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Environ. Res. Lett. **8** (2013) 035022 (11pp) doi:10.1088/1748-9326/8/3/035022

Microbial growth under humic-free conditions in a supraglacial stream system on the Cotton Glacier, Antarctica

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Received 12 April 2013
Accepted for publication 4 July 2013
Published 23 July 2013
Online at stacks.iop.org/ERL/8/035022

Abstract
During the austral summers of 2004 and 2009, we sampled a supraglacial stream on the Cotton Glacier, Antarctica. The stream dissolved organic matter (DOM) was low (44–48 μM C) and lacked detectable humic fluorescence signatures. Analysis of the excitation emissions matrices (EEMs) indicated that amino-acid fluorophores dominated, consistent with DOM of microbial origin, with little humic-like fluorescence. In most aquatic ecosystems, humic DOM attenuates harmful UV radiation and its absence may represent an additional stressor influencing the microbial community. Nonetheless, the stream contained an active microbial assemblage with bacterial cell abundances from 2.94×10^4 to 4.97×10^5 cells ml^{-1}, and bacterial production ranging from 58.8 to 293.2 ng C l^{-1} d^{-1}. Chlorophyll-*a* concentrations ranged from 0.3 to 0.53 μg l^{-1} indicating that algal phototrophs were the probable source of the DOM. Microbial isolates produced a rainbow of pigment colors, suggesting adaptation to stress, and were similar to those from other cryogenic systems (*Proteobacteria* and *Bacteroidetes* lineages). Supraglacial streams provide an example of contemporary microbial processes on the glacier surface and a natural laboratory for studying microbial adaptation to the absence of humics.

Keywords: dissolved organic matter (DOM), extracellular emission matrices (EEMs), ice nucleation activity, fluorescence

S Online supplementary data available from stacks.iop.org/ERL/8/035022/mmedia

1. Introduction

Dissolved organic matter (DOM) plays an important role in conditioning the microbial environment in aquatic ecosystems. DOM is comprised of a complex mixture of organic compounds produced by the degradation of higher plant matter and senescent microbial biomass, or actively excreted by microorganisms. Some DOM fractions directly support growth of heterotrophic microbes (Allan 1995). In contrast, the humic DOM fraction influences the quality of the milieu surrounding organisms by absorbing visible and UV light and buffering pH, for example. In temperate

ecosystems, runoff from the landscape conveys DOM rich in humic materials to lakes and streams and the contribution of autochthonous DOM from microbial processes may become more important in summer. Because of the absence of vascular plants in continental Antarctic ecosystems, DOM in these surface waters is primarily comprised of microbially derived materials (McKnight *et al* 1994). The glacial ice that provides the water source for the streams generally has exceedingly low DOC concentrations and does not contain spectral evidence of humic substances (Barker *et al* 2006, Pautler *et al* 2011). The conditioning of this glacial meltwater by the accumulation of microbially derived humic DOM in the dry valley lakes represents an ecological feedback that may enhance overall productivity. Characterization of DOM by fluorescence spectroscopy can provide insight into the relationships between microbial communities and the DOM pool in aquatic ecosystems and may be advantageous in supraglacial fluvial systems where DOM levels are expected to be low.

Supraglacial streams are one class of aquatic ecosystem in the McMurdo Dry Valleys where ecological feedback involving microbial production of DOM may be particularly weak. Supraglacial streams, such as the Cotton Glacier stream (figure 1), are located on the surface of glaciers and commonly occur in ablation zones (Sharp 1969). In contrast to cryoconite holes and supraglacial lakes, supraglacial streambed communities are subjected to high flushing rates when the streams are flowing. The transient nature of supraglacial streams may limit the establishment of microbial communities in perennial microbial mats and the conditioning of the overlying water by production of microbial DOM.

Supraglacial streams may offer insights to environmental requirements for the growth of microbes adapted to extremely transient and humic-free conditions. Although microbes have been found to inhabit a wide range of permanently cold temperature environments in polar regions, alpine lakes, super-cooled cloud droplets and sea ice (Psenner and Sattler 1998, Abyzov *et al* 2001, Sattler *et al* 2001), no previous study has examined the geomicrobiology of supraglacial streams on a pristine Antarctic glacier. Several characteristics (i.e., tolerance to high levels of UV radiation, production of extracellular polysaccharides to avoid desiccation, modified phospholipids, spore formation, novel enzyme systems and/or the ability to form antifreeze proteins or nucleate ice) may enable organisms to survive under low temperatures and long distance transfer. Determining which of these characteristics are important for survival may best be studied at such physically dynamic sites as the Cotton Glacier stream network because annual community development will most likely occur independent of the previous years' events.

We examined the microbial assemblage and spectral characteristics of the DOM of a remote supraglacial stream system that forms seasonally on the Cotton Glacier in the Transantarctic Mountains. Fluorescence spectroscopy was used to characterize the DOM in the stream. Microbial abundance, activity and community structure were investigated in conjunction with the biogeochemical characterization of the site to understand how the microbial ecology is influenced by DOM quality.

Figure 1. Map showing the location of the Cotton Glacier, Antarctica and the sampling site, the beginning of the supraglacial feature and the terminal moulins at the end of the feature. The image was created by the Polar Geospatial Center using imagery from WorldView-2.

2. Materials and methods

2.1. Site description

Supraglacial streams are common features of glacier surfaces of the coastal glaciers in Antarctica. The flow regime of supraglacial streams is influenced by solar radiation, convection of air currents and condensation. We examined a supraglacial stream on the Cotton Glacier in the Transantarctic Mountains north of Cape Roberts (77°07′S, 161°40′E) on the south side of the Clare Range, flowing eastward between Sperm Bluff and Queer Mountain in Victoria Land for ∼16 km (figure 1). The Cotton Glacier was discovered by the Western Geological Party led by Griffith Taylor of the 1910–1913 British Antarctic Expedition (Wright and Priestly 1922). The Cotton Glacier has a limited catchment area in the Clare and St Johns ranges, but receives a large amount of sedimentary deposits of unknown origin (fluvial, glacial or aeolian) from surrounding areas. The bedrock geology is dominated by basement granite and Ferrar dolerite sills, with minor amounts of amphibolite and schist sandwiched between granite bodies. Beacon siliciclastic strata occur at higher elevations to the west (Elliot 2005).

