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Microbiology of the subglacial Lake Vostok: first results of borehole-frozen lake water analysis and prospects for searching for lake inhabitants

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This article examines the question of the possible existence of microbial life inhabiting the subglacial Lake Vostok buried beneath a 4 km thick Antarctic ice sheet. It represents the results of analysis of the only available frozen lake water samples obtained upon the first lake entry and subsequent re-coring the water frozen within the borehole. For comparison, results obtained by earlier molecular microbiological studies of accretion ice are included in this study, with the focus on thermophiles and an unknown bacterial phylotype. A description of two Lake Vostok penetrations is presented for the first time from the point of view of possible clean water sampling. Finally, the results of current studies of Lake Vostok frozen water samples are presented, with the focus on the discovery of another unknown bacterial phylotype w123-10 distantly related to the above-mentioned unknown phylotype AF532061 detected in Vostok accretion ice, both successfully passing all possible controls for contamination. The use of clean-room facilities and the establishment of a contaminant library are considered to be prerequisites for research on microorganisms from Lake Vostok. It seems that not yet recorded microbial life could exist within the Lake Vostok

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water body. In conclusion, the prospects for searching for lake inhabitants are expressed with the intention to sample the lake water as cleanly as possible in order to make sure that further results will be robust.

1. Introduction

The subglacial Lake Vostok is a well-known giant lake in Eastern Antarctica [1, fig. 1]. Many works have been performed on clarifying its geophysics, geology, chemistry, gas content, biogeochemistry and biology, and microbiology in particular [2,3]. The main scientific objective of the lake entry is to search for unusual microbial life that could cope with its extreme conditions—pressure reaching 400 bar, temperature close to freezing point, no light, no dissolved organic carbon [4], very dilute major chemical ions, long-term isolation from the above surface biota (at least 14 Ma) [5] and very probable excess of dissolved oxygen (in the range of 700–1300 mg l^{-1}) [6,7].

It is likely that the lake existed before Antarctic glaciations, which could be suggested based on interpretation of its geological setting [8,9], and numerous life forms could have flourished in the lake before glaciation started. Therefore, it is a big challenge to discover the life, if any, that remained to thrive in the water body of the lake and its sediments. The present-day lake access technique is based on electromechanical ice drilling with the use of kerosene drilling fluid [10,11], which is chemically and microbiologically dirty [12]. Taking into account such a contamination threat and the very small amounts of microbial cells detected in the accretion (naturally frozen lake water) ice [13], further research requires the development of special clean lake entry technologies as well as strict decontamination procedures. It is also crucial to discover a way to prevent contamination of the devices to be lowered into the lake in order to record its physical and chemical parameters, to measure dissolved oxygen content and, finally, to provide researchers with clean samples of water/sediments.

The deepest part of the 5G borehole-recovered ice, starting from the depth of 3539 m, consists of accretion ice, i.e. naturally frozen lake water accreted to the glacier floating above the lake. Drillers cored this ice for a long time and many samples were provided for different studies, including research of French [14–17] and US scientists [18–22]. This ice consists of two layers—accretion ice type I with entrapped minerals, mostly mica–clay (along with small rock fragments) inclusions, originating from the lake shore, and accretion ice type II composed of very clean giant monocrystalline ice originating from the deep part of the lake [2].

Comprehensive analyses (constrained by ancient DNA research criteria and performed with the use of clean-room facilities and microbial DNA-free consumables and reagents) have showed that the microbial biomass of accretion ice is generally very low. Only ice type I containing mica–clay inclusions allowed the discovery of a few bacterial phylotypes all passing numerous contaminant control criteria. They include a well-known chemolithoautotrophic thermophile *Hydrogenophilus thermoluteolus* (100% sequence similarity) (β-Proteobacteria), an actinobacterium related to *Ilumatobacter fluminis* (95% similarity), along with an unidentified unclassified bacterium AF532061 (92% similarity to closest relatives) [23–25]. By contrast, the deeper accretion ice type II with no entrapped sediments gave no reliable signals (no polymerase chain reaction (PCR) signals or only contaminants [25]). It is worth noting that archaeal DNA was not detected in both types of accretion ice.

2. Lake Vostok entry and sampling of borehole-frozen lake water

The first entry into Lake Vostok was performed at the depth of 3769.3 m on 6 February 2012. The water rose up into the borehole to 363 m and froze. With regard to the research programme, a small sample of frozen water that became stuck in a drill bit during this entry, hereafter drillbit water, was provided for biological studies. During the next season, 2012–2013, drillers re-cored the

fast frozen lake water and were able to obtain only 32 m of the ice core. Further drilling operations of re-coring of this frozen lake water were influenced by the old borehole geometry and went out into the pristine glacial ice. Three ice samples of this 32 m long ice core (from the topmost full-cylinder ice core containing kerosene in bubbles to the bottommost moon-shaped 'milky' ice), hereafter borehole-frozen water, were provided for microbiological analyses.

The second entry into the lake occurred at the depth of 3769.15 m some 3 years later, on 25 January 2015. The water rose up again into the borehole to the level of about 70 m and was left to freeze. In 4 days, drillers re-cored the icy 'cork' and obtained about 12 m of the new fast frozen lake water core before the water came up again into the borehole and finally froze at the level of 65–67 m above the ice–water interface. The bottommost 10 cm (of 12 m core) frozen water ice segment was provided for microbiological studies.

