RESEARCH ARTICLE

Evidence of form II RubisCO (*cbbM*) in a perennially ice-covered Antarctic lake

Weidong Kong[1], Jenna M. Dolhi[1], Amy Chiuchiolo[2], John Priscu[2] & Rachael M. Morgan-Kiss[1]

[1]Department of Microbiology, Miami University, Oxford, OH, USA and [2]Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT, USA

Correspondence: Rachael Morgan-Kiss, Department of Microbiology, Miami University, Oxford, OH 45045, USA. Tel.: +1 513 529 5434; fax: +1 513 529 2431; e-mail: morganr2@muohio.edu

Present address: Weidong Kong, Department of Medicine, University of California, San Francisco, CA, 94143, USA.

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Abstract

The permanently ice-covered lakes of the McMurdo Dry Valleys, Antarctica, harbor microbially dominated food webs. These organisms are adapted to a variety of unusual environmental extremes, including low temperature, low light, and permanently stratified water columns with strong chemo- and oxy-clines. Owing to the low light levels during summer caused by thick ice cover as well as 6 months of darkness during the polar winter, chemolithoautotrophic microorganisms could play a key role in the production of new carbon for the lake ecosystems. We used clone library sequencing and real-time quantitative PCR of the gene encoding form II Ribulose 1, 5-bisphosphate carboxylase/oxygenase to determine spatial and seasonal changes in the chemolithoautotrophic community in Lake Bonney, a 40-m-deep lake covered by *c.* 4 m of permanent ice. Our results revealed that chemolithoautotrophs harboring the *cbbM* gene are restricted to layers just above the chemo- and oxi-cline ($\leq$ 15 m) in the west lobe of Lake Bonney (WLB). Our data reveal that the WLB is inhabited by a unique chemolithoautotrophic community that resides in the suboxic layers of the lake where there are ample sources of alternative electron sources such as ammonium, reduced iron and reduced biogenic sulfur species.

Introduction

The McMurdo Dry Valleys represent the largest ice-free region (*c.* 4000 km^2) on the Antarctic continent (Priscu, 1998). A number of permanently ice-covered lakes located in the dry valleys have been investigated since the International Geophysical Year (1957–1958). While environmental conditions within these lakes can be extreme (including year-round low temperatures, hypersalinity, extreme shade, and seasonal extremes in light availability), the water column beneath the ice cover is one of the few sources of perennial liquid water on the Antarctic continent. As such, these lakes provide the only year-round habitable environments on the continent. Three lakes (Bonney, Fryxell, and Hoare) located in Taylor Valley, a major valley within the McMurdo Dry Valleys, have been studied intensively as part of the McMurdo Dry Valleys Long-Term Ecological Research (McM-LTER; http://www.mcmlter.org/) program since 1993. The water columns of each of the dry valley lakes are isolated by a year-round ice cover (3–6 m thick) which prevents wind-driven turbulence and produces strong vertical stratification in biogeophysical parameters to exist. Each lake supports a distinct stratified microbial community containing bacteria, microalgae, as well as flagellated, and ciliated protozoans that interact to form truncated food webs dominated almost exclusively by microorganisms (Priscu *et al.*, 1999). A stratified phototrophic population, including cryptophytes in the shallow waters as well as chlorophytes, haptophytes, and stramenopiles within the deeper photic waters, plays a key role in primary productivity in the food webs of these lakes during the summer (Lizotte & Priscu, 1998; Priscu *et al.*, 1999; Bielewicz *et al.*, 2011; Kong *et al.*, 2012). Owing to minimal allochthonous inputs and atmospheric gas exchange as well as a lack of higher trophic levels, the microorganisms residing in this environment strongly influence the biogeochemistry of the carbon, nitrogen, and sulfur cycles in the lakes (Lee *et al.*, 2004a, b).

Priscu *et al.* (1999) showed that photosynthetic primary production (P)-to-respiration (R) ratios in Lake Bonney were < 0.5 on an annual basis, indicating that photoautotrophic carbon production was inadequate to support the

level of respiration in the lakes. However, a water column P/R ratio of < 1 should eventually lead to a system with low dissolved oxygen and little to no reduced carbon: phenomena which have not been observed in this lake. This conundrum, in concert with high levels of nitrous oxide, and reduced iron and biogenic sulfur compounds, indicates that chemolithoautotrophic fixation of carbon dioxide may plan an important role within this lake and perhaps others in the McMurdo Dry Valleys (Voytek *et al.*, 1999; Priscu *et al.*, 2008). These reports led to a recent study of the abundance and diversity of the *rbcL* gene encoding the major subunit of the enzyme RubisCO (Kong *et al.*, 2012). The study by Kong *et al.* (2012) revealed *rbcL* sequences related to chemolithoautotrophic *Proteobacteria* from form I A/B *rbcL* sequence libraries generated from sampling depths collected below the chemocline (i.e. 15 and 20 m) in the west lobe of Lake Bonney (WLB). In contrast, no putative chemolithoautotroph *rbcL* sequences were recovered from libraries generated from the east lobe of Lake Bonney (ELB; Kong *et al.*, 2012). In other Antarctic lakes, chemolithoautotrophic bacteria have been detected based on cultivation and molecular methods (Karr *et al.*, 2003, 2005; Clocksin *et al.*, 2007; Sattley & Madigan, 2007). The presence of chemolithoautotrophic bacteria is also suggested by high rates of light independent fixation of inorganic carbon in the dry valley lakes (J. Priscu, unpublished).

Form II RubisCO is one of two forms of RubisCO that are directly involved in fixation of CO_2 in autotrophic organisms through the Calvin–Benson–Basham (CBB) cycle, and this gene is adapted to functioning in low-O_2 and high-CO_2 environments (Tabita, 1999; Tabita *et al.*, 2007). The form II RubisCO gene, *cbbM*, has been used in a variety of environments as a functional marker for chemolithoautotrophic organisms (Giri *et al.*, 2004; Naganuma *et al.*, 2005; Hall *et al.*, 2008; Chen *et al.*, 2009; Tourova *et al.*, 2010). Given recent reports of chemolithoautotrophs in nearby Blood Falls, which flows into WLB (Mikucki & Priscu, 2007; Mikucki *et al.*, 2004, 2009), as well as the detection of putative chemolithoautotrophic *rbcL* sequences in the WLB water column (Kong *et al.*, 2012), we designed our current study to focus on the diversity and abundance of the form II RubisCO gene in the WLB.

