Journal of Microbiology (2019) Vol. 57, No. 12, pp. 1095–1104
DOI 10.1007/s12275-019-9366-2
eISSN 1976-3794
pISSN 1225-8873

H_2 Metabolism revealed by metagenomic analysis of subglacial sediment from East Antarctica[§]

Zhifeng Yang[1,2,3], Yu Zhang[2,3], Yongxin Lv[2,3], Wenkai Yan[2,3], Xiang Xiao[2,3], Bo Sun[1], and Hongmei Ma[1*]

[1]*SOA Key Laboratory for Polar Science, Polar Research Institute of China, P. R. China*
[2]*State Key Laboratory of Ocean Engineering, Shanghai Jiao Tong University, P. R. China*
[3]*School of Oceanography, Shanghai Jiao Tong University, P. R. China*

(Received Jul 30, 2019 / Revised Oct 14, 2019 / Accepted Nov 6, 2019)

Subglacial ecosystems harbor diverse chemoautotrophic microbial communities in areas with limited organic carbon, and lithological H_2 produced during glacial erosion has been considered an important energy source in these ecosystems. To verify the H_2-utilizing potential there and to identify the related energy-converting metabolic mechanisms of these communities, we performed metagenomic analysis on subglacial sediment samples from East Antarctica with and without H_2 supplementation. Genes coding for several [NiFe]-hydrogenases were identified in raw sediment and were enriched after H_2 incubation. All genes in the dissimilatory nitrate reduction and denitrification pathways were detected in the subglacial community, and the genes coding for these pathways became enriched after H_2 was supplied. Similarly, genes transcribing key enzymes in the Calvin cycle were detected in raw sediment and were also enriched. Moreover, key genes involved in H_2 oxidization, nitrate reduction, oxidative phosphorylation, and the Calvin cycle were identified within one metagenome-assembled genome belonging to a *Polaromonas* sp. As suggested by our results, the microbial community in the subglacial environment we investigated consisted of chemoautotrophic populations supported by H_2 oxidation. These results further confirm the importance of H_2 in the cryosphere.

Keywords: subglacial, Antarctic, hydrogen, chemoautotrophy, nitrate, metagenome

Introduction

The Antarctic ice sheet accounts for over 80% of the global glacier area and remains an unexplored area of Earth that elicits immense curiosity (Cockell *et al.*, 2011). Microbial communities surviving in niches beneath the Antarctic ice sheet have been proposed to have diverse metabolic potential (Wadham *et al.*, 2013). High rates of chemoautotrophic activity relative to chemoheterotrophic activity have been observed in a subglacial lake as a result of limited organic carbon (Christner *et al.*, 2014). Thus, chemoautotrophic metabolism by subglacial microbes has gained increasing attention for its significance in supporting local communities. Biochemical oxidation of pyrite from a glacial bed was proven to enhance development of mineral-associated biomass in a subglacial environment (Mitchell *et al.*, 2013). In addition, chemoautotrophic bacteria able to oxidize reduced sulfur compounds have been detected in the Blood Falls, a subglacial outflow (Mikucki and Priscu, 2007). Environmentally available inorganic reducing compounds, which are determined by glacier hydrology and bedrock lithology, have shaped subglacial communities with diverse chemoautotrophic potential.

Numerous studies have suggested that H_2 could be a potential energy source for subglacial microbes. Silicate mineral weathering linked to glacial erosion produces sufficient H_2 to maintain hydrogenotrophic metabolism in the subglacial environment (Telling *et al.*, 2015; Macdonald *et al.*, 2018). Moreover, it has been verified that even trace amounts of atmospheric H_2 can support rich microbial communities in energy-limited ecosystems (King, 2003; Ji *et al.*, 2017). In recent studies, H_2 has typically been found to be consumed by hydrogenotrophic methanogenesis beneath glaciers (Michaud *et al.*, 2017; Ma *et al.*, 2018). However, electron acceptors other than CO_2, such as O_2 (Boga *et al.*, 2007), SO_4^{2-} (Badziong *et al.*, 1978; Boga *et al.*, 2007), NO_3^- (Suzuki *et al.*, 2001), and Fe(III) (Caccavo *et al.*, 1992), can be reduced by H_2 to conserve energy in facultatively anaerobic conditions. Furthermore, these oxidants are available to meet the demand for H_2 oxidation in the subglacial environment (Mikucki *et al.*, 2009; Christner *et al.*, 2014). Despite the advantages of these oxidants over CO_2 in oxic conditions, the abundance and diversity of microbes able to carry out H_2 oxidation with these oxidants are still poorly understood in subglacial environments.

In addition to energy from H_2 oxidation, coupled carbon fixation is also important in generating the biomass on which all other organisms thrive. Other than biochemical studies on chemoautotrophic rates (Boyd *et al.*, 2014; Christner *et al.*, 2014), studies on the genes involved in CO_2 fixation in subglacial microbes have been restricted to analyses of key enzyme genes in the Calvin cycle commonly used by iron- and sulfur-oxidizing bacteria (Boyd *et al.*, 2014). A total of six classic CO_2 fixation pathways have been found in CO_2-

*For correspondence. E-mail: mahongmei@pric.org.cn; Tel.: +86-21-587 11801
[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

assimilating microorganisms (Fuchs, 2011; Saini *et al.*, 2011). Recently, a seventh CO_2 fixation pathway, the reductive glycine pathway, was proposed for microbial chemolithoautotrophic phosphite oxidation under an energy-limited condition (Figueroa *et al.*, 2018). A detailed survey of the carbon fixation pathways beneath ice sheets, especially within autotrophic hydrogenotrophs, will provide a clearer blueprint for the lifestyle of microbes in subglacial ecosystems.

Here, we supplied sufficient H_2 to subglacial sediment from the East Antarctic for seven months and analyzed the metagenomes of raw and incubated sediments. This incubation strategy enabled us to target the active populations and pathways that could benefit from H_2. Through metagenomic analysis of the subglacial communities, we intended to answer the following questions: 1, What kinds of hydrogenase genes are dominant in subglacial sediment and are enriched by H_2 supplementation?; 2, Which oxidants and carbon fixation pathways are possibly used and enriched by H_2 supplementation?; 3, What types of bacteria oxidize H_2?

Materials and Methods

Study site

The study site (76°16′11.85″E, 69°24′57.93″S) is located on the Ingrid Christensen Coast of Princess Elizabeth Land, East Antarctica, near the Prydz Bay. Subglacial sediment was collected approximately 0.5 m inside the edge of the glacier (covered by nearly 1 m of snow/ice layers) by the 28th Chinese National Antarctic Research Expedition on 20 January 2012. The subglacial sediment was then stored in a sterile sealed bag (CLEANWRAP) from which the air had been pushed out by hand. The sediment was immediately frozen at -20°C for transportation and storage in the laboratory.

Incubation experiment

The subglacial sediment was precultured with an equal volume of basal salt medium (Zhang *et al.*, 2010) (modified to contain NaCl [10 g/L] and no Na_2SO_4) overnight to homogenize the slurry. Then, 4 ml of precultured sample was mixed with 11 ml of basal salt medium including a 0.1% trace element mixture, 5 mM $NaHCO_3$ solution, 0.1% vitamin mixture, 0.1% thiamin solution, and 0.1% vitamin B12 solution (Balows *et al.*, 2013) in a 38 ml glass serum bottle (Wheaton glass serum bottle; Sigma-Aldrich). No additional oxidants were added to the incubated sediment after the original ones. Next, 25 ml of 10% Na_2S was added to the bottle after the headspace of the bottle was flushed with 0.02 MPa N_2 to create anoxic conditions. H_2 was supplied through a connected gastight gas bag (Leiqi) with 0.5 L of H_2. The serum bottle was incubated at 1°C covered with aluminum foil to maintain dark conditions for seven months to establish a stable H_2-metabolizing community.

