1st Workshop on *'The Geomicrobiology of Glacial Environments'*

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Abstracts

Day 1

Cryoconite Ecosystems upon the Greenland and East Antarctic Ice Sheets

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The Greenland and East Antarctic ice sheets contain about as much prokaryotic biomass as all of Earth's freshwater lakes combined, at least according to the sparse data described in Priscu and Christner (2004). These data and others have also been used to suggest that about 10 million trillion microorganisms are liberated annually by glacier melt (Castello and Rogers, 2005). It is therefore important to assess where and when these ice sheets support microbial activity upon their surfaces, to consider which microorganisms are active and to assess their combined impact upon atmospheric CO_2. This presentation therefore describes the application of DNA sequencing and in-situ incubation methods to the study of cryoconite ecosystems upon the margins of both the Greenland and the East Antarctic Ice Sheets, and compares them to similar data collected from small glaciers. We show how net ecosystem carbon transfers may be easily deduced from light and dark incubations, which yield estimates of primary production and respiration without resort to isotope incorporation. Our results always reveal a close balance between primary production and respiration, which means that significant CO_2 transfers into and out of the ice largely cancel each other out. The implications are important for the net carbon balance of the Greenland Ice Sheet (GIS) because it means that respiration in the slush zone, where burial prevents photosynthesis, forces the ice sheet to be a carbon source. Calculations of the magnitude of this net flux will be presented that use modelled extents of bare ice, wet slush and dry snow for recent summers.

On the EAIS, we find that cryoconite ecosystems are restricted to the so-called Blue Ice Areas, which represent 1 – 2% of the ice sheet surface and, like the GIS, have well-balanced carbon fluxes. However, low rates of production are observed when biological activity in cryoconite is normalised for the dry mass present and then compared to data from elsewhere. However, the rates are not low when data

are normalised for the organic mass in the cryoconite debris. Clear sky radiation conditions enable deep penetration of the cryoconite into the blue ice (80 cm), and isotope incorporation results show that photosynthesis is adapted to low light conditions at these depths. The snow-covered area of the EAIS is a long way from harbouring slush ecosystems, and so the locus, duration and activity of cryoconite upon it are sensitive to rather different meteorological factors than the GIS. For example, the windfield is critical for allowing development of BIAs and for governing the deposition of debris upon them. Since the latter process initiates cryoconite hole formation and enables radiative warming to provide a vital water supply, the position of debris sources relative to katabatic air drainage is critical for enabling life to become established upon the surface of the EAIS.

Castello, J.D. and Rogers, S.O. 2005. *Life In Ancient Ice*, Princeton University Press, New Jersey, USA, Chapter 1.

Priscu, J.C., and B.C. Christner. 2004. Earth's icy biosphere. *In* Bull, Alan T. (ed.), *Microbial Diversity and Bioprospecting*, pp. 130-145. American Society for Microbiology, Washington, D.C.

Microbial diversity in cryoconite: From quality to quantity.

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The context of our research goals on cryoconite is the fate of atmospheric nitrogen depositions in the Arctic (project NSINK). Microbial communities in cryoconite represent a crucial factor affecting the pathways of nitrogen in the glacial ecosystem due to relatively high metabolic activity. Our purpose is not only to detect organisms present in cryoconite using molecular methods but also to asses the abundance of particular groups using FISH (fluorescence in situ hybridization). This approach can provide the information about quantity of particular functional groups affecting the fate of nitrogen. During the summer season 2009 four glaciers (Midtre Lovénbreen, Austre Brøggerbreen in the Kongsfjorden area, Alegondabreen and Grønfjordbreen in Grønfjorden area; Svalbard) were investigated with the focus on the development of cryoconite community in time and we expect to reveal also variability caused by different nutrient availability at different sites (effect of bird colonies).

Arctic cryoconites: autotrophic or heterotrophic ecosystems?

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Microbial activity on the surface of glaciers is largely associated with sediment grains, known as cryoconite. Photosynthetic microorganisms such as cyanobacteria, green algae and diatoms contained in the cryoconite have the potential to fix organic carbon into the sediment from carbon dioxide in the atmosphere, and this organic carbon could then be transported from the glacier surface to help support the carbon cycle in ecosystems at the margins of glaciers. This study examines the net microbial carbon budget of cryoconite debris on 3 different glaciers situated in NW Spitsbergen, Svalbard, over the entire summer melt season (beginning of July to mid September 2009). Field results indicate that the microbial communities in arctic ecosystems, such as those in Svalbard, are much closer to carbon balance than previously thought (rates of autotrophy closely matched by rates of heterotrophy), with the net balance strongly dependent on sediment depth. Results from nutrient addition and light limitation field experiments will also be discussed.