The unique fluvial system (see figure 2) that forms on the Cotton Glacier results from its location in the Transantarctic Mountains. The prevailing winds converge and deposit debris on the glacier surface. Solar heating of the dark debris warms the surface, generating much greater meltwater production

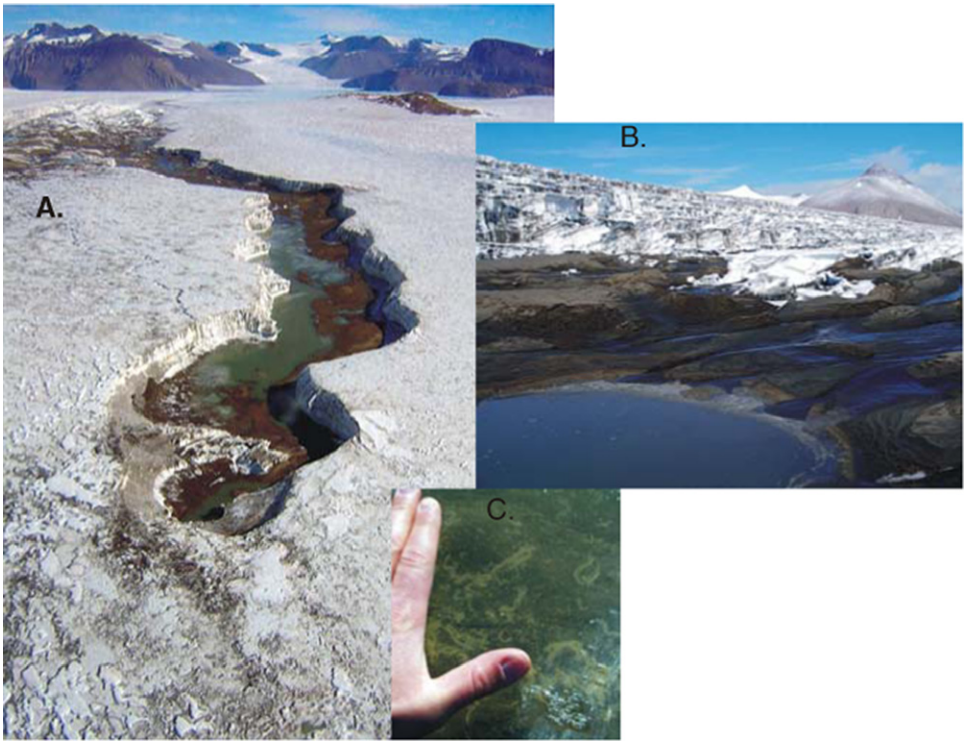

Figure 2. Photographs from the Cotton Glacier, Antarctica. (A) An aerial photograph of the supraglacial stream on the Cotton Glacier. (B) Image of the stream taken from the glacier surface. (C) A Close-up view of the benthos with a hand present to indicate scale.

than for other glaciers in Victoria Land. During the austral summers of 2004–2005 and 2009–2010, we sampled along a braided stream that flowed from mid glacier into a series of crevasses (figure 2). The stream was approximately 16 km long and varied from areas where the stream bed was entirely composed of ice to areas where sandy sediments were present. Stream depth ranged from tens of centimeters to several meters, with areas of still water and high flow.

2.2. Sample collection

Samples were collected in three locations along the channel network (Site CG1 77°05′S, 161°58′E; Site CG2 77°01′S, 162°09′E; Site CG3 77°04′S, 162°01′E), that were accessed via helicopter on 30 December 2004. At site CG1 the stream was flowing and had sediment present, CG2 was an undercut icy stream bank without sediments and high water flow and CG3 was in the midst of a large sand dune with a series of upstream ponds, low flow and potentially benthic biofilms present. Two liters of surface water were collected from CG1 and CG2, and 62 liters were collected from CG3. An additional sample was collected in December of 2009 (CG2009) near the location of CG3 (CG2009 77°04′S, 162°01′E). Temperature, pH, conductivity and total dissolved solids (TDS) measurements were measured on site using a Hydrolab mini-sonde. Surface water was collected into acid-washed, deionized water (DIW) rinsed (6×) Nalgene containers and crimp sealed serum vials and stored in dark coolers for transport to McMurdo Station.

2.3. Chemical analysis

Water samples were analyzed according to the protocols of the McMurdo Dry Valleys Long Term Ecological Research Group (Priscu and Wolf 2000). Samples for non-purgeable dissolved organic carbon (DOC) and total nitrogen analysis were filtered in the dark through 25 mm pre-combusted GF/F filters, acidified to pH 2 and analyzed on a Shimadzu TOC-V. Filters were frozen until subsequent extraction for chlorophyll-*a* analysis. Nutrients and ions were analyzed on a Lachatt autoanalyzer and a Dionex DX-300 ion chromatography system. Infrared gas analysis was used to measure dissolved inorganic carbon on acidified samples (Priscu and Wolf 2000).

