Metagenomic Analysis of Stress Genes in Microbial Mat Communities from Antarctica and the High Arctic

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Polar and alpine microbial communities experience a variety of environmental stresses, including perennial cold and freezing; however, knowledge of genomic responses to such conditions is still rudimentary. We analyzed the metagenomes of cyanobacterial mats from Arctic and Antarctic ice shelves, using high-throughput pyrosequencing to test the hypotheses that consortia from these extreme polar habitats were similar in terms of major phyla and subphyla and consequently in their potential responses to environmental stresses. Statistical comparisons of the protein-coding genes showed similarities between the mats from the two poles, with the majority of genes derived from *Proteobacteria* and *Cyanobacteria*; however, the relative proportions differed, with cyanobacterial genes more prevalent in the Antarctic mat metagenome. Other differences included a higher representation of *Actinobacteria* and *Alphaproteobacteria* in the Arctic metagenomes, which may reflect the greater access to diasporas from both adjacent ice-free lands and the open ocean. Genes coding for functional responses to environmental stress (exopolysaccharides, cold shock proteins, and membrane modifications) were found in all of the metagenomes. However, in keeping with the greater exposure of the Arctic to long-range pollutants, sequences assigned to copper homeostasis genes were statistically (30%) more abundant in the Arctic samples. In contrast, more reads matching the sigma B genes were identified in the Antarctic mat, likely reflecting the more severe osmotic stress during freeze-up of the Antarctic ponds. This study underscores the presence of diverse mechanisms of adaptation to cold and other stresses in polar mats, consistent with the proportional representation of major bacterial groups.

Microbial mats dominated by cyanobacteria are commonly found in extreme environments, such as geothermal springs, hypersaline basins, ultraoligotrophic ponds, and hot and cold desert soils (10, 14). Cyanobacterial mats are also a dominant feature of polar lake, pond, and river ecosystems, with some of the most luxuriant communities growing on the thick ice shelves that float on Arctic and Antarctic seas (55). The stresses encountered by organisms on polar ice shelves include sparse nutrients, freeze-thaw cycles, bright sunlight exposure during summer, prolonged darkness during winter, salinity fluctuations, desiccation, and persistent low temperatures (29, 56). Extreme cold is an overarching stress in the polar regions because it drastically modifies the physical-chemical environment of living cells, with effects on biochemical reaction rates, substrate transport, membrane fluidity, and conformation of macromolecules, such as DNA and proteins (45, 61). Once the freezing point is crossed, there are additional physical and chemical stresses imposed by ice crystal formation, water loss, and increasing solute concentrations.

Although polar ice shelf mats are visually dominated by cyanobacteria, other microorganisms, including *Bacteria*, *Archaea*, and protists, live within these mats, supporting microinvertebrates, such as nematodes, rotifers, and tardigrades (4). Previous studies on the mats have shown that they contain much higher concentrations of nutrients than the overlying ultraoligotrophic waters (54). Furthermore, proteins involved in diverse scavenging and recycling processes are coded for within the mat metagenome (53), suggesting that the cyanobacteria profit, in terms of recycled nutrient supply, from the close proximity to other microorganisms.

Heterotrophic *Bacteria* and *Archaea* isolated from polar environments appear to be true psychrophiles in that they show evidence of cold adaptation strategies, synthesizing common stress proteins (cold shock proteins, chaperone proteins, and antifreeze proteins) and producing cryoprotection substances, including exopolysaccharides (EPS), all of which may enable their optimal growth at low temperatures (40, 60). However, cyanobacteria isolated from both the Arctic and Antarctica are psychrotolerant rather than psychrophilic, with growth optima at temperatures that are well above those of the ambient environment (51). Consistent with these observations, *in situ* measurements of Arctic ice shelf mats have shown that photosynthesis increases with increasing temperatures up to the limit tested (20°C, well above the maximum ambient water temperature of 1.7°C), while bacterial production showed no such trend, with rates at 2.6°C that were as high as or higher than those at warmer temperatures (29). These differences led us to hypothesize that mat communities dominated by different major phyla could have differences in their potential responses to stress at a genetic level. We investigated this hypothesis by way of metagenomic analyses of ice shelf mats from both the Arctic and Antarctica. In addition, we tested the notion that consortia occupying similar extreme habitats, but on opposite sides of the planet, were genetically similar in terms of potential responses to environmental stress, irrespective of geographic origin.

TABLE 1 Environmental and metagenomic comparisons for the three sampling sites[a]

Environmental and mat data	Value for indicated sampling site		
	MIS	WHI	MCM[b]
Environmental data			
Latitude	83º02′N	83º05′N	78º01′S
Longitude	71º31′W	74º26′W	165º33′E
Temp (ºC)	1.8 (0.9)	0.9 (0.6)	7.4
pH	6.5 (0.3)	6.5 (0.5)	9.6
Conductivity (μS cm^{-1})	637 (144)	385 (345)	1568
Mat Chl a (μg cm^{-2})	9	40	24
Classes found in mat (% of total sequences)			
Alphaproteobacteria	26	20	9
Betaproteobacteria	17	20	25
Other *Proteobacteria*	12	11	9
Cyanobacteria	17	25	38
Planctomycetes	2.5	2	4
Actinobacteria	10.5	10	3
Archaea	0.28	0.26	0.25
Eukaryota	0.56	0.55	0.67
Virus	0.02	0.01	0.02

[a] For MIS and WHI, the environmental data are the means (standard deviations [SDs]) of three meltwater ponds from which the mat samples were pooled for metagenomics analysis. The percentages are the percentages of significant matches to taxonomic groups for all assigned genes. The comparisons among the metagenomes from the Markham Ice Shelf (MIS), Ward Hunt Ice Shelf (WHI), and McMurdo Ice Shelf (MCM) used BLASTX against the SEED protein-coding gene database (E value of $\leq 10^{-5}$; alignment length of ≥ 50 bp; percentage of identity of $>65\%$).

[b] Data are for the closest date of sampling of Fresh Pond (24 Jan 2008) (I. Hawes, personal communication).