DNA sequencing and read processing

Raw and incubated sediment samples were used for DNA extraction and sequencing. First, 0.3 g of sediment was subjected to DNA extraction using a FastDNA SPIN Kit for Soil (MP Biomedicals). The concentration of the extracted DNA was subsequently measured using a Qubit Fluorometer (Thermo Fisher Scientific), while the integrity of the DNA was evaluated by agarose gel electrophoresis. The qualified DNA was then used for library construction following the method previously described (He *et al.*, 2016) and was later sequenced on an Illumina HiSeq 2000 platform to obtain the paired-end sequences of 100 bp. The sequences have been deposited in the SRA database under accession numbers SRR8820263 and SRR8820264.

The raw sequence reads were quality filtered with bbduk v37.81 (Bushnell, 2014) with thresholds of a quality score > 20 and a length > 90 bp. The filtered reads were then assembled into scaffolds with idba_ud v1.1.1 (Peng *et al.*, 2012), and gene prediction was performed for the assembled scaffolds with Prodigal v2.6.3 (Hyatt *et al.*, 2012) using the default parameters. In order to calculate the relative abundance of annotated genes in our samples, we first mapped the raw reads back to the scaffolds using bbmap v37.81 (Bushnell, 2014). The read counts for each gene were then normalized into transcripts-per-million (TPM) counts. By replacing the transcripts with reads here, TPM values can be applied to metagenomes to eliminate the influence of gene lengths and total read counts when genes are compared between samples (Ribicic *et al.*, 2018; Ali *et al.*, 2019). The sum of TPMs of all genes in each metagenome was one million. The predicted genes were then annotated with the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000). To assign the taxonomy of reads, metagenomic sequence reads were analyzed using the Kraken 2 taxonomic sequence classification approach based on National Center for Biotechnology Information (NCBI) taxonomy assignment (Wood and Salzberg, 2014; Wood *et al.*, 2019).

Metagenomic analysis

The hydrogenase genes responsible for H_2 oxidation and production in the metagenomes were identified and classified by their conserved sequence patterns. Two very conserved regions surrounding the two pairs of cysteine ligands of the [NiFe] centers in [NiFe]-hydrogenase large subunits were used to search for [NiFe]-hydrogenase genes in our metagenomes with ps_scan (Gattiker *et al.*, 2002). Based on the differences in the conserved sequence pattern, the detected [NiFe]-hydrogenase genes were classified into different groups corresponding to different types of H_2 metabolism according to a review on hydrogenase (Vignais and Billoud, 2007). To further confirm the classification of [NiFe]-hydrogenase, evolutionary relationships were analyzed using a neighbor-joining phylogenetic tree constructed with MEGA X (Kumar *et al.*, 2018). In the tree, the reference sequences with classification information were also from the review (Vignais and Billoud, 2007). [FeFe]-hydrogenases have three conserved segments encompassing the cysteine ligands of the metal site, and these segments were also applied to identify [FeFe]-hydrogenase genes using ps_scan. The [Fe]-hydrogenase Hmd was identified according to KEGG annotations.

To elucidate the oxidants used by microbes in subglacial sediment, genes for dissimilatory nitrate reduction, denitrification, dissimilatory sulfate reduction, O_2 reduction, and CO_2 reduction were identified using KEGG annotations. Carbon fixation potential was evaluated based on the pro-

portion of total genes and key enzyme genes in six classic CO_2 fixation pathways (Fuchs, 2011; Saini *et al.*, 2011) using KEGG annotations. The proportion of total genes was weighted by the average gene abundance in each pathway (the sum of 6 pathways is normalized to 1 for each sample), while the relative abundance of key enzyme genes was estimated by TPM. Fisher's exact test was used to test if H_2 supplementation significantly influenced the proportions of hydrogenase genes, reductase genes and carbon fixation enzyme genes (Parks and Beiko, 2010).

To reconstruct the metagenome-assembled genomes (MAGs) containing hydrogenases, we predicted bins (MAGs) using CONCOCT (ver 1.0.0) (Alneberg *et al.*, 2013) and manually corrected it by mmgenome2 (Karst *et al.*, 2016). The quality of bins was assessed by checkM (Parks *et al.*, 2015). ORFs were called by Prodigal (ver 2.6.3) and annotated by BlastKOALA (Kanehisa *et al.*, 2016). MAGs containing hydrogenases with completeness over 50% were selected for analysis if they were able to carry out H_2 oxidation as chemolithotrophs. The average nucleotide identity (ANI) between the MAG and all published genomes (the closest genus determined by Tetra Correlation Search of JSpecies WS) was performed with the ANIm algorithm via the JSpecies WS web service (Richter *et al.*, 2015). Taxonomic identification of these MAGs was also performed by GTDB-Tk based on concatenated protein phylogeny (Parks *et al.*, 2018). The genomic coverage of a MAG was estimated by the sum of bases (sequence length * coverage) mapped to this MAG divided by the sum of bases in the whole metagenome. These MAGs have been deposited in the National Genomics Data Center under accession number PRJCA001827.

Results

Overview of the metagenomic data

In the metagenomes, a total of 355,917,252 reads from the subglacial raw sediment (RS) sample and 194,042,960 reads from the H_2-incubated sediment (HS) sample that met the quality control criteria were subjected to further assembly. When 500 bp was used as the cutoff, 108,184 scaffolds (N50: 8,093) and 25,176 scaffolds (N50: 15,490) were generated from the RS and HS metagenomes, respectively. Furthermore, 478,934 genes were predicted in RS, among which 60.59% were successfully annotated against the KEGG; 136,393 genes were predicted in HS, among which 44.6% were successfully annotated against the KEGG.

Taxa, including bacteria, archaea, and viruses, were assigned to 27% of reads from RS and 28% of reads from HS by Kraken 2. Nearly all annotated reads were attributed to bacteria, while the archaeal reads represented 0.08% of RS reads and 0.06% of HS reads. Additionally, 0.01% of reads were attributed to viruses. The bacterial communities of RS and HS mainly comprised Betaproteobacteria (RS: 32% of bacterial reads, HS: 36% of bacterial reads), Bacteroidetes/Chlorobi group (RS: 22%, HS: 32%), Actinobacteria (RS: 15%, HS: 5%), Alphaproteobacteria (RS: 14%, HS: 8%), and Gammaproteobacteria (RS: 8%, HS: 11%) (Supplementary data Figs. S1 and S2). Flavobacteriia were enriched after H_2 supplementation, increasing in abundance from 12 to 28%. For archaeal communities, Halobacteria made up 67% of archaeal reads in RS and 39% of archaeal reads in HS (Supplementary data Figs. S3 and S4). The fraction of reads from methanogenic groups, including Methanomicrobia, Methanococci, and Methanobacteria, increased from 17 to 37%.

Identification and classification of hydrogenase genes

A total of 11 genes from RS and 18 genes from HS were identified as 5 subgroups of [NiFe]-hydrogenase and [FeFe]-hydrogenase genes (Supplementary data Table S1), while no [Fe]-hydrogenase Hmd genes were detected in either sample upon KEGG annotations. A phylogenic tree of [NiFe]-hydrogenases further confirmed the existence of 5 groups and the accuracy of the method for predicting hydrogenases (Supplementary data Fig. S5). The relative abundances of hydrogenases were expressed as TPM, and they are summarized in Fig. 1. In general, the hydrogenases were selectively enriched upon H_2 supplementation in our experiment (in HS), especially [FeFe], [NiFe] group 2b, and [NiFe] group 3d hydrogenases. Each group represented a different type of hydrogen metabolism. [FeFe]-hydrogenases are mostly used to produce H_2, with some exceptions linked to H_2 uptake; in contrast, [NiFe] group 2b hydrogenases are capable of sensing H_2, and [NiFe] group 3d hydrogenases are referred to as bidirectional hydrogenases linked to NAD(P). H_2 supplementation significantly influenced the proportion of all hydrogenase genes (Fisher's exact test: $P < 0.05$). Considering the lower number of total genes (HS: 108,184, RS: 478,934) and higher number of hydrogenase genes (HS: 18, RS: 11) detected in HS, the proportion of hydrogenases was 5.7 times higher in HS than in RS. The other genes detected in RS and HS were classified as [NiFe] group 1, [NiFe] group 2a, and [NiFe] group 3b. [NiFe] group 1 hydrogenases are referred to as membrane-bound H_2 uptake hydrogenases that perform respiratory H_2 oxidation linked to quinone reduction. [NiFe] group 2a hydrogenases are used for H_2 photoproduction in Cyanobacteria. [NiFe] group 3b hydrogenases are referred to as bifunctional (NADP) hydrogenases, which are capable of reducing S^0 to H_2S and oxidizing H_2.

