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SHORT COMMUNICATIONS

Investigation of Microbial Communities of Viluchinskaya Hydrothermal System (Kamchatka) by Methods of Optical and Electron Microscopy

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The hydrothermal systems of Kamchatka are unique natural laboratories where minerals are formed under the natural conditions at the present time. Elucidation of the role of bacteria, algae, and diatoms from thermal waters and bacterial mats in the geochemical transformation of rocks has attracted the special attention of researchers. The involvement of microorganisms in the processes of precipitation and formation of new mineral forms has been studied for a long time and has remained an essential piece of evidence for their active role in the functioning of thermal ecosystems [1].

In the present work, we studied certain characteristics of the waters of the Viluchinskaya hydrothermal system. We analyzed the chemical composition of the water, the total number of bacteria and the proportion of microorganisms in the aquatic microbial community that exhibited enzymatic activity. Actual evidence of the formation of amorphous precipitates of silicon, iron, and calcium on the surface of bacterial cells is also given.

Water samples were collected in March and October, 2002 from hydrogeological wells nos. 1 and 2, located on the right bank of the Spokoinyi stream, from natural thermal outlets 1 and 2 of the travertine cupola and from the arsenical spring, located on the travertine terrace along the right bank of the Vilucha river. The physicochemical parameters of the water (water temperature, pH, and concentration of dissolved oxygen) were determined under field conditions. The chemical composition of the water was analyzed by methods of colorimetry, a combination of photometry and atomic absorption and atomic emission spectrophotometry, mass-spectrometry (ICP-MS), and X-ray fluorescence analysis. To assay the total number of bacteria (TNB), the samples were prepared as described earlier [2]. The numbers of bacteria exhibiting enzymatic activity (BEA) were determined with 5-carboxyfluorescein diacetate (CFDA); its intracellular decomposition by esterases results in the formation of a fluorochrome carboxyfluorescein. CFDA (Molecular Probes Inc.) was added directly during collection of thermal water samples up to the final concentration of 0.1 mM [3]. The samples of autoclaved thermal water served as a negative control. Cell counts were performed with an epifluorescent microscope (Nikon EFD-3; digital camera system: Nikon COOLPIX E995, Japan) equipped with a set of appropriate color filters at 100× magnification. The average and the standard deviation were assayed by the results of cell counts in 10–20 random microscope fields in all filters. The portion of EAB was expressed as a percentage of the TNB. The samples were prepared for scanning electron microscopy (SEM) by the method of cryophilic drying [4]. The samples were analyzed by the SEM equipped with energy spectrometer (SEM: JEOL-JSM-5200LV; EDS: Philips-EDAX PV9800 STD, Japan). The grids for transmission microscopy were fastened on the slides with a carbonic suspension to assess the morphological diversity of microorganisms of thermal waters. The slides were incubated under the natural conditions for a day, fixed with 2.5% glutaric aldehyde, dried in the air, and analyzed with a JEOL 2000EX transmission microscope (Japan) at accelerating voltage 80–120 kV.

All samples were characterized by low concentrations of dissolved oxygen, which did not exceed 1.63 mg/ml (at well 1, the temperature 57°C), and high temperatures (57–74°C). All samples had pH values close to neutral (from 6.85 to 7.75). The waters varied

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Total bacterial numbers and numbers of bacteria exhibiting enzymatic activity in the waters of Viluchinskaya hydrothermal system

Sample	Total number of bacteria ($\times 10^4$ cells/ml) (average ± SD)[a]	Number of BEA ($\times 10^4$ cells/ml) (average ± SD)	Portion of BEA (%)[b]
Well 1	1.63 ± 0.15	0.75 ± 0.09	46
Travertine cupola, outlet 1	0.99 ± 0.04	0.89 ± 0.02	91
Travertine cupola, outlet 2	1.56 ± 0.04	1.12 ± 0.08	72
Arsenical spring	1.67 ± 0.16	1.07 ± 0.17	64

[a] Average and standard deviation (SD) were assayed from the cell counts of 10–20 random microscope fields on each filter.
[b] Percent of bacteria exhibiting enzymatic activity (BEA) was assayed as a fraction of the total number of bacteria detected by DAPI fluorescence.

slightly in their ionic composition and belonged to the sodium carbonate type. High concentrations of iron (0.33–6.14 μg/l), arsenic (0.79–0.95 μg/l), and strontium (0.82–0.99 μg/l) were detected in all samples by ICP-MS.

Maximum numbers of bacteria were found in the waters of the arsenical spring (1.67×10^4 cells/ml) and in the well 1 (1.63×10^4 cells/ml) (table). The TNB was 0.99×10^4 and 1.56×10^4 cells/ml in the thermal waters of the travertine cupola. Different authors estimate the total number of bacteria in thermal wells and natural outlets from 10^2 to 10^3 cells/ml depending on method [5–7]. Importantly, these values are much lower than the values for other natural ecosystems. For example, the TNB in freshwater lakes and rivers varies from 1×10^6 to 7×10^6 cells/ml [2, 8, 9]. However, the portion of BEA in the microbial community was high. The lowest content of EAB was determined in the water of the well 1, but it reached 46% of TNB. In natural thermal outlets of the travertine cupola, the proportion of BEA was higher and reached 72–91% (outlets 1 and 2) and in the arsenical spring, 64%. The estimation of BEA in thermal waters was performed for the first time, but these data are in agreement with the earlier results of analysis of natural freshwater ecosystems, where the portion of BEA was lower, but reached 12.9–62.6% of TNB [8, 9].