MATERIALS AND METHODS

Study site and sample collection. Sampling was undertaken from 12 to 14 July 2007 on the Ward Hunt Ice Shelf (WHI) and the Markham Ice Shelf (MIS), located along the northern coastline of Ellesmere Island in the Canadian High Arctic (29, 30), and on 8 March 2008 on the McMurdo Ice Shelf (MCM), located in the Ross Sea sector of Antarctica (Table 1). Mats sampled from the respective regions were visually representative of the mats in their area. The Arctic samples were collected from shallow (<25-cm-deep) unnamed ponds on the ice shelves. The mats were 1-cm-thick, loosely cohesive aggregates that were olive green in color with a thin (≤ 100-μm), more cohesive orange layer at the surface, as reported earlier (29). The Arctic mats were collected from three 10- to 20-cm-deep meltwater ponds on each ice shelf and combined to produce one composite sample for MIS and another for WHI. The samples were placed directly into sterile 50-ml Falcon tubes and stored in the dark at 0 to 4°C for a day before being transported to the field laboratory, where they were frozen at −20°C until further processing. The Antarctic sample (MCM) was collected from Fresh Pond, a 1-m-deep, perennial meltwater pond on the McMurdo Ice Shelf, which was above freezing in summer and had a higher conductivity and pH than the Arctic ponds (Table 1). The Antarctic mats were cohesive, 3- to 4-mm-thick biofilms that were green-gray at the surface and gray at depth (54). Several samples of the MCM mat from Fresh Pond were combined in a single tube and stored in the dark at 0°C for 3 days before being transferred to −20°C. Physical characteristics of the source ponds, pH, conductivity, and temperature (Table 1), were determined at each site during the maximum growth period in the summer using a portable pH/Con 10 Series instrument (Oakton Instruments, Vernon Hills, IL). Chlorophyll a (Chl a) concentrations were determined by high-performance liquid chromatography (HPLC) (methods as reported by Hawes et al. [13] and Jungblut et al. [18]).

DNA extraction and sequencing. The DNA extraction and sequencing protocols were as described by Varin et al. (53). Briefly, mat samples were freeze-dried to avoid interference from exopolymeric substances during subsequent steps and extracted in XS buffer (52). DNA was purified with four phenol-chloroform-isoamyl alcohol (25:24:1) (Sigma-Aldrich) wash steps, precipitated in isopropanol (Sigma-Aldrich) with 1/10 volume of ammonium acetate (4 mM; Sigma-Aldrich), and rinsed with 70% ethanol. RNA was removed from the extracts by addition of 2 μl of RNase A (10 mg ml^{-1}; Roche Lifesciences). DNA was then washed with phenol-chloroform-isoamyl alcohol (25:24:1), precipitated, and resuspended in 1× Tris-EDTA (TE) buffer. For each site in the Arctic (MIS and WHI) and Antarctic (MCM), ca. 5 μg of total DNA was used for each pyrosequencing run (26) using a 454 Sequencing System (Roche 454 Life Sciences) at the McGill University and Genome Québec Innovation Centre (Montreal, Quebec, Canada).

Bioinformatics and statistical analyses. The 454 replicate filter proposed by Gomez-Alvarez et al. (9) (http://microbiomes.msu.edu /replicates/) was used to screen for potential artificial pyrosequencing replicates. Short or low-quality sequences with ambiguous bases (multiple internal N′s) were not included in our analysis. All metagenomic sequences were compared to protein-coding gene databases using the Meta-Genome Rapid Annotation with Subsystem Technology (MG-RAST) server, version 2.0 (http://metagenomics.nmpdr.org) (28, 37). MG-RAST used Basic Local Alignment Search Tool X (BLASTX) (2) algorithms for comparisons with protein-coding gene databases. Taxonomic analyses in MG-RAST consisted of comparing our metagenomic sequences with those in the SEED protein-coding gene database (http://www.theseed.org /wiki/index.php/Home_of_the_SEED), where we have deposited the three metagenomes (MIS, WHI, MCM). Only matches of >50 nucleotides and >65% similarity to a taxonomic group or a subsystem (subsets of sequences showing similarities to each major metabolic process) and with an E value of $\leq 10^{-5}$ were included. The best match for each sequence was automatically selected and classified as "known" if its match against the relevant database was significant, or the sequence was classified as "unknown" if no significant match was found in the database. We used the metagenomic SEED viewer of the MG-RAST server to identify sequences matching functions of interest. The metabolic comparisons performed within MG-RAST were conducted on the SEED subsystems. The tabular view filter option was used to narrow searches within subsystems and retrieve the number of matches for specific genes. Percentages of matches for a given gene were calculated according to the total number of significant sequences found with MG-RAST for each metagenome. BLAST output files were parsed by our custom scripts written in Ruby (www.ruby-lang.org/) as needed. We employed the Statistical Analysis of Metagenomic Profiles (STAMP) (version 1.08; Faculty of Computer Science, Dalhousie University) statistical probability model to identify biologically relevant differences between metagenomic communities (http: //kiwi.cs.dal.ca/Software/STAMP) (38). This model takes into account the sampling effort, defined here by the total number of reads per metagenome, to evaluate differences in the proportions of gene groups (subsystems) found with MG-RAST. In order to determine biologically significant differences between the Arctic and Antarctic ice shelves using STAMP, which is valid only for two-way comparisons, we initially compared the two Arctic metagenomes separately with the Antarctic metagenome. Since results were similar in the two-way comparisons, we then combined the two Arctic samples (MIS-WHI) and all subsequent analyses were Arctic and Antarctic comparisons for both taxonomic and functional distributions. Statistically significant differences between subsystems of metagenomes were identified by Fisher's exact test combined with the Newcombe-Wilson method for calculating confidence intervals (nominal coverage of 95%). As a multiple-hypothesis test correction, a false-discovery-rate (FDR) method was applied (either the Storey or Benjamini-Hochberg FDR approach) to indicate the percentages of false positives (reported by q values) that should be expected among all signif-
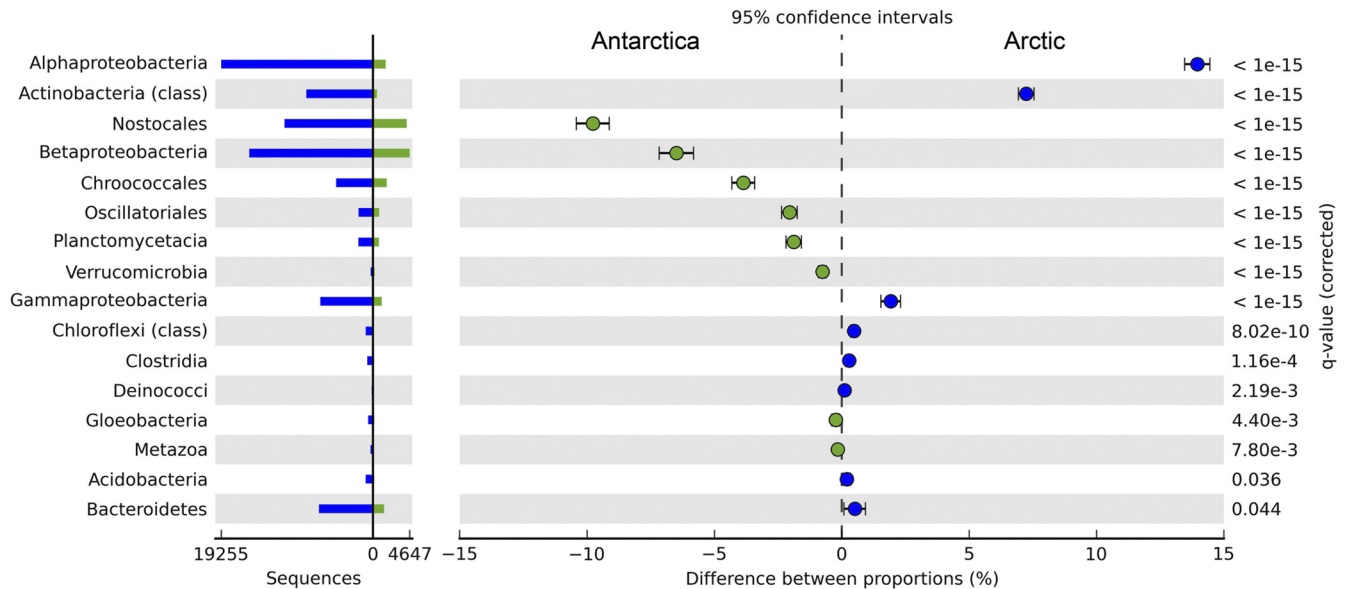
FIG 1 Statistical analyses of taxonomic profiles for the Arctic (combined MIS and WHI samples) and Antarctic (MCM sample) metagenomes. Orders or classes overrepresented in the Antarctic have a negative difference between proportions (green dots); those overrepresented in the Arctic community have a positive-value difference between proportions (blue dots). Features (orders or classes) with a q value of >0.05 were considered significant.

