MICROBIAL LIFE IN EXTREME SUBGLACIAL ANTARCTIC LAKE ENVIRONMENTS: LAKE VOSTOK

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The objective of the study was to estimate microbial content in accretion (frozen lake water) ice from Lake Vostok buried beneath 4-km thick East Antarctic ice sheet. The ultimate goal of the study is to discover microbial life in this extreme icy environment featured by very long isolation, no light, high pressure, close to zero temperature, ultra-low DOC and excess of oxygen. The PCR based prokaryotic 16S ribosomal RNA gene sequencing constrained by Ancient DNA research criteria of authentication was used as a main approach. The Vostok accretion ice consists of two layers - upper ice 1 with sediment inclusions and deeper ice 2 which is very clean. Both layers feature ultra-low total gas content with no oxygen, methane and hydrogen sulfide but with carbon dioxide a bit enriched (anaerobic conditions) and ultra-low DOC content (ultra-oligotrophic conditions). The only ice 1 shows mostly oxidized sulfur compounds while no nitrate. This means that possible life-supporting redox couples seem to be limited to hydrogen as the only reducer and sulfates and carbon dioxide as e-acceptors and carbon source. Thus, for the accretion ice 1 we could expect to discover anaerobic chemoautotrophic piezophilic psychrophiles while for the lake water – 'oxygenophilic' chemoautotrophic piezophilic psychrophiles. However, the DNA study showed that accretion ice is essentially bacterial and archaeal DNA-free. A few bacterial phylotypes were recovered from accretion ice 1 while the cleaner ice 2 showed no reliable biological signals as did the deep old glacial ice horizons. Supporting this, the flow cytometry succeeded in estimating 0.6 - 9 cells/ml in both accretion ice types while direct microscopy failed to detect any cells. This indicates ultra low biomass in accretion ice and highlights the importance of stringent decontamination measures. Of findings from accretion ice 1, the only chemolithoautotrophic bacterium detected proved to be the true thermophile *Hydrogenophilus thermoluteolus* which seems to originate from within deep faults in the lake bedrock. Thus, the Vostok accretion ice 1 contains very low biomass indicating that the water body should also be hosting a highly sparse life, if any.

Keywords: microbial ecology, molecular phylogenetics, exobiology, subglacial lakes, Antarctica, accretion ice, bacteria, extremophiles, thermophiles, redox reactions, high oxygen tension, low biomass, contamination.

Abbreviations: SALE – subglacial Antarctic lake environments; DOC – dissolved organic carbon; PCR – polymerase chain reaction.

Introduction

Since subglacial Antarctic lake environments (Fig. 1) and especially the biggest of known Lake Vostok (Fig. 2) are thought to analogues for extraterrestrial environments such as Europa and Enceladus (Fig. 3), the ability of these environments to support microbial life is hotly discussed. While the presence of microbial cells in the Lake Vostok, East Antarctica has been inferred from accretion ice (frozen lake water) analyses [1, 2], (Fig. 4), the likelihood of 'forward-contamination' of these samples (e.g., [3]) necessitates caution when interpreting the results [4, 5].

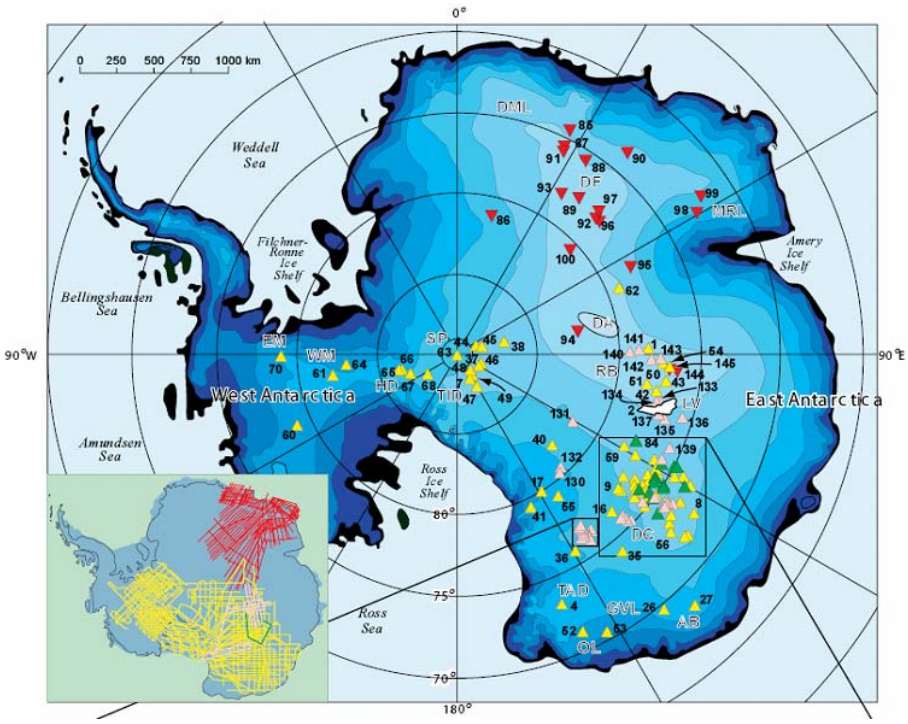

Fig. 1. Subglacial Antarctic lake (aquatic) environments discovered by remote sensing technologies until now [6]