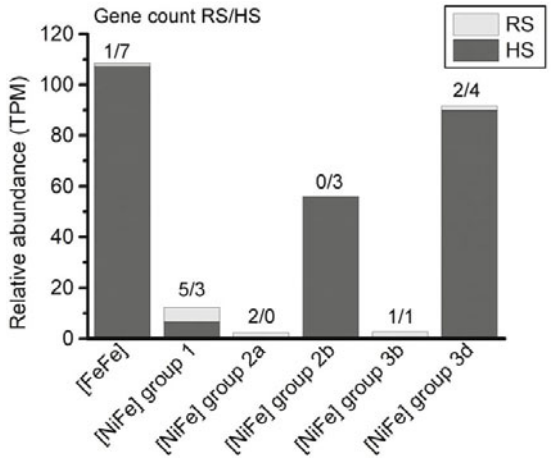

Fig. 1. Relative abundance of each group of [NiFe]- and [FeFe]-hydrogenase genes in raw sediment (RS) and H_2-incubated sediment (HS). The number x/y above each bar represents x genes found in RS and y genes found in HS.

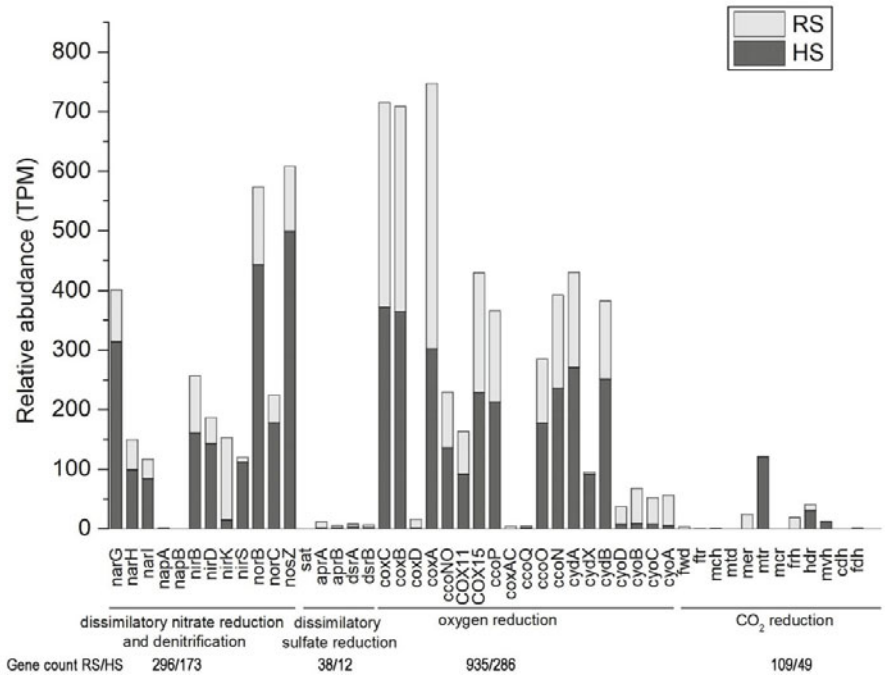

Fig. 2. Reductases for various electron acceptors. The relative abundance of genes involved in dissimilatory nitrate reduction, denitrification, dissimilatory sulfate reduction, O_2 reduction and CO_2 reduction from raw sediment (RS) and H_2-incubated sediment (HS) is shown. The abundances of multiple subunit genes for each enzyme in the methanogenesis pathway were summed to represent the total abundance of each enzyme. The number x/y beneath each type of metabolism represents x genes found in RS and y genes found in HS.

Genes for aerobic and anaerobic respiration

To explore the potential oxidants used as electron acceptors to convert energy for use in the ecosystem, enzymes that reduce NO_3^-, SO_4^{2-}, and O_2 and CO_2 were searched for via KEGG annotation (Fig. 2 and Supplementary data Table S2). Genes involved in denitrification and nitrate reduction were already abundant in RS and were even more abundant in HS. In RS, genes for all enzymes involved in denitrification and nitrate reduction were found, including nitrate reductase (EC 1.7.5.1) genes (*narG*, *narH*, and *narI*), nitrite reductase (NADH) (EC 1.7.1.15) genes (*nirB* and *nirD*), nitrite reductase (NO-forming) (EC 1.7.2.1) genes (*nirK* and *nirS*), nitric oxide reductase (EC 1.7.2.5) genes (*norB* and *norC*) and a nitrous oxide reductase (EC 1.7.2.4) gene (*nosZ*). In particular, the *nirK* and *norB* genes involved in reducing NO_2^- to NO_2 showed the highest abundance at 137.80 and 131.28 TPM, respectively. After supplementation with H_2 for seven months, significant increases in the gene abundances for nitrate reduction and denitrification from 742.72 to 2,047.68 TPM in total were observed. In addition, the genes for dissimilatory sulfate reduction were identified, suggesting that SO_4^{2-} served as an electron acceptor, although these genes showed relatively low abundance. The genes transcribing enzymes to reduce O_2 to H_2O were mainly cytochrome c oxidase, cytochrome d oxidase and cytochrome o oxidase subunit genes in prokaryotes (Ludwig, 1987), and they showed high abundances in the samples before and after H_2 augmentation without apparent changes (Fig. 2). CO_2 could serve as an electron acceptor in the methanogenic pathway. This pathway in both metagenomes was low in abundance, and there was a lack of genes for key enzymes such as methyl-coenzyme M reductase (MCR) (EC 2.8.4.1) and methylenetetrahydromethanopterin dehydrogenase (MTD) (EC 1.5.98.1). In addition, genes for CO_2 reduction to CO and HCOOH in the reductive acetyl-CoA pathway were minimally detected (Fig. 2). H_2 supplementation significantly influenced the proportions of genes involved in NO_3^- and CO_2 reduction (Fisher's exact test: $P < 0.05$), whereas no significant association of H_2 supplementation and genes involved in O_2 and SO_4^{2-} reduction was found (Fisher's exact test: $P = 0.29$ and 0.73). The proportion of genes involved in nitrate reduction (HS: 0.13%, RS: 0.06%) was 2.5 times higher in HS than in RS.

