Functional Groups of Bacteria and Their Involvement in Geochemical Processes in Microbial Communities of Mineral Sources in Sakhalin Island (Far East, Russia)

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Abstract. The chemical composition of mineral waters as well as the diversity, quantity and distribution peculiarities of different functional groups of bacteria in microbial mats in Sakhalin mineral springs was studied. The prevalence of sodium cations in the composition of bicarbonate anions and chloride ions was noticed. The studied waters were substantially enriched with silicone, iodine, boron, iron and strontium. It was established that the main morphogenetic bacteria in the mats of cold springs were the colorless sulfur bacteria Thiotrix sp.

The microbial mats formed at the outlets of Sakhalin mineral springs contain a high quantity of different functional bacteria groups that play an important role in the geochemical cycles of carbon, nitrogen, sulfur, iron and manganese. Prevalence of bacteria of sulfur and carbon cycles is a peculiarity of microbial mats in Antonovsky and Nevelsky mineral waters. The important role of sulfur cycle bacteria is confirmed by high quantities of thionic, sulfate-reducing bacteria and colorless sulfur bacteria. The mat of Alexandrovsky thermal spring contains a high amount of bacteria of carbon and nitrogen geochemical cycles; an important role of these microorganisms in mat’s functioning is shown. Pure bacterial cultures were isolated; they mostly include gram-positive, spore-forming motile rods Bacillus sp.

1. Introduction

More than 20 mineral springs, mainly cold springs with temperatures at 10°С (nearly 90%) are known at Sakhalin Island. The thermal springs have the temperature less than 50°С. Many manifestations of cold mineral water do not discharge as springs and could be found only during the core-drilling. Most mineral spring systems are distributed within the West Sakhalin uplift between two main tectonic dislocations of the island - West-Sakhalin and Central-Sakhalin faults systems [1]. The biggest basins and occurrences are situated within North Sakhalin hydrogeological basin of pressure waters (Daginsk thermal springs basin, Lunsk and Paromaik thermal springs). Chemical composition of Sakhalin ground waters has been studied insufficiently; the major contribution into their studies has been made by V. Ivanov, N. Tsitenko, M. Stein, I. Zavadsky, T. Rozoriteleva and others [2-5].

Mineral springs are known to be a natural habitat of various microorganisms. Bacteria in water and in biomats that are formed at the outlets of mineral waters are instrumental in ecosystem functioning by producing and destroying organic matter and participating in geochemical cycles of elements [6]. However, the distribution of microorganisms of different physiological groups in microbial mats of Sakhalin mineral springs has not been studied before. Therefore, the work was aimed at studying the
chemical composition of waters as well as of their composition, structure, quantity and distribution peculiarities of different functional bacteria groups in the microbial mats of Sakhalin mineral springs.

2. Material and methods

2.1. Sampling
The samples of mineral waters and microbial mats were collected in Sakhalin Island in June 2015 from Daginsk thermal springs (Alexandrovsky spring, 45.2° C) and cold Nevelsky and Antonovsky springs (temperature 8.0-10.0° C). All samples of mineral waters at outlets had microbial mats. Two types of microbial mats were sampled in Antonovsky springs (A1B, A14). Nevelsky and Antonovsky springs are distributed within the West Sakhalin uplift; between two main tectonic dislocations of the island – West-Sakhalin and Central-Sakhalin faults systems. The structural denudation landscape is typical for the West Sakhalin Mountains; the absolute heights vary between 300 m and 1300 m. Alexandrovsky spring is located in the East Sakhalin and circulated within Late Cretaceous (Senoman-Danian) sediments presented by sandstones, siltstones, and mudstones.

Samples of water and microbial mats were collected under sterile conditions into sterile 200 ml glass tubes. Some samples were on site fixed with 40% formalin. The rest of the samples were stored in the refrigerator for less than 12 hours before they were analyzed in the lab. Chemical analysis of samples was conducted at the facilities of the analytical center within FEGI FEB RAS.