The objective of our study was to estimate microbial content and diversity in accretion ice from Lake Vostok buried beneath 4-km thick East Antarctic ice sheet [7] (Fig. 5). The ultimate goal of the study is to discover microbial life in this extreme icy environment featured by ~15 Ma long isolation, no light, high pressure (about 400 bars), low temperature (close to the freezing point), ultra-low DOC content, and predicted excess of dissolved oxygen (Fig. 6).

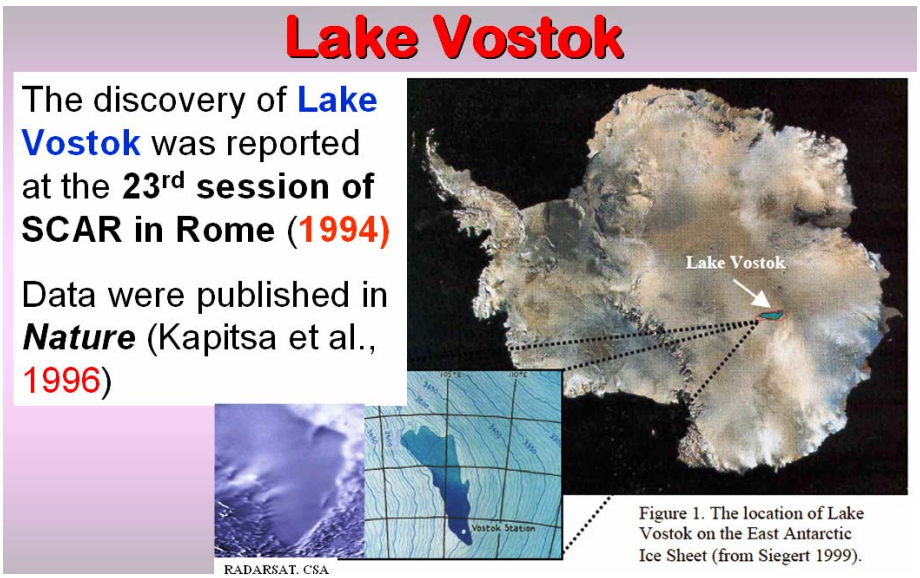

Fig. 2. Discovery of the Lake Vostok in Antarctic and its view from space (radar altimetry from satellite)

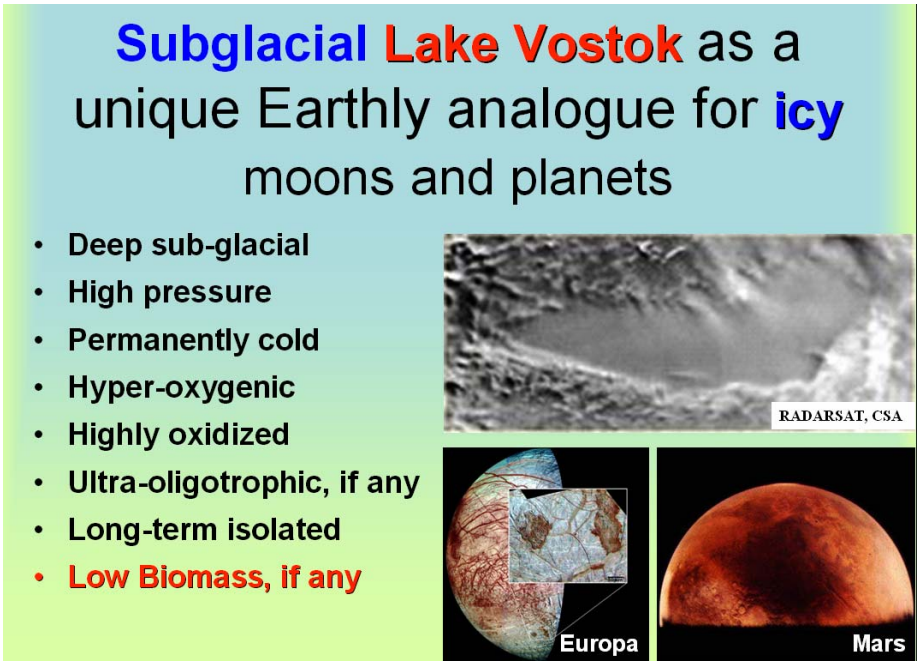

Fig. 3. Subglacial Lake Vostok as an analogue for icy planets and moons

The PCR based bacterial (and archaeal) 16S ribosomal RNA gene sequencing constrained by Ancient DNA research criteria of authentication [8] was used as a main approach (Fig. 7, Fig. 8). The flow cytometry was implemented for cell enumerating.