2.4. Optical analysis

All DOM fluorescence signatures were analyzed according to the procedures of Cory and McKnight (2005). EEMs were collected over an excitation range of 240–450 nm, by 5 nm and 10 nm increments, respectively, while emission was monitored from 300–600 nm and 300–560 nm by 2 nm increments on a Fluoromax-3 fluorometer (Jobin-Yvon Horiba). No inner filter corrections were necessary; absorbance scans (Shimadzu UV-1601 PC) confirmed samples were optically dilute (<0.02 AU in excitation range 240–450 nm). All EEMs data were corrected for instrument-specific bias using manufacturer-generated correction files for excitation and emission and blank corrected. The blank for the Nalgene bottle control was obtained by storing lab grade water for 24 h and when

the EEM for this sample was blank corrected there were no detectable fluorophores (see supplemental information, figure S1 available at stacks.iop.org/ERL/8/035022/mmedia). Intensities were normalized to the areas under the water-Raman curves at an excitation of 350 nm (Stedmon *et al* 2003). EEMs of the samples were examined in the context of the three major emission maxima, which are commonly observed in aquatic ecosystems (Coble *et al* 1990, figure 3). Peaks A and C, thought to be associated with the fulvic and humic fraction of DOM, have excitation/emission wavelength maxima (Ex_{max}/Em_{max}) of 240–260/380–460 nm and 320–360/420–460 nm, respectively. The third region of emission maxima (Ex_{max}/Em_{max} = 270–280/310–360 nm) is commonly called the protein or amino-acid region, because aromatic amino acids such as tryptophan, tyrosine and phenylalanine have excitation and emission maxima in this region (Coble *et al* 1993, Yamashita and Tanoue 2003, Cory and McKnight 2005). The amino-acid region of DOM EEMs is commonly split into peaks B and T, tyrosine-like and tryptophan-like, respectively (Coble *et al* 1993).

2.5. Microbiological analysis

Water samples for bacterial and bacteriophage enumeration were collected in sterile 125 ml screw cap flasks. All flasks were immediately flash frozen in liquid nitrogen in the field and stored at −80 °C as recommended by Wen *et al* (2004). Prior to sample processing, all flasks were removed from −80 °C storage and allowed to thaw in the dark at room temperature overnight. Samples were first filtered through a 25 mm diameter, 0.20 μm pore size filter to retain the bacteria. Filtrate from each sample was aseptically collected and then filtered through a 25 mm diameter, 0.02 μm pore size filter to retain the bacteriophage. Filters were stained with SYBR Gold as previously described by Lisle and Priscu (2004). All filters were counted for bacteria and bacteriophage abundance using an Olympus BX51 epifluorescent microscope equipped with a UPlanApo oil immersion objective (total magnification: 1000×) and a filter cube set (#31001, Chroma Technology Corp.) optimized for the detection of SYBR Gold. A calibrated ocular reticle was used to count at least 20 systematically selected fields, with each field containing a minimum of 20 cells per reticle grid (Lisle *et al* 2004). Replicate filters were prepared and counted for each sample.

Heterotrophic bacterial productivity was measured via the incorporation of ^{3}H-thymidine (20 nM final conc.) into DNA following the methodology of Takacs and Priscu (1998). Triplicate ^{3}H-thymidine assays and duplicate formalin-killed controls (5% final concentration, 30 min prior to ^{3}H-thymidine addition) were analyzed per sample.

To cultivate organisms from the Cotton Glacier, liquid enrichment cultures and agar solidified media plates were prepared. A general-recovery low-nutrient media, R2A (Difco) has been proven successful in isolating ice bound organisms (Christner *et al* 2000, 2003). All media were pre-incubated for at least 72 h to test for contamination before inoculation. All cultures were grown in an illuminated incubator at 4–6 °C until signs of growth appeared (∼3 weeks, with some organisms only appearing after several months).

Genomic DNA was extracted from cells that formed isolated colonies on agar plates after several transfers. Samples were sent to Laragen (www.laragen.com) for 16S ribosomal gene sequence analysis using an Applied Biosystems ABI3730 (www.appliedbiosystems.com) automated sequencer. Nearly full-length 16S sequences were obtained with multiple reads (1250–1450 bp). Nucleotide sequences were assembled and aligned in BioEdit (Hall 1999) using the ClustalX function with data obtained from GenBank using the BLAST search tool (Version 2.0; www.ncbi.nlm.nih.gov/BLAST.cgi, Altschul *et al* 1990). Sequences were filed with GenBank under accession numbers FJ152549-FJ152553.

A culture independent approach was also employed with stream water (200 ml) from site CG3 filtered onto a 47 mm Supor®-200 0.2 μm pore size, sterile membrane under low pressure (<7 psi). Filters were transferred to cryovials containing 4 ml TES buffer (100 mM Tris, 100 mM EDTA and 2% SDS), flash frozen in liquid nitrogen, and stored at −80 °C. Biomass collected on the filter was extracted and purified using the UltraClean Soil DNA kit (MO BIO, Carlsbad, CA, USA). Using universal bacterial 16S rRNA primers 9F (5′-GAGTTTGATCCTGGCTCAG) and 1492R (5′-GGTTACCTTGTTACGACTT) (Stackebrandt and Liesack 1993) purified genomic DNA was amplified. Each 50 μl PCR reaction contained approximately 2 ng μl^{-1} DNA, 0.2 μM of each primer, $MgCl_2$ buffer (final conc. 1X), TAQ master (final conc. 1X), Taq DNA Polymerase (final conc. 0.025 u μl^{-1}) (all components from 5 Prime, Eppendorf). The amplification protocol included a hot start (94 °C for 4 min) and a touchdown program (Mastercycler ep, Eppendorf). The touchdown program began with an initial annealing temperature of 65 °C and decreased by 1 °C every cycle for eight cycles, followed by 22 cycles at 58 °C and a final extension at 72 °C for 10 min. A negative PCR control without DNA template was run in parallel with the sample. PCR product was confirmed by band visualization. Clone libraries were constructed using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA, USA) following the specified protocol. Clone transformants were screened with DGGE (Burr *et al* 2006) using primers 341F (5′-CCTACGGGAGGCAGCAG-3′) and 534R (5′-AATACCGCGGCTGCTGG-3′) (Muyzer *et al* 1996) with a 40 base pair GC clamp at the 5′ end of the 341F primer. Based on DGGE migration patterns, 27 clones from each clone library were selected and sent to Functional Bioscience Inc. on LB agar plates for DNA preparation and sequencing. DNA sequences were determined with a Big-DYE Terminator v3.1 cycle sequencing kit and sequence reaction products were run on an ABI 3730 DNA sequencer. DNA sequences were aligned and assembled in BioEdit (Hall 1999). Sequences were analyzed for chimeras with Bellerophon (Huber *et al* 2004), and chimeric sequences were removed. All sequences were deposited in GenBank accession numbers: HQ221513–HQ221542. Gene sequence identification was obtained through a comparison with known sequences in the GenBank data base using BLASTN (www.ncbi.nlm.nih.gov/blast/Blast.cgi, Altschul *et al* 1990). All 16S

Table 1. Water quality of streamwater from the Cotton Glacier, Antarctica. (Note: DOC = dissolved organic carbon, TN = total nitrogen, DIC = dissolved inorganic carbon, ND = not detected.)