The importance of basal ice studies in assessing microbial activity in glacial environments and its consequences for the ice core community

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« Basal ice » refers to the portion of an ice body that is in contact and interacts with the glacial bed. The ice present at the bottom of e.g. a glacier, an ice sheet or an entirely frozen lake meets this definition. As a result of its specific location within the ice body, basal ice generally displays a higher debris content as well as a more variable structure as compared to the overlying ice. These characteristics confer to the basal zone very distinctive physical and chemical properties, which will often influence the behaviour of the entire ice body. For these reasons, the study of basal ice properties is essential to the understanding of past and modern glacial dynamics.

Another reason that makes basal ice so important for environmental studies is that, during the last decade, viable microbes have been detected in significant concentrations at the base of various types of ice bodies. Whereas subglacial environments were long thought to be "abiotic", i.e. devoid of life, they are now considered as local oases of microbial activity relative to the glacial ice overlying them, owing mostly to the availability of liquid water and finely ground rock debris that can provide the nutrients and electron acceptors necessary for life in such extreme conditions. In turn, the metabolism of such microbial consortia can have determining consequences for the interpretation of important ice core proxies, which will be discussed here.

Ice Sheets as extensive methanogenic wetlands: Impact on the global carbon budget

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Subglacial environments are a previously neglected component of the Earth's carbon cycle; a reflection of the view held until recently that they are dominated by abiotic and oxic conditions. Here we provide a realistic assessment of the theory that the basal regions of the ice sheets that formed over North America and Europe during glaciations were host to significant populations of anaerobic microorganisms, including methanogens, able to metabolize organic carbon sequestered during interglacials and overridden during Quaternary glacials. In doing so, we estimate the potential organic carbon sequestered during ice sheet growth and assess the potential for the development of anoxic conditions within subglacial debris. We go on to analyse the concentrations of methane, carbon dioxide and oxygen in basal ice from three different glacier/ice sheet systems (Lower Wright Glacier, Antarctica; Russell Glacier, Greenland Ice Sheet and Finsterwalderbreen, Svalbard). Concentrations of methane in debris rich basal ice exceed atmospheric concentrations by up to three orders of magnitude, carbon dioxide concentrations are also elevated with respect to atmospheric values and oxygen is depleted. These observations are consistent with a diverse and active subglacial microbial population, including heterotrophs and methanogens. Long term incubation experiments conducted on debris/meltwater microcosms from the same field sites and conducted at low temperature (0, 4 and 10 ºC) also support the presence of a viable methanogenic community and enable rates of carbon turnover to be calculated. We subsequently employ these data to model the microbial conversion of overridden organic carbon to methane gas beneath northern hemisphere ice sheets over a glacial/interglacial cycle, in order to assess the potential impact of methane release on atmospheric concentrations during ice sheet wastage.

Organic chemistry of basal ice -- What's on menu for microbes?

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Recent studies show that subglacial environments previously thought to be devoid of life contain a host of active microbial organisms. Presence of liquid water due to overburden pressure, chemical erosion of bedrock releasing nutrients and occurrence of carbon source in overridden sediments facilitate life in this extreme environment. Little is known of concentrations and diversity of labile organic compounds essential for sustaining microbial metabolism. This study investigates three subglacial ecosystems that considerably differ in range and amount of available organic compounds; 1-Engabreen Glacier in northern Norway overlying high-grade metamorphic rocks with low organic carbon content; 2-Finsterwalderbreen Glacier in Svalbard that has overridden ancient black shales with relatively high carbon content yet recalcitrant to microbiological consumption; and 3-Russell Glacier in western Greenland with recently overridden quaternary organic rich paleosols. Presence of higher concentration labile organic compounds in Greenland basal ice suggest that recently overridden paleosols have highest potential for sustaining microbial populations present within and underneath basal ice. High concentration of total organic carbon in basal ice from Finsterwalderbreen doesn't correlate with presence of labile organic compounds. This indicates lack of ability to digest ancient recalcitrant carbon in cold temperatures. In all three investigated environments higher concentrations of labile organic compounds are connected with debris rich basal ice.

