

Diversity and possible metabolic activity of the microbial community in nitrate- and radionuclide-contaminated groundwater

Babich T.L.^{1*}, Kalmykov S.N.², Nazina T.N.¹

¹ Winogradsky Institute of Microbiology, Research Center of Biotechnology, RAS, Moscow, Russia

² Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia

* microb101@yandex.ru

Key words: nitrate- and radionuclide-contaminated groundwater, 16S rRNA gene, high-throughput sequencing, denitrification, radionuclides

Motivation and Aim: Since 1951, Lake Karachai located at the north of the Chelyabinsk region (Russia) has been used for the disposal of low- and intermediate-level liquid radioactive waste (RW) [1, 2]. Radioactive and other toxic components of radioactive waste were filtered through the permeable lake floor and concentrated in groundwater. Special attention has been paid to hydrochemical and radiochemical monitoring of the groundwaters around Lake Karachai. However, microbiological studies of this object have not been carried out. It is known that microorganisms affect the migration of radioactive and non-radioactive waste components [3–6]. Investigation of radionuclide-contaminated media is important both for determination of the composition and functional activity of microbial communities of these extreme environments, and for the development of biotechnologies for bioremediation of contaminated areas. The aim of the work was to determine the physicochemical and radiochemical conditions, the number and phylogenetic diversity of microorganisms, the isolation of dominant bacteria and the assessment of the possibility of their application for *in situ* bioremediation of groundwater from nitrate and reduction of radionuclides migration.

Methods and Algorithms: In this study were used analytical methods for analysis of the chemical and radiochemical composition of groundwater; microbiological methods – for enumeration and isolation of pure cultures of microorganisms; radioisotope methods – for estimation of the rate of microbial processes, sorption and reduction of radionuclides by microorganisms. The prokaryotic community of contaminated groundwater samples was analyzed by sequencing of 16S rRNA genes and Q-PCR of the functional genes of denitrification, nitrite reductase genes (*nirS* and *nirK*).

Results: Groundwater samples were collected from observation wells (at the 20–100 m deep) at a distance of 2.2–4.0 km from Lake Karachai. Water samples contained high concentrations of acetate, oxalate, nitrate and sulfate, as well as radionuclides (especially strontium) and heavy metals. The high redox-potential of the medium contributed to the preservation of radionuclides in a mobile oxidized form. Due to oxidative conditions in groundwater, high abundance of aerobic bacteria (up to 10^5 cells/ml) was detected. Fermentative (up to 10^5 cells/ml), denitrifying (up to 10^4 cells/ml) and iron-reducing bacteria (up to 10^3 cells/ml) were also present, while the number and activity of sulfate-reducing and methanogenic prokaryotes were low. By high-throughput sequencing of 16S rRNA gene, in contaminated groundwater was revealed a diverse microbial community represented mainly by *Proteobacteria*, uncultivated *Patescibacteria* of the

candidate phyla *Microgenomates* (OP11), *Parcubacteria* (OD1), and *Gracilibacteria* (GN02), and ammonium oxidizing archaea of the phylum *Thaumarchaeota* ('*Candidatus Nitrosopumilus koreensis*', '*Candidatus Nitrosotalea devanaterrea*'). More than 50 pure cultures isolated from groundwater samples belonged to 23 genera and 44 species of aerobic organotrophic and anaerobic denitrifying and iron-reducing bacteria. Bacteria of the genera *Pseudomonas*, *Pusillimonas*, *Rhizobium*, *Shewanella*, *Acinetobacter*, *Roseomonas*, *Pantoea*, and *Dechloromonas* reduced nitrates to nitrites and/or molecular nitrogen. The potential of microbial denitrification *in situ* was confirmed by the detection of *nirS/nirK* genes of denitrifying bacteria in the studied water samples. The genomes of strains of the genera *Pusillimonas* and *Roseomonas* contained genes that determine the reduction of nitrates, resistance to heavy metals and metalloids. These strains were also resistant to ionizing radiation. Bacteria of the genera *Shewanella* and *Pantoea* reduced Fe(III), U(VI) and Np(V) in media with acetate or lactate. Under neutral conditions, the isolated bacteria were able to sorb radionuclides (americium, uranium, plutonium, and neptunium) from the solution. The strains differed from each other by the spectrum of mainly sorbed radionuclides. Ultramicrobacteria and ultramicroforms belonging to the genera *Microbacterium*, *Salinibacterium* and *Chryseobacterium* have been isolated from groundwater.

Conclusion: The nitrate- and radionuclide-contaminated groundwater contained diverse population of denitrifying bacteria capable of reducing nitrates to dinitrogen and decreasing the redox-potential of the medium. This contributed to the growth of bacteria that reduced radionuclides and decreased the migration of radionuclides in groundwater. The data obtained are necessary for development of biotechnologies for *in situ* bioremediation of groundwater from nitrate and reduction of radionuclides migration.

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