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# Isolation of Microbes from Lake Vostok Accretion Ice[▽]

Tom D'Elia, Ram Veerapaneni, and Scott O. Rogers*

*Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403*

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**Bacteria from seven Lake Vostok accretion and two deep glacial Vostok ice core sections were characterized. The cell concentrations were low, but many of the cells were viable. From the hundreds of cultures, 18 unique bacterial rRNA gene phylotypes were determined. Lake Vostok may contain a complex microbial ecosystem.**

Subglacial Lake Vostok, the eighth largest lake on Earth (area = 14,000 km$^2$, volume = 5,600 km$^3$) (9, 16), is covered by a 4-kilometer-thick layer of glacial ice. As the glacier traverses the lake over a period of 18,000 years, ice freezes (or accretes) to the bottom surface of the glacier, eventually forming a 200-m layer of accretion ice that has retained a linear and temporal record of the contents of the upper surface of the lake. The glacier passes over a shallow embayment, near an island (or peninsula), and then over part of the main lake basin. As the glacier passes through the embayment, initially it is grounded on the lakebed, and partly because of this, it collects mineral inclusions, making the ice silty (termed type I accretion ice) (13). Melting and freezing in this area, as well as a possible influx of material from a river system and/or from hydrothermal activity, may contribute to the characteristics of the type I ice (3, 14). The glacier is suspended over open water in portions of the embayment and over most of the main parts of the lake. The ice that forms over open water contains far fewer inclusions and lower concentrations of ions, organic carbon, and biomass (6, 10, 14). This ice is very clear ice and has been termed type II accretion ice (13). The top section (from 3,538 to 3,595 m), which accreted within and near the embayment, primarily consists of type I ice, although there are some regions of type II ice (2). Bacteria from this ice, including potentially psychrophilic and psychrotolerant species as well as the molecular signature of a thermophilic bacterium, have been reported (1, 3, 4, 5, 7, 10). Within the lake, temperatures average −2°C, pressures approach 400 atmospheres, high oxygen levels exist, there are low nutrient levels, and it is completely dark.

We isolated and characterized microbes from Lake Vostok type I and II accretion ice from the embayment and the main basin as well as from glacial ice immediately above the accretion ice layers. Sequence results from the rRNA small subunit genes and internal transcribed spacers indicate that at least 18 species are represented in the accretion ice. All are psychrotolerant in that they grew at 4°C, although optimal growth was often at higher temperatures.

**Descriptions and cell concentrations.** Nine Vostok 5G ice core sections were assayed. Five sections represented ice that accreted over the shallow embayment (depths of 3,540, 3,563, 3,582, and 3,584 m, all type I ice, and 3,591 m, type II ice) (2) approximately 3,800 to 5,100 years ago (2, 6, 13, 14), two accreted 3,400 to 3,500 years ago over the main lake basin (3,606 m, type I ice, and 3,610 m, type II ice), and two were glacial ice cores near the bottom of the glacier (3,501 and 3,520 m, approximately 1 to 2 million years old) (13, 14). The surfaces of all of the ice core sections were decontaminated prior to melting, as described previously (12). Ice core meltwater initially was analyzed using a live/dead stain (*Bac*Light viability kit; Molecular Probes, Eugene, OR) to count cells from 10 1-ml samples for each core section by using fluorescence microscopy. The concentrations (means ± standard deviations) of viable and nonviable cells ranged from 2.33 ± 0.29 to 12.33 ±

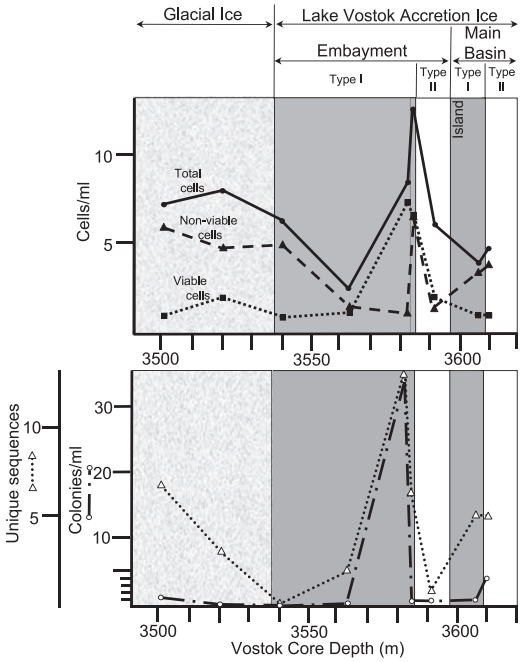


FIG. 1. Cell concentrations (upper panel), colonies of viable cells, and numbers of unique sequences (lower panel) in the ice core sections. Cell concentrations were based on cell counts of fluorescently stained cells visualized via microscopy from 10 ml of meltwater from two glacial and seven accretion ice core sections. The graph also shows the region of the lake that is represented in each ice core section. The highest concentrations of cells, viable cells, cultures, and unique sequences are in the areas near the transitions between type I and type II accretion ice.