Diversity, abundance and activity of methanogenic archaea beneath the Antarctic and Greenland ice sheets

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The presence of liquid water beneath ice sheets, together with the anoxia caused by the microbial oxidation of organic carbon and sulphide minerals, create favourable conditions for methanogenic archaea in subglacial environments, and hence the process of methanogenesis. This is of significance for the role of ice sheets in global biogeochemical cycles, and is consistent with hypotheses that

organic carbon sequestered beneath ice sheets during period of ice advance may be converted to methane during glacial periods and released rapidly during retreat. Here, we provide first evidence of the presence, diversity and abundance of archaea beneath the Antarctic and Greenland ice sheets, determined using a combination of microscopic and molecular techniques. The abundance of archaea in the subglacial sediment samples from Antarctica and Greenland was between 10^3 – 10^5 cells per gram of sediment, and most archaea specific 16S rDNA clones were found to be close to uncultured clones from other types of cold environments, such as ice-covered lakes and permafrost peat. Between 40 and 60% of the archaeal clones clustered with the methane producing groups of Methanosarcinales and Methanomicrobiales. Significant growth of methanogens and methane production was detected in long-term laboratory incubations of the subglacial samples with various substrates. Hence, methanogenic archaea can cope with the harsh conditions underneath ice sheets, and may produce significant amounts of methane over long periods of time, potentially using all three – ie hydrogenotrophic, acetotrophic and methylotrophic – pathways of methanogenesis.

Molecular diversity of cyanobacteria in glacial environments

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Cyanobacteria are photosynthetic bacteria that need only solar light, liquid water, air and some mineral nutrients for growth. Several cyanobacterial groups are also capable to light-dependent and light-independent N2-fixation. Cyanobacteria function as primary producers in glacial environments and serve as primary colonizers of soils in areas of retreating glaciers thus playing an important role in the succession of biological communities caused by global or local warming. The innovation of oxygenic photosynthesis in cyanobacteria caused the Great Oxidation Event (2.3 Ga). Also cyanobacteria associated with ice could be abundant during the 2.3-2.2 Ga Makganyene ‘‘snowball Earth’’ global glaciation and played yet non recognized role in its termination. This presentation summarizes data on the distribution and molecular diversity of cyanobacteria in different types of glacial environments: cryoconites on the surface of glaciers and permanently frozen lakes, and ice shelf ponds.

The First High-Resolution Time-Series of Microbial Load and Composition in Subglacial Meltwaters

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Little is known about the temporal variation in the microbiology of glacial environments. By using in-field sample processing techniques and especially in-field analytical techniques, a high-resolution time-series was collected for the first time from subglacial outlet waters – in this case from the Leverett Glacier in western Greenland during summer 2009. A suite of four analytical techniques was used on samples taken daily or twice daily, and analysed in the field. The first three techniques were based on the detection of biomarkers: ATP bioluminescence, lipopolysaccharide endotoxin detection and real-time PCR. The fourth technique was microbial enumeration via fluorescent microscopy. Real-time PCR primers targeted taxonomic (bacteria and archaea) and functional pathway sequences (methanogenisis, sulphate reduction, methanotrophy, nitrogen fixation and denitrification). In addition, samples from the glacier surface were taken at intervals throughout the season to provide contextual information about supraglacial inputs.

Early analysis of the time-series indicates a degree of correlation between the outputs of the various independent analytical techniques. Variations on two scales are seen; variation at diurnal timescales and variation over a few days. Some variation is expected to be due to sample processing, whilst comparison of the analytical data with water sediment load measurements suggests a positive correlation with sediment load.

More generally, the experience of implementing in-field sample collection, processing and analysis, using multiple and complementary techniques, has demonstrated the advantages this approach exhibits; in particular efficient resource utilisation and maximising scientific returns.

Posters

Biogeochemistry of Nitrogen in a High Arctic Glacial Catchment

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In the high Arctic, the biogeochemistry of nitrogen is strongly influenced by a seasonal snow cover depletion and longer-term deglaciation. Watershed biogeochemical dynamics are therefore best understood by taking a nested approach using microcatchments that are representative of the different functional units of the system. This project therefore examines the biogeochemistry of nitrogen along flow-paths activated by melt in glacial, talus, moraine and pro-glacial (soil) components of the well-known Bayelva watershed (Ny Alesund, Svalbard).