icant subsystems. A filter was applied to remove features with a q value of >0.05.

In order to identify the likely taxonomic source of cold and stress gene content in metagenomes from both poles, an additional statistical analysis of Arctic and Antarctic metabolic profiles was performed with STAMP that considered only sequences related to cold adaptation-specific genes and not all sequences of each metagenome (45, 50). Exceptionally for this analysis, all matches obtained using MG-RAST (BLASTX) with an E value of $\leq 10^{-5}$ were selected, regardless of alignment length and percentage of similarity thresholds.

Metagenome sequence accession. The sequence data are available under "Public Metagenomes" at http://metagenomics.nmpdr.org/. The individual files are named mis_wdu for the Markham Ice Shelf, whi_wdu for the Ward Hunt Ice Shelf, and McMurdo for the McMurdo Ice Shelf sequences.

RESULTS

Mat metagenomes. The average guanine-cytosine (GC) contents of the polar metagenomes were similar, with 55% for MIS, 53% for WHI, and 52% for MCM. The pyrosequencing yields were 256,849 sequences from MIS, 335,705 sequences from WHI, and 83,271 sequences from MCM. The average lengths were 208 bp, 184 bp, and 372 bp, respectively, giving an overall average of 255 bp and a total of 146,080,402 bp. The metagenomes represent a sampling of hundreds of thousands of bacteria, belonging to many genera. The low number of significant matches reflects the fact that these are short reads and most do not contain sufficient taxonomic information to be of use. Around 15% of the total retained sequences from Arctic data sets had matches against the SEED phylogenetic profile database (alignment length of >50 bp; percent identity of $>65\%$; E value of $\leq 10^{-5}$; 37,521 matches for the MIS samples and 47,423 matches for the WHI samples), whereas about 25% of the total MCM sequences were significantly assigned, with 18,607 matches. There were 23,184 significant matches for MIS, 29,505 for WHI, and 11,753 for MCM against the SEED metabolic profile subsystems database (alignment length of >50 bp; percent identity of $>65\%$; E value of $\leq 10^{-5}$).

Taxonomic and functional comparisons of polar microbial mats. The protein-coding gene sequences indicated that there were diverse microbial taxa in all three polar mat communities, with similarities but also significant differences between the Arctic and Antarctic metagenomes (Fig. 1). In all three metagenomes, the most dominant sequences were *Proteobacteria* (Table 1). *Cyanobacteria* contributed the second-most dominant sequences but made a greater contribution to the Antarctic metagenome, with 38% of MCM sequences being from *Cyanobacteria* compared to 17% of MIS and 25% of WHI sequences. The *Planctomycetes* contributed a 2-fold-higher percentage of the total Antarctic sequences than of the Arctic sequences (Table 1). Conversely, *Actinobacteria* were over 3-fold more common in the Arctic, with ca. 10% of the total Arctic reads versus 3% of the total Antarctic reads. Within the *Proteobacteria*, there were also differences between the two polar regions. *Betaproteobacteria* contributed to a much greater percentage of *Proteobacteria* in the Antarctic mat (59%) than in the Arctic mats (31 to 39%), while *Alphaproteobacteria* were proportionately more important in the Arctic (40 to 48% of total *Proteobacteria*) than in Antarctica (20%). *Archaea* accounted for 0.61% (MIS), 0.58% (WHI), and 0.80% (MCM) of the sequences. Similarly, less than 1% of the total gene sequences were assigned as eukaryotes and viruses in the three samples. In this taxonomic analysis, 85% (219,328) of MIS, 86% (288,282) of WHI, and 78% (64,664) of MCM total sequences were not assigned to any taxon.

