ЭКОЛОГИЯ, БИОЦЕНОЛОГИЯ И БИОГЕОГРАФИЯ
ECOLOGY, BIOCENOLOGY AND BIOGEOGRAPHY

Оригинальная статья / Original paper

https://doi.org/10.30758/0555-2648-2024-70-4-554-564

УДК 579; 57.08; 575.8; 55; 910

Dedicated to Nikolay Vasiliev —
a brilliant person who unsealed Lake Vostok

The uppermost water horizon of subglacial Lake Vostok could be microbial DNA-free, as shown by Oxford Nanopore sequencing technology

Sergey A. Bulat[1,2]✉, Oksana I. Anosova[3], Anna Yu. Tsvetkova[1,4], Alexey V. Shvetsov[1,4]

[1] *Petersburg Nuclear Physics Institute named after B. P. Konstantinov of National Research Centre "Kurchatov Institute", Gatchina, Russia*

[2] *Institute of Physics and Technology, Ural Federal University, Ekaterinburg, Russia*

[3] *Lobachevsky First University Lyceum, Lomonosov Moscow State University branch campus, Ust-Labinsk, Russia*

[4] *Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia*

✉*bulat_sa@pnpi.nrcki.ru*

SAB, 0000-0002-2574-882X; OIA, 0009-0000-0533-7284; AYT, 0009-0005-0749-3420; AVS, 0000-0003-1140-5751

Abstract. The research aimed to search for microbial life in subglacial Lake Vostok. This was done by examining the uppermost layer of water that entered the borehole and froze after the lake was accessed. The sample was collected from a depth of 3721 m and consisted of water-frozen re-cored ice. It underwent thorough decontamination and was melted successively in cold and cleanroom facilities. Genomic DNA was then isolated and amplified using v3-v4 16S rRNA bacterial gene region-specific degenerate primers. The Sanger method and high-throughput Oxford Nanopore sequencing were used to sequence the amplicons generated. The Sanger DNA analysis revealed 16 bacterial phylotypes, and only one of them, 3721v34-24, met all the contamination criteria. This phylotype was the dominant one, making up 41.4 % of the clones and consisting of three allelic variants. However, it remained unclassified and showed 87.7 % similarity to the closest GenBank entry, *Mucilaginibacter daejeonensis* NR_041505 of *Bacteroidota* (family *Sphingobacteriaceae*). The Oxford Nanopore technology generated 21067 reads for the 3721m sample and 3780 for the control one. Among these, 7203 (34 %) and 1988 (53 %) reads for the ice sample and the control one were classified with 93 % accuracy. For the 3721m sample, 21 bacterial phylotypes were identified with an abundance above 0.5 %. Fifteen were identical to the Sanger findings and identified as contaminants. The remaining six were different, either found in the control Nanopore trial or were apparent contaminants. The discovery of phylotype 3721v34-24 in the lake water by Sanger sequencing was unexpected. However, it was later detected in the 3721m sample and control experiments using nanopore sequencing, indicating it was also a contaminant. Thus, the research suggests that the topmost

water layer in Lake Vostok may not contain any microbial DNA. Additional frozen-water samples are currently being analyzed to investigate the issue further.

Keywords: Antarctica, contamination, deep ice coring, frozen lake water, lake unsealing, microbial communities, nanopore sequencing, subglacial Lake Vostok

For citation: Bulat S.A., Anosova O.I., Tsvetkova A.Yu., Shvetsov A.V. The uppermost water horizon of subglacial Lake Vostok could be microbial DNA-free, as shown by Oxford Nanopore sequencing technology. *Arctic and Antarctic Research*. 2024;70(4):554–564. https://doi.org/10.30758/0555-2648-2024-70-4-554-564

Received 08.04.2024 **Revised 13.07.2024** **Accepted 02.09.2024**

Introduction

Lake Vostok is a giant (270 × 70 km, 15800 km^2 area), deep (up to 1.3 km) freshwater liquid body buried in a graben beneath a 4-km thick East Antarctic Ice Sheet with the temperature near the ice melting point (around –2.5 °C) under 400 bar pressure. It is exceptionally oligotrophic and poor in primary chemical ions (compared with the surface snow), under high dissolved oxygen tension (in the range of 320–1300 mg/L), with no light, and sealed from the surface biota about 15 Ma ago [1, 2]. Lake Vostok has a breathtaking history of discovery [3] — starting from assumptions in the 1960s and finishing with complete certainty [4].