Materials and methods

Site description

Lake Bonney is separated into two 40-m-deep basins by a shallow (*c.* 13 m) sill that allows exchange of oxygenated surface waters between the basins but eliminates exchange of deeper nutrient rich, suboxic waters. The water columns lack wind-driven turbulent mixing, which has produced stable gradients in temperature and conductivity, with bottom waters being saline and cold. Less than 0.1% of incident radiation reaches the depth of the chemocline, and no light penetrates the ice cover during the period of polar darkness (*c.* 6 months). The two lobes of Lake Bonney have a complex history. Long separation and differential evaporative histories between the two lobes have led to distinctive water chemistry in the isolated bottom waters: in WLB, oxygenated surface waters overlay anoxic layers where measureable rates of denitrification occur (Priscu *et al.*, 1996; Priscu, 1997; Ward & Priscu, 1997), while ELB exhibits suboxic waters below the chemocline with high nitrate and supersaturated nitrous oxide levels (> 700 000% over air saturation; Voytek *et al.*, 1999; Ward & Priscu, 1997). WLB is also fed by glacial melt water during the summer from the terminus of the Taylor Glacier, a major outlet glacier of the East Antarctic Ice Sheet. A unique geochemical feature known as Blood Falls is located at the northern end of the Taylor Glacier terminus and delivers iron-rich, hypersaline subglacial brine to the deep waters of WLB. Blood Falls is a subglacial outflow thought to originate from an ancient pool of marine brine located under the Taylor Glacier (Mikucki *et al.*, 2004, 2009; Mikucki & Priscu, 2007). The saline deep waters of WLB are thought to be very old (> 10^4 years) while the east lobe has undergone recent evaporative and refilling events and has been ice-covered for < 300 years (Poreda *et al.*, 2004).

Field sampling

Water samples were collected over three field seasons (2008, 2009 and 2011) at selected depths throughout the water column of WLB. Water samples were collected weekly during the summer–winter transition between 2 and 30 March 2008, a period when incident photosynthetically available radiation (PAR) was dropping rapidly and averaged 1-μmol photons m^{-2} s^{-1} at 10 m in the lake. To assess the presence of chemolithoautotrophs during the polar summer, samples were also collected during mid-summer (16 December 2009, 1 January 2010, and 23 November 2011). All sampling depths were measured from the piezometric water level in the ice hole (*c.* 30 cm below the ice surface). Water samples were collected using a 5-L Niskin bottle (General Oceanics, FL) and were filtered (1–5 L; n = 2–4 replicate filters) through 47-mm 0.45-μm Durapore polyvinylidene fluoride membrane filters (Millipore, MA) or 47-mm GF/C filters (Whatman, UK) for phylogenetic analyses or enzyme assays, respectively, using a vacuum of 0.3 mBar. The filters were frozen immediately in liquid nitrogen before being transported on dry ice to our US laboratory, where they were stored at −80 °C until processing. Conductivity, dissolved organic carbon (DOC), dissolved inorganic

carbon (DIC) PAR, light-mediated primary productivity (PPR), and nutrients (SO_4^{-2}, NH_4^+, NO_3^-, NO_2^-, and PO_4^{-3}) were measured as part of the NSF-funded McM-LTER program using methods described in Priscu (1997) and outlined in the McM-LTER limnology manual (http://www.mcm.lter.org). Briefly, inorganic nitrogen species were determined with a Lachat autoanalyzer, and soluble reactive phosphorus was analyzed manually using the antimony-molybdate method (Strickland & Parsons, 1972). PAR was measured with a LICOR LI-193 spherical quantum sensor (LI-COR Biosciences, NE). PPR was measured as ^{14}C-bicarbonate incorporation into particulate matter over a 24-h *in situ* incubation (Priscu, 1997).

Nucleic acid isolation and real-time quantitative PCR

Environmental DNA was isolated using FastDNA® spin kit for soil (MP Biomedicals, OH) following the manufacturer's protocol and according to Kong *et al.* (2012). The PCR products for Form II RubisCO gene (*cbbM*) were amplified using the primer set (cbbM-F: TTC TGG CTG GGB GGH GAY TTY ATY AAR AAY GAC GA and cbbM-R: CCG TGR CCR GCV CGR TGG TAR TG) (Campbell & Cary, 2004). Primer specificity and *cbbM* sequence verification were confirmed in sequence clone libraries.

Gene copy number (i.e. copies of *cbbM* DNA in 1 L of lake water) was quantified by real-time quantitative PCR (qPCR) according to Kong *et al.* (2012) on a Bio-Rad iCycler coupled with SYBR Green kit (Bio-Rad, CA). The PCR conditions were an initial 5-min period at 95 °C, followed by 40 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, and 78 °C for 10 s to collect data. To determine the PCR product specificity, a melting curve was acquired by heating from 50 to 95 °C. Data analysis was carried out using iCycler iQ Optical System software version 3.01 (Bio-Rad). The threshold cycle (C_t) was defined as the cycle number at which a statistically significant increase in fluorescence was detected.

Standard curves for qPCR were developed from plasmids containing the target inserts according to Kong & Nakatsu (2010). Copy numbers of the target genes were calculated directly from the concentration of the isolated plasmid DNA assuming 1.096×10^{-12} g per bp. All standard curves were generated from tenfold serial dilutions of DNA with known copy numbers and were subjected to qPCR assay in duplicate.

