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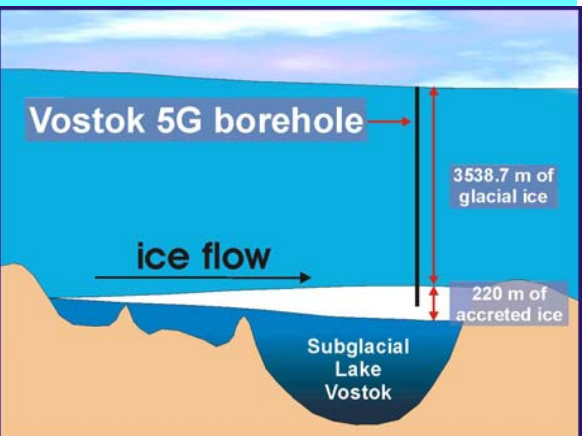
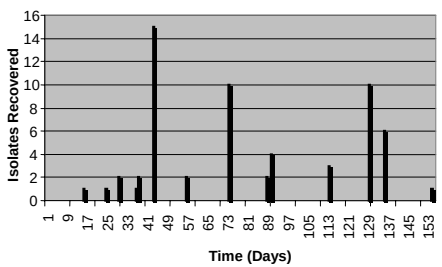
ISOLATION AND CHARACTERIZATION OF MICROORGANISMS IN GLACIAL AND ACCRETED ICE FROM THE VOSTOK ICE CORE: IS THERE LIFE IN LAKE VOSTOK?

An ice core was recovered in 1998 at Vostok Station that contains ice >420,000 years old, and the deepest ~80 m represents water from Subglacial Lake Vostok accreted to the bottom of the ice sheet. This ancient ice provides samples to examine microbial longevity and inhabitants of the lake, which has been isolated from the atmosphere for at least 15 million years. Before drilling into the lake, it is important and practical to first conduct microbiological studies on the overlying glacial and accreted ice. Using verifiable decontamination protocols, 28 and 20 unique isolates have been obtained from melted samples of the glacial and accretion ice, respectively. Based on 16S rDNA sequencing, the bacterial isolates classify within the alpha, beta, and gamma Proteobacteria, Actinobacteria, and Low G+C Gram Positives; 3 yeast isolates have also been identified. Although no true psychrophiles have been obtained, 93% of the isolates tested are capable of growing below 5 degrees C. These results document microbial survival in ancient ice and demonstrate that viable bacteria exist within Subglacial Lake Vostok.



Cross sectional schematic of Subglacial Lake Vostok along the flow line of the overlying ice sheet. The lake consists of 2 basins; the southern basin, shown here, is twice the spatial area of the northern basin. Subglacial Lake Vostok is estimated to have a volume of ~5400 km^3 and lies beneath 3700-4300 meters of ice. The depth of the lake water is >500 m beneath Vostok Station and ~1000 m to the north of the station. For illustrative purposes, the thickness of the accreted ice is exaggerated.



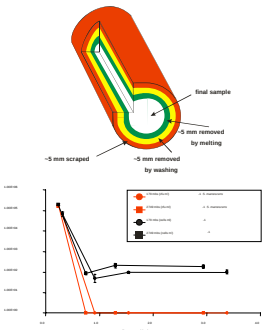
Resuscitation time for recovered isolates. Liqud encrichment media was incubated at 4°C before plating on solid media. It took at least 90 days of incubation at 4°C for >50% of the isolates to initiate growth. Once isolated, most species grew to saturation in <1 week.

Recovery of Viable Microorganisms from the Vostok Ice Core (5G)