Regarding microbiological studies, only our team has worked on 'borehole-frozen' lake water following several cases of lake unsealing. In addition, we are the first to use high-throughput sequencing technologies to recover possible microbial communities in Lake Vostok. Nevertheless, cell concentrations and Sanger finds were reported a decade or more ago. However, regrettably, these studies on natural accretion ice did not adequately address the issue of "foreign" contamination, which is crucial when analyzing such pristine low-biomass samples [5, 6]. At the same time, there are papers by the S. Rogers team [7–9] that reported data (cell counts and DNA study) that appear to be misleading because of contamination issues linked to the limitation of optical microscopy (number of fields to scan) and inappropriate methods implemented (e. g., for DNA isolation, MinElute Virus Spin Kits (QIAGEN, Valencia, CA) were used, which, however, cannot break down all the bacteria). This applies to a recent paper [10], which used an unclear ice sample source and flawed methodology (again, for DNA isolation, MinElute Virus Spin Kits (QIAGEN, Valencia, CA) were employed). Anyway, it would be incorrect to compare studies performed on natural accretion lake ice (due to the features of ice formation—about 1cm per year, and consequent matter fractionation). Those of "borehole-frozen" lake water speedily flow into a borehole from the upper-most water horizon (as with our sample 3721).

Water-frozen (in a borehole) samples have been shown to feature very dilute cell concentrations — from 167 to 38 cells per ml. So far, the 16S rRNA gene Sanger sequencing has yielded three bacterial phylotypes, all meeting numerous contamination criteria. Two phylotypes were reported earlier [11] — the still unidentified and phylogenetically unclassified phylotype w123-10, likely belonging to *Parcubacteria Candidatus Adlerbacteria*, and 3429v3-4, which shows below-genus level (93.5 %) similarity with *Herminiimonas glaciei* of *Oxalobacteraceae* (*Betaproteobacteria*). The third find (phylotype 3698v46-27) has proved to be conspecific with several species of *Marinilactobacillus* of *Carnobacteriaceae* (*Bacillota*), featuring very similar 16S rRNA

genes. Among them is *M. piezotolerans*, isolated from a 4.15 m deep sub-seafloor sediment core collected at 4790.7 m deep Nankai Trough [12].

Our purpose was to search for microbial life in the subglacial Antarctic Lake Vostok by analyzing the uppermost layer of the water that entered the borehole following the lake unsealing at a depth of 3769 m from the surface [13]. The current study aims to re-evaluate microbial finds in a 3721 m borehole-frozen lake water sample obtained with Sanger sequencing applying the high throughput Oxford Nanopore sequencing technology. This technology is now regarded as an attractive tool for studying microbial communities (metataxonomics), even in field conditions [14 and references within], including Antarctica [15].

Materials and methods

The water sample studied was 3721 m deep borehole-frozen re-cored ice (Fig. 1). It was thoroughly decontaminated and melted in cold and cleanroom facilities [16], and the genomic DNA extracted [16] was amplified with 16S rRNA bacterial gene v3-v4 region-specific degenerate primers Merk-341F and Merk-805R [17] for 31+26 cycles using FastStart polymerase (Roche, USA) at 53 °C annealing temperature. The amplicons generated were sequenced by the Sanger technique (Beagle, Saint-Petersburg, Russia). The negative PCR was used as a control.

It is worth noting that the ice segment was rather clear/transparent but had a faint smell of kerosene.

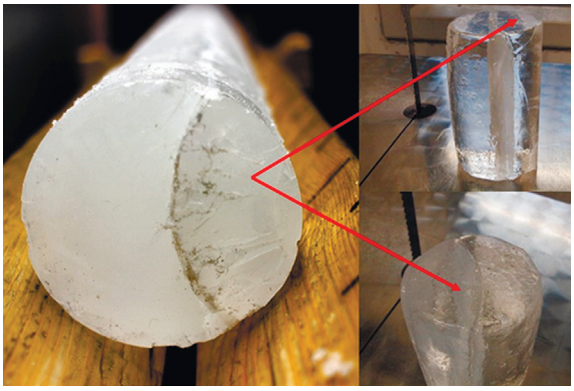

Fig. 1. 5G-3N borehole frozen lake water sample (3720.32–3720.75) as a moon-shape segment. The arrows point to the same ice segment (frozen water)

Рис. 1. Керн льда замерзшей воды (3720,32–3720,75 м) из скважины 5Г-3Н.
Видна «полулунная» структура в результате отклонения скважины при повторном бурении. Стрелки указывают на сегмент льда замершей воды

The MinION device equipped with Flow Cell R9.4 was used for nanopore sequencing. The sequencing run was operated by MinKNOW software (Oxford Nanopore, UK). The sequencing was performed with libraries prepared for 16S rRNA gene v3-v4 region amplicons (about 485 bp). The corresponding kits were implemented — to repair amplicon ends (NEBNext Ultra II End repair/dA-tailing Module reagents E7546), ligate barcodes (Native Barcoding Expansion 1-12 EXP-NBD104), and then sequencing adapters (Adapter Mix II AMII). All these and further steps (like loading the libraries in a flow cell, etc.) followed instructions provided by Oxford Nanopore Technologies. The sequencing was

run for 72 hours. The Fast5 files obtained were basecalled under the "high accuracy" option with trimming barcodes using MinKNOW software. The resulting FastQ files were processed with EPI2ME software (Oxford Nanopore, UK). The Min similarity score was settled at 88 % (given the PCR primers comprise about 9 % of the total amplicon length). Further work was performed on classified reads using GenBank entries.