Clone library construction and sequencing

The PCR products containing the target fragment (328 bp in length) of *cbbM* gene were amplified from environmental DNAs to generate clone libraries from environmental samples collected during the 2008 field season. A total of 16 transformants from each library were randomly selected and sequenced on an Applied Biosystems 3730 × l DNA Analyzer (Applied Biosystems, CA). All sequences obtained from each library were aligned using ClustalW from the MEGA 4.1. The resulting alignment was used to calculate rarefaction curve with a cutoff value at 0.02 (sequence differences do not exceed 2%) using the MOTHUR program (Schloss *et al.*, 2009). Sequences with more than 98% nucleotide similarity were grouped into the same operational taxonomic unit (OTU). BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/) was used to search GenBank for nearest relative sequences to OTUs. Phylogenetic trees were constructed by neighbor-joining method with a Kimura two-parameter distance model using MEGA 4.1 software. Bootstrapping was used to estimate reliability of phylogenetic trees with 1000 replicates. Sequences generated in this study have been deposited in the National Center for Biotechnology GenBank database under the accession numbers JN091926–JN091960.

RubisCO carboxylase activity filter assay

Maximum carboxylase activity of the enzyme RubisCO was estimated using a modified radioisotope assay for filtered samples (Tortell *et al.*, 2006; Dolhi *et al.*, 2012). Enzyme activities from flash-frozen field samples were assayed within 2 months after collection. Briefly, frozen GF/C filters were extracted in bicine extraction buffer and soluble fractions were produced using a Minibead beater (Biospec, CA) followed by centrifugation. Soluble fractions were collected and used for enzyme assays. Maximum RubisCO activity was performed using a ^{14}C-based assay which measured rate of ^{14}C-incorporation into acid-stable products. A detailed protocol as well as a video of the RubisCO filter assay is described in Dolhi *et al.* (2012).

Results and discussion

Water column chemical characteristics

Physical and chemical characteristics of the WLB water column have changed relatively little over the past decade (compare Priscu, 1995, Spigel & Priscu, 1996, Priscu *et al.*, 2008). A dominant feature of the water column is the steep salinity gradient between 13 and 18 m where salts (primarily NaCl) increase from freshwater levels to about 2.3 times seawater (Fig. 1a). Temperatures above the chemocline are near 2 °C and decrease to −4 °C in the deep saline waters coinciding with a sharp decrease in oxygen from supersaturated levels (1 mM) to suboxic

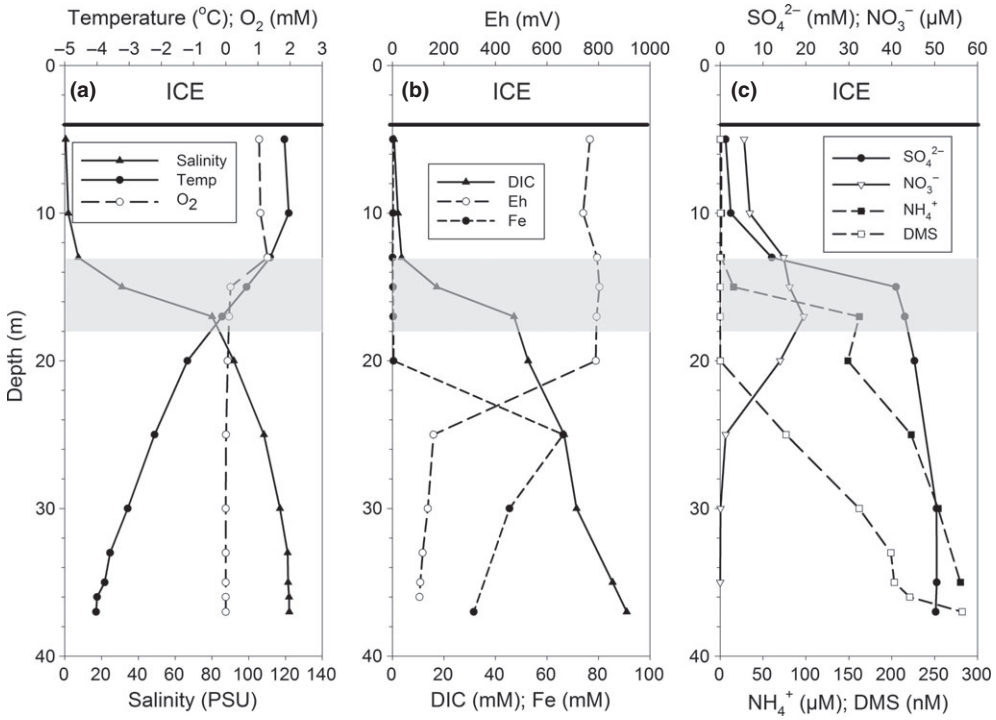

Fig. 1. Typical water column characteristics in the WLB. Depths were measured from the piezometric water level within the sampling hole. The ice cover was between 3.5 and 4.0 m thick.

conditions below 14 m. The suboxic conditions within the deep water are reflected by lower redox (Eh) levels, which decrease from a maximum of 800 mV in the surface to 105 mV in the deep saline waters (Fig. 1b). The reducing conditions in the deep waters result in high levels of ferrous iron, NH_4^+, and reduced biogenic sulfur (Fig. 1c; see also Lee *et al.*, 2004a, b). Active denitrification and the presence of denitrifying bacteria have been shown to be present below the chemocline (Priscu, 1997; Ward & Priscu, 1997), leading to depletion of NO_3^- in the deeper waters. Despite the suboxic reducing environment that exists below the chemocline, the suboxic waters are not sulfidic; H_2S is not measurable below the chemocline despite high levels of SO_4^{2-} (> 40 mM; Fig. 1b and c) and reducing conditions. We currently have no simple biochemical explanation to explain the lack of SO_4^{2-} reduction to H_2S, but a similar scenario has been shown to exist in subglacial water from the Taylor Glacier that flows into WLB via Blood Falls (Mikucki & Priscu, 2007; Mikucki *et al.*, 2009). These authors were unable to detect dissimilatory sulfate reductase genes and concluded that SO_4^{2-} was reduced to reduced sulfur intermediates which were then oxidized by ferric iron back SO_4^{2-} by a consortium of unknown microbial species. The geochemical gradients in the region of the chemocline (shaded area in Fig. 1), in concert with a strong gradient in dissolved inorganic carbon, provide appropriate redox couples to support chemolithoautotrophic metabolism driven by dimethyl sulfide (DMS), reduced iron, and ammonium (Lee *et al.*, 2004a, b; Priscu *et al.*, 2008). Any chemolithoautotrophs metabolizing within this geochemically distinct ecotone must be able to cope with cold and saline conditions.