Protein-coding sequences of polar bacterial isolates with available complete genomes had matches with the metagenomes (see Table S1 in the supplemental material). The match with the highest frequency in all metagenomes was to the marine *Actinobacterium* strain PHSC20C1, isolated from the surface waters of the western Antarctic Peninsula (32). Other high-frequency matches in all metagenomes were to *Desulfotalea psychrophila* LSv54 from cold marine Arctic sediments (43), *Polaribacter irgensii* from sea ice, and *Flavobacterium psychrophilum* from cold-water fish (7,
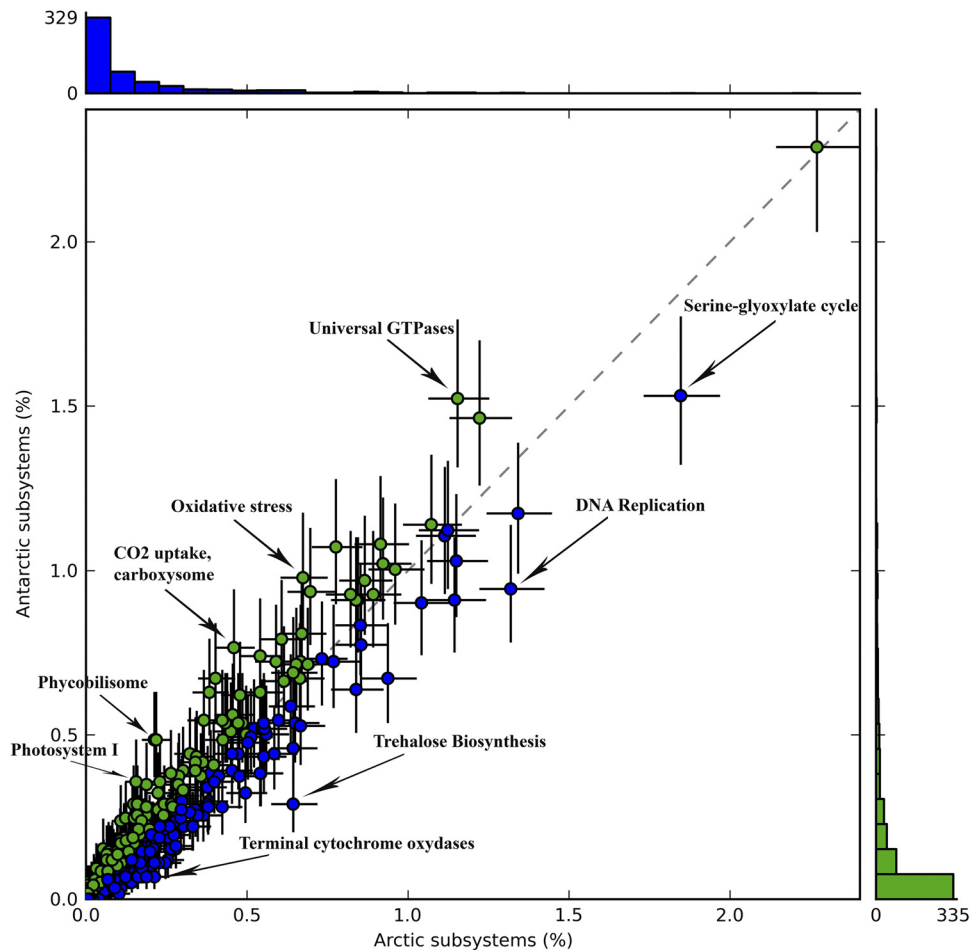
FIG 2 Comparison of metabolic profiles for the Arctic (combined MIS-WHI samples) and Antarctic (MCM sample) metagenomes. Scatter plot of metabolic profiles of Arctic and Antarctic subsystems. Blue dots represent Arctic subsystems, and green dots represent Antarctic subsystems. Dots on either side of the dashed trend line are enriched in one of the two samples. Labeled dots indicate subsystems with the greatest distances from the dashed trend line; these subsystems had the greatest proportional differences (%) between Arctic and Antarctic metagenomes. Significant differences are shown in Fig. 3. The green bar graph (right) indicates the numbers of Antarctic subsystems as proportions of the total number of sequences in the Antarctic metagenome. The blue bar graph (top) indicates the numbers of Arctic subsystems as proportions of the Arctic metagenome, indicating that most subsystems represent a low proportion of the total number of sequences. The point in the upper right-hand corner is the tRNA aminoacylation subsystem, which accounted for a relatively high proportion of identified subsystems; however, since this was the same in both metagenomes, it was not labeled.

11). There were a small number of matches to protein-coding sequences from the psychrotolerant archaeal isolate *Methanococcoides burtonii* DSM 6242 from an Antarctic lake (8). Additionally, there were matches to *Synechococcus* strain WH5701 protein sequences associated with cold stress responses; although WH5701 is not a polar species, it falls in the same 16S rRNA gene clade as several isolates from Antarctic lakes (cluster 5.2 in reference 42).

The functional analysis also revealed a high degree of similarity between the Arctic and Antarctic metagenomes (Fig. 2). However, again there were some differences, with significantly more reads in the Antarctic mat assigned to Photosystems I and II, phycobilisome proteins, and components of the cyanobacterial circadian clock (17). These results are consistent with the greater abundance of cyanobacterial genes in general in the Antarctic mat (Fig. 1 and 3). Other genes with significant overrepresentation in the Antarctic samples relative to Arctic samples included those for oxidative stress and universal GTPases (Fig. 3). In the Arctic mats, the trehalose biosynthesis coding genes were statistically more numer-

ous than in the Antarctic, as were genes associated with DNA replication and terminal cytochrome oxidases (Fig. 3).