Results and discussion

Sanger sequencing

The DNA analyses revealed 16 different bacterial phylotypes with a low gene library coverage of 55.2 %, indicating significant biodiversity (Table 1). Of these, only one phylotype, 3721v34-24, met all the contamination criteria [16], including the Contaminant Library, which consists of 329 16S rRNA gene phylotypes.

Phylotype 3721v34-24 was the dominant one, making up 41.4 % of the clones and consisting of three allelic variants. It was taxonomically unclassified — showed 87.7 % similarity (below the family level) with *Mucilaginibacter daejeonensis* NR_041505 of *Bacteroidota* (family *Sphingobacteriaceae*). Some DNA clones with identical sequences were found in GenBank, such as uncultured unidentified *Bacteroidetes* DQ316809 from uranium-contaminated sediment in the USA. Additionally, more sediment clones were found with only a single mismatch, for example, KC431957 and DQ404664, both unidentified. As a result, the new lake-water phylotype was identified as an unclassified "sediment-loving" bacterium and was assigned to the new *Bacteroidota* phylum for the lake inhabitants. Therefore, the newly discovered bacterial phylotype [18] and the three previously recorded phylotypes may represent indigenous cell populations in Lake Vostok.

Table 1

3721 m sample vs. Control amplicons in Sanger vs. Nanopore sequencing

Таблица 1

Образец льда 3721 м в сравнении с контролем нанопорового секвенирования и результатом секвенирования по Сэнджеру

No.	Sanger 3721 m	NANOPORE Control (reads/%)	Taxa classified (closest by DNA similarity)	NANOPORE 3721m (reads/%)	Conclusion Status
1	Cont_+2_3721v34-63 (-12)	15 0.85	*Hyphomicrobium denitrificans*	844 12.70	Cont
2	Cont_+2_3721v34-60 (-19)	136 7.69	*Sphingobium yanoikuyae*	839 12.62	Cont
3	Cont_3721v34-30	172 9.73	*Sphingomonas echinoides*	822 12.37	Cont
4	Cont_3721v34-86	235 13.29	*Cloacibacterium normanense*	784 11.79	Cont
5	Cont_3721v34-105	26 1.47	*Novosphingobium gossypii*	303 4.56	Cont
6	Cont_3721v34-111	1 read	*Corynebacterium tuberculostearicum*	300 4.51	Cont
7	Cont_3721v34-122	ND	*Psychrobacter cibarius* Fermented seafood	246 3.70	Cont
8	Cont_3721v34-29	13 0.74	*Acinetobacter junii*	141 2.12	Cont

End of Table 1

Окончание табл. 1

No.	Sanger 3721 m	NANOPORE Control (reads/%)	Taxa classified (closest by DNA similarity)	NANOPORE 3721m (reads/%)	Conclusion Status
9	95.5 %* Cont-drill_3721v34-17	8 reads	*Novosphingobium Naphthalenivorans* (Genus level)	120 1.81	Cont
10	Cont_3721v34-117	24 1.36	*Cutibacterium acnes*	99 1.49	Cont
11	Cont_3721v34-1	50 2.83	*Diaphorobacter polyhydroxybutyrativorans (1546149)*	94 1.41	Cont
12	Cont_3721v34-89	12 0.68	*Phenylobacterium koreense*	88 1.32	Cont
13	Cont_3721v34-114	1 read	*Methylobacterium jeotgali*	87 1.31	Cont
14	ND	6 reads	*Sphingobium scionense*	79 1.19	Cont
15	Cont_excSK24	ND	*Staphylococcus warneri*	68 1.02	Cont
16	ND	ND	*Psychrobacter immobilis* Human clinical, infection	66 0.99	Cont
17	Cont_w22-59 Cont_+16_w23-22	17 0.96	*Rothia amarae*	54 0.81	Cont
18	ND	8 reads	*Cloacibacterium rupense*	46 0.69	Cont
19	ND	23 1.30	*Acinetobacter tjernbergiae*	43 0.65	Cont
20	ND	11 0.62	*Sphingomonas kyungheensis*	40 0.60	Cont
21	ND	ND	*Veillonella rogosae* Human oral microbiome	37 0.56	Cont
	87.7 %* +12_3721v34-24	ND	*Mucilaginibacter daejeonensis* Rice straw	3 (88.8%*) 0.04	Cont
	85.6 %* +12_3721v34-24	5 reads (88.8%*)	*Mucilaginibacter jinjuensis* Rotten wood	14 (89.4%*) 0.2	Cont