Analysis of form II RubisCO gene diversity and distribution

Few studies have reported on functional gene diversity in the McMurdo Dry Valley lakes, and even less regarding genes associated with chemoautotrophy, despite prolonged winter periods where the water column is completely dark and respiratory carbon oxidation has been shown to exceed photosynthetic production of new carbon (Priscu *et al.*, 1999). In a recent paper, we detected RubisCO form IA gene (*rbcL*) sequences related to known chemolithoautotrophic *Proteobacteria* in WLB waters at depths below the chemocline (i.e. 15 and 20 m). These *rbcL* sequences were related to an endosymbiont of *Oligobrachia haakonmosbiensis* as well as *Thiobacillus* sp. (Kong *et al.*, 2012). In this current study, we further investigated the presence of chemolithoautotrophic organisms in WLB waters by developing clone libraries for the form II RubisCO gene large subunit encoded by the *cbbM* gene.

Sequence libraries were constructed from depths spanning the chemocline (15 and 20 m) during the polar night transition from three sampling time points (T1 = 2 March; T3 = 16 March; T5 = 30 March). Rarefactions curves revealed a relatively high diversity of *cbbM* genes in WLB (Fig. 2). The *cbbM* gene diversity was fairly constant in all samples collected from a depth of 15 m but higher in 20-m samples, with the highest diversity observed in the last time point (i.e. 30 March; Fig. 2). A total of 35 representative OTUs across all sequences from the six clone libraries were revealed with > 98% similarity in *cbbM* gene sequence (Fig. 3). The phylogenetic relationships of the *cbbM* sequences in WLB waters fell into four groups related to *Rhodopseudomonas* and *Rhodovulum* spp. (Accession No. AF416674 and HQ877080; 79–82% similarity), *Thiobacillus* sp. (Accession No. EU746412; 82–86% similarity), *Thiomicrospira* sp. (Accession Nos. DQ272537 and DQ272535; 75–82% similarity), as well as an endosymbiont of the Arctic tubeworm, *O. haakonmosbiensis* (Accession No. AM883191; 82% similarity). The first three groups belong to *Alpha-*, *Beta-* and *Gammaproteobacteria*, respectively, and together with the endosymbiont have been associated with biogeochemical transformations in extreme environments (Badger & Bek, 2008; Rogers & Schulte, 2012). *Thiomicrospira*, frequently detected in extreme environments such as deep-sea hydrothermal vents (Brinkhoff *et al.*, 1999; Dobrinski *et al.*, 2005, 2010; Ghosh & Dam, 2009; Niederberger *et al.*, 2009), are wide-spread sulfur-oxidizing bacteria in oxic zones of these extreme environments. The *Thiomicrospira*-like *cbbM* sequences dominated the 15-m water in WLB (> 70% of the clone libraries; Fig. 4). Previous studies have detected the presence of sulfur-oxidizing and sulfur-reducing bacteria in other dry valley lakes (Karr *et al.*, 2003, 2005; Jung *et al.*, 2004; Clocksin *et al.*, 2007). Moreover, Mikucki & Priscu (2007) reported that the most abundant 16S rRNA gene sequence in clone libraries constructed from Blood Falls samples (which flows into WLB) were related to a psychrophilic marine *Thiomicrospira arctica*. Our results as well as others collectively indicate that sulfur might be the dominant element in supporting chemolithoautotrophic communities in WLB and other dry valley lakes in waters with high levels of SO_4^{2-}. In the deeper waters (20 m), both *Thiomicrospira*- and *Thiobacillus*-like *Proteobacteria* dominated in WLB. *Thiobacillus* are obligately autotrophic, obtaining energy for CO_2 fixation by oxidizing iron and sulfur with O_2 and have been reported to combine inorganic sulfur-compound oxidation with denitrification (Beller *et al.*, 2006). A psychrotolerant *Thiobacillus thioparus*-like bacterium has been isolated from nearby Lake Fryxell (Sattley & Madigan, 2006) which utilizes hydrogen sulfide and elemental sulfur as electron donors. Cell numbers of the *Thiobacillus* sp. peaked in the oxycline of the water column in Lake Fryxell where both dissolved oxygen and sulfide are present (Sattley & Madigan, 2006). Lake Fryxell differs from WLB in that high levels of H_2S are present the waters below the chemocline in the former, whereas no measureable H_2S is present in WLB, despite low redox and oxygen levels (Fig. 1a and b).

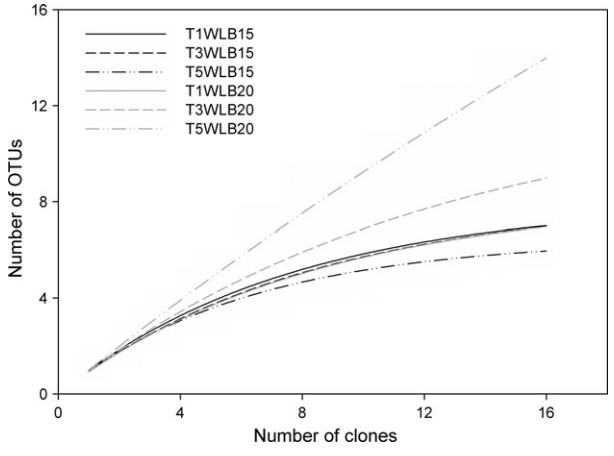

Fig. 2. Rarefaction analysis of *cbbM* gene clone libraries obtained from WLB sampling depths 15 and 20 m. The rarefaction curves plotted as number of observed phylotypes as a function of number of clones were calculated using the program MOTHUR with a cutoff value set to 0.02 for the analysis. Sampling times were as follows: T1, 2 March; T3, 16 March; T5, 30 March.