2.2. Sample preparation and analysis
Water samples for analysis of cations and sulfates were filtered through membrane filters (cellulose nitrate, 0.45 μm, Sartorius) at the site of sampling for removing the suspended matter; then they were acidulated with nitric acid. Water samples for analysis of anions were also filtered through membrane filters (cellulose nitrate, 0.45 μm, Sartorius) and then collected into plastic tubes (without acidulation). The main cations and anions were identified with liquid ion chromatography (HPLC-10AVp, SHIMADZU). Unstable parameters were measured at the site of water sampling.

Bacteria were discovered and cultivated with traditional methods of practical microbiology [7]. The quantity of different functional bacteria groups was measured by method of limiting dilution culture and Koch’s method [8]. The quantity of different physiological groups of bacteria was defined at selective media described earlier [9-10]. The microorganisms were grown in thermostats at 25° C (Antonovsky, Nevelskoy springs) and 45° C (Alexandrovsky spring). Anaerobic forms of bacteria were cultivated in an anaerobic jar with gas-generating bags BD GasPak EZ. The morphology, sizes, mobility, and spore production of separated pure cultures of bacterial cells were studied with a light microscope AxioStar plus (Carl Zeiss, Германия) equipped with photo-documenting system Axiovision and phase-contrast attachment (x1000). Microorganisms were identified in accordance with Bergey’s Manual of Systematic Bacteriology as well as with API-tests produced by bioMerieux (France) [11-12].

3. Results and discussion

3.1. Chemical composition of Sakhalin mineral springs
Here, we present a short description of chemical composition for three springs (table 1). Groundwaters are represented with Na-Cl-HCO₃ (Nevelsky & Antonovsky springs) and Na-HCO₃-Cl (Alexandrovsky spring) water types with TDS varying from 0.25 to 1.67 g/l. Two springs are cold, the pH changes from 6.5 to 9.1, and Eh values vary from -42 to -224 mV. Na⁺ is prevailed cation species ranging from 66.9 to 587 mg/l. The concentrations of Si, Mn, I, Ba are moderate (table 1).
Methane is the main component (69 vol.%) of the associated free gases in the Antonovsky and Alexandrovsky springs, N₂ and O₂ are less than 28 vol% and 1.5 vol%, respectively. The H₂S concentration can reach 1.1% in cold mineral springs with shallow geochemical characteristics (Nevelsky and Antonovsky springs). H₂S (aqueous) is possible to transform to 2H⁺ + SO₄²⁻ through oxidation of H₂S in the vadose zone [13]. It could also be associated with sulphur-bearing bacteria below the water surface, suggesting biogenically mediated SO₄²⁻-reduction [14]. Helium content varies with the full range of the first tens of ppm. Also, in the Antonovsky spring a high amount of Rn (up to 2400 Bq/m³) was detected.

### 3.2. Composition, structure and quantity of functional bacterial groups and their biodiversity in microbial mats of Sakhalin mineral springs

Income of endogenous substrates, low oxidation-reductive potential and presence of gases (hydrogen sulfide, nitrogen, methane) at the outlet of mineral springs create favorable conditions for the formation of microbial mats. Cold mineral waters of Antonovsky and Nevelsky springs contain white microbial mats in the form of white toulus. The springs emit a strong smell of hydrogen sulfide. Diatomic algae and colorless sulfur bacteria are the major morphogenetic components. At the outlet of thermal waters of Alexandrovsky spring (Daginsky water supply) grey-green microbial mats were found; cyanobacteria and different cells of diatomic algae prevailed in their composition.