Note. Blastn data (classified reads above 0.5% abundance). Nanopore Control — control trial (sham DNA isolation/negative PCR, etc); Cont — contaminant ('Cont_' in the 'Sanger' column means the phylotype is in our Contaminant Library); ND — not detected; * — similarity score (%); if there is no indication, the percentage is 98 % or more. Despite the very low similarity score, the Epi2Me Oxford Nanopore software classified these reads as *Mucilaginibacter ssp.*

Примечания. Ампликоны классифицированы с использованием программы NCBI Blastn. Данные приведены для встречаемости таксонов 0.5 % и выше. В предпоследней колонке приведен окончательный статус таксонов. Нанопоровый контроль — «холостая» экстракция ДНК/негативная ПЦР; Cont — контаминант ('Cont_' в первой колонке "Sanger 3721 m" означает, что данный филотип присутствует в нашей библиотеке контаминантов); ND — не обнаружено; * — сходство (%); если нет указаний, то значение 98 % и выше; несмотря на очень низкое сходство (ниже уровня семейства); программа для классификации (Epi2Me Oxford Nanopore) с использованием данных в GenBank, классифицировала нанопоровые прочтения как *Mucilaginibacter ssp*.

Nanopore sequencing

To clarify the latest finding, phylotype 3721v34-24 from the ice-frozen water core 3721 m was re-tested using high-throughput nanopore sequencing with the same amplicon for the v3-v4 region of the 16S rRNA gene. This study included nanopore controls (sham DNA isolation/negative PCR and 'carry-over' contamination during nanopore library preparation) for the first time (see Fig. 2).

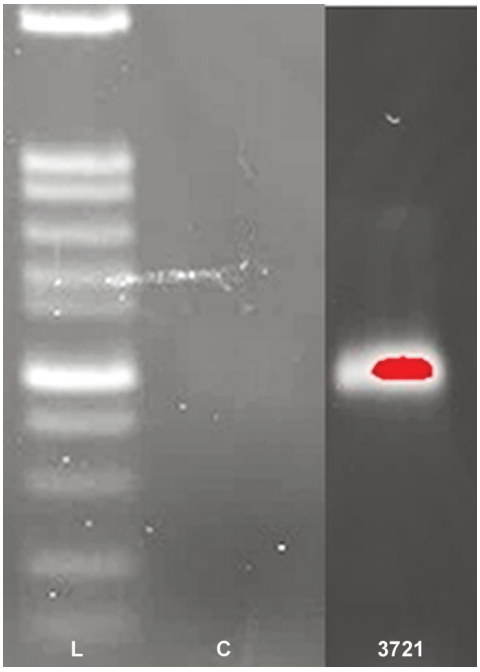

Fig. 2. True signal vs. Control in Nanopore sequencing.

1.7 % composite agarose gel stained with Ethidium Bromide. L — 100 bp ladder (bright band below — 500 bp in size); C — sham DNA isolation followed by negative PCR (Ambient RNA-free water); 3721 m sample amplicon. The recording was performed with ChemiDoc (Thermo Fisher Scientific, USA), which allowed us to highlight the band intensity (in red — overexposure) in trying to detect a signal in the control lane

Рис. 2. «Истинный» сигнал ампликона образца 3721 м в сравнении с сигналом контроля (фактически его отсутствием) в нанопоровом секвенировании.

Использовали 1,7 % композитный агарозный гель с окрашиванием этидием бромидом. L — 100 п. о. (пар оснований) маркер молекулярный весов (яркий фрагмент снизу — размер 500 п. о.); C — контрольный ампликон («холостая» экстракция ДНК в ПЦР); ампликон образца 3721 м. Детекция сигнала выполнена с использованием прибора ChemiDoc (Thermo Fisher Scientific, USA) с переэкспозицией сигнала (красный цвет) с целью выявить какой-либо сигнал в контроле

After performing "high accuracy — trim barcodes" basecalling, we obtained 21067 reads for the 3721 m sample and 3780 for the control one (no visible amplicon). Of these, 7203 reads (34 %) for the ice sample and 1988 reads (53 %) for the control sample were classified with 93 % accuracy. For the 3721 m sample, we identified 21 bacterial phylotypes above 0.5 % abundance (Table 1). Among these, 15 phylotypes matched Sanger's findings, while the remaining six phylotypes were unique to nanopore sequencing and were found in the control Nanopore trial, indicating contamination. One phylotype unique to nanopore sequencing, *Psychrobacter immobilis*, was also considered a contaminant due to its known origin from a human clinical source [19]. The same was true with *Veillonella rogosae* [20]. Therefore, all the 21 phylotypes discovered in nanopore sequencing above 0.5 % abundance were identified as contaminants (identical to either Sanger contaminants or control findings). In terms of accuracy, nanopore sequencing (> 97.69 similarity) was found to be superior compared to Sanger readings (100 % accuracy) for our amplicons (Fig. 3).