Taxonomy of genes involved in cold and other stresses. Numbers presented in tables were normalized to the metagenome with the highest total number of sequences (WHI). The STAMP comparison adjusts for sampling coverage and hence reports only on the biological relevance of a feature between samples and, despite 5 times more Arctic sequences than Antarctic sequences, compensates for sampling artifacts. Genes implicated in adaptation to cold stress (45) were present in all three polar metagenomes, as shown in Table 2. These included the genes encoding DNA transcription and replication regulators (DnaA), recombination factor A (RecA), and topoisomerases (GyrA). RNA chaperones (Csp proteins) were rare and only sporadically recovered. Trehalose phosphate synthesis proteins (OtsA and OtsB) and fatty acid desaturases were present. Other sequences related to molecular chaperones identified as adaptations to psychrophilic lifestyles (6), such as DnaK, DnaJ, and peptidyl prolyl *cis-trans*
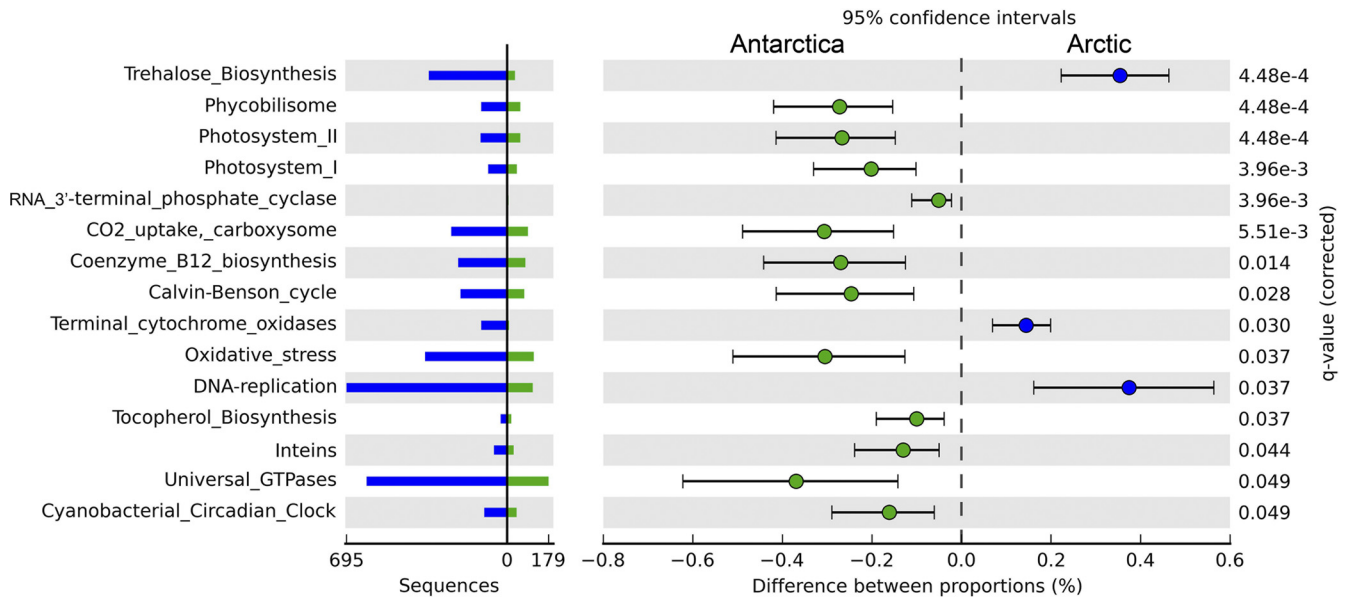
FIG 3 Statistical analyses of metabolic profiles for the Arctic microbial mats (combined MIS-WHI samples) and the Antarctic metagenome (MCM sample). Total numbers of sequences in the different categories are shown in the left bar graph; the left side (blue) represents the Arctic mats, while the right side (green) represents the Antarctic mat. Subsystems in the Antarctic microbial mat community have negative differences between proportions (green dots). Subsystems overrepresented in the Arctic microbial mat samples have positive differences between proportions (blue dots). Features (orders or classes) with a *q* value of >0.05 were considered significant.

isomerase, involved in protein folding, were more common. Translation initiation factor 1 (IF1) and ribosome-binding factor A (RbfA), both involved in protein biosynthesis, were present. Sequences associated with exopolysaccharide biosynthesis were also abundant in polar metagenomes (Table 2), as were fragments of genes coding for known cryo- and osmoprotectants, such as glutamate, glycine, betaine, and choline (50)(Table 2). Sequences matching other genes implicated in adaptation to cold stress (45), such as those encoding purine nucleoside phosphorylase (PNP), implicated in RNA degradation, and the AceE and AceF proteins (pyruvate dehydrogenases), were present in all three metagenomes. Sequences with nearest matches to several enzymes from cold-adapted *Archaea* were also recovered, including sequences coding for the archaeal tRNA modification process, such as the tRNA dihydrouridine synthase (Table 2).

Among non-cold-related stress genes, copper homeostasis and the sigma B stress response subsystems differed significantly. The Arctic metagenome was overrepresented in genes belonging to the copper homeostasis group (Table 3). Other non-cold-related stress genes were recovered from the metagenomes, most notably in subsystems from the heat shock *dnaK* gene cluster, flavohemoglobin, periplasmic stress, and acid resistance mechanisms, none of which were significantly more abundant in the metagenomes from either pole (Table 3).

Taxonomy of functional differences. Among the assumed cold-adaptive genes, there were several biologically significant differences between mats from the two polar regions. The di- and oligosaccharide genes involved in trehalose biosynthesis, those encoding OstA and OstB, were overrepresented in the Arctic and contributed by different bacterial groups than in the Antarctic (Table 2), with the majority of the Arctic sequences from *Alphaproteobacteria*, whereas in the Antarctic, *ostA* was most often found within the *Deltaproteobacteria* and *ostB* was most often

found in the *Bacteroidetes*. Genes assigned as those encoding fatty acid desaturases were statistically overrepresented in Antarctica, and both *Cyanobacteria* and *Actinobacteria* were identified as important sources of the genes (Table 2). Similarly, *Cyanobacteria* contributed to the EPS biosynthesis gene pool in both poles. *Betaproteobacteria* represented the second-most abundant source of EPS genes (Table 2).

Among non-cold-related stress genes, the Arctic metagenome was overrepresented in genes belonging to the copper homeostasis group. *Cyanobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Bacteroidetes* contributed to the copper homeostasis pool at both poles, but *Actinobacteria* were also an important source in the Arctic. The alternative sigma factor (sigma B) stress response regulation genes were overrepresented in the Antarctic, and *Cyanobacteria* accounted for most of these genes in both the Arctic and Antarctic metagenomes (Table 3).

DISCUSSION

Ice shelves provide similar habitats for microbial colonization and growth in the North and South polar regions, with liquid water conditions that persist for only a few weeks to months each year. Aqueous temperatures may occasionally rise above 5°C but are more typically around 0°C. During freeze-up, salts are excluded from the ice and the resultant brines may have temperatures that fall well below zero (13, 14, 29, 48). Calculations based on production-to-biomass ratios have shown that the ice shelf mats are perennial, with the standing stock representing many years of microbial biomass accumulation (29). The whole-mat metagenomes are therefore likely to reflect genetic responses to the ensemble of environmental conditions, including persistent cold, freeze-up, and variable salinities.