However, despite the fact that the Russians were the first to enter a subglacial Antarctic lake at such a great depth (3769 m) and were able to perform the lake entry twice, the drilling technology used (electromechanical drill along with kerosene drilling fluid with the use of foranes (namely Freon 141B, 1,1-dichloro-1-fluoroethane, as densifier)) proved to be inappropriate to collect liquid water in general and clean samples in particular. More technological developments are needed to face such a challenge, including the development of sophisticated systems consisting of well-sealed transport modules preventing the contamination of in-module-encapsulated devices/samplers with the drilling fluid [11] as well as the use of thermal drilling just before the next lake entry, which is now under discussion.

3. First results from borehole-frozen water of Lake Vostok upon first lake entry

The main objective of this study was to search for resident microbial life in the subglacial Lake Vostok by studying the uppermost layer of the water that entered the borehole upon the first lake entry (5 February 2012) and then was frozen, in comparison with thoroughly studied accretion ice [23,24]. The fast frozen water samples included the drillbit water along with three samples of re-cored borehole-frozen water. The fast frozen lake water collected upon the second lake entry in 2015 is still waiting to be analysed.

Both types of fast frozen lake water samples proved to be contaminated with the drilling fluid. The drillbit water sample was heavily polluted with drilling fluid (at ratio 1 : 1) while borehole-frozen water samples were rather clean but still contained numerous microdroplets of drilling fluid, giving the ice a 'milky' appearance. The cell concentrations measured by flow cytofluorometry showed 167 cells per millilitre in the drillbit water sample while borehole-frozen water samples contained from 5.5 to 38 cells per millilitre. At the same time, drill fluid analyses showed about 100 cells per millilitre [12].

The ice samples were strictly decontaminated in the cold-room facilities of LGGE UJF-CNRS, Université J. Fourier, Grenoble, France (with outer core cut out, rinsed with GC-grade pentane and ozone treated—depending on the ice sample facture), and meltwater was processed in clean-room facilities using centrifugal filtering through 5–10 kDa membranes (5 kDa (45.9 nm) allows us to collect 135 kb DNA fragments). Genomic DNA was also extracted in clean-room facilities using bead-based cell lysis kits, plastic ware and solutes that were made DNA-free using 100 kDa membrane filtering and ozone treatment. Primary PCR reactions targeted different regions (v3–v5, v4–v8, v4–v6, v4 and full gene) of 16S rRNA genes and were again processed in clean-room facilities while amplicons generated were analysed outside of these facilities and even not in the same building/country to prevent the clean-room facilities from amplicon carry-over contamination.

A total of 49 bacterial phylotypes were discovered by sequencing of bacterial 16S rRNA genes. Of them, only two phylotypes have successfully passed all contamination criteria, including our own contaminant library [23] consisting of 278 phylotypes (as of June 2014) originating from various contamination sources (e.g. negative PCR, sham DNA extraction, human-associated bacteria, drilling fluid, even dust microparticles in clean-room facilities). With no such library the work on tiny cell biomass is meaningless.

The first remaining phylotype, hereafter w123-10, proved to be a hitherto-unknown type of bacterium showing less than 86% sequence similarity to known taxa. Its phylogenetic assignment to bacterial divisions was also unsuccessful except for the fact that it showed reliable clustering with the above-mentioned unidentified bacterium AF532061 earlier detected in accretion ice. The second phylotype is still dubious in terms of contamination. It showed 93% similarity to *Janthinobacterium* sp. of Oxalobacteraceae (β-Proteobacteria)—well-known 'water-loving' bacteria, though we do not expect to meet a known representative of Oxalobacteraceae in Lake Vostok because of its hitherto known physical–chemical conditions. Notably, no Archaea were detected in all tested samples of frozen lake water.

Regarding 47 contaminant phylotypes detected in drillbit and borehole-frozen lake water samples, they proved to belong to several bacterial divisions Proteobacteria (33 phylotypes), Actinobacteria (six phylotypes), Firmicutes (seven phylotypes) and Bacteroidetes (one phylotype) with predominance of γ-Proteobacteria (19 phylotypes), which indicates that the main sources of contamination were human-associated and soil bacteria. It means that, even working in clean-room facilities and trying to avoid bacterial contamination of the human/soil source, we still face a big challenge and personnel should be taught properly to follow the rules of working with traces of DNA under clean-room conditions. A low amount of Archaea, if any, inhabit human skin, which is why they were reasonably not detected in our PCR trials. At the same time, it means that with no access to clean-room facilities, which includes the use of ultra-clean water, there is no chance to cope with the bacterial contamination while working with valuable dilute DNA samples.

4. Expectations and prospects for searching for lake inhabitants

At present, the most important finding is the unidentified unclassified bacterial phylotype w123-10, which along with another one (AF532061 [23]) may represent indigenous cell populations in the subglacial Lake Vostok provided they are able to cope with the high dissolved oxygen content/highly oxidized environment (at least 320 mg l^{-1}) (V. Lipenkov, personal communication, 2015, at this Theo Murphy meeting), which can represent the main constraint for microbial life in the lake water. At the same time, a kind of proof may come with analyses of a new sample of fast frozen lake water obtained upon second lake entry (25 January 2015).

However, it is worth noting that there is little hope of finding active (propagating) microbial populations at the uppermost lake water level, which contacts directly the overcooled water–glacier interface where the ice accretes. A true challenge would be to collect the water within the whole water column (680 m below the borehole) and especially closer to sediments where the water is expected to be warmer and enriched with mineral nutrients. For this, we need to sample the lake water as cleanly as possible upon further clean lake entry using special water sampling devices well protected from contamination with drilling fluid. Such devices are planned to be developed and manufactured at PNPI NRC KI (Russia) based on the experience of UK 'Ellsworth' project engineers.

Competing interests. The author declares that he has no competing interests.

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