Phylotype 3721v34-24 was identified in Sanger sequencing with 12 clones, which may indicate a population of living cells. This phylotype was detected in the 3721 m sample with three nanopore reads (0.04 % abundance) and approximately 20 reads for all the three closely related species. Additionally, it was found in the control sample studied with five

1: +12_3721v34-24_87.68-Mucilaginibacter_daejeonensis	100.00	99.04	99.52	99.28	99.28
2: da6fc4b1-3b6a-4293-98ec-d1545f8f087b_CONTROL	99.04	100.00	98.64	97.69	97.95
3: r-c_b89629ae-7402-420b-8fee-de2c8de0573a_CONTROL	99.52	98.64	100.00	98.15	98.19
4: f9cd4690-d61c-476f-a134-9ce78793252a_NANO-LV3721	99.28	97.69	98.15	100.00	97.91
5: 56d03a9b-24fa-42a9-8dcf-b62f580d4599_NANO-LV3721	99.28	97.95	98.19	97.91	100.00

Fig. 3. Distance matrix of Nanopore reads (sequences) related to Sanger clone 3721v34-24.

+12_3721v34-24 — Sanger phylotype sequence from 3721 m sample; CONTROL — Nanopore reads from the control; NANO-LV3721 — Nanopore reads from 3721 m sample; r-c — reverse complement strand

Рис. 3. Матрица дистанций (%) последовательностей нанопоровых прочтений в сравнении с клоном по Сэнджеру 3721v34-24.

+12_3721v34-24 — филотип по Сэнджеру образца 3721 м; CONTROL — нанопоровые прочтения контрольного апликона; NANO-LV3721 — нанопоровые прочтения ампликона образца 3721 м; r-c — «обратно-комплементарная» нить ДНК

reads, although this species was not identical but only closely related to M*ucilaginibacter jinjuensis.* Even when using the "super-accurate" option for basecalling, the 3721 m sample yielded only five reads.

In contrast, when seven additional nanopore controls from other sequencing experiments were examined for *Mucilaginibacter spp.*, these species were represented by significantly higher read numbers, with 23 reads for *Mucilaginibacter daejeonensis* and 424 reads for the closely related *Mucilaginibacter jinjuensis* (Table 2). This suggests that they may be contaminants.

Table 2

***Mucilaginibacter spp.* — related phylotypes in Sanger and Nanopore sequencing, including control trials**

Таблица 2

Филотипы, сходные с *Mucilaginibacter spp.*, в секвенировании по Сэджеру и нанопоровом секвенировании, включая нанопоровый контроль

Controls Max reads	Taxa	LV3721 Nanopore reads	LV3721 Sanger finds
23	*Mucilaginibacter daejeonensis**	3 (88–91 % similarity)	87.7 % + 12_3721v34-24
424	*Mucilaginibacter jinjuensis**	14 (88–91 % similarity)	85.6 % + 12_3721v34-24

*— Despite a very low similarity score (below family level), the Epi2Me Oxford Nanopore software classified these reads as *Mucilaginibacter ssp.*

* — Несмотря на очень низкое сходство (ниже уровня семейства), программа для классификации (Epi2Me Oxford Nanopore) с использованием данных GenBank классифицировала нанопоровые прочтения как *Mucilaginibacter ssp.*

Phylotype 3721v34-24, linked to *Mucilaginibacter daejeonensis*, was discovered in Sanger sequencing [18]. However, it cannot represent findings from Lake Vostok. The lake's uppermost water layers may be free of microbial DNA [21]. Additional frozen water samples, 16S rRNA gene regions, and other Sanger findings are currently being processed through nanopore sequencing to resolve this issue.

The assumption that the Lake Vostok water body, especially its uppermost layers, could be free of microbes is not as unusual as many people might think. The belief that

microbes inhabit the Earth in all possible places may not apply to exceptional cases such as Lake Vostok. The only factor that can prevent microbes from living there is the extremely high oxygen levels, which have been calculated but not yet measured. If the estimated values of around 800 mg/L [22] are accurate, then no life as we know it, including environmental DNA, should be expected to be found there. The lake could be considered a "cold oxygen reactor" (quoting Chris McKay). Despite life's ability to adapt quickly to environmental changes (e.g., the lake's ice cover), it may not withstand them and could become extinct following the Gaian bottleneck hypothesis [23].