Consistent with the similar extreme conditions imposed by the ice shelf environments, the protein-coding genes indicated largely

TABLE 2 Genes implicated in the adaptation to cold environments in the three metagenomes[a]

Functional classification	Protein or subsystem name	No. of genes implicated in:		Sig[b]	Phyla (no. of hits) found in indicated polar region[c]	
		MIS-WHI	MCM		Arctic	Antarctic
DNA replication	GyrA (DNA gyrase A)	97	90	N	*Alphaproteobacteria* (28) *Bacteroidetes* (14) *Betaproteobacteria* (9) *Planctomycetes* (9) *Actinobacteria* (8)	*Betaproteobacteria* (36) *Planctomycetes* (18) Undetermined phylum (14) *Alphaproteobacteria* (9) *Bacteroidetes* (5)
	RecA (recombination factor A)	40	41	N	*Alphaproteobacteria* (8) *Betaproteobacteria* (4) *Deltaproteobacteria* (1) Undetermined phylum (1)	*Betaproteobacteria* (23) Undetermined phylum (9)
	DnaA (replication initiator protein)	134	117	N	*Alphaproteobacteria* (24) *Cyanobacteria* (19) *Bacteroidetes* (17) *Betaproteobacteria* (14) *Actinobacteria* (11)	*Cyanobacteria* (27) *Gammaproteobacteria* (14) *Betaproteobacteria* (14) *Bacteroidetes* (9) *Verrucomicrobia* (9)
DNA metabolism	HU-β (DNA supercoiling)	8	18	—		
Di- and oligosaccharides	OstA (trehalose phosphate synthase)	84	50	Arc	*Alphaproteobacteria* (17) *Actinobacteria* (15) *Betaproteobacteria* (10) *Gammaproteobacteria* (10) *Deltaproteobacteria* (3)	*Deltaproteobacteria* (14) *Cyanobacteria* (9) *Actinobacteria* (5) *Bacteroidetes* (5) *Planctomycetes* (5)
	OstB (trehalose phosphatase)	54	9	Arc	*Alphaproteobacteria* (27) *Actinobacteria* (5) *Betaproteobacteria* (4) *Deltaproteobacteria* (2) *Bacteroidetes* (2)	*Bacteroidetes* (5)
Unsaturated fatty acids	Fatty acid desaturases	18	54	Ant	*Actinobacteria* (7) *Cyanobacteria* (4) *Bacteroidetes* (2)	*Cyanobacteria* (41) Undetermined phylum (5) *Actinobacteria* (5)
Protein folding	Chaperone DnaK and DnaJ	363	432	N	*Cyanobacteria* (121) *Actinobacteria* (29) *Alphaproteobacteria* (29) *Betaproteobacteria* (26) *Bacteroidetes* (25)	*Cyanobacteria* (198) *Betaproteobacteria* (41) *Alphaproteobacteria* (23) *Actinobacteria* (18) *Planctomycetes* (9)
	Peptidyl-prolyl cis-trans isomerase	149	198	N	*Betaproteobacteria* (32) *Bacteroidetes* (13) *Gammaproteobacteria* (12) *Alphaproteobacteria* (12) *Cyanobacteria* (10)	*Betaproteobacteria* (50) *Gammaproteobacteria* (18) *Cyanobacteria* (18) *Verrucomicrobia* (18) *Bacteroidetes* (9)
Protein biosynthesis	Translation initiation factor 1 (IF1)	33	27	N	*Bacteroidetes* (7) *Alphaproteobacteria* (6) *Actinobacteria* (5) *Cyanobacteria* (4) *Planctomycetes* (2)	*Betaproteobacteria* (9) *Cyanobacteria* (5) *Verrucomicrobia* (5) *Actinobacteria* (5) *Bacteroidetes* (5)
	Ribosome-binding factor A (RbfA)	24	18	—		
Clustering-based subsystems	Exopolysaccharide biosynthesis	57	113	Ant	*Cyanobacteria* (10) *Betaproteobacteria* (6) *Alphaproteobacteria* (4) *Gammaproteobacteria* (3) *Verrucomicrobia* (2)	*Cyanobacteria* (27) *Betaproteobacteria* (23) *Verrucomicrobia* (14) *Deltaproteobacteria* (5) *Alphaproteobacteria* (5)

TABLE 2 (Continued)

Functional classification	Protein or subsystem name	No. of genes implicated in: MIS-WHI	MCM	Sig[b]	Phyla (no. of hits) found in indicated polar region[c] Arctic	Antarctic
Capsular and extracellular polysaccharides	Glycine biosynthesis	107	108	N	*Bacteroidetes* (25) *Alphaproteobacteria* (16) *Gammaproteobacteria* (11) *Actinobacteria* (10) *Cyanobacteria* (10)	*Cyanobacteria* (41) *Bacteroidetes* (18) *Gammaproteobacteria* (9) *Alphaproteobacteria* (9) *Betaproteobacteria* (5)
Amino acids and derivatives	Glutamate biosynthesis	20	5	—		
	Choline and betaine uptake, betaine biosynthesis	316	252	N	*Alphaproteobacteria* (103) *Actinobacteria* (43) *Betaproteobacteria* (37) *Cyanobacteria* (18) *Bacteroidetes* (17)	*Alphaproteobacteria* (63) *Betaproteobacteria* (45) *Cyanobacteria* (45) *Actinobacteria* (14) *Bacteroidetes* (9)
Nucleosides and nucleotides	Purine nucleoside phosphorylase (PNP)	20	41	—		
Pyruvate metabolism II	AceE (pyruvate dehydrogenase E1 component)	170	162	N	*Actinobacteria* (41) *Alphaproteobacteria* (27) *Cyanobacteria* (27) *Betaproteobacteria* (24) *Gammaproteobacteria* (14)	*Alphaproteobacteria* (41) *Cyanobacteria* (32) *Betaproteobacteria* (27) *Verrucomicrobia* (14) *Actinobacteria* (9)
	AceF (dihydrolipoamide acetyltransferase)	65	81	N	*Actinobacteria* (15) *Cyanobacteria* (11) *Alphaproteobacteria* (9) *Betaproteobacteria* (8) *Bacteroidetes* (4)	*Cyanobacteria* (36) *Planctomycetes* (9) *Betaproteobacteria* (9) *Gammaproteobacteria* (9) *Bacteroidetes* (5)
tRNA modification *Archaea*	tRNA dihydrouridine synthase	78	81	N	*Cyanobacteria* (14) *Bacteroidetes* (14) *Actinobacteria* (13) *Alphaproteobacteria* (10) Undetermined phylum (8)	*Cyanobacteria* (23) *Verrucomicrobia* (14) *Bacteroidetes* (14) Undetermined phylum (9) *Alphaproteobacteria* (5)

[a] Number of genes in the mat metagenomes implicated in adaptation to cold environments (40, 45), number of other genes assumed to be adaptive to polar conditions, and number of genes in exopolysaccharide pathways. Using BLASTX with an *E* value of $\leq10^{-5}$, metagenomic sequences were compared to those of genes present in the SEED database. Numbers were normalized to the combined Arctic metagenome with the highest number of BLASTX hits.