The results of microbiological studies show that the microbial mats contain a relatively high quantity of functional bacterial groups that varied between 0 and 4.8×10⁵ cells/cm². The microbial mats of Antonovsky and Nevelsky springs showed a significant prevalence of bacteria of sulfur and carbon cycle (table 2). The amount of microorganisms of sulfur cycle in the mats of cold sulfur hydrogen springs was the highest; colorless sulfur bacteria prevailed in all samples (7.4×10⁵ (A14) - 4.8×10⁶ (Nevelsky) cells/cm²). The highest quantity of colorless sulfur bacteria was found in Nevelsky mineral spring that has a lower level of water mineralization, higher alkaline conditions and absence of methane in the gas composition of waters (table 1, 2). Identification of thread sulfur bacteria cultures shows that in all samples they are represented by Thiothrix sp. microorganisms. Cells of colorless sulfur bacteria on the surface formed white mineral sediments. Elemental analysis of white sediments shows that they consist of elemental sulfur: its content goes up to 99%.

The biological role of colorless sulfur bacteria in microbial mats is in the oxidation of hydrogen sulfide that comes with volcanic fluids as well as from bottom sediments and microbial mats caused by microbe sulfate reduction to elemental sulfur that accumulates in the cell cytoplasm and then acetifies to sulfates. These sulfates reduce to hydrogen sulfate involving anaerobic sulfate reducing bacteria. The quantity of sulfate reducing bacteria in the samples of microbial mats in cold springs was quite high and varied between 6.4×10⁵ (A14) and 5.2×10⁶ (Nevelsky) cells/cm² that confirms the

### Table 1. Physico-chemical composition of the mineral springs of Sakhalin island.

| Sampling point | t °C | pH | Na | Ca | Mg | K | Cl | SO₄ | HCO₃ | F |
|---------------|-----|----|----|----|----|----|----|-----|-----|----|
| 1             | 10  | 9.1| 0.067 | 0.005 | 0.0005 | 0.0003 | 0.032 | 0.016 | 0.11 | 0.0002 |
| 2             | 8   | 6.5| 0.091 | 0.0115 | 0.0023 | 0.0009 | 0.065 | 0.034 | 0.116 | 0.0001 |
| 3             | 45.2| 6.53 | 0.587 | 0.0198 | 0.0013 | 0.0045 | 0.709 | 0.001 | 0.405 | 0.0006 |

| Sampling point | TDS g/l | Eh mV | Fe | Mn | Si | Al | Ba | Sr | I | B |
|---------------|---------|-------|----|----|----|----|----|----|---|---|
| 1             | 0.25    | -219  | 0.1 | 0.001 | 8.7 | 0.007 | - | 0.001 | 0.5 | 0.47 |
| 2             | 0.33    | -224  | 0.1 | 0.06 | 10 | 0.01 | 0.001 | 0.16 | 0.01 | 0.76 |
| 3             | 1.67    | -42   | 0.25 | 0.001 | 19.7 | 0.015 | 0.04 | 0.6  | 4.1 | 0.27 |

1- Nevelsky spring.  
2- Antonovsky spring.  
3- Alexandrovsky spring.
partly biogenic origin of hydrogen sulfide. Presence of sulfates in cold mineral waters causes a vigorous activity of sulfate reducing bacteria (table 1). Such prevalence of sulfur cycle bacteria in the structure was previously found in carbonic acid mineral waters of Primorye [15]. In addition to colorless sulfur bacteria, the oxidation of hydrogen sulfide involves thionic bacteria which oxidize it to elemental sulfur that is laid outside cells. Thionic microorganisms were found only in the mats of Antonovsky spring (1.8-4.7×10⁵ cells/cm³) where the amount of colorless sulfur bacteria was lower (table 2).

Table 2. The number of different physiological groups of bacteria in the microbial mats of mineral springs Sakhalin island.