Location	DOC (mg l^{-1})	TN (mg l^{-1})	F^- (mg l^{-1})	Cl^- (mg l^{-1})	SO_4 (mg l^{-1})	$N–NH_3$ (μg l^{-1})	$N–NO_3$ (μg l^{-1})	$P–PO_4$ (μg l^{-1})	DIC (mg C l^{-1})
CG1	0.57	0.11	0.022	0.66	0.58	4.2	27.1	ND	0.61
CG2	0.56	0.14	0.038	1.34	1.10	7.8	38.0	5.4	0.94
CG3	0.53	0.08	0.020	1.31	0.87	4.0	33.9	ND	0.51

rRNA clone sequences, Cotton Glacier isolate sequences, and reference sequences were aligned with Clustal W (Thompson *et al* 1994). Phylogenetic trees were constructed in MEGA v.4.0 (Tamura *et al* 2007) using the neighbor-joining method (Saitou and Nei 1987) and the Jukes–Cantor distance model (Jukes and Cantor 1969) with bootstrap values of 1000 replicates.

Pure cultures and mixed enrichment cultures from the benthic and glacial water samples were assayed for ice nucleation activity (INA), as the ability to cause nucleation at warmer temperatures may provide a significant survival advantage to organisms in these extreme environments (see supplemental information available at stacks.iop.org/ERL/8/035022/mmedia). An ice nucleation active fluorescent pseudomonas strain was isolated from two separate enrichment cultures by testing the INA of randomly sampled colonies growing on King's Medium B (KB) (King *et al* 1954). The purified strains are referred to as CGina-01 (from glacial water) and CGina-02 (from benthic samples). The frequency of INA among cells was determined using the droplet-freezing method (Vali 1971). INA of CGina-01 and CGina-02 was compared to that of two strains of ice nucleation active pseudomonads isolated from freshly fallen snow in Europe and maintained as reference cultures in our laboratory: strains CC1475 (*P. syringae* from central France) and GrX-01 (*P. fluorescens* from southern Greece).

CGina-01 and CGina-02 were characterized for a range of phenotypic properties pertinent to the fluorescent pseudomonads group: presence of cytochrome C oxidase; reduction of nitrate; production of arginine dihydrolase to hydrolyze esculin, gelatinase, levan sucrase and fluorescent pigments; and growth at 41 °C as described elsewhere (Lelliott *et al* 1966). These strains were also typed for their rate of resistance to a range of antibiotics: 25–200 mg l^{-1} of streptomycin, kanamycin, gentamycin, ampicillin or rifamycin.

3. Results and discussion

3.1. Biogeochemical characteristics

Supraglacial stream meltwater from the Cotton Glacier during the summer of 2004–2005 had very low ionic strength and was moderately acidic, ranging from a pH of 5.79 at site CG3 to 6.17 at site CG2. Water temperature at the three sites was variable (site CG1 = 4 °C, site CG2 = 0.05 °C, site CG3 = 5.2 °C). The concentration of anions was lowest at the most upstream site (CG1) and highest at CG2 (table 1). Nutrient concentrations were low and followed the same pattern. Dissolved organic carbon was 0.53–0.57 mg l^{-1} (44–48 μM C), which is similar to the low range of concentrations measured in terrestrial streams during mid-summer in the McMurdo Dry Valleys (Aiken *et al* 1996), but higher than typical DOC concentrations in glacial ice (Christner *et al* 2006). Total dissolved solids in the stream ranged from 0.005–0.016 mg l^{-1}. At the time sample CG2009 was collected, the Cotton Glacier stream demonstrated temperature, pH and DOC values of 1.3 °C, 6.70 and 0.70 mg l^{-1} (58 μM C), respectively; similar to those measured during the 2004–2005 season.

The fluorescence spectrum for the CG3 and CG2009 samples were unique in that they *lacked* the characteristic humic fluorescence in the region of peaks A and C (figure 3), and exhibited emission only in the amino-acid region (i.e., closest to the 'B' region; Ex/Em 240–270/300–350 nm) (figure 3). These spectra also contrast with those in the EEM of Pony Lake whole water, where peaks A, C, B and T are clearly visible, (figure 3). Further, there is a lack of detectable emission at an excitation of 370 nm, such that the fluorescence index cannot be applied. Finally, EEMs could not be modeled using either the Cory and McKnight global model (2005) or a specific model developed using DOM derived from other Antarctic lakes and streams. Given the ubiquity of humic substances and their distinctive fluorophores in freshwater and marine environments, it is exceedingly rare to observe a DOM fluorescence signature that lacks a contribution from humic material.

Our results on the lack of detectable humic fluorophores in the Cotton Glacier meltwater are similar to results for DOM in glacier ice. Barker *et al* (2006) compared DOM in glacial systems in Canada and Antarctica and found that the fluorescence spectra of DOM from ice in the Victoria Upper Glacier was dominated by blue-shifted peaks in the amino-acid region, and did not exhibit detectable emission in the region of peaks A and C. Work by Pautler *et al* (2011) demonstrated that glacial ice DOM from the Victoria Glacier in Antarctica was comprised mainly of amino acids, small organic acids and biomolecules, simple sugars and amino sugars, differentiating it from DOM in other aquatic environments.