[b] The biological significance (Sig) of differences between proportions using the STAMP protocols is indicated as nonsignificant (N) or significant, with Arc indicating overrepresentation in the Arctic and Ant indicating overrepresentation in the Antarctic. —, too few data to test.

[c] Phyla or subphyla related to the gene representatives are indicated for the Arctic and Antarctica, and the number of hits belonging to those taxa, normalized to the Arctic metagenomes, are given in parentheses. The cold shock proteins CspA, CspE, CspG, and CspI had <20 matches per metagenome and are not listed in the table; CspB was not detected. Only the top five phyla, ranked by numbers of hits, are given; when fewer than five were present, all are listed.

similar taxonomic compositions in the Arctic and Antarctica. Most of the matched sequences could be attributed to *Proteobacteria*, which likely profit from the organic carbon-rich environment within the mats, and *Cyanobacteria*, which provide the phototrophic energy source and structural biomass of the mats (55) while likely benefiting from the decomposition and nutrient recycling activities of the *Proteobacteria* (53) (Table 1). *Archaea* were a minor but detectable component of all three metagenomes, as were eukaryotes, including metazoans.

Cyanobacterial mats are generally found anywhere that larger metazoan grazers are absent or marginalized because of extremes in temperature, salinity, and UV (10, 16, 55). The vast range of temperatures where cyanobacterial mats are found, from continental polar regions to geothermal hot springs, suggests a diversity of strategies for coping with extremes in temperature (1, 59). Previous work has shown a relative absence in the High Arctic of cyanobacterial ribotypes from warmer latitudes and that many

High Arctic 16S rRNA gene cyanobacterial sequences are >99% similar to sequences from Antarctica, including taxa previously assumed to be endemic to Antarctica (18). This apparent bipolar distribution might also imply similarities in the mechanisms of stress tolerance throughout the cold biosphere.

Despite the similarities, there were some conspicuous differences between the polar regions, both in the relative abundances of bacteria and in the proportions of the major classes of bacteria within the mats. The Antarctic mats had a higher representation of *Cyanobacteria*, and this was also reflected in the functional analysis, with a higher percentage of genes coding for photosynthetic functions found in MCM samples than in MIS-WHI samples. The greater proteobacterial and actinobacterial representation in the Arctic may reflect increased inputs of bacteria and terrigenous materials from the adjacent ice-free land. In addition to terrestrial inocula, the Arctic ice shelves are also exposed to diasporas from marine sources (12). This was reflected in the higher representa-

TABLE 3 Comparison of the numbers of genes implicated in non-cold stress responses in polar metagenomes[a]

| Subsystem name | No. of genes implicated in: | | Sig[b] | Phyla (no. of hits) found in indicated polar region[c] | |
	MIS-WHI	MCM		Arctic	Antarctic
Acid resistance mechanism	196	108	N	*Cyanobacteria* (59) *Alphaproteobacteria* (28) *Bacteroidetes* (25) *Gammaproteobacteria* (14) *Verrucomicrobia* (10)	*Cyanobacteria* (54) *Verrucomicrobia* (14) Undetermined phylum (14) *Alphaproteobacteria* (9) *Bacteroidetes* (5)
Detoxification	19	14	—		
Periplasmic stress	139	162	N	*Cyanobacteria* (23) *Alphaproteobacteria* (20) *Betaproteobacteria* (16) *Bacteroidetes* (15) *Planctomycetes* (6)	*Cyanobacteria* (36) *Alphaproteobacteria* (14) *Betaproteobacteria* (9) *Gammaproteobacteria* (9) *Planctomycetes* (5)
Bacitracin stress response	20	27	—		
Copper homeostasis	1422	941	Arc	*Betaproteobacteria* (235) *Alphaproteobacteria* (202) *Bacteroidetes* (201) *Cyanobacteria* (147) *Actinobacteria* (84)	*Cyanobacteria* (180) *Betaproteobacteria* (153) *Bacteroidetes* (104) *Alphaproteobacteria* (45) *Planctomycetes* (36)
Sigma B stress response regulation	145	279	Ant	*Cyanobacteria* (63) *Alphaproteobacteria* (8) *Bacteroidetes* (7) *Actinobacteria* (6) *Gammaproteobacteria* (5)	*Cyanobacteria* (108) *Bacteroidetes* (23) *Verrucomicrobia* (9) *Alphaproteobacteria* (9) *Planctomycetes* (5)
Universal stress protein family	28	41	—		
Phage shock protein (psp) operon	33	14	—		
Flavohemoglobin	114	122	N	*Alphaproteobacteria* (27) *Betaproteobacteria* (21) *Actinobacteria* (10) *Gammaproteobacteria* (8) *Bacteroidetes* (8)	*Betaproteobacteria* (27) *Cyanobacteria* (18) *Actinobacteria* (14) *Planctomycetes* (9) *Verrucomicrobia* (9)
Bacterial hemoglobin	25	27	—		
Heat shock *dnaK* gene cluster extended	367	342	N	*Alphaproteobacteria* (63) *Bacteroidetes* (57) *Betaproteobacteria* (48) *Cyanobacteria* (41) *Actinobacteria* (22)	*Bacteroidetes* (59) *Betaproteobacteria* (45) *Cyanobacteria* (45) *Deltaproteobacteria* (23) *Alphaproteobacteria* (23)
Hfl operon	83	63	N	*Alphaproteobacteria* (33) *Betaproteobacteria* (21) *Gammaproteobacteria* (7) *Deltaproteobacteria* (4) *Fusobacteria* (1)	*Betaproteobacteria* (45) *Deltaproteobacteria* (5)
Carbon starvation	41	54	N	*Betaproteobacteria* (14) *Verrucomicrobia* (4) *Gammaproteobacteria* (4) *Actinobacteria* (4) *Deltaproteobacteria* (1)	*Planctomycetes* (18) *Betaproteobacteria* (14) *Gammaproteobacteria* (9) Undetermined phylum (9)

[a] Using BLASTX with an E value of $\leq 10^{-5}$, metagenomic sequences were compared to those of genes present in the SEED database. Numbers were normalized to the combined Arctic metagenome with the highest number of BLASTX hits.