Carbon fixation pathways

The relative proportions of six carbon fixation pathways were estimated, and they are shown in Fig. 3. The Calvin cycle appeared to be the most abundant in the original sediment and was even more enriched after H_2 supplementation. The reductive acetyl-CoA pathway and 3-hydroxypropionate/4-hydroxybutyrate cycle were relatively low in abundance, while the reductive TCA cycle, dicarboxylate/4-hydroxybutyrate cycle, and 3-hydroxypropionate bi-cycle made up nearly 20% of the total 6 pathways before and after incubation. A similar pattern was confirmed by calculating the abundance of the key enzyme genes for these pathways (Fig. 3). In the Calvin cycle, the ribulose-bisphosphate carboxylase (RuBisCo) (EC 4.1.1.39), which catalyzes the carboxylation of ribulose-1,5-biphosphate (RuBP) to 3-phosphoglycerate (PGA), showed an abundance of 24.75 TPM in RS and increased 3-fold in abundance after H_2 supplementation. In the reductive TCA cycle, three key enzymes, ATP-citrate lyase (EC:2.3.3.8), 2-oxoglutarate ferredoxin oxidoreductase (EC:1.2.7.3), and fumarate reductase (EC:1.3.1.6), catalyze three reductive steps to reverse TCA cycles. Only the first two enzymes were identified, while the fumarate reductase genes were not detected in RS and HS. The formate dehydrogenase (NADP+) (EC: 1.17.1.10) of the reductive acetyl-CoA pathway was detected in RS and not detected in HS. The dicarboxylate/4-hydroxy-

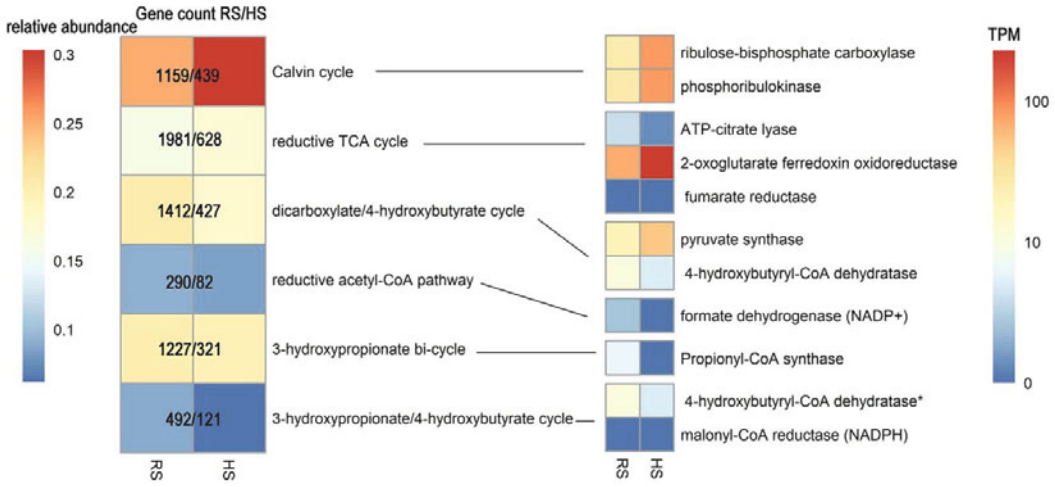

Fig. 3. Carbon fixation pathways. The proportions of total genes and relative abundances of key enzyme genes involved in six carbon fixation pathways from raw sediment (RS) and H_2-incubated sediment (HS) are shown. The proportions of total genes for each sample are normalized to a sum of 1. The relative abundance of key enzyme genes is estimated based on the average abundance (TPM) of subunit genes detected. The number x/y in the boxes of each carbon fixation pathway represents x genes found in RS and y genes found in HS.

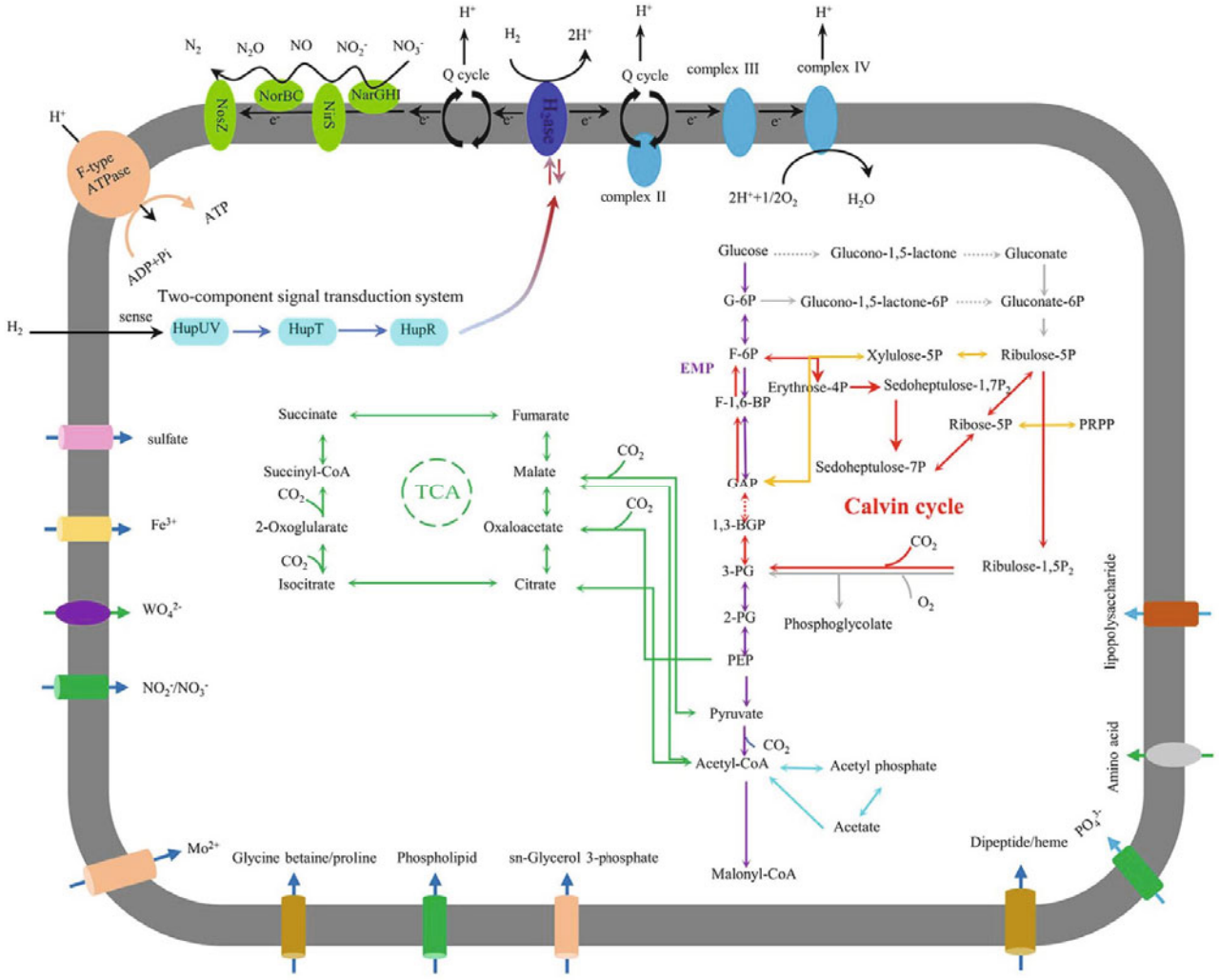

Fig. 4. The hypothetical metabolic characteristics of MAG bin2_6. The enzymes involved in or related to H_2 oxidation, H_2 sense, respiration, carbon fixation and other important functions are shown. The dashed arrow indicates a lack of one enzyme at this step.

butyrate cycle starts with the reductive carboxylation of acetyl-CoA to pyruvate, which is catalyzed by pyruvate synthase (EC:1.2.7.1), which increased in abundance by factor of 2.6 after H_2 supplementation. However, although the 4-hydroxybutyryl-CoA dehydratase was shared by the dicarboxylate/4-hydroxybutyrate cycle and the 3-hydroxypropionate/4-hydroxybutyrate cycle, its gene was barely detected (3.94 TPM) in HS. Despite the considerable abundance of total genes in the 3-hydroxypropionate bi-cycle, the key enzyme propionyl-CoA synthase, which catalyzes three key steps in this cycle, was not detected in HS and detected at only 5.43 TPM in RS, indicating a small abundance of this pathway. Malonyl-CoA reductase (NADPH) (EC:1.2.1.75), which is also shared by the 3-hydroxypropionate bi-cycle and the 3-hydroxypropionate/4-hydroxybutyrate cycle, was not detected in either sample. H_2 supplementation significantly influenced the proportion of genes in the Calvin cycle and the reductive TCA cycle (Fisher's exact test: $P < 0.05$), whereas no significant association of H_2 supplementation and genes in the other four pathways was found (Fisher's exact test: $P = 0.16–1$). The proportion of genes in the Calvin cycle (HS: 3.2%, RS: 2.4%) was 1.3 times higher in HS than in RS.