Conclusions

Interpreting the findings carefully when analyzing ice/snow samples for microbial content with very low biomass is essential. The high throughput Oxford Nanopore sequencing technology allows one to more precisely identify previously discovered phylotypes. Thus, our research has revealed that phylotype 3721v34-24, previously recovered by the Sanger technique and thought to be related to organisms from the lake, has turned out to be a contaminant. The study also suggests that the status of three bacterial phylotypes identified earlier (100 % — *Marinilactobacillus sp*. of *Bacillota*, family *Carnobacteriaceae*) (< 86 % known taxa, *Parcubacteria* Candidatus *Adlerbacteria*), 3429v3-4 (93.5 % — *Herminiimonas* sp. of *Betaproteobacteria*, family *Oxalobacteraceae*) and thought to represent native cell populations in Lake Vostok [11, 12] is unclear and needs to be further investigated.

Competing interests. No conflict of interests is involved.

Funding. The research was carried out partly within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme №1023031500033-1-1.6.7;1.6.4;1.6.8) and was partially funded by the Ministry of Science and Higher Education of the Russian Federation (Ural Federal University Program of Development within the Priority-2030 Program).

Acknowledgements. We want to express our gratitude to RAE (V. Lukin), AARI (V. Lipenkov, A. Ekaykin), and a group of colleagues from the Mining Institute for providing us with ice samples and relevant information for the study. We especially thank Prof Nikolay Vasiliev, whose outstanding contribution and activity in Vostok ice drilling made this work possible.

Конфликт интересов. Конфликт интересов отсутствует.

Финансирование. Работа выполнена частично в рамках государственного задания Министерства науки и высшего образования Российской Федерации (тема №1023031500033-1-1.6.7;1.6.4;1.6.8 «Функциональная и структурная организация сложных, мультикомпонентных биологических систем и их динамика») и частично при финансовой поддержке Министерства науки и высшего образования Российской Федерации в рамках Программы развития Уральского федерального университета имени первого Президента России Б.Н. Ельцина в соответствии с программой стратегического академического лидерства «Приоритет-2030».

Благодарности. Выражаем благодарность РАЭ (В.В. Лукину), ААНИИ (В.Я. Липенкову и А.А. Екайкину), а также группе буровиков из Санкт-Петербургского горного университета за предоставление образцов льда и необходимую информацию. Особая признательность Николаю Ивановичу Васильеву, без вклада и активности которого работа была бы невозможна.