[b] The biological significance (Sig) of differences between proportions using the STAMP protocols is indicated as nonsignificant (N) or significant, with Arc indicating overrepresentation in the Arctic and Ant indicating overrepresentation in the Antarctic. —, too few data to test.

[c] Phyla or subphyla related to the gene representatives are indicated for the Arctic and Antarctic, and the number of hits belonging to those taxa, normalized to the Arctic metagenomes, are given in parentheses. Only the top five phyla, ranked by numbers of hits, are given; when fewer than five were present, all are listed.

tion of bacteria, such as marine *Alphaproteobacteria*, in the Arctic mats than in the Antarctic. In addition, the shallower, more ephemeral ponds of the Arctic may be more prone to invasions by new groups (49) than the deeper, longer-persisting ponds in Antarctica.

The microbial mats showed broadly similar functional gene repertoires for acclimation to environmental stress. Among the detected genes were sequences coding for EPS production, cold shock proteins, and membrane modification. There were, however, some differences between the mats. The Arctic mats had higher representation of copper homeostasis genes, possibly indicating their greater exposure to long-range pollutants, including metals (for examples, see reference 31). Conversely, the Antarctic mat had a greater representation of the alternative sigma factor (sigma B) gene, which appears to be a general stress regulon that induces more than 100 genes in response to a great variety of stresses, including heat, acid, salt, and starvation (58). The greater frequency of this gene in Antarctica might reflect greater osmotic stresses at freeze-up in the more saline waters of the Antarctic ponds, which are more persistent and subject to salt accumulation over time.

Many studies have shown the important role of EPS in buffering and cryoprotection for diverse microorganisms against ice crystal damage and high salinity (25, 34, 35). *Cyanobacteria* produce large amounts of EPS (24, 36) and were the primary source of EPS genes in the mats from both Antarctica and the Arctic. EPS allow bacterial aggregate formation, which in turn provides opportunities for close biogeochemical interactions (34). In the cryophilic gammaproteobacterium *Psychromonas ingrahamii*, production of EPS may sequester water from the ambient saltwater, lowering the freezing point (44). Junge et al. (19) demonstrated a significant correlation between concentrations of local bacteria and EPS in Arctic winter sea ice. In harsh environments, such as the polar regions, it is likely that EPS contributes to the physical stability of microbial communities (40). Sulfate-reducing bacteria in the *Deltaproteobacteria* may also produce large amounts of EPS (5, 57). Nichols et al. (34) showed that *Proteobacteria* (especially *Gammaproteobacteria*) and *Bacteroidetes*, which were common phyla in all three polar metagenomes, are able to synthesize EPS in response to low temperatures, implying that this is a cold-adaptation process.

In all three polar samples, genes coding for xylose, mannose, rhamnose, and fucose synthesis were among the most abundant monosaccharide synthesis genes. These sugars are typically found in bacterial EPS (21), but the exact monosaccharide composition of EPS varies largely among bacterial strains (35). EPS produced by marine bacteria generally contains 20 to 50% of the total polysaccharide as uronic acid (22). Sequences assigned to uronic acid synthesis were rare in all three microbial mat samples. This is consistent with the presence of taxa, such as *Pseudoalteromonas* and *Flavobacterium*, that are known to produce EPS rich in neutral sugars (especially mannose and fucose) but with little uronic acid (35).

All of the polar metagenomes contained genes encoding cold shock proteins, which are a common feature of prokaryotes growing in low temperatures (47). RNA chaperones (cold shock proteins CspA, CspB, CspE, CspI, and CspG) are essential for proper protein folding, especially at low temperatures, guiding nascent polypeptides into functional three-dimensional configurations (44). All three polar metagenomes contained cold shock protein sequences. Matches assigned to the genes of more-constitutive

proteins associated with cold adaptation, such as DNA transcription regulators (DnaA), recombination factors (RecA), topoisomerases (GyrA), trehalose synthesis proteins (OstA and OstB), and chaperones DnaK and DnaJ, were all numerous in the three polar microbial communities. These genes are known to be induced in bacteria upon exposure to cold temperatures (20, 45, 46, 61), and DnaA and GyrA are involved in the maintenance of functional DNA topology at cold temperatures (45).

It has long been known that exposure of microorganisms to lower temperatures results in substantial alteration of their membrane compositions, with changes in the ratio of saturated to unsaturated fatty acids (for examples, see reference 27). Saturation of the membrane fatty acids decreases at low temperatures in the psychrophilic gammaproteobacterium *Psychrobacter arcticus* (41), a widespread cold-adapted species that can survive for long periods under harsh conditions, including deep permafrost (3, 41). *Gammaproteobacteria* sequences with close similarity to those of *P. arcticus* were identified in the MIS, WHI, and MCM metagenomes. In cold environments, maintenance of cell membrane integrity requires an increased proportion of unsaturated and branched fatty acids (15, 23, 33). In cyanobacteria, this membrane composition adjustment occurred via desaturases (39), and the genes coding for these enzymes were prevalent in the three polar mat metagenomes. In *P. arcticus*, the effects of low temperatures on enzyme activity are compensated for by structural modifications that increase the flexibility of at least 50% of its proteome, thereby reducing energetic requirements (3).

In summary, this metagenomic analysis of polar microbial mat consortia has revealed the presence of many cold stress genes that to date have mostly been known only from laboratory studies on isolated microorganisms. Consistent with our hypothesis, the analyses showed diverse mechanisms of potential responses to cold and other stresses, and this reflects the taxonomic diversity within the mats. In both polar regions, *Proteobacteria* and *Cyanobacteria* dominated the sequences, including the cold stress genes. However, there were distinct differences in terms of taxonomy and preferred biological functions between the Antarctic and Arctic mats. For example, the greater representation of *Cyanobacteria* in MCM was reflected by a significantly higher percentage of genes coding for photosynthetic functions. Factors such as habitat stability and the connectivity to marine and terrestrial sources of microbiota may account for the differences between the Arctic and Antarctic ice shelf mats noted here; however, additional data from a broader range of sites and habitats are required to evaluate whether these reflect fundamental, consistent differences between the two poles of the cold biosphere.

ACKNOWLEDGMENTS

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