Metabolic characteristics of H_2 oxidizers

To obtain a comprehensive understanding of H_2 oxidation by a representative microbe in a subglacial ecosystem, MAGs containing hydrogenase genes were reconstructed. A total of 10 MAGs (2 of RS and 8 of HS) were reconstructed with a high completeness of over 75.47% and at least one hydrogenase gene (Supplementary data Table S3). Among the MAGs, bin2_6 (completeness of 98.73% and contamination of 0.68%) of HS was verified with nearly complete pathways for both hydrogen uptake and carbon fixation ability, whose hypothetical metabolism related to H_2 oxidation is plotted in Fig. 4. This MAG presented at a relative abundance of 1.83% in HS. The ANI between bin2_6 and *Polaromonas* sp. strain CG9_12 (Smith *et al.*, 2014), which was isolated from an Antarctic supraglacial stream, is 90.08%, which is the highest ANI for bin2_6 among all accessible *Polaromonas* genomes and the genomes of other genera. Additionally, a taxonomic assignment by phylogeny of proteins confirmed bin2_6 as *Polaromonas* (Supplementary data Table S3). The hydrogenase of bin2_6 is classified as a member of [NiFe]-hydrogenase group 1 and is encode by three subunit genes: a hydrogenase large subunit, a hydrogenase small subunit, and a Ni/Fe-hydrogenase 1 B-type cytochrome subunit. The bimetallic NiFe center of the active site is located in the large subunit, while the small subunit and cytochrome subunit not only transport electrons to electron carriers, such as coenzyme Q, but also bind the enzyme to the membrane. In addition, the [NiFe]-group II hydrogenase HupUV and HupT (EC:2.7.13.3) and HupR, which can sense hydrogen and regulate the transcription of H_2-uptake hydrogenase, were found in the genome (Dischert *et al.*, 1999). A complete denitrification pathway and genes involved in oxidative phosphorylation, which transport electrons to NO_3^- and O_2, respectively, for energy conversion, were found in bin2_6. The key enzymes RuBisCo and phosphoribulokinase (PRK) (EC:2.7.1.19) and other enzymes in the Calvin cycle except glyceraldehyde-3-phosphate dehydrogenase (NADP+) (EC:1.2.1.13) were detected in bin2_6. For other types of carbon metabolism, bin 2_6 contains all enzymes involved in the TCA cycle and a nearly complete glycolytic pathway that lacks glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12].

Discussion

Since H_2 can be generated abiogenically through erosion, it may serve as an electron donor to fuel subglacial microbial populations (Telling *et al.*, 2015; Macdonald *et al.*, 2018). Considering that H_2 generation occurs only when rocks are intensely eroded under thick ice sheets (Herman *et al.*, 2011), the H_2 concentration is 200–550 nmol/L in the central area beneath the Greenland Ice Sheet, which is 700-fold higher than the concentration in air-equilibrated water (Christner *et al.*, 2012); it is supposed to be much higher than that at the front of the subglacial zone, where there is little erosion. There was no report for H_2 concentration in the front subglacial zone, while a similar environment, underground water, possesses H_2 at typical concentrations of 1–10 nmol/L (Lovley and Goodwin, 1988). To illustrate how microbial communities respond to high and low H_2 concentrations in subglacial ecosystems, we compared genomic information from sediment samples taken from the front of the subglacial zone with and without long-term supplementation of H_2. As a result of H_2 supplementation, the relative abundance of major bacterial and archaeal groups changed, whereas most clades remained present in both samples (Supplementary data Fig. S1–S4). The subglacial sediment we sampled from the Ingrid Christensen Coast of Princess Elizabeth Land, East Antarctica, possessed various hydrogenases to allow the microbial communities to participate in the local H_2 cycle through H_2 utilization and evolution, but this occurred to a limited extent at the front of the glacier. The relative abundance of hydrogenases detected in RS was much lower than that in permafrost soil, the deep ocean and the Antarctic desert (Greening *et al.*, 2016; Ji *et al.*, 2017). However, the method we applied to screen the metagenomes for hydrogenases was somewhat stricter than that used in other studies; our study allowed no mismatch with conserved patterns, while other studies used a sequence identity of 60% as the threshold. Therefore, our method led to fewer false positive results and fewer detected hydrogenase genes than other methods. In fact, in previous studies, subsequent examination of the conserved patterns after identification of hydrogenases using BLAST removed nearly 3/4 of putative hydrogenases (Khdhiri *et al.*, 2017). Even considering this, the abundance of hydrogenases in the original sediment, where the H_2 concentration was low, was still very limited, suggesting that H_2 is a minor energy source in the ecosystem and that only a small population is supported by H_2 therein.

An apparent enrichment in [NiFe] group 2b and group 3d genes was observed when the subglacial sediment was incubated under a H_2 atmosphere (Fig. 1). [NiFe] group 2b hydrogenases can sense H_2 to regulate the two-component HupT/HupR system to activate or deactivate [NiFe] group 1 hydrogenases in the presence or absence of organic carbon (Dischert *et al.*, 1999). The enrichment suggested that the facultative autotrophs living on either H_2 or organic carbon

from glacial debris could respond quickly to abundant H_2 for growth. In different redox states, [NiFe] group 3d enzymes can consume and produce H_2, suggesting that its host is a superior population for utilizing H_2 when the level of reducing compounds in the ecosystem is insufficient. In contrast, another H_2 addition experiment showed no enrichment in H_2-oxidizing bacteria in soil (Khdhiri *et al*., 2017); the lower levels of energy sources in the original sediment than in the soil allowed H_2-oxidizing bacteria to outcompete other organisms more easily in the sediment. As a result, we hypothesize that sufficient H_2 production may support H_2-oxidizing bacteria more in the central parts of glaciers than at the edge of the ice sheet we surveyed. Nevertheless, a clear enrichment in [FeFe]-hydrogenase genes also suggested that the anaerobic incubation conditions we applied are quite suitable for fermentation to produce H_2 using the original organic carbon in the sediment.

Methanogenesis has been considered the primary hydrogenotrophic microbial activity in the subglacial ecosystem (Stibal *et al*., 2012; Michaud *et al*., 2017; Ma *et al*., 2018). As previously reported, the addition of H_2 and CO_2 stimulates methanogenic activity and increases the expression of *mcrA* genes (Ma *et al*., 2018). However, genes encoding the methanogenesis pathway were rarely detected in our metagenomic data (Fig. 2). This result is consistent with the minimal proportions of metagenomic reads belonging to methanogenic archaea (0.01–0.02%), though they increased as expected (Supplementary data Figs. S3 and S4). Similar results have been found for other potential electron acceptors, such as SO_4^{2-} and Fe(III) (nearly no Fe(III)-reducing genera were detected). SO_4^{2-} and Fe(III) have previously been demonstrated to be produced in subglacial environments because of weathering of bedrock minerals such as pyrite (Mitchell *et al*., 2013). In particular, SO_4^{2-} has been observed at higher concentrations than NO_3^- in subglacial water (Christner *et al*., 2014). However, SO_4^{2-} was not selected as the main electron acceptor in our samples because of the relatively low abundance of the genes in the dissimilatory sulfate reduction pathway (Fig. 2). The Gibbs free energy (estimated according to previously reported environmental parameters [Ma *et al*., 2018]) for each mol of H_2 oxidized using SO_4^{2-} as the electron acceptor was much smaller than that when NO_3^- was used as the acceptor, even considering the high concentrations of SO_4^{2} (Table 1). In addition, the H_2 threshold demanded by sulfate-reducing species was estimated to be approximately one order of magnitude higher than that demanded by nitrate-reducing species in the subsurface environment (Gregory *et al*., 2019). Consistent with the fact that O_2 levels are refreshed by melting ice (Siegert *et al*., 2003), abundant microorganisms capable of aerobic respiration were detected in our samples. Aerobic H_2 oxidation can be carried out by species such as *Bradyrhizobium japonicum*, *Cupriavidus necator*, *Mycobacterium smegmatis*, *Rhodococcus equi*, *Streptomyces* spp., and *Pyrinomonas methylaliphatogenes* (Khdhiri *et al*., 2017), whose genes were all detected in RS. As no apparent decreases or increases in the abundance of genes for O_2 reduction were observed without O_2 supplementation for seven months of incubation, the cytochrome oxidase genes likely belonged to facultatively anaerobic species.