Fig. 4. Two types of Vostok accretion ice (type 1 and type 2) and microbial cell levels as detected by flow fluorocytometry (D. Marie, pers. comm.)

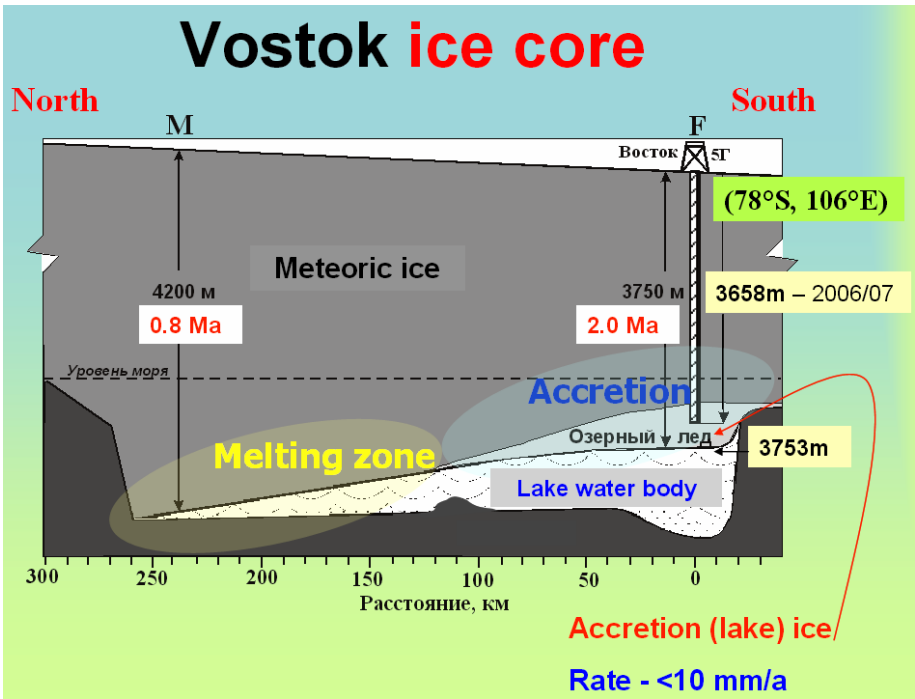

Fig. 5. Sketch of Vostok ice core at longitudinal sectioning of Lake Vostok

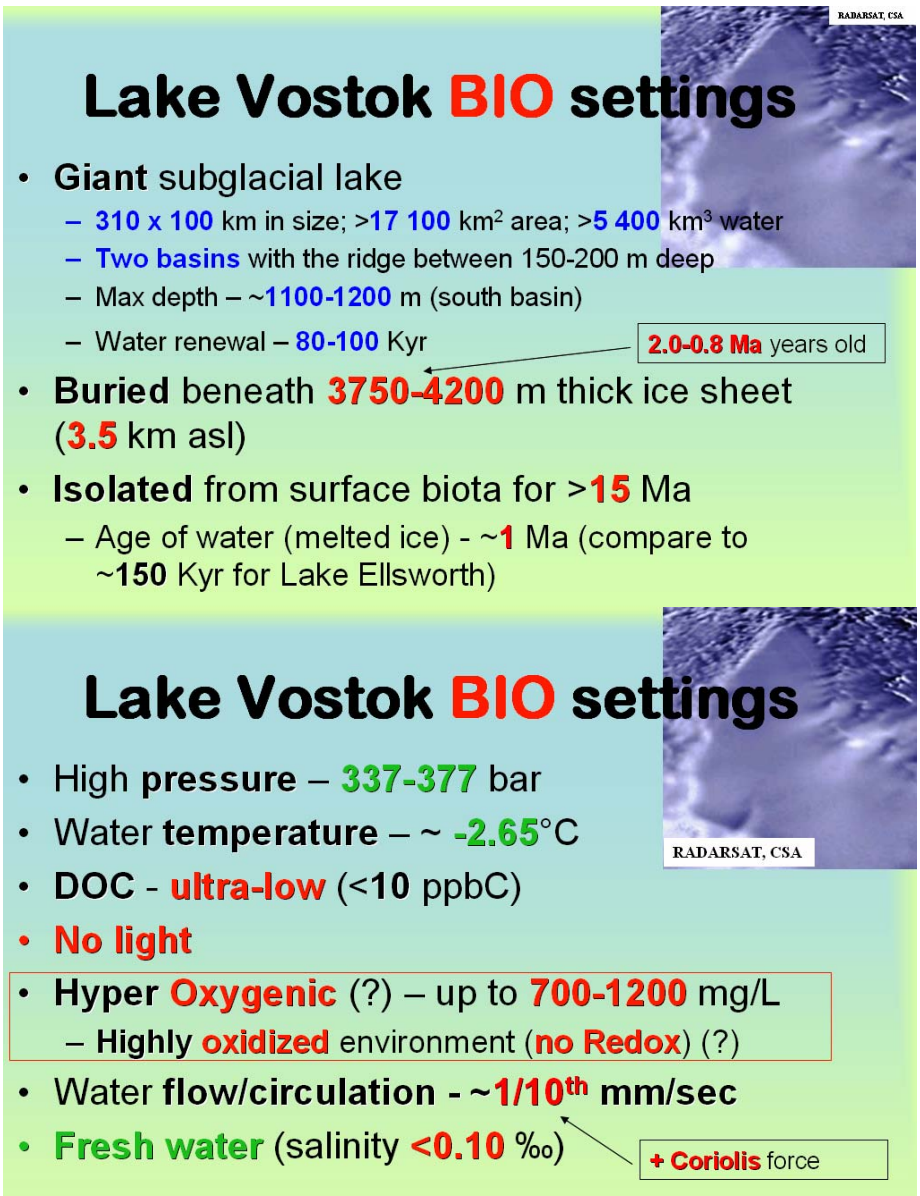

Fig. 6. Lake Vostok (water column) biological features

Biogeochemical assessment of lake ice and water column

The accretion ice retrieved by deep ice coring at Vostok [9] (Fig. 9) provides the best present-day template and unique opportunity for searching for life in the subglacial lake environment. This ice consists of two layers – type 1 at the top featured by presence of small inclusions of mica-clay sediments and thought to originate from a shallow depth bay along the glacier flow line towards Vostok, and type 2 at the bottom containing nothing of prominent (very