There are several reports of *Thiobacillus* strains utilizing DMS as an electron source under both anaerobic and aerobic conditions (Visscher & Taylor, 1993; Arellano-Garcia *et al.*, 2009; Ramirez *et al.*, 2011). DMS in the deep waters of WLB (> 330 nM) are among the highest recorded in a natural aquatic ecosystem, yet no simple biogeochemical explanation exists for its presence (Lee *et al.*, 2004a). Lee *et al.* (2004b) used thermodynamic constraints to examine potential biogeochemical transformations of biogenic sulfur in WLB and hypothesized that the microbial reduction of dimethyl sulfoxide (DMSO) produced by phytoplankton was the most feasible source of DMS in the suboxic waters of this lake. Oxidation of DMS by *Thiobacillus* generally produces carbon dioxide and sulfate, and certain strains can oxidize DMS to carbon dioxide while reducing nitrate to nitrite (e.g. Kim *et al.*, 2007; Schafer, 2007; Schafer *et al.*, 2010). These reports, together with our results, indicate that biogenic sulfur may provide an important energy source for chemolithoautotrophic metabolism within the chemocline of WLB.

Form II RubisCOs related to purple nonsulfur bacteria (*Rhodopseudomonas* and *Rhodovulum* spp.) and a chemolithoautrophic endosymbiont were the least abundant sequences (< 10%) in the *cbbM* sequence libraries (Fig. 4). *Rhodopseudomonas palustris* is a highly metaboli-

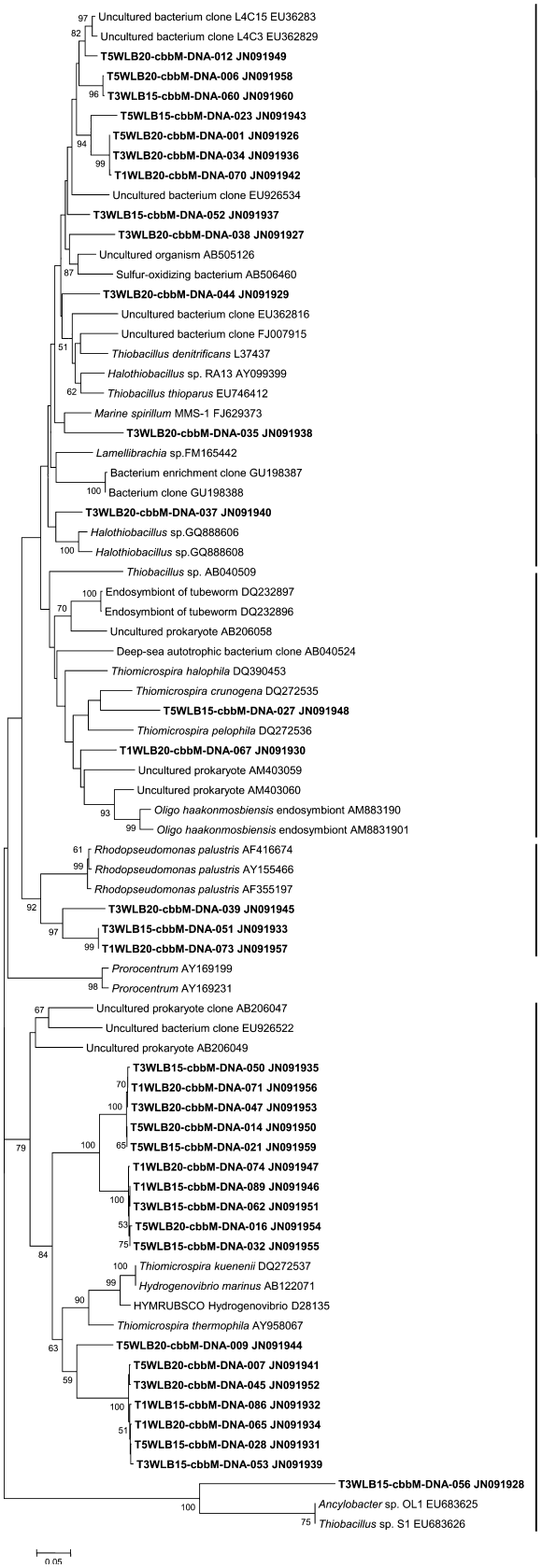

Fig. 3. Neighbor-joining phylogenetic tree of *cbbM* gene sequences (328 bp) retrieved from environmental DNA (in bold) in WLB. Scale bar indicates 0.2 substitutions per nucleotide position. The bootstrap consensus tree was inferred from 1000 replicates. Clones are named by sampling time point_lake name_depth_primer name_ template type_index number. GenBank accession numbers are listed after each sequence name. Sampling times were as follows: T1, 2 March; T3, 16 March; T5, 30 March. Bootstrap values of < 50% are not shown.

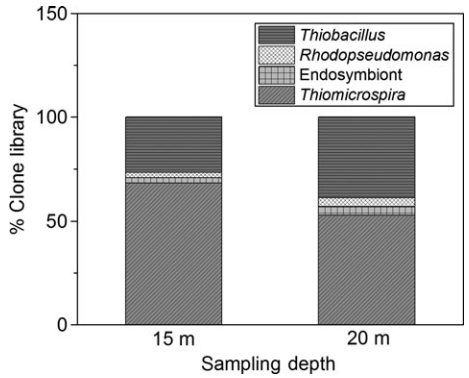

Fig. 4. Distribution of *cbbM* gene sequences generated from environmental DNA clone libraries. Samples were collected from sampling depths of 15-m and 20-m water depths from the WLB between 2 and 30 March 2008. Percentages of each group were determined from sequence data.

cally versatile bacterium capable of anoxygenic photosynthesis under anaerobic conditions as well as oxidative respiration under aerobic and anaerobic conditions using a variety of carbon sources (Larimer *et al.*, 2004). *Rhodovulum sulfidophium* is found in marine and high salt environments and can utilize both reduced sulfur compounds such as sulfide and thiosulfate as well as oxidize DMS to DMSO (McDevitt *et al.*, 2002; Creevey *et al.*, 2008). Two *cbbM* sequences detected in the current study were most closely related to *O. haakonmosbiensis* endosymbiont (Fig. 3). These endosymbiont *Proteobacteria* have been reported in chemolithoautotrophic sulfur oxidation (Pimenov *et al.*, 2000; Lenk *et al.*, 2011).