This poster describes how various processes influence melt season biogeochemistry of nitrogen and presents preliminary data from the 2009 summer. It is shown that, like maritime Antarctic and other Arctic glacial catchments, there is a tendency for increasing NO3-production as the summer progresses. Thus, after a strong snowmelt-driven phase, the influence of microbial nitrogen production in young glacial sediments and older pro-glacial soils becomes increasingly apparent. Future research will identify the processes that are responsible and constrain them within a mass balance scheme.

Isotopic characterization of the provenance and fate of nitrogen in high Arctic catchments

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Previous studies (Hodson et al., 2005; Wynn et al, 2007), of nitrogen dynamics in high Arctic glacial catchments indicate a major knowledge gap with respect to nitrogen biogeochemistry following the onset of melt. Biological activity, especially photosynthesis and respiration, in cryoconite holes, has been reported to be comparable with that found in warm nutrient rich soils (Anesio et al., 2009). Heterotrophic activity beneath glacier, especially within subglacial pressure melting zone, is also significant and cause anoxia, low nitrate values (with relatively high $\delta^{15}N_{NO3}$ values; +2.3 to +4.0‰),

sulphide oxidation and sulphate reducing activity (Wadham et al 2004; Lanoil et al., 2009). This leads to imbalances in the yields and loading of atmospheric nitrogen and sulphure that is also detectable in the isotopic composition of runoff. For example the isotopic values of $\delta^{18}O_{NO3}$ (+20.3 to +72‰) and $\delta^{15}N_{NO3}$ (-9.9 to +2.3 ‰) reported by Wynn et al., (2007) reveal the diverse nature of nitrogen sourcing (atmospheric as well biological) and its further processing (microbial and physical) in subglacial flow paths following glacial melt. The biotic processes are poorly understood as are physical processes involving reactive nitrogen phases such as, NH_4 (e.g. adsorption on the negatively charged sediment surface; Schubert et. al., 2001).
This study is committed to tracking the fate of nitrogen following melt in a high Arctic glacier basin (Midtre Lovenbreen, Svalbard) using a novel approach to the measurement of stable isotope tracers (N15, O17, O18, S34) at very low abundance level. This presentation will show how these isotopes may be coupled with solute chemistry to offer a direct means of source identification within glacial system and to reveal different biological processes that take place.

Key words: Autotrophy, Anoxia, Heterotrophy, Isotopic tracer.

Determination of Trace Organic Carbon in Subglacial Systems by Ion Chromatography (IC) and Spectrofluorimetry

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Glaciers and ice sheets remain one of the least explored sectors of the Earth's biosphere. The basal regions of these ice masses (the "subglacial" environment) represent one of the harshest habitats for life. Here, microbial organisms are active despite the cold temperatures, lack of sunlight and scarcity of food sources and bioelements. This means they are potentially "active" components of the Earths biogeochemical cycles. For example, the net carbon content of the Earth's glaciers and subglacial environments is comparable to the ocean (~10 Pg C), advocating a revision of traditional global carbon cycles. Subglacial environments were previously neglected due to assumptions of predominantly abiotic, oxic conditions, limited carbon production and transformation. Now, significant stores of bioavailable carbon are thought to be present in glacially-overridden material, providing a potential substrate for *in situ* microbial metabolism and biotic mediation of geochemical weathering. However, little is known about the range of compounds present, their availability to in situ micro-organisms or the pathways and rates of carbon turnover that occur. Here, we successfully utilise ion chromatography (IC) and fluorescence spectroscopy to infer the molecular characteristics of dissolved organic carbon (DOC) across a range of glacial sample types. Qualitative analysis of the fluorescence spectra suggests glacial DOC is derived from both terrestrial sources and microbial production and is highly dynamic spatially and temporally. A range of key metabolic substrates (e.g. volatile fatty acids, VFAs) have also been identified and quantified by IC. The provision of bioavailable VFAs is expected to be a principal control of subglacial microbial activity, influenced by the age and organic composition of overridden material and the delivery of OC from supraglacial sources. Results suggest that there is dynamic microbial cycling of OC within these environments, despite the extreme conditions.