clean) (Fig. 4). Both accretion ice types features ultra-low total gas content (2-3 order of magnitude lower than in glacier ice) including absence of oxygen, methane and hydrogen sulfide but a bit enriched with carbon dioxide (meaning anaerobic conditions) and ultra-low DOC content (< 10 ppbC) (meaning ultra-oligotrophic conditions) (Fig. 10). Additionally, the only ice 1 shows mostly oxidized sulfur compounds while no nitrate. Hydrogen is suspected in the lake ice but remains to be measured. This means that possible redox couples capable to support the life seem to be limited to hydrogen as the only reducer and sulfates and carbon dioxide as electron acceptors and carbon source.

Fig. 7. Performing reliable analysis of ice core biological content

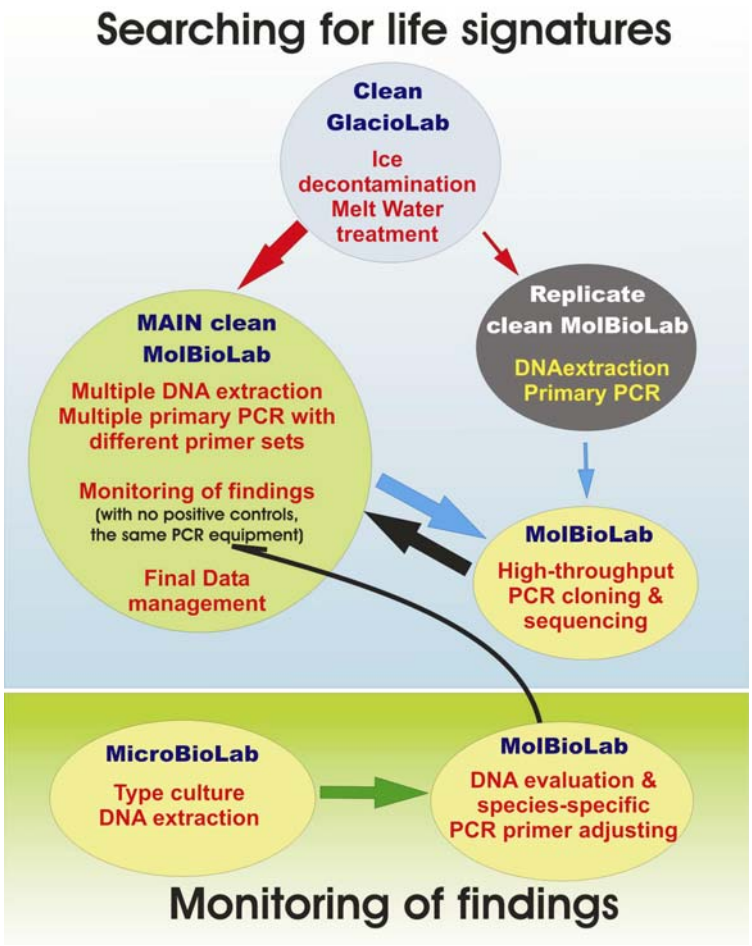

Fig. 8. Laboratory network for reliable analysis of biological content of ice core