The morphological diversity of microbial communities was analyzed by the methods of light optical, scanning (SEM), and transmission electron microscopy (TEM). The predominant morphotype of the bacterial community of the waters from well 1 was represented by rod-shaped bacteria with diameter about 0.5 μm and length 5–7 μm, which often formed aggregates—microcolonies. Microbial communities of the natural outlets of the travertine cupola and the arsenical spring had a more considerable diversity of microorganisms. Small rods (with length up to 2–3 μm), cocci, and long filamentous bacteria occurred as single cells or in aggregates with mineral particles. Cells with different extent of encrustation with new mineral formations were detected both as single cells and in mineral aggregates. Rod-shaped bacteria of the dominating morphotype in the microbial community of well 1 were studied in more detail with TEM (Figs. 1a, 1b) and SEM (Fig. 1c). Microorganisms and mineral particles were examined by TEM, and electron diffraction was analyzed both from the bacterial cell wall and from mineral particles of different size. It should be noted that only amorphous matter, which does not give clear diffraction patterns, was detected on bacterial surfaces. However, a combination of SEM and chemical analysis allowed us to reliably determine the presence of Fe, Si, and Ca in the composition of some mineral formations and bacterial capsules (Fig. 1c, insert). Thus, formation of an amorphous phase of oxides and/or hydroxides of iron, silicon, and calcium on the surface of bacterial cells was demonstrated.

Very rapid sedimentation of iron- and silicon-containing minerals in Viluchinskaya hydrotherms was recently determined [10]. The chemical analysis of thermal waters revealed high total concentrations of iron (6.14 μg/l) and siliceous acid (260 mg/l). Obviously, those rates of chemical oxidation of ferrous iron and formation of iron hydroxides are low at low concentrations of dissolved oxygen, high temperatures, and neutral media pH. However, under natural condi-

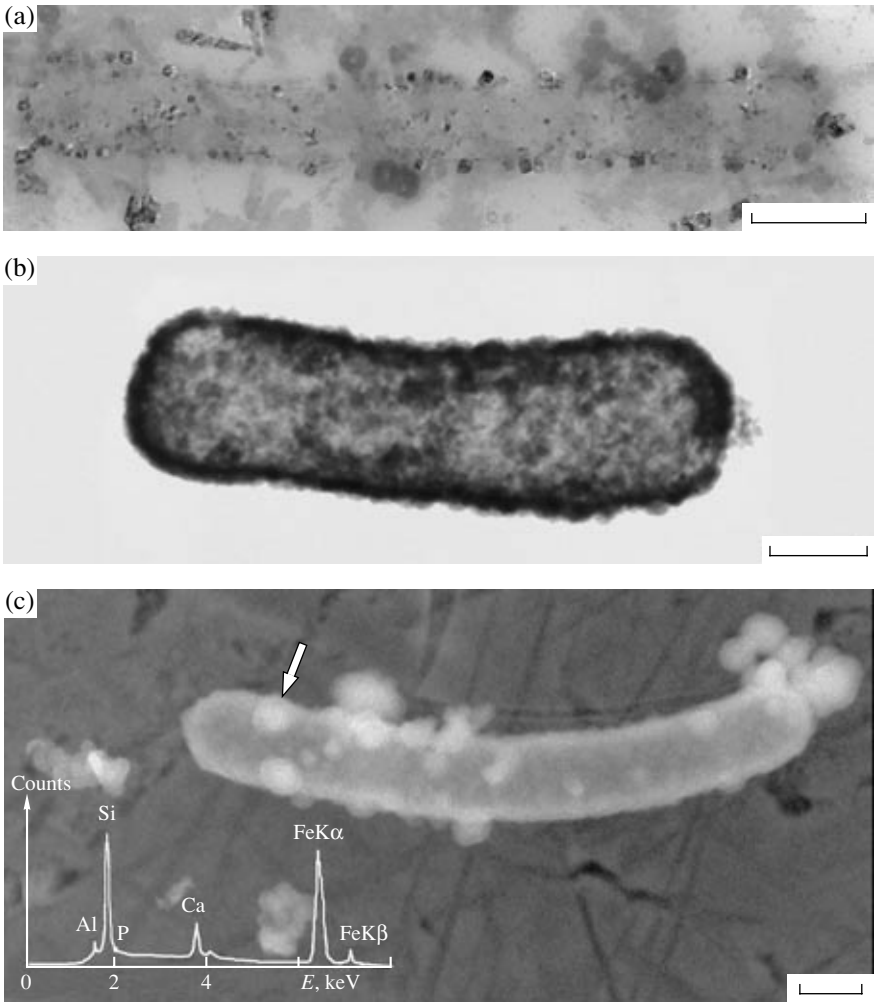

Fig. 1. Transmission (a, b) and scanning (c) micrographs of bacterial cells, detected in water (a, c) and bacterial mats (b) of well 1. The chemical analysis of mineral formations on bacterial cell walls (SEM-EDX spectrum on c) revealed the presence of silicon, calcium, and iron in their composition. Scale bar, 500 nm.

tions this process can occur with the participation of microorganisms.

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