Thus, the supraglacial stream of the Cotton Glacier stands out as an oligotrophic, transient, *humic-free* aquatic ecosystem, representing a 'rapidly assembled' ecosystem at the edge of survivability. It appears that the DOM arises primarily from the glacier ice and from growth of seasonally established microbial communities without accumulation of humic materials over time as in other aquatic ecosystems. We hypothesize that at the end of the bioactive season (i.e., before

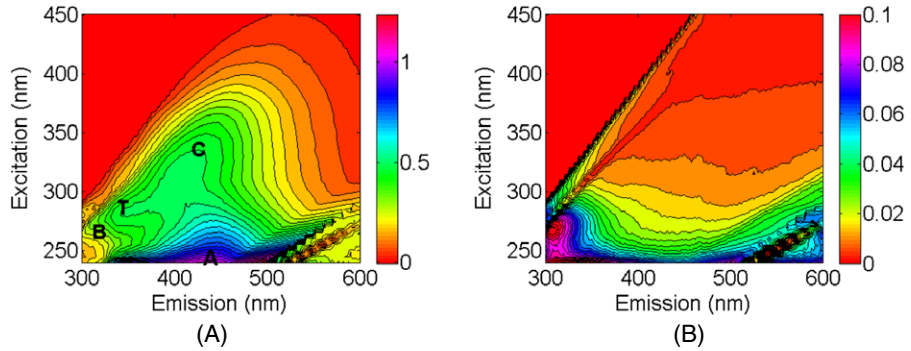

Figure 3. (A) Excitation emission matrix (EEM) from Pony Lake (sample 6 January 2005) compared to (B) an EEM from the Cotton Glacier sample. The color contours represent equal emission intensities following the legend on the right of each EEM. The location of peaks for fluorophores commonly observed in natural waters are indicated for the EEM presented in (A); peaks A and C are thought to be associated with the fulvic and humic fractions of DOM, while peaks B and T are referred to as the amino-acid region with peak B resembling a tyrosine-like peak and peak T a tryptophan-like peak. Note the absence of A and C regions from the CG sample.

Table 2. Chlorophyll-*a*, bacteria and virus-like particle (VLP) data from the Cotton Glacier, Antarctica.

Location	Chl-*a* (μg l^{-1})	Bacteria (Cells ml^{-1})[a]	Bacterial productivity (ng C l^{-1} d^{-1})	Bacterial productivity (cells d^{-1})	Carbon demand (BP/BGE) (ng l^{-1} d^{-1})	VLP (VLP ml^{-1})[a]
CG1	0.42	4.97×10^5 (1.89×10^5)	176.98	1.61×10^7	7.69×10^3	1.08×10^4 (7.97×10^3)
CG2	0.30	2.94×10^4 (1.06×10^4)	58.84	5.35×10^5	2.80×10^3	1.06×10^4 (6.43×10^3)
CG3	0.53	1.19×10^5 (3.51×10^4)	293.18	2.67×10^7	1.17×10^4	1.36×10^4 (8.15×10^3)

[a] Mean bacteria, VLP and V:B ratio values ($\pm$1 standard deviation).

freeze-up) the DOM pools in the system are essentially flushed out by the high flow rates. Because the geomorphic features of the stream system are altered on a yearly basis (based in part on sediment deposited on the glacier the previous winter) the DOM formed from microbial activity in the stream does not undergo mixing with humic materials generated in the stream system from previous years. In other aquatic systems, where the water bodies are permanent or semi-permanent, humic and fulvic acids accumulate over time and are a significant fraction of the DOM pool. Indeed their fluorescence signal dominates the typical spectra measured in natural waters (Cory and McKnight 2005, Coble 2007).

The undetectable humic content in the Cotton Glacier stream system is likely to be an outcome of low rates of production by the microbial community, rapid dilution during the period of flow, and high rates of photo-degradation of any humic-like chromophores. Such a role can be anticipated in high latitude regions given the continuous sunlight and almost nonexistent light attenuation in the Cotton Glacier stream. Furthermore, photo-degradation has been shown to selectively remove the humic/fulvic fluorophores relative to the amino-acid-like fluorophores in Arctic surface waters (Cory *et al* 2007). From a broader perspective, photo-degradation has been shown to be the most important sink for humic substances in several other low DOC environments (Coble 2007 and references therein).

3.2. Microbial communities

There were measurable concentrations of chlorophyll-*a* in all three samples, indicating the presence of an autotrophic community that could be a source of DOM. Bacterial cell counts ranged from 2.49×10^4 (CG2) to 4.97×10^5 (CG1) cells ml^{-1} between the three sites (table 2) with rods, cocci and filaments present. No yeast or filamentous fungi were observed. The highest bacterial abundance was associated with CG1. The lowest bacterial abundance among the three sites occurred in samples from CG2, the undercut stream site, which had no sediments present and the coldest temperature. Bacterial productivity values provided evidence of active microbial metabolism in the streamwater samples. The lowest productivity was found at CG2 (58.84 ng C l^{-1} d^{-1}) followed by CG1 (176.98 ng C l^{-1} d^{-1}) and CG3 (293.18 ng C l^{-1} d^{-1}). Our bacterial abundance values are similar to those recovered from other supraglacial streams (1.00×10^4–3.70×10^5 cells ml^{-1}) (Battin *et al* 2001, Skidmore *et al* 2005, Mindl *et al* 2007), and Taylor Valley cryoconites (range 1.27×10^4–7.94×10^4 cells ml^{-1} Foreman *et al* 2007), but greater than those found in South Pole surface snows (200–5000 cells ml^{-1} Carpenter *et al* 2000).

