfate-reducing bacteria, on Postgate B medium, and heterotrophic nitrifiers, on the medium by Gode and Overback. The composition of these media was exactly as prescribed in manuals [4, 5].

Autotrophic thionic bacteria were enumerated on a modified Beijerinck medium of the following composition (g/l): $Na_2S_2O_3 \cdot 5H_2O$, 5; $NH_4Cl$, 0,1; $NaHCO_3$, 1; $MgCl_2 \cdot 6H_2O$, 0.1; 10% phosphate buffer (pH 7.5–7.8), 1 ml; Pfennig's trace elements and vitamins, 0.2 ml; and original Baikal water, 1 l. Each medium component except magnesium salt was sterilized separately and added (upon cooling) to sterile water containing $MgCl_2$; pH of the medium was 8.0–8.2.

To enumerate lithoheterotrophic thionic bacteria, a modified Rittenberg medium composed of (g/l) $Na_2S_2O_3 \cdot 5H_2O$, 2; $NH_4Cl$, 1; $KH_2PO_4$, 1; $MgCl_2 \cdot 6H_2O$, 0.5; Pfennig's trace elements and vitamins, 1 ml; yeast extract (Difco), 0.1; acetate, 0.1; and original Baikal water, 1 l was used. Each medium component except magnesium and ammonium salts was sterilized separately as 10% solutions and added to sterile water containing $MgCl_2$ and $NH_4Cl$; the pH of the medium was 8.0–8.2.

Denitrifying bacteria were enumerated on Taylor's medium of the following composition (g/l): $KNO_3$, 2; $NH_4Cl$, 1; $KH_2PO_4$, 2; $NaHCO_3$, 2; $MgSO_4 \cdot 7H_2O$, 0.8; $Na_2S_2O_3 \cdot 5H_2O$, 5; Pfennig's trace elements and vitamins, 1 ml; and distilled water, 1 l. The medium was prepared by adding the above components to sterile water containing $KNO_3$, $NH_4Cl$, and $MgSO_4$; the pH of the medium was 7.0.

Autotrophic and lithoheterotrophic thionic bacteria were cultured in tubes with cotton plugs, and denitrifying bacteria were cultured in bottles with rubber stoppers and without gas phase. Growth was determined upon two successive culture transfers from the loss of thiosulfate and by microscopic observations. The medium without inoculum was used as the control. The consumption of thiosulfate was determined by separate iodometric titration [6]. Acidification of the media by autotrophic and lithoheterotrophic thionic bacteria was

[1] Corresponding author; e-mail: tzema@lin.irk.ru

Number of bacteria of various physiological groups in bottom sediments of Frolikha Bay (northern Baikal) in October, 1997 ($10^3$ cells/cm$^3$)

| Station | Depth, m | Horizon | Saprophytes | Ammonifiers | Ammonium oxidizers | Heterotrophic nitrifiers | Denitrifiers | Nitrite oxidizers | Sulfate-reducers | Sulfur-oxidizers | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | autotrophic | lithoheterotrophic | denitrifying |
| 1 | 415 | 0–1 cm | 22.0 | – | 1.2 | 0.7 | 0.2 | 0.8 | 1.0 | >10 | 1.0 | 0.1 |
| 3 | 405 | 0–1 cm | 15.0 | >10 | 0 | >10 | 0 | 0.2 | 1.0 | 1.0 | 1.0 | >10 |
| 5 | 400 | 0–1 cm | 49 | >10 | 0 | 0.7 | 0.3 | 0.1 | 0.1 | – | – | – |
| 6 | 215 | Near-bottom water | 86 | 3.5 | 0.5 | 3.1 | 1.3 | 9.5 | – | – | – | – |
| | | 0–1 mm | 95 | – | 1.7 | 2.0 | 1.1 | 0.6 | 0.001 | – | – | – |
| | | 0–1 cm | 32 | – | 6.0 | 0.9 | 0 | 0.5 | 0.1 | >10 | 0.1 | >10 |
| | | 10–15 cm | 2 | 0.4 | 0 | 0.8 | 0 | – | – | – | – | – |
| 7 | 105 | Near-bottom water | 109 | 0.9 | 25.2 | 2.4 | 0 | 28.2 | – | – | – | – |
| | | 0–1 mm | 49 | 0.8 | 0 | 0.5 | 0.7 | 1.4 | 0.001 | – | – | – |
| | | 0–2 cm | 30.8 | 0 | 0 | 0.3 | 8.2 | 1.0 | 1.0 | >10 | 1.0 | >10 |
| | | 10–15 cm | 3.9 | 0.3 | 1.4 | 0 | 0.4 | 0 | – | – | – | – |
| 11 | 42 | 0–3 cm | 71 | 3.1 | 0 | 19.4 | 13.2 | 2.4 | 0.1 | – | – | – |
| 12 | 32 | 0–3 cm | 16 | 1.1 | 0 | 0 | 0.9 | 0 | 0.01 | 0.001 | 0.1 | 1.0 |
| 10 | 23 | 0–3 cm | 29.0 | 2.0 | 0 | 16.0 | 0 | 1.0 | | – | – | – |