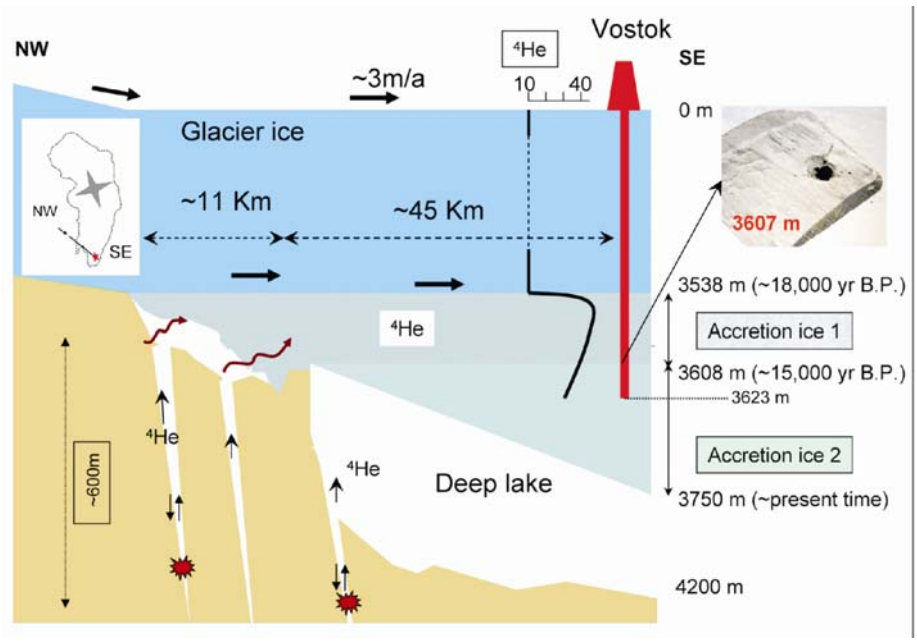

Fig. 9. Sketch of Vostok ice core at perpendicular sectioning of Lake Vostok (ice flow from left to right)

Lake ice BIO features

Accretion ICE	GAS content*	DOC (ppbC)	Major ions*
TYPE 1 Sediments	Very low (10^{-2}) CO_2 (no O_2, CH_4, H_2S)	10 ± 9	Mg^{2+} Ca^{2+} SO_4^{2-} (S^{2-}) (no NH_4^+, nitrate) pH – 7.0?
TYPE 2 Very clean	Ultra-low (10^{-3}) (no O_2, CH_4, H_2S)	5.6 ± 4.6 (n = 14)	- (no NH_4^+, nitrate) pH – 7.0
↓ Water	Low Aerobic →Anaerobic	Ultra-oligotrophic	No Redox? (Hydrogen?)

*enriched as compared to glacial ice

Fig. 10. Vostok accretion ice biological features

With such knowledge about the lake ice and what is predicted for the open lake water (provided the lake ice indeed reflects the lake water) the following expectations in terms of possible microbial life forms can be inferred. For the accretion ice (namely ice 1) we could expect to discover anaerobic chemoautotrophic piezophilic psychrophiles (Fig. 11, Fig. 12) while for the open lake – 'oxygenophilic' (or aerobic – depends on actual oxygen tension [10]) chemoautotrophic piezophilic psychrophiles (Fig. 6, Fig. 13). It is worth to notice that in both cases the microbes supposed to thrive in lake ice and lake water must be chemoautotrophic by energy gaining and psychrophilic and piezophilic by physiology.

OXYGEN in Vostok ice core

Dissolved in crystal lattice

V. Lipenkov, pers. comm.

Ice	Depth (m)	Oxygen (mkM)	Bio features
Glacier ice	100 – 3538	15.0 (dissolved + air bubbles + hydrates)	Aerobic
Lake ice	TYPE 1 3539 - 3608	0.4	Low
	TYPE 2 3609 - 3623	0.0	Anaerobic
Water			

Fig. 11. Dissolved oxygen content in Vostok lake ice

Fig. 12. Expecting to discover in lake ice type 1

Findings

However, the DNA study showed that accretion ice in general is essentially bacterial and archaeal DNA-free. Up to now, a few bacterial phylotypes have been recovered from accretion ice 1 which contains mica-clay sediments while the deeper and cleaner accretion ice 2 showed no reliable biological signals upon thorough examination (Fig. 14). The deep old glacial ice horizons just above the lake also demonstrated no confident DNA signals, thus, serving a life-barrier between Lake Vostok ecosystem and surface biota for at least 15 Ma [4].

