



On the assessment of the potential for life in the subglacial Lake Vostok, East Antarctica

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We attempted to estimate microbial contents of accretion 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 15 Ma long isolation, no light, high pressure (about 400 bars), temperature close to the freezing point, ultra-low DOC contents, and probable excess of oxygen. The PCR based bacterial and archaeal 16S ribosomal RNA gene sequencing constrained by Forensic Biology and Ancient DNA research criteria was used as a main approach. The original ice samples to study were representing about 10 cm long half diameter core increments. The flow cytometry was used for cell enumeration. DNA study showed that the accretion ice is essentially bacterial and archaeal DNA-free. Up to now, a few bacterial phylo-types have been only recovered from accretion ice 1 which is featured by presence of small inclusions of mica-clay sediments. Surprisingly, the same DNA signature of chemolithoautotrophic thermophile *Hydrogenophilus thermoluteolus* was found at two of four studied horizons (3607m and 3561m). In contrast, the deeper and cleaner accretion ice 2 (containing no sediment inclusions and very small amount of trapped gases) revealed no reliable biological signals in the three ice core increments examined. The thermophile microbes detected in accretion ice 1 are supposed to be thriving in rather warm anoxic sediments 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. A few geophysical and geological evidences support this scenario. Sediment-free accretion ice 2, which forms

above a deeper part of the lake, shows no evidence for reasonable source for microbe contribution. Flow cytometry trials on strictly decontaminated ice samples provided supporting results. With meltwater samples concentrated up to 20 times the cytometry actually failed to reveal cells in accretion ice 2 (3613m and 3621m) while succeeded in a preliminary estimate of about 10 cells/ml for accretion ice 1 (3561m). However, given the ratio contaminants to indigenous cells is about 5:1 (from PCR results), the genuine microbial contents for accretion ice samples does not exceed 2 cell/ml, that is practically not far from "sterile" conditions. Thus, the accretion ice 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. Our results suggest that Lake Vostok may be the only near-sterile aquatic system on the Earth that can provide a unique test area in a search for life on icy planets and moons. The search for life in Lake Vostok is constrained by a high chance of forward-contamination which can be minimized by using the stringent decontamination procedures and comprehensive biological controls.