NO_3^- was proposed to be the most effective electron acceptor in our incubation system, supported by the result that the bacterial populations with genes involved in nitrate reduction and denitrification were apparently enriched (Fig. 2). The nitrate reduction and denitrification pathway detected in RS is consistent with the considerable rate of nitrate reduction previously observed in subglacial sediment (Boyd *et al*., 2011). Furthermore, anaerobic respiration using NO_3^- as the electron acceptor was found to result in the highest energy density in a simulated anoxic subglacial environment (Vick-Majors *et al*., 2016), which is similar to our finding that H_2 oxidation using NO_3^- exhibited the highest Gibbs free energy in our incubation under anaerobic conditions (Table 1). NO_3^- beneath an Arctic glacier was found to come from melting water, nitrification of supraglacial ammonium and mineralization of organic nitrogen (Wynn *et al*., 2007). The rare detection of nitrification genes such as methane/ammonia monooxygenase subunit A (*pmoA-amoA*) (RS: 1.51 TPM; HS: 0 TPM) in the metagenomes of both samples suggests the possible involvement of originating from melting glacier water and organic nitrogen.

The existence of key enzyme genes for carbon fixation in RS revealed the catalytic basis of chemoautotrophic activity observed in a previous study (Christner *et al*., 2014). The Calvin cycle was the main carbon fixation pathway in both samples, while H_2 supplementation enriched both total genes and key enzyme genes of this pathway (Fig. 3). In addition, several key enzyme genes of the other five pathways were hardly detected. Therefore, chemoautotrophic microbes in the community mainly applied the Calvin cycle to produce biomass during incubation. Moreover, microbial communities capable of utilizing the Calvin cycle have also been observed in other subglacial environments, further confirming the importance of this pathway in nature (Rogers *et al*., 2013; Kayani *et al*., 2018). The key enzyme gene of the reductive acetyl-CoA pathway, which is commonly found in anaerobic methanogens and acetogens living in energy-limited conditions, was minimally de-

Table 1. Gibbs free energy of the reactions for H_2 oxidation coupled with different oxidants

Reaction	$G^{0'}$ kJ/mol H_2[a]	G kJ/mol H_2[b]
$4H_2\,(aq) + NO_3^-\,(aq) + 2H^+\,(aq) = 3H_2O\,(l) + NH_4^+\,(aq)$	-169.49	-151.44
$5H_2\,(aq) + 2NO_3^-\,(aq) + 2H^+\,(aq) = 6H_2O\,(l) + N_2\,(g)$	-243.45	-218.70
$4H_2\,(aq) + SO_4^{2-}\,(aq) = 4H_2O\,(l) + S^{2-}\,(aq)$	-47.03	-24.8
$2H_2\,(aq) + O_2\,(g) = 2H_2O\,(l)$	-263.67	not determined
$4H_2\,(aq) + HCO_3^-\,(aq) + H^+\,(aq) = CH_4\,(aq) + 3H_2O\,(aq)$	-95.53	-83.74
$4H_2\,(aq) + 2HCO_3^-\,(aq) + 2H^+\,(aq) = CH3COOH\,(aq) + 4H_2O\,(l)$	-136.43	not determined

[a] $\Delta G^{0'}$ was estimated under conditions of 100 kPa, 1°C, pH = 7 and 1 mol/L solute (exception: H^+ concentration = 10^{-7} mol/L). For each reactant state, aq indicates a solute, l indicates a liquid, and g indicates a gas.
[b] ΔG for nitrate, HCO_3^-, and sulfate reduction was estimated based on the actual concentration of each reactant used in the incubation experiment.

tected in our metagenomes. The O_2 sensitivity of the key enzymes was probably the reason for their low abundance in the subglacial oxic environment (Thauer, 2007). Similarly, the reductive citric acid cycle and dicarboxylate/4-hydroxybutyrate cycle are also oxygen-sensitive because of the requirement of oxygen-sensitive reduced ferredoxin in the reductive conversion of acetyl-CoA to succinyl-CoA (Fuchs, 2011). These three anaerobic pathways, in addition to the Calvin cycle, are commonly applied by H_2-oxidizing bacteria in the global marine environment (Hugler and Sievert, 2011). Therefore, the Calvin cycle may be the best choice for H_2-oxidizing bacteria in the subglacial ecosystem beneath the West Antarctic ice sheet, where oxygen (16% in air-saturated water) was ever detected (Christner *et al*., 2014). Certainly, as H_2-oxidizing bacteria were only a minor part of the community, it should be confirmed by MAGs whether these bacteria used the Calvin cycle.

The MAG bin2_6 we constructed from the metagenomic data described a blueprint for the H_2-oxidizing bacteria adapting to the low-energy environment (Fig. 4). For example, the existence of a two-component signal transduction system containing the [NiFe]-group II hydrogenase HupUV, HupT, and HupR indicated that bin2_6 represents a genome capable of encoding for mixotrophic growth (Dischert *et al*., 1999). When the organisms are supplied with organic carbon, the uptake hydrogenase is not synthesized under the regulation of this signal system. When they are not supplied with organic carbon but with H_2, the uptake hydrogenase is synthesized. This strategy enables this clade to expand its niche space over the subglacial environment with different energy sources. It is not surprising that bin2_6 contains genes utilizing both O_2 and NO_3^- as electron acceptors, which is consistent with the main populations of the overall communities (Fig. 2). The Calvin cycle found in this bin2_6 suggests its role in chemolithoautotrophy. The genes coding RuBisCo and PRK in bin2_6 accounted for 5.6 and 4.9% of the genes in the whole community, which represented the rough proportion of H_2-oxidizing bacteria among all autotrophs of HS. In a study on subglacial sediments sampled from Robertson Glacier, the *cbbL* gene (coding RuBisCo) of a H_2-oxidizing bacterium accounted for a similar percentage (6.9%) of the whole *cbbL* library (Boyd *et al*., 2014). Lack of the 16S rRNA gene in this MAG makes it hard to determine its accurate taxonomy, and bin2_6 is likely attributed to *Polaromonas* because of the ANI of 90.08% between bin2_6 and *Polaromonas* sp. strain CG9_12 and because of protein phylogeny (Supplementary data Table S3). *Polaromonas* made up 3–4% of bacterial reads, while bin2_6 accounted for 1.83% of the total communities (Supplementary data Figs. S1 and S2), indicating that this MAG was a main representative of *Polaromonas*. In fact, *Polaromonas hydrogenivorans* and *Polaromonas naphthalenivorans* also possess the ability to grow chemolithotrophically on H_2:CO_2 (Sizova and Panikov, 2007), and the hydrogenase of *Polaromonas naphthalenivorans* was identified as an uptake hydrogenase (Greening *et al*., 2016). As *Polaromonas* is widely distributed in the polar region and glacial ecosystem (Gawor *et al*., 2016), a detailed genomic survey of all strains in this genus targeting hydrogenase genes will reveal the association between H_2 metabolism and the wide adaptation of this genus to the barren environment.