Of findings from accretion ice 1, the only chemolithoautotrophic bacterium detected by DNA signatures proved to be the true thermophile *Hydrogenophilus thermoluteolus* [4]. It was initially found at 3607m depth but now confirmed for another ice 1 horizon (3561m) differed by 47m core depth and about 5 kyr time-scale [8] (Fig. 15).

Fig. 13. Expecting to discover in Lake Vostok water column

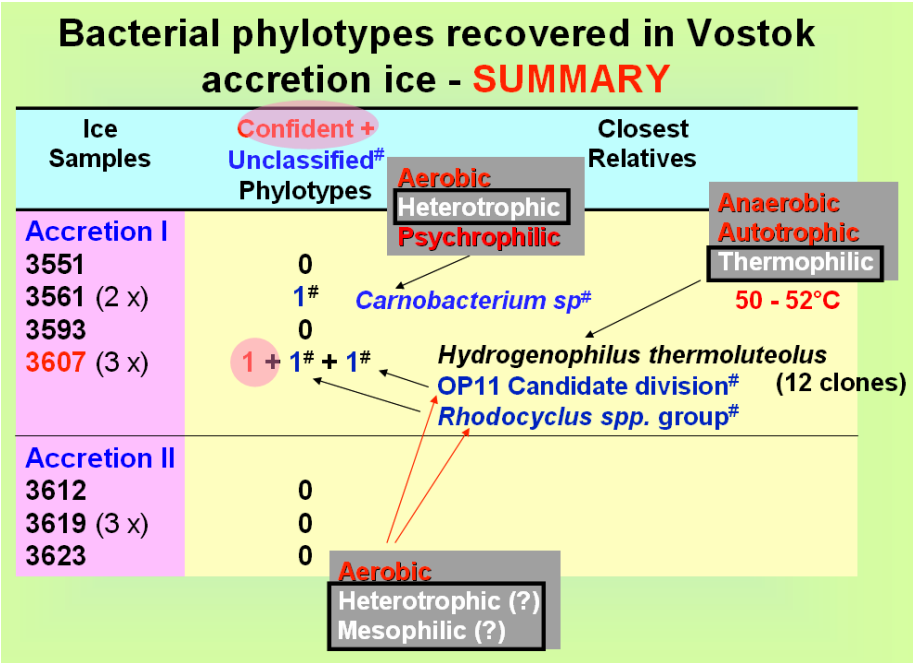

Fig. 14. Bacterial findings in Vostok lake ice [4]. The red font shows bacterial features which fit those predicted (Fig. 10, Fig. 11) while white font – those opposing.

Bacterial phylotypes recovered in Vostok accretion ice - SUMMARY

	Ice Samples	Confident Phylotypes	Closest Relatives
	Accretion I		Thermophile 50 - 52°C
	3551	0	
47m ~5 kyr	3561 (2 x)	1	*H. thermoluteolus* (3 + 6* clones)
	3593	0	
	3607 (3 x)	1	*Hydrogenophilus thermoluteolus* (12 clones)
	Accretion II		
	3612	0	
	3619 (3 x)	0	
	3623	0	

* Celine Lavire/Philippe Normand (Lyon)

Fig. 15. Thermophile findings in Vostok lake ice type 1 [4, 11]

The thermophile microbes detected by DNA signature in accretion ice 1 seem to originate from not open water but rather within the lake bedrock. They are supposed to be living in relatively warm anoxic sediments reach in CO_2 and hydrogen in deep faults at the lake bottom and sporadically flushing out along with sediments to the veins in a shallow depth bay due to a seismotectonic activity likely operating in the lake environment [12] (Fig. 16, Fig. 17). A few geophysical and geological evidences support this scenario [13].

Keeping in mind the possible redox couples in accretion ice 1 a special attempt was made to detect DNA signatures of two other groups of autotrophs which could be thriving there – methanogenic archaea and sulfate-reducing bacteria. However, no PCR amplicons at all were generated for archaea and no DNA sequences for sulfate reducers were recovered from clone libraries which both in agreement with no methane and hydrogen sulfide gas records in this ice (Fig. 18). Thus, the life in the lake remains to be discovered.