| Functional groups of bacteria:                                | Antonovsky spring | Nevelsky spring | Alexandrovsky spring |
|---------------------------------------------------------------|-------------------|-----------------|----------------------|
| **Carbon cycle:**                                             |                   |                 |                      |
| Saprophytic bacteria, aerobes                                 | 1.6×10⁴           | 3.2×10⁵         | 1.5×10⁴              |
| Saprophytic bacteria, anaerobes                               | 2.0×10⁷           | 2.7×10⁸         | 2.0×10⁷              |
| Proteolitic bacteria                                          | 3.5×10⁵           | 8.1×10⁸         | 6.0×10⁷              |
| Cellulose-degrading bacteria                                  | 0                 | 1.9×10⁸         | 0                    |
| Methane-forming bacteria                                      | 0                 | 5.6×10⁸         | 0                    |
| **Nitrogen cycle:**                                           |                   |                 |                      |
| Nitrogen fixing                                               | 0.7×10⁵           | 1.2×10⁸         | 0                    |
| Ammonifiers                                                   | 3.1×10⁷           | 0               | 1.2×10⁷              |
| Autotrophic nitrification                                     | 0                 | 0               | 0                    |
| Heterotrophic nitrifying                                     | 0                 | 5.2×10⁸         | 0                    |
| Denitrifying                                                  | 0                 | 0               | 3.2×10⁸              |
| **Sulfur cycle:**                                             |                   |                 |                      |
| Thionic bacteria                                              | 4.7×10⁴           | 1.8×10⁴         | 0                    |
| Sulfate Reducing                                              | 6.4×10⁵           | 1.2×10⁸         | 5.2×10⁸              |
| Colorless sulfur bacteria                                     | 9.5×10⁷           | 7.4×10⁸         | 4.8×10⁴              |
| **Cycle of iron, manganese:**                                 |                   |                 |                      |
| Iron Oxidizing Heterotrophs                                   | 0                 | 0               | 7.0×10²              |
| Iron reducing bacteria                                        | 0                 | 0               | 0                    |
| Manganese-oxidizing heterotrophs                              | 0                 | 0               | 0                    |
| Manganese reducing bacteria                                   | 0                 | 3.8×10⁷         | 0                    |
| **Silicon cycle:**                                            |                   |                 |                      |
| Silicate bacteria                                             | 0                 | 4.5×10⁷         | 9.5×10⁷              |

