Supporting Online Material

Materials and Methods

Sample processing. The ice samples analyzed in this study were obtained by removing the debris that covers the buried ice (~20 to 75 cm thick), removing the top 15 cm of the buried glacier, and then cutting out a square block, averaging 20 cm on a side; samples were triple wrapped in plastic bags and stored in a freezer at -20°C. Because we could not control for potential chemical and microbiological contamination at the time of ice sampling in the field, we devised a way to decontaminate the samples before analysis. To recover potentially viable microbes and genetic material, we decontaminated the exterior of the ice with a controlled melting process (S*1*). A section of block ice (ca. 1 kg) was thoroughly rinsed with ice-cold ethanol, followed by copious amounts of flowing 0.22 μm-filtered MilliQ® water. These rinses effectively melted the exterior few centimeters of the ice samples. The cleaned ice was thawed in a sterile plastic bag at 4°C, and the meltwater was subsequently handled aseptically in a sterilized laminar flow hood.

Chemical analyses. Assays for dissolved inorganic nitrogen (DIN; NH_4, NO_2, and NO_3) and soluble reactive phosphate were performed by Lachat QuickChem (S*2*), while determinations of dissolved organic carbon were done on a Shimadzu 5000A analyzer. Total dissolved nitrogen (TDN) was analyzed by Antek Model 7000, using a high-temperature combustion followed by chemiluminescent detection of nitric oxide.

Epifluorescence and scanning electron microscropy. Subsamples of fresh meltwater were preserved in 2% formalin and stored at 4°C until processed (within a few days). Samples were thoroughly mixed via vortexing, after which large till particles were allowed to settle for 15 min on

ice, so as not to clog membrane filters. Bacterial cell visualization and enumeration were performed by a modified SYBR Gold (Molecular Probes) staining procedure (S*3*). SYBR Gold (1X, 10,000-fold dilution of stock) was added directly to preserved aliquots (1.8 ml for DLE-98-12 and 5 ml for EME-98-03) and incubated for 15 minutes on ice in the dark. Stained samples were filtered directly onto 0.22 µm black polycarbonate track-etched (PCTE) membrane filters under light vacuum, mounted with antifade mountant (50% glycerin and 0.1% ascorbic acid in phosphate buffered saline) and visualized at 1000X on a Zeiss Axioskop. Cell abundance in thirty randomly chosen fields was enumerated (at least 300 cells total) using a calibrated ocular grid. Filters were stored at –20°C in the dark. For SEM analysis, meltwater was filtered onto sterile 0.22 µm PC filters. Collected material was preserved for 2 h with Trump's fixative (4% formaldehyde, 1% glutaraldehyde in phosphate buffer, pH 7.2), rinsed twice in 0.2 µm-filtered 50 mM phosphate buffer and MilliQ® water, followed by sequential dehydration in ethanol (50%, 70%, 80%, 95%, 100% twice). Dehydrated specimens were critical point dried (Balzers CPD 020), sputter coated with gold palladium (Balzers SCD 004) and visualized on an Amray 1830 I scanning electron microscope.

DNA extraction and PCR amplification. For harvesting of encased microbes for subsequent DNA analysis, the meltwater was centrifuged in sterilized bottles at 15,000 g for 20 min to collect the particle phase and associated cells. The supernatant was vacuum filtered onto sterilized 0.2-µm polycarbonate (PC) filters using a sterilized filtration manifold; filters and sediment were asceptically transferred to sterilized 2.0 ml microfuge tubes and immediately frozen until analysis. Genomic DNA was extracted from both the PC filters and the solid phase using the SoilMaster kit (Epicentre) according to manufacturer's instructions. Extracted DNA was stored in TE buffer (10

mM Tris-HCl, pH 7.8; 1 mM EDTA) at -80°C. The size and integrity of total extracted community DNA was determined by agarose gel electrophoresis (0.8%) followed by staining with SYBR Gold (Molecular Probes) and visualized using a Typhoon 9400 Variable Mode Imager (Amersham Pharmacia Biotech) with ImageQuant Solutions software. The weighted mean DNA size for ice samples was subsequently determined by obtaining densitometry profiles of the entire ice DNA using ImageJ 1.34s (National Institute of Health) and exported to Excel in order to calculate the weighted mean (distance) of the DNA peak with determination of DNA size (bp) calculated from the distance-bp calibration of the molecular weight standards.