Bacteriophage or virus-like particle (VLP) abundance ranged from 1.06×10^4 (CG2) to 1.36×10^4 (CG3) VLP ml^{-1} (table 2). However, differences between VLP abundance among sites are not significant based on the measured variability (i.e., standard deviations) within the respective counts. Due to the limited amount of sample collected at CG1 and CG2, enrichment cultures focused on site CG3. Several highly pigmented as well as opaque and translucent microbial colonies were aerobically cultured and isolated on R2A media. Based on colony morphology and pigmentation characteristics, dominant isolates were chosen for sequence analysis. Nearly full-length 16S rRNA sequences

(1250–1450 bp) of these isolates revealed that they were members of the *β-Proteobacteria* and *Bacteroidetes* lineages (formerly known as Cytophaga-Flavobacteria-Bacteroides (CFB) phylum). The CG3 16S rRNA clone library sequence lineages were consistent with the dominant lineages found in the Cotton Glacier isolates. For clone library and isolate sequences the closest relatives, according to GenBank, came from cold temperature environments (see figure 4).

Two strains of fluorescent pseudomonads (CGina-01 and CGina-02) recovered from an R2A enrichment culture were ice nucleation active. However, none of the sequenced isolates showed INA. In pure culture, these strains had INA that could be detected at −4 °C. These strains produced ice nucleation active cells at a rate of 1 cell per 1.5×10^7 cells at −5 °C and 1 per 2×10^6 at −6 °C. Both strains produced fluorescent pigments on King's B media and possess a terminal cytochrome oxidase C. Neither strain hydrolyzed esculin, liquefied gelatin, produced levan sucrase, reduced nitrate nor grew at 41 °C suggesting that they are similar to *Pseudomonas putida*.

3.3. Physiological adaptations of the microbial assemblage to survival in a harsh, humic-free environment

Further, the Cotton Glacier microbial assemblage may reflect the multiple stresses associated with this ecosystem, as well as the stresses associated with long range aeolian transport. Representatives from the *Bacteroidetes* lineage were strongly represented among the cultured isolates. Several novel species belonging to the genus *Flavobacterium* have been isolated from Antarctica including: *Flavobacterium hibernum*, *F. gillisiae*, F. *tegetincola*, *F. frigidarium* and *F. antarcticum*. Members of this lineage have been found in a variety of cold temperature environments and often dominate in response to inputs of organic substrates, suggesting that these organisms play an important role in the decomposition of organic matter in cold environments (Tamaki *et al* 2003).

The microbial assemblage present in the Cotton Glacier stream water represents one that can function in a humic-free environment, with higher exposure to ultraviolet radiation dependent on the solar radiation and the transparency of Cotton Glacier water. Although detailed radiometric data are not available for the Cotton Glacier when the samples were collected, pyranometer data recorded at meteorological stations at nearby (<50 km away) Lake Brownsworth in the McMurdo Dry Valleys suggest a high solar flux. The average solar flux at Lake Brownsworth for the months of November through January from 2000 through 2004 was 299 W m^{-2} and on 30 December 2004 the average solar flux was 300 W m^{-2} (with minimum and maximum values of 63.9 W m^{-2} and 838 W m^{-2}, respectively, see metadata at www.mcmlter.org). The similar magnitude of recorded fluxes and the close proximity suggests that the average solar flux at the Cotton Glacier will be similar to 300 Wm^{-2}, neglecting local variables such as cloud cover.

The DOM molar absorptivity at $\lambda = 280$ nm of Cotton Glacier DOM was very low at 77 ± 12 l mol C^{-1} cm^{-1} (95% CI; $n = 7$) (Chin, unpublished data), almost half of that measured for Lake Fryxell fulvic acid (150 l mol C^{-1} cm^{-1}) (Chin *et al* 1994). At the level of carbon measured at CG3 and the molar absorptivity, we estimate the spectrophotometric attenuation coefficient (α) of CG3 at $\lambda = 280$ nm to be 0.87 m^{-1}. When corrected to account for the effect of the solar zenith angle at a noon sampling time (54.585°) (http://solardat.uoregon.edu/SolarPositionCalculator.html; accessed 1 March 2010) on water column light penetration, a diffuse attenuation coefficient (K_d) was estimated to be 1.087 m^{-1}, assuming that the refractive index of water at 5.2 °C is 1.36 (www.luxpop.com, accessed 28 February 2010, Schiebener *et al* 1990). This estimate is very similar to the K_ds measured in Lake Fryxell for UV wavelengths of 320 (1.295 m^{-1}), 340 (1.365 m^{-1}), and 380 (1.005 m^{-1}) nm (Vincent *et al* 1998). Lake Fryxell has been characterized as a 'global extreme' in its transparency to solar UV radiation (Vincent *et al* 1998) and the absorbance characteristics of the Cotton Glacier suggests similar extreme transparency as well. Although this estimate only considers DOM absorbance and refraction on light attenuation, given the shallow depth at CG3 (<1 m) and lack of turbidity in the water as shown by the very low UV/vis absorbances, we assume minimal contributions from scattering and predict complete penetration of UV light through the water column.

One mechanism which may help microorganisms avoid damage in a humic-free environment is production of light-screening pigments. Isolates from the Cotton Glacier produced a rainbow of pigment colors. Biological structures such as pigments can absorb UV radiation (Zenoff *et al* 2006), and may provide partial protection from UV exposure during transport. The high frequency of pigment production in recovered isolates from ice cores (Dancer *et al* 1997, Christner *et al* 2000, Priscu *et al* 2007) is consistent with the need to absorb harmful solar irradiation. Using bacterial isolates from the Cotton Glacier and other Antarctic environments Dieser *et al* (2010) has shown that carotenoid pigmentation increases resistance to environmental stressors, such as ultraviolet radiation and freeze–thaw cycles.