In conclusion, microbes with the potential capacity to sense and utilize H_2 constituted a minor part of subglacial communities at the edge of the Antarctic ice sheet, and such populations enriched with sufficient H_2 are supposed to exist beneath the central part of the ice sheet. The enrichment of dissimilatory nitrate reduction and denitrification pathways by H_2 supplementation suggested that NO_3^- was likely to be the most commonly used oxidant in subglacial ecosystems when O_2 is depleted. In addition, the Calvin cycle was the dominant CO_2 fixation pathway among six classical pathways. A MAG attributed to *Polaromonas* was verified to have H_2 oxidation ability and carbon fixation ability using the Calvin cycle. Its trait of functioning as a mixotroph and being a facultatively anaerobic bacterium could expand this clade's niche space in a subglacial environment. These results support the idea that in the subglacial ecosystem, H_2 is an important electron donor that can be utilized for energy generation, for example, to fuel autotrophic microbial populations.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [41476123, 41276202 and 41676177] and the National Key Research and Development Program of China [2016YFC0300709].

Conflict of Interest

None declared.

References

Ali, N., Gong, H., Giwa, A.S., Yuan, Q., and Wang, K. 2019. Metagenomic analysis and characterization of acidogenic microbiome and effect of pH on organic acid production. *Arch. Microbiol.* **201**, 1163–1171.

Alneberg, J., Bjarnason, B.S., de Bruijn, I., Schirmer, M., Quick, J., Ijaz, U.Z., Loman, N.J., Andersson, A.F., and Quince, C. 2013. CONCOCT: clustering contigs on coverage and composition. ArXiv preprint. arXiv:1312.4038v1.

Badziong, W., Thauer, R.K., and Zeikus, J.G. 1978. Isolation and characterization of Desulfovibrio growing on hydrogen plus sulfate as the sole energy source. *Arch. Microbiol.* **116**, 41–49.

Balows, A., Trüper, H.G., Dworkin, M., Harder, W., and Schleifer, K.H. 2013 The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications. Springer Science & Business Media.

Boga, H.I., Ji, R., Ludwig, W., and Brune, A. 2007. *Sporotalea propionica* gen. nov. sp. nov., a hydrogen-oxidizing, oxygen-reducing, propionigenic firmicute from the intestinal tract of a soil-feeding termite. *Arch. Microbiol.* **187**, 15–27.

Boyd, E.S., Hamilton, T.L., Havig, J.R., Skidmore, M.L., and Shock, E.L. 2014. Chemolithotrophic primary production in a subglacial ecosystem. *Appl. Environ. Microbiol.* **80**, 6146–6153.

Boyd, E.S., Lange, R.K., Mitchell, A.C., Havig, J.R., Hamilton, T.L., Lafreniere, M.J., Shock, E.L., Peters, J.W., and Skidmore, M. 2011. Diversity, abundance, and potential activity of nitrifying and nitrate-reducing microbial assemblages in a subglacial ecosystem. *Appl. Environ. Microbiol.* **77**, 4778–4787.

Bushnell, B. 2014. BBTools software package. URL http://sourceforge.net/projects/bbmap.

Caccavo, F., Blakemore, R.P., and Lovley, D.R. 1992. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. *Appl. Environ. Microbiol.* **58**, 3211–3216.

Christner, B.C., Montross, G.G., and Priscu, J.C. 2012. Dissolved gases in frozen basal water from the NGRIP borehole: implications for biogeochemical processes beneath the Greenland Ice Sheet. *Polar Biol.* **35**, 1735–1741.

Christner, B.C., Priscu, J.C., Achberger, A.M., Barbante, C., Carter, S.P., Christianson, K., Michaud, A.B., Mikucki, J.A., Mitchell, A.C., and Skidmore, M.L. 2014. A microbial ecosystem beneath the West Antarctic ice sheet. *Nature* **512**, 310.

Cockell, C.S., Bagshaw, E., Balme, M., Doran, P., McKay, C.P., Miljkovic, K., Pearce, D., Siegert, M.J., Tranter, M., and Voytek, M. 2011. Subglacial environments and the search for life beyond the Earth. *In* Antarctic Subglacial Aquatic Environments. Vol. 192, pp. 129–148, American Geophysical Union Washington, DC, USA.

Dischert, W., Vignais, P.M., and Colbeau, A. 1999. The synthesis of *Rhodobacter capsulatus* HupSL hydrogenase is regulated by the two-component HupT/HupR system. *Mol. Microbiol.* **34**, 995–1006.

Figueroa, I.A., Barnum, T.P., Somasekhar, P.Y., Carlstrom, C.I., Engelbrektson, A.L., and Coates, J.D. 2018. Metagenomics-guided analysis of microbial chemolithoautotrophic phosphite oxidation yields evidence of a seventh natural CO_2 fixation pathway. *Proc. Natl. Acad. Sci. USA* **115**, E92–E101.

Fuchs, G. 2011. Alternative pathways of carbon dioxide fixation: insights into the early evolution of life? *Annu. Rev. Microbiol.* **65**, 631–658.

Gattiker, A., Gasteiger, E., and Bairoch, A.M. 2002. ScanProsite: a reference implementation of a PROSITE scanning tool. *Appl. Bioinformatics* **1**, 107–108.

Gawor, J., Grzesiak, J., Sasin-Kurowska, J., Borsuk, P., Gromadka, R., Gorniak, D., Swiatecki, A., Aleksandrzak-Piekarczyk, T., and Zdanowski, M.K. 2016. Evidence of adaptation, niche separation and microevolution within the genus *Polaromonas* on Arctic and Antarctic glacial surfaces. *Extremophiles* **20**, 403–413.

Greening, C., Biswas, A., Carere, C.R., Jackson, C.J., Taylor, M.C., Stott, M.B., Cook, G.M., and Morales, S.E. 2016. Genomic and metagenomic surveys of hydrogenase distribution indicate H_2 is a widely utilised energy source for microbial growth and survival. *ISME J.* **10**, 761–777.

Gregory, S.P., Barnett, M.J., Field, L.P., and Milodowski, A.E. 2019. Subsurface microbial hydrogen cycling: natural occurrence and implications for industry. *Microorganisms* **7**, 53.

He, Y., Li, M., Perumal, V., Feng, X., Fang, J., Xie, J., Sievert, S.M., and Wang, F. 2016. Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. *Nat. Microbiol.* **1**, 16035.

Herman, F., Beaud, F., Champagnac, J.D., Lemieux, J.M., and Sternai, P. 2011. Glacial hydrology and erosion patterns: A mechanism for carving glacial valleys. *Earth Planet Sci. Lett.* **310**, 498–508.

Hugler, M. and Sievert, S.M. 2011. Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. *Ann. Rev. Mar. Sci.* **3**, 261–289.

Hyatt, D., Uberbacher, E.C., Hauser, L.J., and LoCascio, P.F. 2012. Gene and translation initiation site prediction in metagenomic sequences. *Bioinformatics* **28**, 2223–2230.

Ji, M., Greening, C., Vanwonterghem, I., Carere, C.R., Bay, S.K., Steen, J.A., Montgomery, K., Lines, T., Beardall, J., van Dorst, J., *et al.* 2017. Atmospheric trace gases support primary production in Antarctic desert surface soil. *Nature* **552**, 400–403.

Kanehisa, M. and Goto, S. 2000. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30.

Kanehisa, M., Sato, Y., and Morishima, K. 2016. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. *J. Mol. Biol.* **428**, 726–731.