REFERENCES

1. Bulat S., Petit J.R. Vostok, Subglacial Lake. In: Gargaud M. (ed.) *Encyclopedia of Astrobiology.* Berlin, Heidelberg: Springer; 2023. P. 3206–3212. https://doi.org/10.1007/978-3-662-65093-6_1765
2. Siegert M.J. Lakes beneath the ice sheet: the occurrence, analysis, and future exploration of Lake Vostok and other Antarctic Subglacial Lakes. *Annual Review of Earth and Planetary Sciences.* 2005;33:215–245. https://doi.org/10.1146/annurev.earth.33.092203.122725
3. Kotlyakov V.M., Krenev V.A. Who discovered the Lake Vostok? *Led i Sneg = Ice and Snow.* 2016;56(3):427–432. (In Russ). https://doi.org/10.15356/2076-6734-2016-3-427-432
4. Kapitsa A.P., Ridley J.K., Robin G. de Q., Siegert M.J., Zotikov I.A. A large deep freshwater lake beneath the ice of central East Antarctica. *Nature.* 1996;381:684–686. https://doi.org/10.1038/381684a0
5. Priscu J.C., Adams E.E., Lyons W.B., Voytek M.A., Mogk D.W., Brown R.L., McKay C.P., Takacs C.D., Welch K.A., Wolf C.F., Kirshtein J.D., Avci R. Geomicrobiology of subglacial ice above Lake Vostok, Antarctica. *Science.* 1999;286:2141–2144. https://doi.org/10.1126/science.286.5447.2141
6. Karl D.M., Bird D.F., Bjorkman K., Houlihan T., Shakelford R., Tupas L. Microorganisms in the accreted ice of Lake Vostok. *Science.* 1999;286:2144–2147. https://doi.org/10.1126/science.286.5447.2144
7. D'Elia T., Veerapaneni R., Rogers S.O. Isolation of microbes from Lake Vostok accretion ice. *Applied Environmental Microbiology.* 2008;74:4962–4965. https://doi.org/10.1128/AEM.02501-07
8. Shtarkman Y.M., Koçer Z.A., Edgar R., Veerapaneni R.S., Morris P.F., Rogers S.O. Subglacial Lake Vostok (Antarctica) accretion ice contains a diverse set of sequences from aquatic, marine and sediment-inhabiting bacteria and eukarya. *PLOS ONE.* 2013;8(7):e67221. https://doi.org/10.1371/journal.pone.0067221
9. Rogers S.O., Shtarkman Yu.M., Koçer Z.A., Edgar R., Veerapaneni R., D'Elia T. Ecology of subglacial Lake Vostok (Antarctica), based on metagenomic/metatranscriptomic analyses of accretion ice. *Biology.* 2013;2:629–650. https://doi.org/10.3390/biology2020629
10. Epova E.Y., Shevelev A.B., Akbayev R.M., Biryukova Y.K., Zylkova M.V., Bogdanova E.S., Guseva M.A., Tynio Y.Y., Egorov V.V. Heterotrophic microbiota from the oligotrophic waters of Lake Vostok, Antarctica. *International Journal of Environmental Research and Public Health.* 2022;19:4025. https://doi.org/10.3390/ijerph19074025
11. Bulat S.A. Microbiology of the subglacial Lake Vostok: first results of borehole-frozen lake water analysis and prospects for searching lake inhabitants. *Philosophical Transactions of the Royal Society A* . 2016;374:20140292. https://doi.org/10.1098/rsta.2014.0292
12. Bulat S. Subglacial Antarctic Lake Vostok vs. subglacial South Pole Martian Lake and hypersaline Canadian Arctic Lakes — prospects for life. In: *Proceeding of the JpGU Meeting 2019, Makuhari Messe, Chiba, Japan.* 2019;PPS04-12. https://confit.atlas.jp/guide/event-img/jpgu2019/PPS04-12/public/pdf?type=in
13. Lukin V.V., Vasiliev N.I. Technological aspects of the final phase of drilling borehole 5G and unsealing Vostok subglacial lake, East Antarctica. *Annals of Glaciology.* 2014;55(65):83–89. https://doi.org/10.3189/2014AoG65A002
14. Ciuffreda L., Rodríguez-Pérez H., Flores C. Nanopore sequencing and its application to the study of microbial communities. *Computational Structural Biotech Journal.* 2021;19:1497–1511. https://doi.org/10.1016/j.csbj.2021.02.020
15. National Academies of Sciences, Engineering, and Medicine. *Technology developments to advance Antarctic research: Proceedings of a workshop.* Washington, DC: The National Academies Press; 2022. https://doi.org/10.17226/26699
16. Bulat S.A., Alekhina I.A., Blot M., Petit J.-R., de Angelis M., Wagenbach D., Lipenkov V.Ya., Vasilyeva L.P., Wloch D.M., Raynaud D., Lukin V.V. DNA signature of thermophilic bacteria from the aged accretion ice of Lake Vostok, Antarctica: implications for searching for life in extreme icy environments. *International Journal of Astrobiology.* 2004;3(1):1–12. https://doi.org/10.1017/S1473550404001879

17. Merkel A.Y., Pimenov N.V., Rusanov I.I., Slobodkin A.I., Slobodkina G.B., Tarnovetckii I.Yu., Frolov E.N., Dubin A.V., Perevalova A.A., Bonch-Osmolovskaya E.A. Microbial diversity and autotrophic activity in Kamchatka hot springs. *Extremophiles.* 2017;21:307–317. https://doi.org/10.1007/s00792-016-0903-1

18. Bulat S.A., Doronin M.V., D.A. Sumbatyan D.A. New microbial finds in the subglacial Antarctic Lake Vostok. In: *Proceedings of the The eleventh Moscow solar system symposium 11M-S3*; *October 5-9, 2020, Moscow.* Moscow: Space Research Institute RAS; 2020. P. 102. https://doi/org/0.21046/11MS3-2020

19. Sriaroon P., Elizalde A., Perez E.E., Leiding J.W., Aldrovandi G.M., Sleasman J.W. *Psychrobacter immobilis* septicemia in a boy with X-linked chronic granulomatous disease and fulminant hepatic failure. *J. Clin. Immunol.* 2014;34(1):39–41. https://doi.org/10.1007/s10875-013-9961-7

20. Gomes da Rocha I.M., Torrinhas R., Fonseca D., de Oliveira Lyra C., de Sousa J.L., Neri A., Balmant B.D., Callado L., Charlton K., Queiroz N., Waitzberg D.L. Pro-inflammatory diet Is correlated with high *Veillonella rogosae*, gut inflammation and clinical relapse of inflammatory bowel disease. *Nutrients.* 2023;15(19):4148. https://doi.org/10.3390/nu15194148

21. Bulat S., Doronin M., Sumbatyan D. The uppermost water horizon of the subglacial Antarctic Lake Vostok is microbial DNA-free as proven by Oxford Nanopore sequencing technology. In: Full Abstract Book. *Antarctica in a changing World. SCAR Open Science Conference 2022, 1–10 August, 2022; Hyderabad, India*. 2022; P. 612.