Abundance of the form II RubisCO gene

Seasonal dynamics for form II RubisCO gene were monitored during the summer–winter transition in 2008 (2–30 March) and during mid-summer in the following year (10 December 2009 and 1 January 2010) using real-time qPCR (Fig. 5). Distinct vertical patterns in *cbbM* gene copy number were observed in different field seasons, with maximum levels occurring at 15 m in the 2008 field season and at 20 m in the 2009 season. Levels of *cbbM* were 100–1000 times lower in 2009 field season than 2008 field season (Fig. 5). The dramatic differences between *cbbM* levels in the 2008 versus 2009 samples are difficult to explain but may be related to seasonal differences in the succession of microorganisms caused by differences in under-ice light levels (Lizotte, *et al.*, 1996) or by diffusive flux of important redox couples and episodic inflow from Blood Falls (Mikucki *et al.*, 2004) which may directly impact microbial populations and associated biogeochemistry in WLB.

The *cbbM* gene copy remained relatively constant throughout out the polar night transition (Fig. 5a). Seasonal trends in form II RubisCO gene differed that of form I RubisCO over the same sampling period: form I RubisCO gene generally declined during the summer–winter transition and exhibited a positive correlation with PAR (Kong *et al.*, 2012). The relatively stable *cbbM* levels during the summer–winter transition suggests that the chemolithoautotrophic community harboring form II RubisCO gene is not impacted by the declining light availability during this seasonal transition implying that the availability of favorable redox couples has a dominant role in the selection of these organisms.

Carbon fixation potential

Given our molecular evidence that a chemolithoautotrophic community of bacteria harboring form II RubisCO resides in WLB at depths where light is extremely low or absent (i.e. below the chemocline at 13 m), we investigated whether we could detect RubisCO carboxylase activity *in vitro* at sampling depths where the cbbM gene was detected (see Figs 4 and 5). As expected, the highest levels of RubisCO-specific activity correlated with the depth where

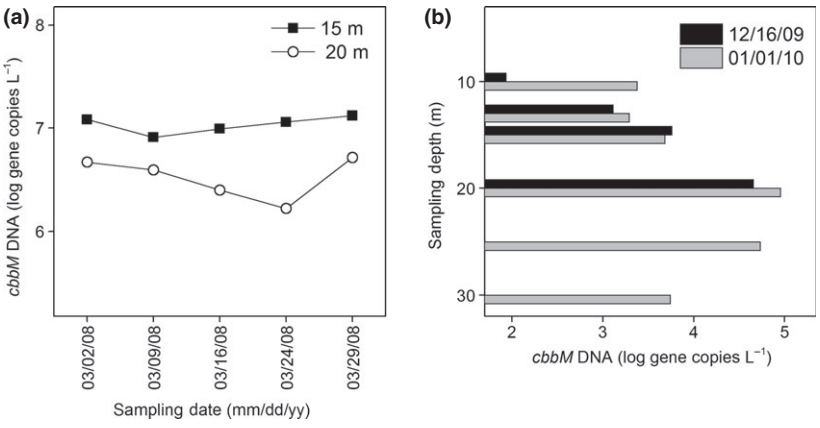

Fig. 5. Seasonal and vertical trends in *cbbM* gene copy number (DNA) in the WLB. (a) Trends in *cbbM* abundance during the transition from summer to winter (sampling dates, 2–30 March 2008) at two sampling depths (15 and 20 m). (b) Trends in *cbbM* abundance during mid-summer (sampling dates, 16 December 2009 and 1 January 2010) at four to six sampling depths. Abundance of *cbbM* gene was quantified using qPCR ($n = 2$).

Table 1. Comparison of light availability, primary productivity rates, and specific activity of the enzyme RubisCO at various sampling depths in the WLB

Sampling depth (m)	PAR (μmol photons m^{-2} s^{-1})	PPR (μg C L^{-1} day^{-1})	RubisCO-specific activity (μmol C h^{-1} mg $protein^{-1}$)
13	5.24	3.22	36.08
15	3.71	1.09	2.55
20	1.41	0.00	1.81
25	0.36	ND	4.17

Values represent the means of 2–6 replicates (n = 2 for PPR; n = 2–6 for RubisCO-specific activity). ND, not determined.

maximum levels of light-dependent primary productivity were detected (13 m, Table 1). This sampling depth occurs just above the chemocline and also correlates with maximum levels of chlorophyll *a* (Kong *et al.*, 2012). However, we also detected RubisCO activity at sampling depths (20 and 25 m) where PPR was below the level of detection. RubisCO-specific activity represents maximum carboxylase levels, thus these data are estimators of carbon fixation potential, rather than *in situ* carboxylation rate. As there are little to no active phytoplankton at these sampling depths, we suggest that carboxylase activity is likely evidence of RubisCO activity in the chemolithoautrophic community.

Lake Bonney has a unique geological evolution that has changed significantly the geochemical gradients in the water column over time. WLB is also influenced by efflux from Blood Falls at depths in and below the chemocline. Thus, the influence of Blood Falls on lake biota and chemistry would be restricted to WLB. Our findings suggest that the unique biogeochemical status of WLB as well as the interactions between Blood Falls and layers at and below the WLB chemocline may regulate the abundance and distribution of chemolithoautotrophs harboring form II RubisCO in Lake Bonney.

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References

Arellano-Garcia L, Revah S, Ramirez M, Gomez JM & Cantero D (2009) Dimethyl sulphide degradation using immobilized *Thiobacillus thioparus* in a biotrickling filter. *Environ Technol* **30**: 1273–1279.

Badger MR & Bek EJ (2008) Multiple Rubisco forms in proteobacteria: their functional significance in relation to CO_2 acquisition by the CBB cycle. *J Exp Bot* **59**: 1525–1541.

Beller HR, Letain TE, Chakicherla A, Kane SR, Legler TC & Coleman MA (2006) Whole-genome transcriptional analysis of chemolithoautotrophic thiosulfate oxidation by *Thiobacillus denitrificans* under aerobic versus denitrifying conditions. *J Bacteriol* **188**: 7005–7015.

Bielewicz S, Bell EM, Kong W, Friedberg I, Priscu JC & Morgan-Kiss RM (2011) Protist diversity in a permanently ice-covered Antarctic lake during the polar night transition. *ISME J* **5**: 1559–1564.