Karst, S.M., Kirkegaard, R.H., and Albertsen, M. 2016. mmgenome: a toolbox for reproducible genome extraction from metagenomes. *BioRxiv* DOI: https://doi.org/10.1101/059121.

Kayani, M.U.R., Doyle, S.M., Sangwan, N., Wang, G., Gilbert, J.A., Christner, B.C., and Zhu, T.F. 2018. Metagenomic analysis of basal ice from an Alaskan glacier. *Microbiome* **6**, 123.

Khdhiri, M., Piché-Choquette, S., Tremblay, J., Tringe, S.G., and Constant, P. 2017. The tale of a neglected energy source: Elevated hydrogen exposure affects both microbial diversity and function in soil. *Appl. Environ. Microbiol.* **83**, e00275-00217.

King, G.M. 2003. Contributions of atmospheric CO and hydrogen uptake to microbial dynamics on recent Hawaiian volcanic deposits. *Appl. Environ. Microbiol.* **69**, 4067–4075.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549.

Lovley, D.R. and Goodwin, S. 1988. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. *Geochim. Cosmochim. Acta* **52**, 2993–3003.

Ludwig, B. 1987. Cytochrome *c* oxidase in prokaryotes. *FEMS Microbiol. Rev.* **3**, 41–56.

Ma, H., Yan, W., Xiao, X., Shi, G., Li, Y., Sun, B., Dou, Y., and Zhang, Y. 2018. *Ex situ* culturing experiments revealed psychrophilic hydrogentrophic methanogenesis being the potential dominant methane-producing pathway in subglacial sediment in Larsemann Hills, Antarctic. *Front. Microbiol.* **9**, 237.

Macdonald, M.L., Wadham, J.L., Telling, J., and Skidmore, M.L. 2018. Glacial erosion liberates lithologic energy sources for microbes and acidity for chemical weathering beneath glaciers and ice sheets. *Front. Earth Sci.* **6**, 212.

Michaud, A.B., Dore, J.E., Achberger, A.M., Christner, B.C., Mitchell, A.C., Skidmore, M.L., Vick-Majors, T.J., and Priscu, J.C. 2017. Microbial oxidation as a methane sink beneath the West Antarctic Ice Sheet. *Nat. Geosci.* **10**, 582–586.

Mikucki, J.A., Pearson, A., Johnston, D.T., Turchyn, A.V., Farquhar, J., Schrag, D.P., Anbar, A.D., Priscu, J.C., and Lee, P.A. 2009. A contemporary microbially maintained subglacial ferrous "Ocean". *Science* **324**, 397–400.

Mikucki, J.A. and Priscu, J.C. 2007. Bacterial diversity associated with Blood Falls, a subglacial outflow from the Taylor Glacier, Antarctica. *Appl. Environ. Microbiol.* **73**, 4029–4039.

Mitchell, A.C., Lafrenière, M.J., Skidmore, M.L., and Boyd, E.S. 2013. Influence of bedrock mineral composition on microbial diversity in a subglacial environment. *Geology* **41**, 855–858.

Parks, D.H. and Beiko, R.G. 2010. Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* **26**, 715–721.

Parks, D.H., Chuvochina, M., Waite, D.W., Rinke, C., Skarshewski, A., Chaumeil, P.A., and Hugenholtz, P. 2018. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat. Biotechnol.* **36**, 996–1004.

Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., and Tyson, G.W. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* **25**, 1043–1055.

Peng, Y., Leung, H.C., Yiu, S.M., and Chin, F.Y. 2012. IDBA-UD: a *de novo* assembler for single-cell and metagenomic sequencing data with highly uneven depth. *Bioinformatics* **28**, 1420–1428.

Ribicic, D., Netzer, R., Hazen, T.C., Techtmann, S.M., Drablos, F., and Brakstad, O.G. 2018. Microbial community and metagenome dynamics during biodegradation of dispersed oil reveals potential key-players in cold Norwegian seawater. *Mar. Pollut. Bull.* **129**, 370–378.

Richter, M., Rosselló-Móra, R., Oliver Glöckner, F., and Peplies, J.

2015. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* **32**, 929–931.

Rogers, S.O., Shtarkman, Y.M., Kocer, Z.A., Edgar, R., Veerapaneni, R., and D'Elia, T. 2013. Ecology of subglacial Lake Vostok (Antarctica), based on metagenomic/metatranscriptomic analyses of accretion ice. *Biology* **2**, 629–650.

Saini, R., Kapoor, R., Kumar, R., Siddiqi, T.O., and Kumar, A. 2011. CO_2 utilizing microbes–a comprehensive review. *Biotechnol. Adv.* **29**, 949–960.

Siegert, M.J., Tranter, M., Ellis-Evans, J.C., Priscu, J.C., and Berry Lyons, W. 2003. The hydrochemistry of Lake Vostok and the potential for life in Antarctic subglacial lakes. *Hydrol. Process.* **17**, 795–814.

Sizova, M. and Panikov, N. 2007. *Polaromonas hydrogenivorans* sp. nov., a psychrotolerant hydrogen-oxidizing bacterium from Alaskan soil. *Int. J. Syst. Evol. Microbiol.* **57**, 616–619.

Stibal, M., Wadham, J.L., Lis, G.P., Telling, J., Pancost, R.D., Dubnick, A., Sharp, M.J., Lawson, E.C., Butler, C.E.H., Hasan, F., *et al.* 2012. Methanogenic potential of Arctic and Antarctic subglacial environments with contrasting organic carbon sources. *Global Change Biol.* **18**, 3332–3345.

Suzuki, M., Cui, Z.J., Ishii, M., and Igarashi, Y. 2001. Nitrate respiratory metabolism in an obligately autotrophic hydrogen-oxidizing bacterium, *Hydrogenobacter thermophilus* TK-6. *Arch. Microbiol.* **175**, 75–78.

Telling, J., Boyd, E.S., Bone, N., Jones, E.L., Tranter, M., MacFarlane, J.W., Martin, P.G., Wadham, J.L., Lamarche-Gagnon, G., Skidmore, M.L., *et al.* 2015. Rock comminution as a source of hydrogen for subglacial ecosystems. *Nat. Geosci.* **8**, 851–855.

Thauer, R.K. 2007. A fifth pathway of carbon fixation. *Science* **318**, 1732–1733.

Vick-Majors, T.J., Mitchell, A.C., Achberger, A.M., Christner, B.C., Dore, J.E., Michaud, A.B., Mikucki, J.A., Purcell, A.M., Skidmore, M.L., Priscu, J.C., *et al.* 2016. Physiological ecology of microorganisms in Subglacial Lake Whillans. *Front. Microbiol.* **7**, 1705.

Vignais, P.M. and Billoud, B. 2007. Occurrence, classification, and biological function of hydrogenases: an overview. *Chem. Rev.* **107**, 4206–4272.

Wadham, J.L., De'ath, R., Monteiro, F.M., Tranter, M., Ridgwell, A., Raiswell, R., and Tulaczyk, S. 2013. The potential role of the Antarctic Ice Sheet in global biogeochemical cycles. *Earth Env. Sci. Trans. Roy. Soc. Edinb.* **104**, 55–67.

Wood, D.E., Lu, J., and Langmead, B. 2019. Improved metagenomic analysis with Kraken 2. *BioRxiv* DOI: https://doi.org/10.1101/762302.

Wood, D.E. and Salzberg, S.L. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol.* **15**, R46.

Wynn, P.M., Hodson, A.J., Heaton, T.H.E., and Chenery, S.R. 2007. Nitrate production beneath a High Arctic glacier, Svalbard. *Chem. Geol.* **244**, 88–102.

Zhang, Y., Henriet, J.P., Bursens, J., and Boon, N. 2010. Stimulation of *in vitro* anaerobic oxidation of methane rate in a continuous high-pressure bioreactor. *Bioresour. Technol.* **101**, 3132–3138.