22. Lipenkov V.Y., Ekaykin A.A., Polyakova E.V., Raynaud D. Characterization of subglacial Lake Vostok as seen from physical and isotope properties of accreted ice. *Philosophical Transactions of the Royal Society A* 2016;374:20140303. https://doi.org/10.1098/rsta.2014.0303

23. Chopra A., Lineweaver C.H. The case for a Gaian Bottleneck: The biology of habitability. *Astrobiology.* 2016;16:7–22. https://doi.org/10.1089/ast.2015.1387

Возможная стерильность верхнего водного горизонта подледникового антарктического озера Восток по данным нанопорового секвенирования

С.А. Булат[1,2] ✉, О.И. Аносова[3], А.Ю. Цветкова[1,4], А.В. Шевцов[1,4]

[1] *Петербургский институт ядерной физики им. Б.П. Константинова Национального исследовательского центра «Курчатовский институт», Гатчина, Россия*

[2] *Институт физики и технологии, Уральский федеральный университет, Екатеринбург, Россия*

[3] *Первый университетский лицей имени Н.И. Лобачевского — филиал Московского государственного университета имени М.В. Ломоносова, Усть-Лабинск, Россия*

[4] *Санкт-Петербургский политехнический университет Петра Великого, Санкт-Петербург, Россия*

✉*bulat_sa@pnpi.nrcki.ru*

САБ, 0000-0002-2574-882X; ОИА, 0009-0000-0533-7284; АЮЦ, 0009-0005-0749-3420; АВШ, 0000-0003-1140-5751

Аннотация. Целью исследования был поиск микробной жизни в подледниковом антарктическом озере Восток путем изучения верхнего слоя воды, попавшей в скважину и замерзшей в ней после того, как озеро было вскрыто. Образец был получен из скважины на глубине 3721 м и состоял изо льда замерзшей

озерной воды. Он был тщательно деконтаминирован, расплавлен в чистом помещении, и выделенная геномная ДНК была амплифицирована с использованием вырожденных праймеров, специфичных для области v3-v4 бактериальных генов 16S рРНК. Для секвенирования полученных ампликонов использовали метод Сэнджера и технологию высокопроизводительного секвенирования Oxford Nanopore. Анализ ДНК по методу Сэнджера выявил в общей сложности 16 бактериальных филотипов, из которых только один филотип, 3721v34-24, прошел все критерии на контаминацию. Этот филотип был доминирующим и включал 41,4 % клонов с тремя аллельными вариантами, но остался неклассифицированным, показав 87,7 % сходства с ближайшим таксоном в GenBank *Mucilaginibacter daejeonensis* NR_041505 из филума *Bacteroidota* (семейство *Sphingobacteriaceae*). Технология Oxford Nanopore секвенирования дала 21067 прочтений для образца 3721 м и 3780 прочтений для контроля. Из них 7203 (34 %) и 1988 (53 %) прочтений для образца льда и контроля, соответственно, были классифицированы с аккуратностью 93 %. Для образца 3721 м был идентифицирован 21 бактериальный филотип с численностью таксонов выше 0,5 %. Пятнадцать из них оказались общими с находками по Сэнгеру, а остальные шесть были уникальными, но присутствовали в нанопоровом контроле или оказались очевидными контаминантами. Пятнадцать филотипов, совпадающих с таковыми по Сэнджеру, были определены как контаминанты. Филотип по Сэнджеру 3721v34-24, который считался истинной находкой для воды озера, в нанопоровом секвенировании был обнаружен как в образце льда 3721 м, так и контроле, т. е. был также отнесен к контаминантам. Таким образом, самый верхний горизонт воды в озере Восток может не содержать микробной ДНК. Для прояснения этого вопроса проводятся дальнейшие исследования замерзших в скважине проб воды.

Ключевые слова: Антарктида, вскрытие озера, глубокое бурение во льду, загрязнение, замерзшая озерная вода, микробные сообщества, нанопоровое секвенирование, подледниковое озеро Восток

Для цитирования: Bulat S.A., Anosova O.I., Tsvetkova A.Yu., Shvetsov A.V. The uppermost water horizon of subglacial Lake Vostok could be microbial DNA-free, as shown by Oxford Nanopore sequencing technology. *Проблемы Арктики и Антарктики*. 2024;70(4):554–564. https://doi.org/10.30758/0555-2648-2024-70-4-554-564

Поступила 08.04.2024 **После переработки 13.07.2024** **Принята 02.09.2024**

Подписано в печать 12.12.2024 Печать цифровая
Формат 70×100 1/16 Печ. л. 9,5
Тираж 85 Заказ №

ООО «Репроцентр»,
170006, Россия, г. Тверь, Беляковский переулок, д. 46, пом. 25.

Signed to print 12.12.2024 Digital Printing
Format 70×100 1/16 P.s. 9,5
Edition 85 Order No

LLC “Reprocenter”,
170006, Russia, Tver, Belyakovsky lane, 46, office 25.