* Corresponding author. Mailing address: 217 Life Sciences Building, Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403. Phone: (419) 372-2333. Fax: (419) 372-2024. E-mail: srogers@bgsu.edu.

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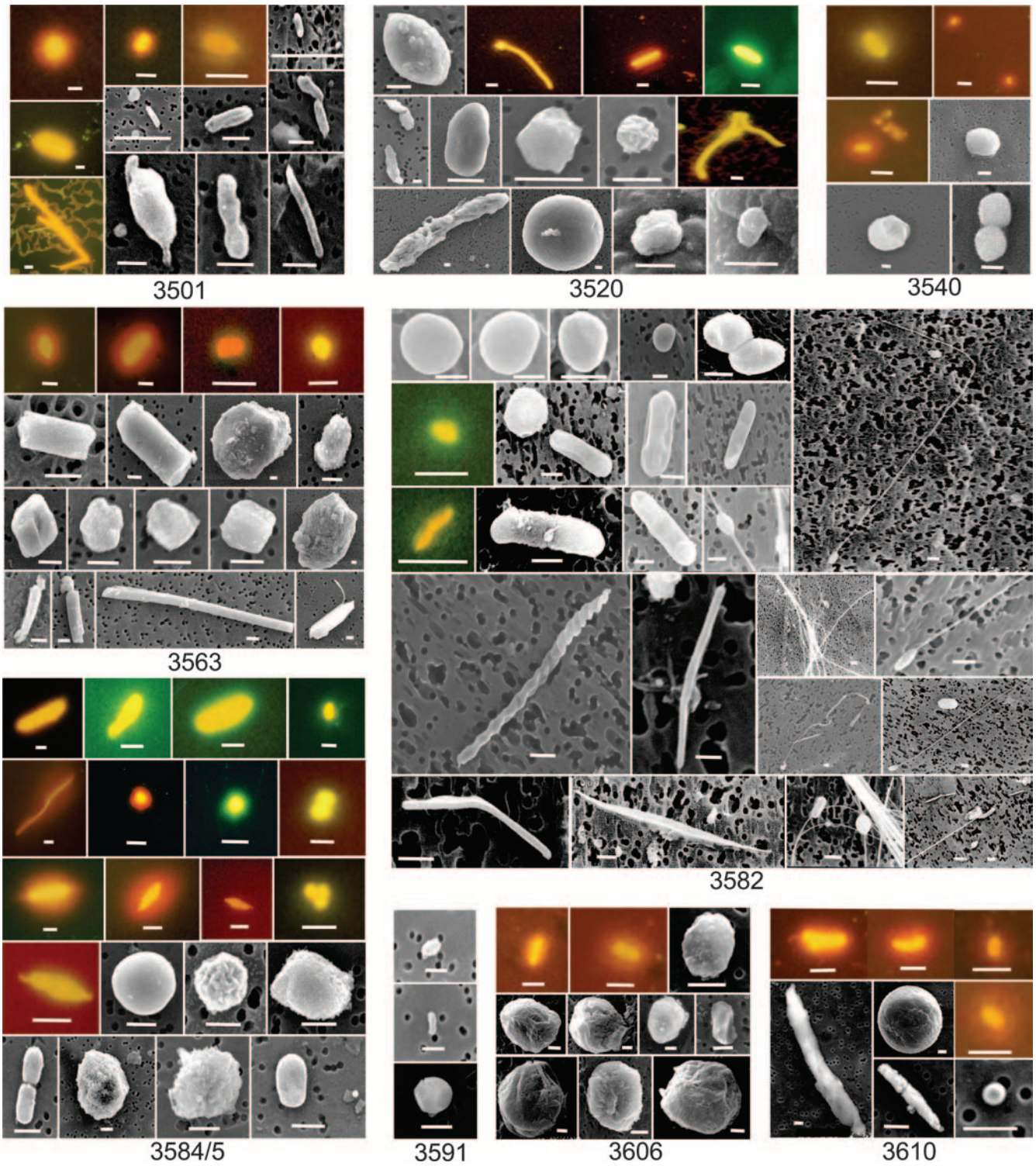