Another important adaptation may be the capability to withstand multiple freeze–thaw cycles. The ability of organisms to readily initiate ice formation can be a means of survival; forming ice rapidly at warmer temperatures may spare their membranes damage associated with the formation of ice crystals. Two isolates from the Cotton Glacier (CGina-01 and CGina-02) were found to possess INA. These bacteria can catalyze ice formation at −4 °C and colder temperatures, which means that they likely possess the most active Type I ice nuclei proteins (Yanofsky *et al* 1981). INA is enhanced under low-nutrient conditions (Nemecek-Marshall *et al* 1993), so the *in situ* activity may actually be higher in the Cotton Glacier than measured in the lab. Microbes found in other McMurdo Dry Valleys environments have also been shown to possess ice active substances (Raymond and Fritsen 2000, Mikucki 2005).

An intriguing possibility related to survival in a humic-free environment is that INA may provide additional protection from exposure to ultraviolet radiation. By initiating the formation of ice at higher temperatures, the organisms

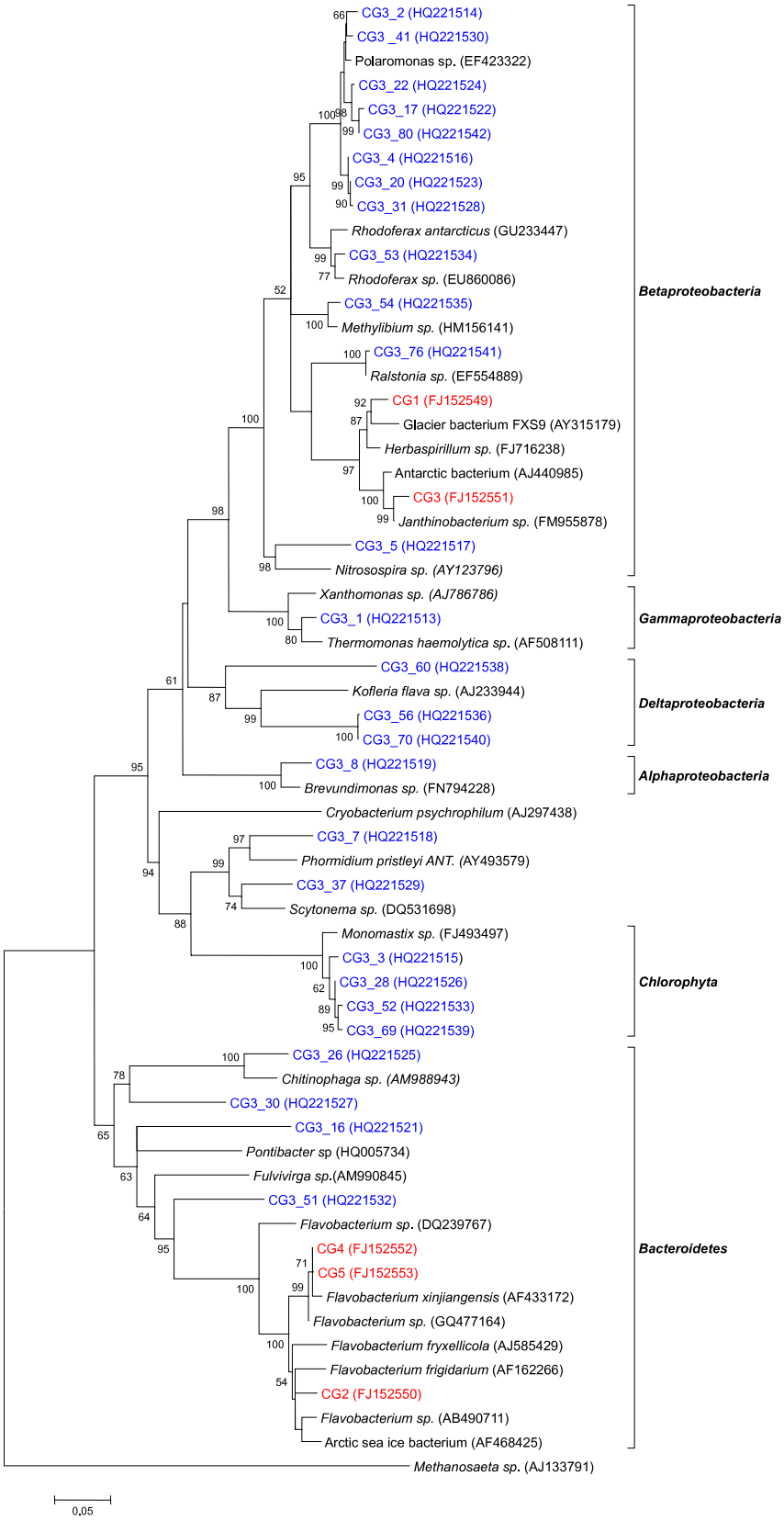

Figure 4. Phylogenetic relationship of cloned 16S rDNA genes (in blue) and isolates from the Cotton Glacier (CG) (in red) to representative 16S rDNA genes (in black). GenBank accession numbers are given in parentheses. The tree was inferred by the neighbor-joining method. Bootstrap values ≥50 are shown.

may be shielded from UV damage. The strains described here possessed robust INA at relatively warm temperatures, suggesting that this property could be expressed by cells adjusting to rapidly fluctuating conditions, such as repeated freeze–thaw cycles occurring in the Cotton Glacier system.

