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Metagenomic Analysis of Microbial Communities of Deep Subsurface Thermal Aquifers of Western Siberia Revealed Abundance of Uncultured Bacterial Lineages

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The deep subsurface habitats are characterised by anaerobic conditions, extremely high pressure and high temperature. Our knowledge of the microorganisms inhabiting these ecosystems is still limited. We analysed the diversity and possible biogeochemical functions of microbial communities inhabiting underground thermal aquifers of Western Siberia (Tomsk region, Russia) by metagenomic analysis of microbial communities. We analysed the microbial communities of the underground thermal waters springing out of three abandoned oil-exploration boreholes. Two approaches were used to characterize the microbial communities: identification of microorganisms based on amplification and pyrosequencing of 16S rRNA genes fragments, and sequencing of the total community DNA.

The borehole 3P was drilled into putatively Mesozoic sedimentary rocks of the Western Siberia basin about fifty years ago but no oil was found. Thermal water (temperature 46-51°C) originates from a depth of about 2.8 km. The 16S rRNA analysis indicated the dominance of *Firmicutes*, represented by sulfate-reducers of the genera *Desulfovibrio* (47% of 16S sequences) and *Desulfotomaculum* (1.3%), as well as by microorganisms related to *Thermacetogenium* (17%) and several uncultured lineages of *Firmicutes* (7%). Methanogenic archaea of the genus *Methanothermobacter* accounted for about 24% of all 16S rRNA gene sequences. Sequencing of the whole metagenome allowed identification of genes and their functional and taxonomic assignment, indicating that microbial community of 3P borehole is characterised by mostly chemolithoautotrophic metabolism based on oxidation of hydrogen and low molecular weight organic compounds coupled to sulfate reduction.

Metagenomic analysis of microbial community at the second borehole, located near the village Bely Yar (water temperature 40-45°C, 2.6 km deep source), revealed quite different and more diverse microbial community, comprising representatives of *Firmicutes*, *Deltaproteobacteria*, *Chloroflexi* and *Nitrospira*, as well as several uncultured bacterial lineages. Archaea were not found. *Firmicutes* were represented by autotrophic sulfate reducers *Desulfurudis audaxviator* (14%) and *Desulfotomaculum* (4%), and by several uncultured groups (8%). Sulfate-reducers were also found among *Deltaproteobacteria* (*Desulfobacca acetoxidans*, 10%) and *Nitrospira* (*Thermodesulfobium*, 2%). *Chloroflexi* were represented by organotroph *Belliinea caldifistulae* (1%) and several uncultured lineages (14% in total). Notably, about 33% of 16S rRNA sequences were not classified even at the phylum level, representing novel bacterial lineages.

Microbial community at the third site, borehole 5P (Chazhemto village, water temperature 20-21°C), comprised approximately equal numbers of Bacteria (49%) and Archaea (61%). Archaea community consisted of methanogenic genera *Methanothermobacter* (20%) and *Methanosaeta* (20%). Bacterial fraction was very diverse and included *Firmicutes* (15%, mostly uncultured lineages), representatives of the phyla *Thermotogae*, *Bacteroidetes*, *Nitrospirae*, *Proteobacteria*, *Chloroflexi*. Bacteria that could not be classified at the phylum level accounted for about 19% of the microbial community.

Overall, our results suggest that the underground thermal aquifers harbour quite different and diverse microbial communities. Some groups, namely sulfate-reducing *Firmicutes* and methanogenic archaea, were found at all three locations, while some sites were dominated by various "uncultured" lineages performing unknown functions in the deep subsurface environments. This work was supported by R3F grant 14-14-01010 (3P and Bely Yar) and RFBR grant 16-34-60124 (5P borehole).