FIG. 2. Fluorescence and scanning electron micrographs of cells found in the glacial (3,501 and 3,520 m) and accretion ice (3,540, 3,563, 3,582, 3,584, 3,591, 3,606, and 3,610 m) core sections. Cells in the fluorographs were stained with BacLight (Molecular Probes, Eugene, OR). Green fluorescence indicates possible viable cells, based on membrane integrity. Red fluorescence indicates possible dead cells based on intracellular staining. Bars represent 5 μm on fluorographs and 1 μm on electron micrographs. 3501, cells characteristic of eubacteria, possibly eukaryotes (in one case, middle right); 3520, probably bacteria (upper two cells; upper right potentially viable), germinating spore (lower left; bacterial or fungal), and unknown (lower right); 3540, all apparently coccoid bacteria; 3563, all apparently bacteria (all nonviable); 3582, filamentous bacteria or fungi (upper portion; some structures may be oogonia [or similar structures]), coccoid bacteria (middle), and a mix of *Bacillus*-type cells, a spiral-shaped cell, and three linear and angular cells (lower portion; the one on the lower right might be related to one type of green algae, and the two angular cells are similar to those reported to occur in core section 3593 [5]); 3584/5 (the cell on the far left in the fourth row may represent a small *Caulobacter*-like cell); 3591, all three apparently bacteria; 3606 and 3610, cells are similar to those in 3582 and 3584/5.