Brinkhoff T, Muyzer G, Wirsen CO & Kuever J (1999) *Thiomicrospira kuenenii* sp. nov. and *Thiomicrospira frisia* sp. nov., two mesophilic obligately chemolithoautotrophic sulfur-oxidizing bacteria isolated from an intertidal mud flat. *Int J Syst Bacteriol* **49**: 385–392.

Campbell BJ & Cary SC (2004) Abundance of reverse tricarboxylic acid cycle genes in free-living microorganisms at deep-sea hydrothermal vents. *Appl Environ Microbiol* **70**: 6282–6289.

Chen Y, Wu L, Boden R, Hillebrand A, Kumaresan D, Moussard H, Baciu M, Lu Y & Murrell JC (2009) Life without light: microbial diversity and evidence of sulfur- and ammonium-based chemolithotrophy in Movile Cave. *ISME J* **3**: 1093–1104.

Clocksin KM, Jung DO & Madigan MT (2007) Cold-active chemoorganotrophic bacteria from permanently ice-covered Lake Hoare, McMurdo Dry Valleys, Antarctica. *Appl Environ Microbiol* **73**: 3077–3083.

Creevey NL, McEwan AG, Hanson GR & Bernhardt PV (2008) Thermodynamic characterization of the redox centers within dimethylsulfide dehydrogenase. *Biochemistry* **47**: 3770–3776.

Dobrinski KP, Longo DL & Scott KM (2005) The carbon-concentrating mechanism of the hydrothermal vent chemolithoautotroph *Thiomicrospira crunogena*. *J Bacteriol* **187**: 5761–5766.

Dobrinski KP, Boller AJ & Scott KM (2010) Expression and function of four carbonic anhydrase homologs in the deep-sea chemolithoautotroph *Thiomicrospira crunogena*. *Appl Environ Microbiol* **76**: 3561–3567.

Dolhi JM, Ketchum N & Morgan-Kiss RM (2012) Assaying carbon fixation potential in microbial eukaryote enrichment cultures isolated from a chemically stratified Antarctic lake. *J Vis Exp* **62**: e3992.

Ghosh W & Dam B (2009) Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea. *FEMS Microbiol Rev* **33**: 999–1043.

Giri BJ, Bano N & Hollibaugh JT (2004) Distribution of RubisCO genotypes along a redox gradient in Mono Lake, California. *Appl Environ Microbiol* **70**: 3443–3448.

Hall JR, Mitchell KR, Jackson-Weaver O, Kooser AS, Cron BR, Crossey LJ & Takacs-Vesbach CD (2008) Molecular characterization of the diversity and distribution of a thermal spring microbial community by using rRNA and metabolic genes. *Appl Environ Microbiol* **74**: 4910–4922.

Jung DO, Achenbach LA, Karr EA, Takaichi S & Madigan MT (2004) A gas vesiculate planktonic strain of the purple non-sulfur bacterium *Rhodoferax antarcticus* isolated from Lake Fryxell, Dry Valleys, Antarctica. *Arch Microbiol* **182**: 236–243.

Karr EA, Sattley WM, Jung DO, Madigan MT & Achenbach LA (2003) Remarkable diversity of phototrophic purple bacteria in a permanently frozen Antarctic lake. *Appl Environ Microbiol* **69**: 4910–4914.

Karr EA, Sattley WM, Rice MR, Jung DO, Madigan MT & Achenbach LA (2005) Diversity and distribution of sulfate-reducing bacteria in permanently frozen Lake Fryxell, McMurdo Dry Valleys, Antarctica. *Appl Environ Microbiol* **71**: 6353–6359.

Kim HG, Doronina NV, Trotsenko YA & Kim SW (2007) *Methylophaga aminisulfidivorans* sp. nov., a restricted facultatively methylotrophic marine bacterium. *Int J Syst Evol Microbiol* **57**: 2096–2101.

Kong W & Nakatsu CH (2010) Optimization of RNA extraction for PCR quantification of aromatic compound degradation genes. *Appl Environ Microbiol* **76**: 1282–1284.

Kong W, Ream DC, Priscu JC & Morgan-Kiss RM (2012) Dynamics of RubisCO gene number and expression during the transition to polar night in Antarctic lake. *Appl Environ Microbiol* **78**: 4358–4366.

Larimer FW, Chain P, Hauser L *et al.* (2004) Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. *Nat Biotechnol* **22**: 55–61.

Lee PA, Priscu JC, DiTullio GR, Riseman SF, Tursich N & deMora SJ (2004a) Elevated levels of dimethylated-sulfur compounds in Lake Bonney, a poorly ventilated Antarctic lake. *Limnol Oceanogr* **49**: 1044–1055.

Lee PA, Mikucki JA, Foreman CM, Priscu JC, DiTullio GR, Riseman SF, deMora SJ, Wold CF & Kester L (2004b) Thermodynamic constraints on microbially mediated processes in lakes of the McMurdo Dry Valleys, Antarctica. *Geomicrobiol J* **21**: 1–17.

Lenk S, Arnds J, Zerjatke K, Musat N, Amann R & Mussmann M (2011) Novel groups of Gammaproteobacteria catalyse sulfur oxidation and carbon fixation in a coastal, intertidal sediment. *Environ Microbiol* **13**: 758–774.

Lizotte MP & Priscu JC (1998) Distribution, succession and fate of phytoplankton in the dry valley lakes of Antarctica, based on pigment analysis. *Ecosystem Dynamics in a Polar Desert: The McMurdo Dry Valleys*, Vol. 72 (Priscu JC, ed), pp. 229–240. American Geophysical Union, Washington, DC.

Lizotte MP, Sharp TR & Priscu JC (1996) Phytoplankton dynamics in the stratified water column of Lake Bonney, Antarctica. I. Biomass and productivity during the winter-spring transition. *Polar Biol* **16**: 155–162.

McDevitt CA, Hanson GR, Noble CJ, Cheesman MR & McEwan AG (2002) Characterization of the redox centers in dimethyl sulfide dehydrogenase from *Rhodovulum sulfidophilum*. *Biochemistry* **41**: 15234–15244.