Fluorescence and laser confocal microscopy, scanning electron microscopy and flow cytometry analyses of strictly decontaminated ice samples provided supporting results. While any microscopy actually failed to detect cells in accretion ice samples the flow cytometry with meltwater concentrated succeeded in recognizing cell populations with estimate of 0.6 – 9.0 cells/ml in both accretion ice 1 and ice 2 samples (Fig. 4). These results based on direct methods indicate very low microbial biomass in accretion Vostok ice core samples, biomass levels two-three orders of magnitude lower than previously published [1,2]. These findings highlight the importance of stringent decontamination measures [4] and leave open the question of whether life exists in subglacial Lake Vostok.

Conclusion and further plans

Thus, the accretion ice, namely ice 1, from Lake Vostok contains very low and unevenly distributed biomass indicating that the water body (at least upper layer) should also be hosting a highly sparse life, if any. These results suggest that Lake Vostok may be the only extremely clean (with ultra-low microbial biomass) - giant aquatic system on the Earth providing a unique test area for searching for life on icy planets and moons.

Finally, the confident life detection in the subglacial Lake Vostok is evidently constrained by a high chance of forward-contamination which can be lessened by applying: a) stringent bio-decontamination protocols in clean (dust-free) room facilities, b) Ancient DNA and Trace DNA analyses regulations along with establishing inter-laboratory network for authentication of findings, c) chemistry controls focused on ions-indicators for contamination and d) comprehensive biological controls including development of contaminant database and inference of relevant indexing contaminant criteria.

The future directions with Vostok include continuous study of newly drilled more close to water accretion ice, the lake water sampled at different water column horizons and finally the lake sediments which unlike the ice and water may have to be inhabited by microorganisms.

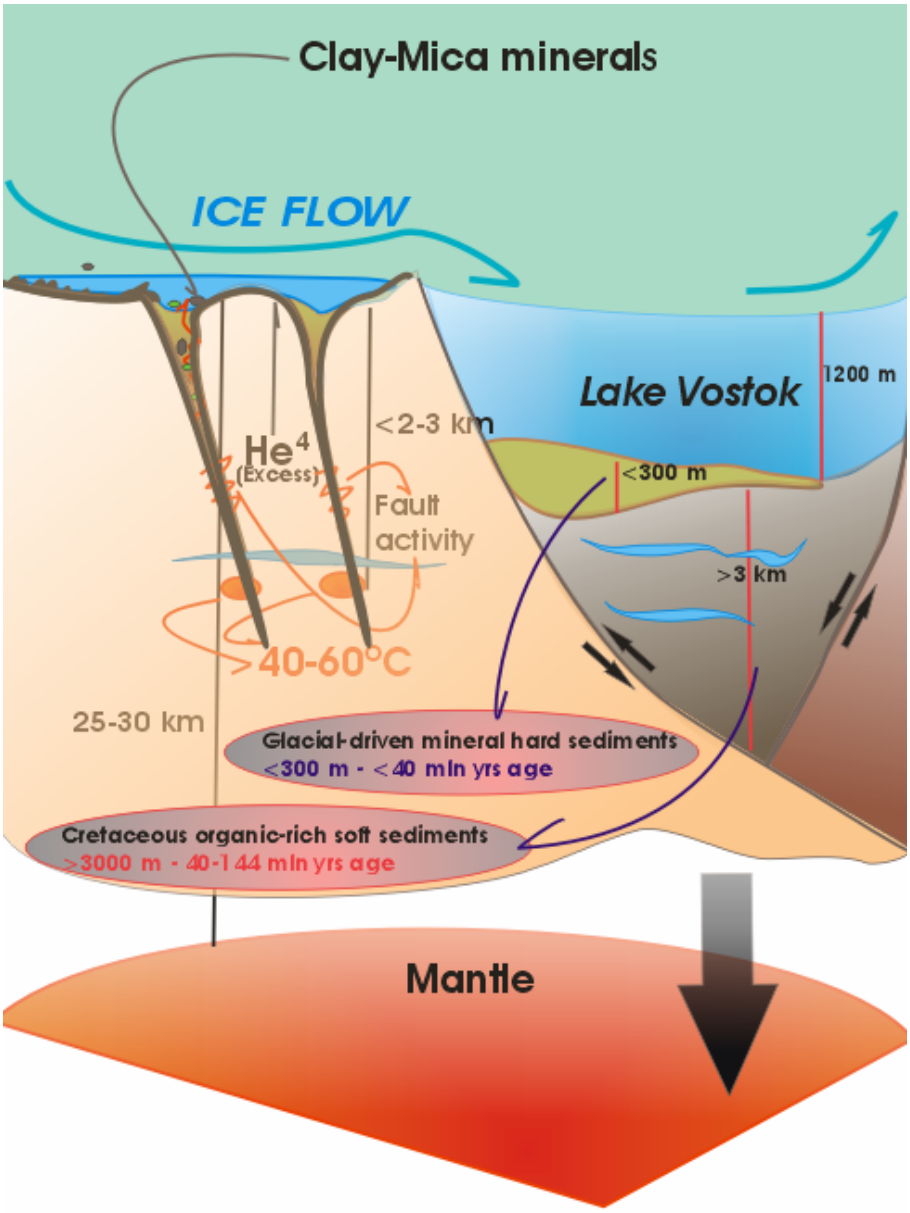

Fig. 16. Proposed geothermal environment within the Lake Vostok bedrock and source for thermophilic bacteria in lake ice type 1