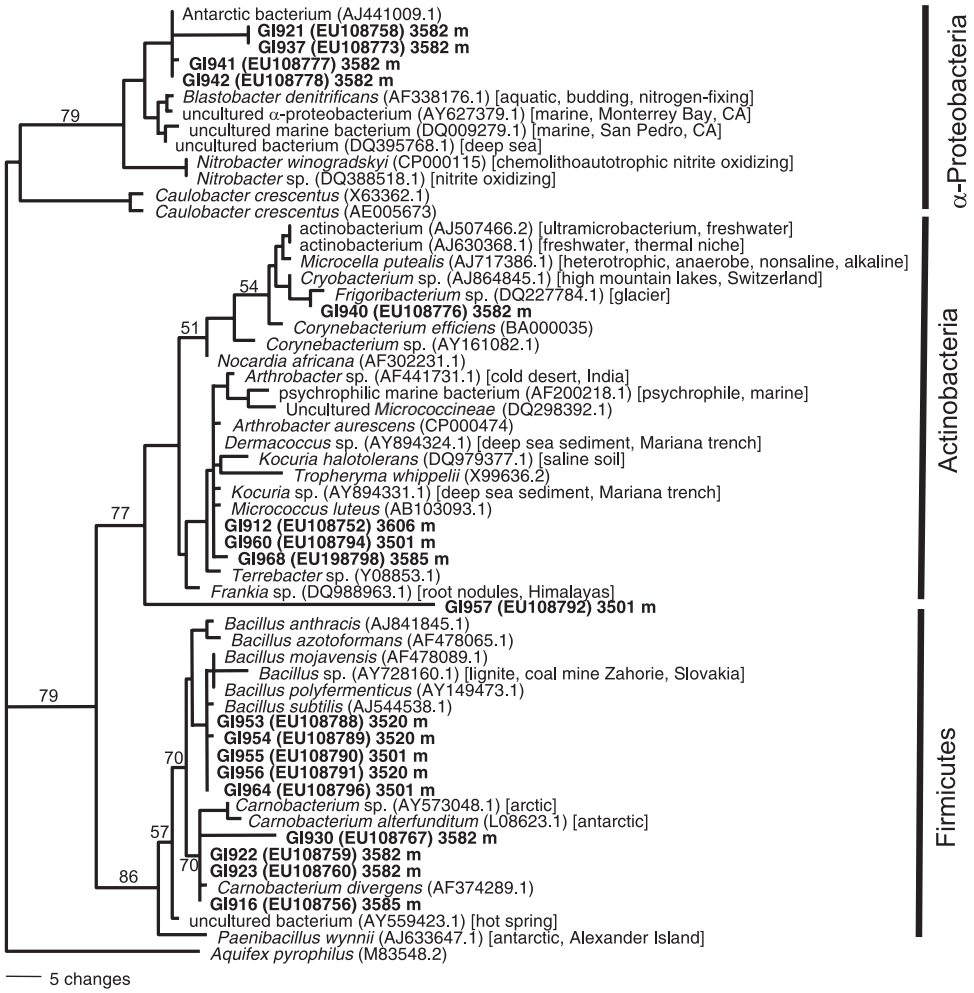


FIG. 3. Maximum-parsimony phylogram (determined using PAUP, version 4 [Sinaur Academic Publishers]) of portions of the small subunit genes from bacterial isolates in this study (GI, glacial isolate numbers) as well as comparable sequences from bacteria that were closest to these sequences in BLAST searches. Brief descriptors are provided for each of the sequences determined by BLAST searches to be the taxa closest to the sequences from this research. NCBI (GenBank) accession numbers are shown for each sequence. Bootstrap values are shown on branches with >50% support.

9.58 cells/ml (Fig. 1). Considering the partition coefficient (0.56) (6), for ice versus water, the concentrations in the lake are approximately 1.78 times higher than these values (4.15 to 21.95 cells/ml). The number of viable cells in each ice core section varied from nearly 0 to a mean of 6.56 cells/ml (Fig. 1). The mean concentrations of nonviable cells were from 1.28 to 5.58 cells/ml. The concentrations of viable cells in glacial ice primarily were lower, between 1.00 and 2.00 cells/ml. This is expected, given the fact that the glacial ice that was examined was between 1 and 2 million years old, while the accretion ice was only 3,400 to 5,100 years old (6, 13, 14).

A 5-ml sample from each core section also was examined by scanning electron microscopy (SEM) (Fig. 2). After filtration, the filter was fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2), rinsed, dehydrated with ethanol, dried (Samdri 780A critical point dryer), sputter coated with gold-palladium (Polaron E500 SEM coating unit), and viewed using an SEM (Hitachi S-2700). Many of the cells in the glacial ice were distorted (Fig. 2), which is consistent with the low cell viabilities indicated by the fluorescence microscopy assays (Fig. 2). Cells in the shallow embayment type I ice also exhibited damage. However, cells in type II ice of the embayment exhibited lower levels of distorted cells, and the diversity of cell shapes was higher. The same pattern was observed for type I and type II ice from the main lake basin, but the numbers of cells and the level of cell diversity were lower than in the ice from the embayment.

**Cultures and sequence analyses.** Approximately 2,000 meltwater aliquots (200 μl each) were spread on agar plates containing 13 different media and were incubated for weeks to months at four temperatures (4, 8, 15, and 22°C). Portions of the rRNA loci were amplified by PCR (as in reference 12, with primers described in reference 11) and sequenced, followed by BLAST searches, CLUSTAL alignment, and phylogenetic analysis (as in reference 8). A total of 665 colonies resulted from the seven Vostok 5G accretion ice core sections, and an additional 22 were isolated from the glacial ice immediately above the accretion ice. The bacteria isolated represent a va-

riety of taxa (Fig. 3), all of which are psychrotolerant (data not shown). All are related to taxa that are aquatic and/or live in lake sediments, soils, or rocks (Fig. 3). In addition to the bacteria, a dozen unique fungi were isolated (data not shown).

**Conclusions about Lake Vostok.** The assembly of microbes that were found in the Lake Vostok accretion ice samples indicates that the lake has a diverse population of microorganisms and potentially a complex ecosystem. Nonetheless, the concentrations of microbes in the subglacial lake are lower than those in most environments on Earth (7). Some have suggested that Lake Vostok is sterile, since parts of the lake may be extremely oligotrophic (3). However, all of our data indicate that the lake supports a diverse microbial assembly, as has been concluded elsewhere (6, 7, 10, 15). There appear to be distinct ecological zones, either spatially or temporally, since different sets of microbes were isolated from each of the four zones (type I and II ice from the shallow embayment and type I and type II ice from the main basin), representing different ages of ice (Fig. 1). Our results indicate that the highest concentrations of viable cells are located close to the transition zones between type I and type II ice, which would correspond to the shoreline of the lake near the grounding line of the glacier. While most research on the accretion ice has focused on bacteria, some fungi have been described (3), and fungi were isolated and photographed in this study (Fig. 2). Therefore, heterotrophs may be present in Lake Vostok. If so, the Lake Vostok ecosystem is more complex than previously thought.

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