Note: The symbol "–" means "no data."

measured with a pH-121 potentiometer; and the formation of sulfates was determined by the spectrophotometric method [7]. Growth of anaerobes was judged from the production of nitrites, determined with the Griess reagent [6].

The results of the enumeration of various physiological groups of bacteria are presented in the table. It can be seen that the number of saprophytes ranges from several thousand to 109 thousand cells per ml. The largest population occurs in the near-bottom water layer, which accumulates fresh organic matter of detritus originating in the water column above. The number of saprophytic bacteria rapidly declines with the depth in the sediment.

The number of ammonium and nitrite oxidizers averaged $10^3$ cells/g, but was as high as $10^4$ cells/g in several samples. The largest number ($2.8 \times 10^4$ cells/g) was observed in the near-bottom water layer. This bacterial group was not found in anaerobic sediment layers. The highest population of heterotrophic nitrifiers was also observed in the near-bottom layer of water. A certain degree of correlation was noted between the distribution of nitrifiers, saprophytic bacteria, and ammonifiers across the bay area. The highest populations of the bacteria of these groups were observed at a depth of 105 m, where *Thioploca* sulfur mats occur. Among unicellular sulfur-oxidizing bacteria, denitrifiers were found in all the tested samples of bottom sediments. The number of aerobic lithoheterotrophic sulfur-oxidizing bacteria was about an order of magnitude lower and amounted to $10^2$–$10^4$ cells/g.

Sulfate-reducing bacteria were detected in all the tested samples of bottom sediments, but their number was typically low (from a few cells to several hundred cells in 1 g), reaching $10^3$ cells/g only at a depth of 400–415 m.

Thus, representatives of all studied physiological groups of bacteria of the sulfur, carbon, and nitrogen cycles were found in all the types of bottom sediments analyzed and were part of the bacterial communities where *Thioploca* was present in the form of dense sulfur mats or as separate filaments. However, a previously made comparison of the biomass of *Thioploca* and the net biomass of aerobic and anaerobic unicellular sulfur-oxidizing bacteria shows that the latter constitute only a very small fraction of the biomass of filamentous sulfur bacteria [7]. Therefore, it can be assumed that the major contribution to the oxidation of reduced sulfur compounds comes from *Thioploca*, and the role played by unicellular sulfur-oxidizing bacteria in these processes is not significant.

The occurrence of nitrifiers in natural water and in the topmost sediment layer is indicative of their role as suppliers of nitrate, which is the final product of their metabolism and is further utilized by *Thioploca* as an electron acceptor in the oxidation of sulfide during autotrophic or mixotrophic growth of this organism in anaerobic zones of sediments.

The obtained results demonstrate that bacterial communities dominated by *Thioploca* have a complex structure and trophic relations exist between their components. The latter shows up in the production of the substrates (electron donors and acceptors) required by both *Thioploca* and other components of the bacterial community.

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