3.4. Carbon cycling in a harsh, humic-free supraglacial stream environment

The Cotton Glacier bacterial communities were physiologically active. Bacterial productivity (BP) values for the Cotton Glacier stream sample sites CG1, CG2 and CG3 were 176.98 ng C l^{-1} d^{-1}, 58.84 ng C l^{-1} d^{-1} and 293.18 ng C l^{-1} d^{-1}, respectively. These values are approximately an order of magnitude less than those recorded in high latitude lakes by Säwström *et al* (2007). They recorded BP rates that averaged 1363.9 ng C l^{-1} d^{-1} (range: 170.40–2440.8 ng C l^{-1} d^{-1}) in two ultraoligotrophic freshwater lakes in Antarctica. The DOC concentrations in these lakes during the austral summer ranged from 0.90–1.90 mg l^{-1}, which is approximately 1.5–3.5 fold greater than those in the Cotton Glacier streams. Foreman *et al* (2007) studied cryoconites on the surface of four glaciers in the McMurdo Dry Valleys, Antarctica. The average BP in the cryoconites (59.6 ng C l^{-1} d^{-1}) was similar to that recorded at CG2, but significantly less than the other Cotton Glacier sites. The DOC concentrations in the cryoconites ranged from 1.9 to 3.2 mg l^{-1}, which is 3–6 fold greater than the DOC concentrations in the Cotton Glacier streams. In another study of microbial activity in cryoconites, but in the Arctic, Hodson *et al* (2007) recorded a mean BP value of 127.20 ng C l^{-1} d^{-1}; range: 84.00–187.20 ng C l^{-1} d^{-1}, which is comparable to the CG1 and CG3 sites.

Bacterial growth efficiency (BGE) is the ratio of bacterial biomass produced (i.e., BP) to the amount of nutrient substrate (e.g., carbon) assimilated through BP and bacterial respiration (BR). The BGE value is regulated by complex interactions between temperature and nutrient quantity and quality (del Giorgio and Cole 1998, Eiler *et al* 2003, Apple *et al* 2006, Lopez-Urrutia and Moran 2007). del Giorgio and Cole (1998) derived a predictive model for BGE using BP values from published data sets ($n = 237$),

$$\mathrm{BGE} = [0.037 + (0.65\mathrm{BP})]/(1.8 + \mathrm{BP})$$

where BP is bacterial production (μg C l^{-1} h^{-1}).

Using this model, the estimated BGE values for sites CG1, CG2 and CG3 are 0.023, 0.021 and 0.025, respectively. These BGE values are similar to those calculated for ultraoligotrophic freshwater lakes in Antarctica (mean: 0.039, range: 0.023–0.054, Säwström *et al* 2007) and cryoconites (mean: 0.022, range: 0.021–0.023 Foreman *et al* 2007; Hodson *et al* 2007). These values are 3–6 fold less than the BGE values for bacterial communities in the relatively more nutrient enriched lakes in the McMurdo Dry Valleys (mean: 0.125, range: 0.081–0.220, Takacs *et al* 2001).

Using the BP values in table 2 and conversion factors described for the bacterial production calculations it is possible to calculate the number of cells produced per day (cells d^{-1}) (table 2). These cell production rates, when expressed per liter, are only 3.2%, 1.8% and 22.4% of the bacterial abundances in CG1, CG2 and CG3, respectively (table 2). These data suggest that the calculated BGE estimates may be too low. However, the BGE values from this study are similar to others derived from ecosystems in the same region of Antarctica. An alternative hypothesis would be that there is a persistent release of bacterial cells from the ice as it melts. These cells would be counted in the abundances for this season, but their growth would have occurred in previous seasons. The BP values are assumed to be relevant to the specific streams during a specific time period, but the release of biomass from previous seasons may artificially increase the abundance data while having little influence on the BP data. This situation would lead to current BP rates representing only a small percentage of the total bacterial abundance in each of the streams, as described. The source of DOC in these glacier streams may be predominantly from photoautotrophic processes. An additional source may be the viral lysis of bacterial biomass. Viral induced lysates of bacterial biomass have been shown to include high concentrations of amino acids and complex carbohydrates that would contribute to the DOC (Mathias *et al* 2003, Middelboe and Jørgensen 2006). The presence of viruses in these glacier streams (table 2) supports this as a possible source of DOC, though lytic infection and lysogenic rates need to be quantified.

Comparison of the DOC requirements for bacterial productivity (i.e., BP/BGE) (del Giorgio and Cole 1998) to the mean CG stream DOC concentration (0.55 mg C l^{-1}) provides another constraint on production of humic materials in this system. Based on the measured BPs and the calculated BGE values the bacterial communities at CG1 and CG3 require 61.9% and 93.5% of the available DOC, respectively, while the communities at CG2 require only 28.2%. Although the harsh and oligotrophic conditions of this stream promote the establishment of bacterial communities that are physiologically active but replicating at very low rates, these rates may be sufficient to rapidly turnover the pool of organic compounds that might otherwise react through condensation reactions to form humic material, thus further circumventing the accumulation of DOM in this system.

4. Summary

The Cotton Glacier supraglacial stream system represents a natural laboratory for studying microbial adaptation to the absence of humics, and chemical processes controlling the genesis of humic DOM. Fresh waters collected from the Cotton Glacier stream demonstrate a unique lack of fluorescence within the humic peak regions. The absence of detectable humics is likely an outcome of low rates of production by the microbial community and rapid flushing during periods of high flow. This rapid flushing and inter-annual reconfiguration of the stream system prevents the conditioning of the streamwater by the production and temporal accumulation of humic substances by microbial communities, eliminating an important ecological feedback in this harsh environment. The continuous and often

intense UV radiation acts as another stressor, photolyzing any chromophoric DOM produced by algal growth in the streamwater. This process prevents the formation and accumulation of UV-screening microbial humic DOM. This absence of humic DOM appears to exert a selective pressure on the microbial community, limiting biodiversity and causing a dominance of highly pigmented species.

Acknowledgments

Helicopter access was provided by Petroleum Helicopters Incorporated, with special thanks to D Black, B James and J Scott Pentecost. Logistical support was provided by Raytheon Polar Services, with B Brunner providing mountaineering advice. Chris Jaros was integral to field sampling and coordination. K Welch provided the ion data. Descriptions of the Cotton Glacier geology were provided by D Elliot. We thank D Georgakopoulos (Athens Agricultural University) for kindly providing strain GrX-01, and to T Wagner for the opportunity to visit this unique system. Funding for this project came from NSF OPP-0338260 to YPC, OPP-0338299 to DMM, OPP-0338121 to PLM, and OPP-0338342 and the Montana Space Grant Consortium to CMF. Any opinions, findings, or conclusions expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

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