Day 2

Biogeochemical processes beneath the Antarctic Ice Sheet inferred from the chemical composition of accretion ice and porewaters in till beneath the Bindschadler and Kamb Ice Streams

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with thanks to many others

Microbial life has been found in every glacier environment that has been examined to date, and the bed of Antarctica is no exception. The chemistry and microbiology of accretion ice ice above Subglacial Lake Vosotok is consistent with this assertion. The chemistry of pore waters found in till beneath the Bindschadler and Kamb Ice Streams, as well as the geomorphology of individual quartz grains, is most easily explained by microbially driven chemical weathering. This talk will present the original data and explain the logic behind the assertions made above.

Geochemistry at Midtre Lovenbreen, Svalbard

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Research conducted by the University of Sheffield, focusing primarily on the hydrology and hydrochemistry at Midtre Lovenbreen, now extends over a 15yr period. A review of this work shows that, through analysis of hydrological time series, processes of dynamic meltwater storage and release from en-and/sub-glacial systems have been identified. These processes have impacts upon the hydrochemistry and geochemical weathering, which have also been explored in an Arctic context. More recently concepts of nutrient budgets and or focus upon nitrogen and sulphate have been utilised and coupled with observations of input and output hydrochemistry and dye tracing experiments. Results show that geochemical enrichment of meltwaters by a number of ionic species occurs and water emmanating from the en/subglacial drainage system changes from anoxic to oxic states over a range of time-scales. These results are taken to demonstrate the significant impact that microbial action has upon the geochemistry of both supraglaical and subglacial runoff from a High-Arctic glacier.

Dissolved carbon and CO2 dynamics along a post-glacial successional gradient; Kårsavagge, Abisko, Northern Sweden (68°21'N, 18°49'E).

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Glacial till represents a reactive veneer of critical importance to the alkalinity and thus inorganic carbon budget of catchments throughout Northern Scandinavia. In places it also provides a chronosequence (successional gradient) of earth surface materials which can be used to study the fundamental problems relating to landscape development and its interaction with the carbon

budget. Understanding how carbon fluxes alter with catchment maturity along a chronosequence is important as recent predictions suggest that the Arctic is one of the regions likely to experience the most rapid and highest magnitude climate change (Houghton, 2001). Increased temperatures over the last 50 years have been linked to glacial retreat and it seems likely that future changes would have a similar effect, exposing more sedimentalogically and ecologically immature ground which may have a knock on effect on carbon cycling (Anderson, 2000; Engstrom, 2000; Hodson, 2000; Anderson, 2002; Hodson, 2002, Anderson, 2007).

Understanding and attempting to quantify the pools and fluxes of carbon within the environment has become increasingly important due to the pivotal role of CO2 in global warming and climate change (Cole, 2001; Christensen, 2007; Jonsson, 2007). The need for a more complete understanding of carbon fluxes from polar environments has become even more imperative as measurements in the Arctic over the past 25 years have shown that the Arctic tundra systems have shifted from being a net sink to a source of CO2 due to the warmer and drier conditions (Loya, 2004). Christensen et al. (2007) showed that the birch forest ecosystems within the Abisko area are an important sink for atmospheric CO2. In contrast the aquatic ecosystems of the region are thought to be sources of CO2 to the atmosphere, however few quantitative estimates of CO2 emissions from Arctic lakes and streams are available (Cole, 1994; Oechel, 1994; Algensten, 2003; Boström, 2007; Christensen, 2007; Torgersen, 2008).

This research project sought to better understand the geochemical controls upon fluvial carbon transfers and their variability during landscape maturation. In order to access the coupling of inorganic and organic carbon fluxes during landscape maturation the pCO2, DOC, DIC and the stable carbon isotopic ratio of the DIC of a glacial melt-water fed stream was measured at regular intervals along a glacial succession gradient. Sampling took place over two runoff seasons, 2006 and 2007, during both peak flow and base flow conditions so that the temporal variations in carbon fluxes could also be considered. The research formed part of a wider project within the Kårsavagge which explored the extrinsic controls upon mineral weathering, including the biological, climatic and hydrological processes that govern rock water contact time (for more information see Plant, 2009; Plant et al., in press).