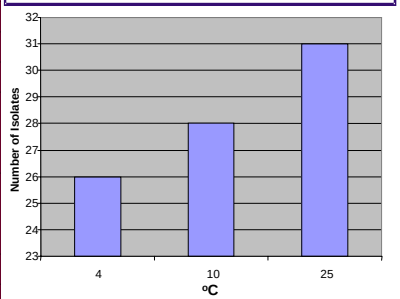
Strain ID/ Depth	# of bases	Alignment with closest relative	% Similarity	Closest Relative	Isolation Location
V171-1	1378	1386/1394	99	*Spingomonas* sp. V1	Lake Vostok accretion ice
V171-4	1397	1345/1368	98	*Kaistobacter koreensis*	Alphaproteobacteria
V171-5	1473	1456/1461	99	*Bacillus thuringiensis*	Ubiquitous in the environment
V179-2	1224	1079/1104	97	*Rhodosporidium bebjevae*	Yeast
V179-4	1048	945/975	96	*Cryptococcus* sp.	Yeast from Antarctica
V1686-1	467	463/466	99	*Paenibacillus* sp.	No information
V1686-5&6	1510	1297/1316	98	*Nocardioides* sp.	No information
		1283/1318	97	Glacial Ice Bacterium	Glacial and subglacial environments
V2303-1&2	1467	977/986	99	*Pseudomonas psyphrotolerans*	Cold environments
			98	Glacial Ice bacterium	Antarctic glacial ice
V2303-3	940	889/939	94	*Agrobacterium*	Common in soil
V2303-5	88	86/87	98	Glacial Ice Bacterium	Glacial ice from Sajama, Bolivia
V2303-6	80	80/80	100	*Sphingomonas* sp.	Lake Vostok accretion ice
V2334-5	614	542/546	99	Jellyfish degrading bacterium	Marine environments
		541/546	99	*Brachybacterium* sp.	No information
V2334-6	344	249/270	92	*Acinetobacter* sp. Muzt-B31	Glacial ice from Himalayas
		249/270	92	*Sphingobacterium antarcticum*	Isolate from Antarctica
V2334-7	253	235/249	94	*Shingomonas* sp.	Antarctic endolithic communities
V3519-1	515	514/516	99	*Paenibacillus* sp.	No information
V3519-5	580	573/576	99	Arctic Sea Ice Bacterium	Arctic Sea ice
V3519-6	596	270/277	97	Marine bacterium	Marine environments
V3519-7	NA	NA	93	*Arthrobacter* sp.	Marine environments (North Sea)
V3519-9	114	95/98	96	*Arthrobacter*	Marine environments (North Sea)
V3572-1	1197	1097/1110	98	*Bacillus circulans*	Ubiquitous
V3572-3	NA	NA	96	*Agrobacterium* sp.	Common in soil
V3572-4	1328	1323/1328	99	*Agrobacterium albertimagni*	Common in soil
V3605-1	67	60/63	95	*Chelatococcus asaccharovorans*	Soil crusts of the Colorado Plateau
V3605-2	NA	NA	98	*Devosia neptunia*	Aquatic root nodule
			95	*Glacial Ice bacterium*	Subglacial Sediments from New Zealand
V3605-5	246	242/246	98	*Kytococcus sp.*	Aquatic ecosystem
V3605-6	842	809/837	96	Unclassified bacterium	Antarctic soils
V3605-7	878	830/870	95	Unclassified bacterium	No information
V3605-8	NA	NA	90	Uncultured bacterium clone	Ross Island, Antarctica
V3622-3	NA	NA	90	*Subtercola pratensis*	Actinobacteria
			89	Antarctic bacterium	
V3622-5	1396	1358/1370	99	*Dietzia* sp.	No information

NA = not available; identification based on <200 bp of 16S rDNA nucleotide sequence

ICE CORE DECONTAMINATION



Our experiments indicate that removal of 1.5 cm of the core exterior is sufficient to obtain a clean sample, in terms of removing external cells on the ice core surface.



Temperature growth range for the characterized Vostok isolates. None of the isolates recovered are obligately psychrophiles, but 90% classify as phycrotrophs (i.e., are capable of growth at <10°C.

Conclusions

- A verifiable decontamination protocol was used to sample ice cores.
- Microorganisms remain viable for >420,000 years entrapped in glacial ice.
- The damaged cells apparently require a resuscitation period before initiating growth.
- The accretion ice contains viable bacteria, suggesting a sustained ecosystem exists in Lake Vostok.
- Glacial and subglacial environments provide an experimentally tractable analog to examine microbial longevity and survival in icy extra-terrestrial habitats (i.e., Mars and Europa).

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