The ice community DNA was amplified via PCR using 16S rDNA *Bacterial* primers (Eubac-27F, 5'-AGAGTTTGATCMTGGCTCAG-3'; Eubac-1492R, 5'-TACGGYTACCTTGTTACGACTT-3') (S*4, S5*) We also utilized *Archaea*-specific (Arch-21F, 5'-TTCCGGTTGATCCYGCCGGA-3'; Arch-958R, 5'-YCCGGCGTTGAMTCCAATT-3') and *Eukaryotic*-specific (EukF, 5'-AACCTGGTTGATCCTGCCAGT-3'; EukR, 5'-TGATCCTTCTGCAGGTTCACCTAC-3') (S*5*) primer sets to amplify 16S rDNA, but obtained no PCR product for these domains from either ice sample. Due to the high concentration of glacial till particles and the co-extraction of PCR-inhibitory impurities, PCR was optimized by diluting the template (at least10^{-1}) and by increasing the number of thermal cycles. PCR amplicons were visualized by agarose gel electrophoresis (0.8%) and by ethidium bromide staining (0.5 μg ml^{-1}). To control for false-positive PCR signals due the contamination, one liter of MilliQ® water was frozen, thawed, and filtered on a polycarbonate (PC) filter, and subjected to the same DNA extraction procedure. The material extracted from the contamination control filter was used as a template in

PCR with the *Bacterial*-specific primers, and subsequently examined by agarose gel electrophoresis for any amplified product attributable to PCR artifact (Fig. 2A).

Generation of clone libraries. Clone libraries were generated from the 16S rDNA PCR amplicons using TOPO TA Cloning kit (Invitrogen). Bidirectional sequencing was performed on extracted plasmid DNA from picked clones (118 EME clones, 140 DLE clones) using the ABI BigDye sequencing kit (Applied Biosystems) using an automated ABI 3100-Avant capillary sequencer (Applied Biosystems). Clones were de-replicated based on the partial sequences (ca. 700 bp) generated with the *Bacterial*-specific 341F primer (5'-CCTACGGGAGGCAGCAG-3'*)*, with identical or similar (> 98% identity) clones being grouped together. Unique clones were selected for complete sequencing of the ca. 1450 bp amplicon using Bact-27F and Bact-1492R primers. Sequences that were suspected to be of heteroduplex or chimeric origin after analysis with CHIMERA-CHECK program (Ribosomal Database Project website) were discarded. An independent analysis using different portions of each 16S rDNA sequence provided additional assurance against chimeras influencing the robustness of phylogenetic tree branching order.

For metagenomic analysis, a bacterial artificial chromosome (BAC) library was generated for DLE-98-12 by ligating BamH1 digested DNA into the copy-control pCC1BAC vector (Epicentre Copy Control BAC cloning kit), This system combines the clone stability afforded by single copy cloning with the advantages of high yields of DNA obtained by "on-demand" induction of the clones to high copy number. In order to maximize the complete recovery of small size DNA fragements, community DNA for EME-98-03 was end-repaired and converted to blunt-end DNA by exploiting the 5'to 3' polymerase and the 3' to 5' exonuclease activities of T4 DNA Polymerase (End-It™ DNA End-Repair Kit, Epicentre). End repaired community DNA was subsequently

ligated into the blunt cloning-ready Copy Control pCC1™ Vector (Epicentre), following treatment with T4 DNA polynucleotide kinase and ATP phosphorylation of the 5'-ends. Ligated inserts were transformed into Electrocompetent TransforMax™ EPI300™ *Escherichia coli* (Epicentre) following manufacturer's instructions. All clones were grown on Lauria Broth agar under chloramphenicol selection and blue-white screening was used to identify positive clones upon incubation with IPTG and XGAL. Colonies on agar plates, frozen ligation reactions and frozen glycerol stocks of transformants for each ice sample were hand-delivered on dry ice to the Microbial Genomics group at The Institute for Genomic Research (TIGR) for clone picking, griding, cataloging, plasmid extraction and sequencing.

Analysis of metabolic activity. For radiotracer uptake experiments, 60 ml aliquots of each meltwater sample were transferred to three acid-cleaned (10% HCl), sterile glass 125 ml Erlenmyer flasks. Aliquots were spiked with either with 6.78 μCi ^{3}H-thymidine (Specific activity= 1.13 Ci/mmol) or 9.12 μCi ^{3}H-leucine (Specific activity= 1.13 Ci/mmol). Due to inherently different DOC concentrations for DLE-98-12 (317 μM) and EME-98-03 (23 μM) (Table S1), 2.4 μCi and 0.68 μCi of ^{14}C-glucose were added, respectively. These substrate additions were equal to 0.7 μM and 0.2 μM, respectively. The samples were mixed and 3 ml was dispensed into acid-washed, sterile, 10 ml glass Erlenmyer flasks (Kontes), which were fitted with a gas-tight stopper containing a sterile plastic center well (Kontes) with fluted filter paper(Whatman no. 2) wick. The samples were incubated at 4°C in the dark.