Thus the contribution of sulfur cycle bacteria into the generation and oxidation of hydrogen sulfide and sulfates in Sakhalin cold mineral springs has been shown. Various ecologo-trophic groups of bacteria were involved into the carbon cycle in microbial mats of Antonovsky and Nevelsky cold mineral springs (table 2). The amount of bacteria varied between 0 and 2.7×10⁸ cells/cm³; however, aerobic and anaerobic saprophytic microorganisms prevailed showing the processes of organic matter destruction in microbial mats. The quantity of anaerobic forms of saprophyte bacteria in the springs under study was a sequence higher thus showing the prevailing anaerobic processes of organic matter decomposition. We had previously shown the prevalence of anaerobic saprophytes over aerobic in the ground acidulous mineral waters of Kamchatka that contain low content of oxygen [16]. The sample of A14 mat from Antonovsky spring that has no subsoil contained anaerobic methanogenic bacteria; their amount was not high and comprised 5.6×10⁵ cells/cm³. Methanogenic bacteria perform the final stage of anaerobic destruction of organic substances; their presence in mineral waters shows the presence of organic substances and anaerobic conditions.
Organic matter in the microbial mats of Alexandrovsky thermal spring (Daginsky basin) is produced by different types of cyanobacteria and other microorganisms; they assimilate carbon dioxide and use the energy of methane and nitrogen oxidation. Destruction of organic matter is caused by different physiological groups of bacteria – saprophytes, hydrolytic, fermentative and methanogenic (Table 2). The amount of microorganisms of carbon cycle in the mats varied between $1.0 \times 10^7$ and $3.7 \times 10^8$ cells/cm$^3$; anaerobic forms of saprophyte bacteria prevailed. Hydrolytic microorganisms that decompose polymeric compounds are instrumental at early stages of destruction. Cellulose-decomposing bacteria were predominant reaching $7.2 \times 10^8$ cells/cm$^3$. The mats of thermal springs also contained relatively high amounts of methanogenic bacteria $8.4 \times 10^4$ cells/cm$^3$. Methanogenic bacteria are obligate anaerobic microorganisms; they obtain growth energy from carbon dioxide, hydrogen and acetates that are generated by an assembly of saprophyte, hydrolytic, fermentative, and acetogenic microorganisms. Presence of methanogens in mat samples shows the processes of biogenic generation of methane. The isotopic data obtained previously in these waters confirm a possible biogenic character of methane from a thermal zone of Daga [1]. The microbial mats of Alexandrovsky thermal springs also contained a high quantity of nitrogen cycle bacteria. Ammonifiers $(1.2 \times 10^3$ cells/cm$^3$), anaerobic denitrifying bacteria $(3.2 \times 10^3$ cells/cm$^3$) and heterotrophic nitrifiers $(6.0 \times 10^4$ cells/cm$^3$) prevailed in the nitrogen cycle. High content of anaerobic manganese-reducing bacteria was found in the cycle of iron and manganese that shows the presence of manganese-reducing compounds in the water. Iron-oxidizing bacteria were discovered in the mat of Alexandrovsky spring in the amount of $7.0 \times 10^2$ cells/cm$^3$ that was congruent with the higher content of iron in these waters (Table 1). We had previously discovered the prevailing manganese-reducing and iron-oxidizing bacteria in the thermal waters of Kuldur that is caused by the enrichment of water with iron and manganese [17]. Pure cultures of thermophile, mesophile and psychrophile bacteria were isolated from enrichment cultures of microbe mats sampled from Sakhalin mineral springs. The strains mostly contained gram-positive, spore-forming, primarily motile rods with different behavior towards oxygen. The majority of strains are chemoorganotrophs in terms of feeding. The microbial mats mostly contain various cultured bacteria types; *Bacillus* sp. microorganisms prevailed in all mineral sources under study. *Bacillus* sp. are widespread; they are isolated from different thermal springs and had been previously isolated in the technogenic waters and soils of tailing dumps as well as in the ground high-mineralized waters of Primorsky Krai [18-20]. Bacteria *Bacillus* sp., *Thiobacillus* sp., *Desulfobacter* sp. (A1B) и *Bacillus* sp., *Thiobacillus* sp., *Desulfomicrobium* sp. (A14) were found in the mats of Antonovsky cold hydrosulfuric springs. In Nevelsky springs the bacteria were mostly represented by *Bacillus* sp., *Pseudomonas* sp., *Desulfovibrio* sp. The biggest variety of microorganisms was observed in the mats of Alexandrovsky thermal springs where the microorganisms *Bacillus* sp., *Pseudomonas* sp., *Clostridium* sp., *Vibrio* sp. prevailed. The bigger variety of bacteria is probably caused by the higher temperature in the springs $(45.2^\circ \mathrm{C})$ that is optimal for development of microorganisms.

### 4. Conclusions

Sodium is a prevailing cation; hydrocarbonate and chloride-ions are predominant anions in the mineral springs under study. Sulfate-ions also prevail in the cold mineral waters. Silicone, iodine, boron, iron and strontium also prevailed in the microelement composition of the studied waters. The microbial mats formed at the outlet of Sakhalin mineral springs contain relatively high amounts of functional groups of bacteria that play an important role in the geochemical cycles of carbon, nitrogen, sulfur, iron and manganese. The mats of cold waters showed a quantitative prevalence of sulfur and carbon cycle microorganisms in the structure; bacteria of carbon and nitrogen cycle were predominant in the microbial communities of thermal springs. The study has also shown the contribution of sulfur cycle bacteria to the production and oxidation of hydrogen sulfide and sulfates in the cold mineral springs of Sakhalin Island. The presence of methanogenic anaerobic bacteria has been stated in the composition of microbial mats of Antonovsky and Alexandrovsky springs. Pure bacterial cultures have been isolated; they mostly contained gram-positive, spore-forming motile rods with prevalence of *Bacillus*.
sp. The biggest variety of bacteria has been found in the mats of thermal waters of Alexandrovsky springs.

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