Mikucki JA & Priscu JC (2007) Bacterial diversity associated with Blood Falls, a subglacial outflow from the Taylor Glacier, Antarctica. *Appl Environ Microbiol* **73**: 4029–4039.

Mikucki JA, Foreman CM, Priscu JC, Lyons WB, Sattler B & Welch KA (2004) Geomicrobiology of Blood Falls: a saline, iron-rich subglacial feature of Taylor Glacier, Antarctica. *Aquat Geochem* **10**: 199–220.

Mikucki JA, Pearson A, Johnston DT *et al.* (2009) A contemporary microbially maintained subglacial ferrous "ocean". *Science* **324**: 397–400.

Naganuma T, Elsaied HE, Hoshii D & Kimura H (2005) Bacterial endosymbioses of gutless tube-dwelling worms in nonhydrothermal vent habitats. *Mar Biotechnol* **7**: 416–428.

Niederberger TD, Perreault NN, Lawrence JR *et al.* (2009) Novel sulfur-oxidizing streamers thriving in perennial cold saline springs of the Canadian high Arctic. *Environ Microbiol* **11**: 616–629.

Pimenov NV, Savvichev AS, Rusanov II, Lein A & Ivanov MV (2000) Microbiological processes of the carbon and sulfur cycle in cold methane seeps in the North Atlantic. *Mikrobiologiia* **69**: 831–843.

Poreda RJ, Hunt AG, Lyons WB & Welch KA (2004) The helium isotopic chemistry of Lake Bonney, Taylor Valley, Antarctica: timing of late holocene climate change in Antarctica. *Aquat Geochem* **10**: 353–371.

Priscu JC (1995) Phytoplankton nutrient deficiency in lakes of the McMurdo Dry Valleys, Antarctica. *Freshw Biol* **34**: 215–227.

Priscu JC (1997) The biogeochemistry of nitrous oxide in permanently ice-covered lakes of the McMurdo Dry Valleys, Antarctica. *Glob Change Biol* **3**: 301–305.

Priscu JC (1998) *Ecosystem Dynamics in a Polar Desert: the McMurdo Dry Valleys, Antarctica*. American Geophysical Union, Washington, DC.

Priscu JC, Downes MT & McKay CP (1996) Extreme supersaturation of nitrous oxide in a poorly ventilated Antarctic lake. *Limnol Oceanogr* **41**: 1544–1551.

Priscu JC, Wolf CF, Takacs CD, Fritsen CH, Laybourn-Parry J, Roberts JKM & Berry-Lyons W (1999) Carbon transformations in the water column of a perennially ice-covered Antarctic Lake. *Bioscience* **49**: 997–1008.

Priscu JC, Christner BC, Dore JE, Westley MB, Popp BN, Casciotti KL & Lyons WB (2008) Extremely supersaturated N_2O in a perennially ice-covered Antarctic lake: molecular

and stable isotopic evidence for a biogeochemical relict. *Limnol Oceanogr* **53**: 2439–2450.

Ramirez M, Fernandez M, Granada C, Le Borgne S, Gomez JM & Cantero D (2011) Biofiltration of reduced sulphur compounds and community analysis of sulphur-oxidizing bacteria. *Bioresour Technol* **102**: 4047–4053.

Rogers KL & Schulte MD (2012) Organic sulfur metabolisms in hydrothermal environments. *Geobiology* **10**: 320–332.

Sattley WM & Madigan MT (2006) Isolation, characterization, and ecology of cold-active, chemolithotrophic, sulfur-oxidizing bacteria from perennially ice-covered Lake Fryxell, Antarctica. *Appl Environ Microbiol* **72**: 5562–5568.

Sattley WM & Madigan MT (2007) Cold-active acetogenic bacteria from surficial sediments of perennially ice-covered Lake Fryxell, Antarctica. *FEMS Microbiol Lett* **272**: 48–54.

Schafer H (2007) Isolation of *Methylophaga* spp. from marine dimethylsulfide-degrading enrichment cultures and identification of polypeptides induced during growth on dimethylsulfide. *Appl Environ Microbiol* **73**: 2580–2591.

Schafer H, Myronova N & Boden R (2010) Microbial degradation of dimethylsulphide and related C1-sulphur compounds: organisms and pathways controlling fluxes of sulphur in the biosphere. *J Exp Bot* **61**: 315–334.

Schloss PD, Westcott SL & Ryabin T (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *App Environ Microbiol* **75**: 7537–7541.

Spigel RH & Priscu JC (1998) Physical limnology of the McMurdo Dry Valley lakes. *Ecosystem Dynamics in a Polar Desert: The McMurdo Dry Valleys, Antarctica Antarctic Research Series*, Vol. 72 (Priscu JC, ed), pp. 153–187. American Geophysical Union, Washington, DC.

Strickland JDH & Parsons TR (1972) *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, Ottawa, Canada.

Tabita FR (1999) Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: a different perspective. *Photosynth Res* **60**: 1–28.

Tabita FR, Hanson TE, Li H, Satagopan S & Chan S (2007) Function, structure and evolution of the RubisCO-like proteins and their RubisCO homologs. *Microbiol Mol Biol Rev* **71**: 576–599.

Tortell PD, Martin CL & Corkum ME (2006) Inorganic carbon uptake and intracellular assimilation by subarctic Pacific phytoplankton assemblages. *Limnol Oceanogr* **51**: 2102–2110.

Tourova TP, Kovaleva OL, Sorokin DY & Muyzer G (2010) Ribulose-1,5-bisphosphate carboxylase/oxygenase genes as a functional marker for chemolithoautotrophic halophilic sulfur-oxidizing bacteria in hypersaline habitats. *Microbiology* **156**: 2016–2025.

Visscher PT & Taylor BF (1993) A new mechanism for the aerobic catabolism of dimethyl sulfide. *Appl Environ Microbiol* **59**: 3784–3789.

Voytek MA, Priscu J & Ward BB (1999) The distribution and relative abundance of ammonia-oxidizing bacteria in lakes of the McMurdo Dry Valley, Antarctica. *Hydrobiologia* **401**: 113–130.

Ward BB & Priscu JC (1997) Detection and characterization of denitrifying bacteria from a permanently ice-covered Antarctic lake. *Hydrobiologia* **347**: 57–68.