High resolution monitoring of dissolved oxygen dynamics on glaciers: are supraglacial ecosystems net producers or net consumers?

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Dissolved oxygen is a key parameter in the estimation of biological activity in aquatic ecosystems, including those in the supraglacial and proglacial environment. Traditional methods for measuring oxygen concentration, such as Winkler titration or the use of a Clark-type electrode, are difficult to employ effectively in the field. We report the results from laboratory tests and the first glacial field trials of a fibre optic sensor for dissolved oxygen. The sensor performed well in laboratory tests for response, drift and accuracy. One type of sensor survived simulated freeze-thaw cycles in the lab and was deployed in the field, in snowmelt and glacially fed streams in the Arolla Valley, Switzerland, and in cryoconite holes on Canada Glacier, McMurdo Dry Valleys, Antarctica. We present the first dataset from these deployments, which illustrates nutrient-limited primary production in an otherwise net heterotrophic system. We find that fibre optic sensors are a viable tool for the long term monitoring of glacial biogeochemical conditions, with the ability to enhance our understanding of biological processes in the extreme cold.

Assessment of Viability of Particular Microorganisms in Complex Microbial Communities

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Development of the tools for measuring viable target microorganisms in complex microbial communities has become a very challenging task for environmental microbiologists all over the word. Several methods have been developed as alternatives to plate count to enumerate viable bacteria at single-cell level; however, these methods do not provide information on the identity of the non-culturable cells or still require some incubation or sample preparation (1).
A rapid epifluorescence staining method, the Live/Dead® Bacterial Viability Kit based on the measurement of membrane integrity can be applied to enumerate both viable and total bacteria without incubation. However, this live/dead staining method alone is not suitable for determining the viability of specific microorganisms in environmental samples because of no information of bacterial identity. To overcome this problem, the live/dead staining procedure can be successfully combined with phylogenetic staining such fluorescent in situ hybridization (FISH) to simultaneously analyze the phylogenetic affiliation and the metabolic potential (viability) of single cells in complex microbial communities.
Therefore, the aims of this study were, **(i)** to develop a new fluorescence-based staining method for direct enumeration of viable and total bacteria that are phylogenetically identified by FISH (i.e., *Bacteroides* spp. and *Clostridium* strains, in this study) in complex microbial communities without a need of cultivation and **(ii)** to apply a novel combination for a) evaluation of survival rate of *Bacteroides* spp. as an alternative anaerobic fecal indicator in oligotrophic waters at different temperatures by using the proposed method, and b) delivery of useful information about the diversity and the dominance of a particular *Clostridium* strain (species) in a bioreactor consortium and its contribution to overall biohydrogen production.
To achieve these aims, we combined a fluorescence-based live/dead staining method using ViaGram™ Red+ Bacterial gram stain and viability kit with FISH technique (referred as LDS-FISH method). To our knowledge, this is the first report on such combination.
In the context of microbiology of glacial ice, numerous studies attest to the presence of viable bacteria in ice cores hundreds of thousands of years old (http://brent.xner.net/index.htm). Perhaps the bacteria possess molecular and physiological adaptations that enhance their survival under nongrowth conditions (e.g., enhanced DNA repair), but it is also possible that cells carry out a slow rate of metabolism in the ice to offset macromolecular damage (e.g., to DNA). These issues will also be discussed during presentation.

(1) Savichtcheva, O., Okayama, N., Ito, T., Okabe, S. (2005) Application of a direct fluorescent-based live/dead staining combined with fluorescent *in situ* hybridization for assessment of survival rate of *Bacteroides* spp. in oxygenated water. *Biotechnology and Bioengineering* **92**, 356-363.

Viral lysis in glacial ecosystems

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Several studies have demonstrated that viruses are the most numerous and dynamic members of microbial communities in both marine and freshwaters, but information on viruses in the polar regions is still scarce. Glaciers are no exception and we show the presence of an abundant and dynamic community of viruses in ice cores, proglacial lakes, and cryoconite holes. We show that bacterial lysis mediated by viruses may play a significant role in the cycling of nutrients and organic matter in different glacial ecosystem, where the extent of viruses to bacteria ratios is controlled by nutrient availability, temperature and microbial diversity. Such processes may be relevant to researchers interested in the possibility of extraterrestrial life and the survival and proliferation of life forms on our early Earth (e.g. during the Snowball Earth).

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