After the designated time periods, triplicate samples were removed and processed for ^{3}H-leucine, ^{3}H-thymidine and ^{14}C-incorporation (S*6, S7*). First, 60 μl of 0.22 μm-filtered formalin was injected into each experimental flask using a sterile syringe and swirl mixed in order to terminate

the reaction. For ^{3}H incubations, flasks were chilled for 1 minute on ice followed by the addition of 5% trichloroacetic acid (TCA). ^{3}H-thymidine flasks were chilled on ice for 15 min while ^{3}H-leucine flasks were heated at 80°C for 15 min. After temperature incubations, the entire sample was filtered onto a 13-mm diameter, 0.45-µm pore-size mixed cellular membrane filter using a sterile syringe and a Luer-lock Swinnex (Millipore) filter housing. Filters were rinsed twice with 1 ml of ice-cold 5% TCA, followed by three rinses with 80% ice-cold ethanol. Filters were dissolved in 250 µl of ethyl acetate prior to the addition of ScintiVerse cocktail and liquid scintillation counting.

For ^{14}C-measurements, the fluted filter paper was wetted with 200 µl of □-phenethylamine, using a gas tight syringe, followed by the injection of 100 ml of 5 M H_2SO_4 directly into the sample. Acidification served to precipitate cellular macromolecules and forced any ^{14}C-CO_2 present in meltwater into the head-space. Samples were placed at room temperature for an overnight passive distillation of ^{14}C-CO_2 onto the B-phenethylamine soaked wicks. The stoppers were removed and the wicks were placed directly into liquid scintillation vials containing 4 ml of ScintiVerse cocktail. The formalin-preserved, acidified samples were filtered onto a 13-mm diameter 0.22 µm pore-size polycarbonate filter using a sterile syringe and a luer-lock Swinnex (Millipore) filter housing, followed by rinsing three times with sterile, 0.22 µm filtered MilliQ® water. Filters were dissolved in 250 µl of ethyl acetate prior to the addition of ScintiVerse cocktail and liquid scintillation counting.

Recovery of viable microorganisms. In an attempt to resuscitate viable cells, the ice melt water was amended with 4 types of nutrient enrichments and incubated at 4°C in the dark. Nutrient formulations were as follows in g liter^{-1} (T1: 5 g peptone, 0.15 g ferric ammonium citrate, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.05 g $CaCl_2$, 0.05 g $MnSO_4 \cdot H_2O$, 0.01 g $FeCl_3 \cdot 6H_2O$ 0.01; T2: 1 g glucose, 1 g

peptone, 0.5 g yeast extract, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.05 g $MnSO_4 \cdot 4H_2O$; T3: 1 g glucose, 0.5 g casamino acids, 0.5 g yeast extract, 1 g. KH_2PO_4, 0.5 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MnCl_2 \cdot 4H_2O$; T4: R2A) (S*8*). All nutrient formulations were made as concentrated stocks (20-200X), sterilized by autoclaving and directly added (<1/50 volume ratio) to meltwater at 1X augmented concentration. Growth was monitored by measuring the optical density (OD 600 nm). Blanks consisting of autoclaved MilliQ® water amended with the same nutrients and agar plates were performed to control contamination for the liquid cultures and the cell isolations, respectively. No contamination growth was detected for >1 year of incubation. In liquid cultures, subsamples were removed at specified time points during growth for PCR amplification of 16S rDNA and plating onto solid media of the same nutrient type. Visible colonies started to form on the plates after > 1 month of incubation at 4^oC in the dark. Phylotypes of the isolates were identified via their 16S rDNA sequences. Verification of the presence of resuscitated cells in the original ice microbial community was performed by nested PCR amplification with unique signature 16S rDNA primer sequences to these isolates (Table S4) and the original 16S rDNA amplicons as a template. The primer set, Arose-232F (5'-GAATTTTGGTTTTGGATGGACTCGC-3') and Arose-1008R (5'-TGTCTCCAGGTGTTTCCAGTCC-3'), is specific to DLE011i and *Arthrobacter roseus* CMS90. In addition, an Arthro primer set, Arthro-486F (5'-GACATTCCACGTTTTCCGCG-3') and Arthro-1286R (5'-CTCCACCTCACAGTATCGCAAC-3'), was designed for the unique sequences in common among the *Arthrobacter* species isolated in this study as well as *A. roseus* CMS90 and *Arthrobacter* 19503. Primer sets were tested for the specificity, and used for the detection of the target 16S rDNA in the ice DNA.

References

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