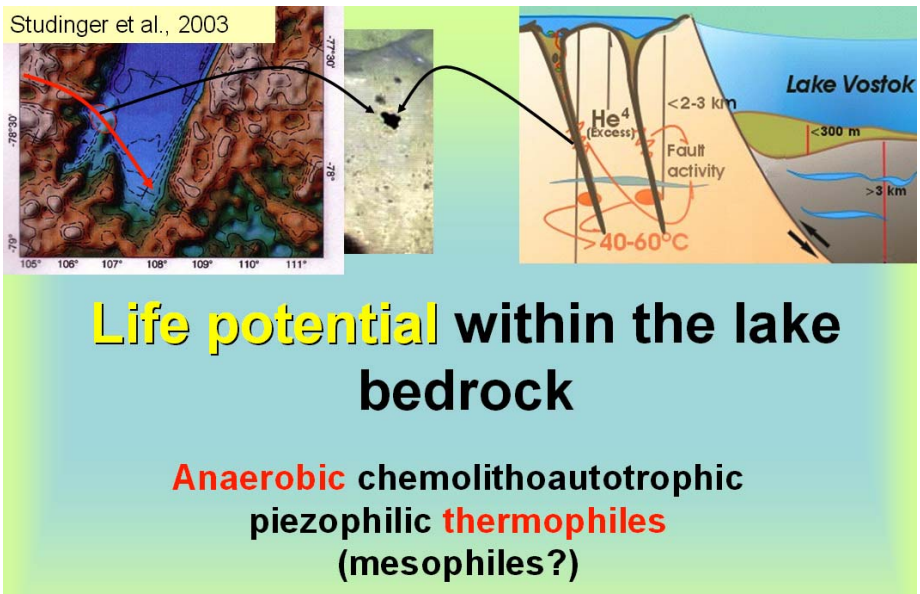

Fig. 17. Expecting to discover within the Lake Vostok bedrock

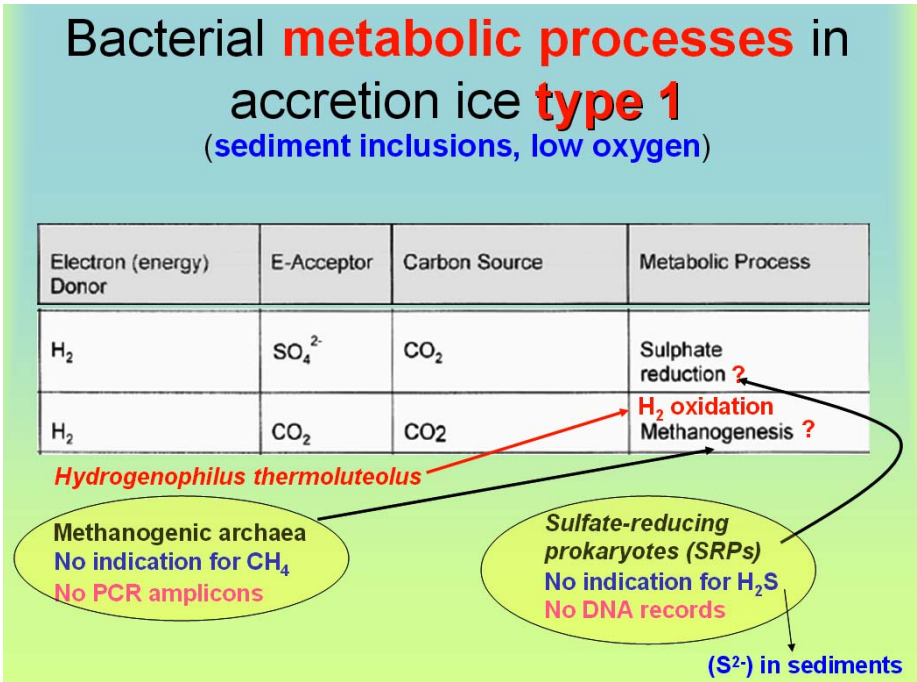

Fig. 18. Expected (and ongoing) redox reactions supporting bacterial metabolism in lake ice type 1

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