SCAR fellowship report

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Research project
Implications of microbial processes for modifying gas records in polar ice cores

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Introduction

Polar environments are a unique habitat because it preserves microbial life and past climate records (gases, chemical species and particulates) chronologically for hundreds of thousands of years. Measurements of trace gases in ice cores are the primary means for reconstructing the composition of the atmosphere. The analysis of these gases is based on the assumption that the air bubbles trapped in ice cores represent the atmospheric composition at the time they were formed and that their composition has been undisturbed since their deposition in ice. However, there is now evidence that unusually high concentration of bacteria living at isolated depths in glacial ice produce gaseous metabolic products that can distort the climate records of trace gases like CO_2 (Ahn et al. 2004, Tung et al. 2006), CH_4 (Tung et al. 2005) and N_2O (Campen et al. 2003, Sowers 2001).

Biogeochemical studies of debris-rich basal ice from Taylor glacier, Antarctica, have demonstrated that such basal ices are a viable subglacial microbial habitat and that microbial activity is both a source and sink for dissolved gases in these environments (Montross et al. 2014). Several studies have highlighted that 1) basal sediments of ice sheets can be a potential source of methane during deglaciation (Zeng, 2003; Weitemeyer and Buffett, 2006) and 2) methanogenic bacteria in basal ice are metabolically active, producing methane, thereby altering the gas chemistry of the basal ice (Tung et al. 2005). *In-situ* metabolism of microbes especially methanogens have also been reported to account for the huge excess of CH_4 at several depths of the GISP2 ice core which was up to an order of magnitude higher than at other depths (Tung et al. 2005). However, despite the circumstantial evidence for *in situ* CH_4 production, no attempt has been made so far to identify the methanogenic archaea involved in this process. The implied role of microbial activity in altering the gas composition of bubbles in the ice, and therefore skewing paleoclimatic inferences makes it imperative to study their distribution and activity in ice. The objective of this research therefore is to use molecular methods to probe for methanogens in debris-rich Taylor Glacier Basal ice and in the GISP2 ice with anomalous CH_4 signals.

Basal Ice, Taylor glacier

Field sampling and sample preparation are described in Doyle et al (2013). Genomic DNA was extracted from 20g of the debris rich basal ice using a MoBio PowerSoil DNA extraction kit. DNA concentration of the extract was determined using Quant-iT PicoGreen kit (Invitrogen). A two-step PCR procedure was used to amplify 16S rRNA from the extracted genomic DNA sample. In the first step, archaeal and methanogen 16S rRNA genes were amplified using primers A571F/UA1406R and 146F/1324R respectively. An aliquot (2 µl) of the resulting product was used as the template in a second PCR, with archaeon-specific primers A751F/UA1204R. The PCR products obtained were examined by agarose gel electrophoresis. PCR products of expected length (~453 bp) were purified using the QIA quick gel extraction kit (Qiagen) and ligated into the pGEM T-Easy plasmid (Promega) according to the manufacturer's protocol. The cloned inserts were sequenced using primers for the flanking T7 and SP6 promoter sequences. Twenty eight sequences were obtained and were edited and compiled using the Bioedit software package and identified using the EzTaxon-e server. All sequences showed between 98 and 99 % similarity with *Candidatus Nitrosoarchaeum limnia* SFB1 (Thaumarchaeota). Controls consisting of template-free reaction mixtures did not generate any amplicons using these PCR procedures.

As attempts to amplify methanogens by conventional PCR-based approaches were unsuccessful, a technique (developed by Kelley G. Nunez) for selectively capturing DNA sequences of interest was used. The method concentrates DNA samples from low biomass environments and allows a researcher to retrieve DNA sequences from complex communities. The method uses a biotinylated oligonucleotide that targets a sequence of interest removing that sequence and at least 5000 base pairs of adjacent DNA with high specificity. While the method was initially developed for targeting 16S rDNA from low biomass samples, it is not limited to only the 16S rDNA. In this work, the method was used for targeted retrieval of the mcrA gene sequences from basal ice. A biotinylated primer was made that would target the methyl coenzyme M reductase I gene (mcrA). McrA is a key functional gene known to be conserved among all methanogens. Selective capture was performed on 1 µl of DNA extracted from Taylor glacier basal ice. The captured fragments were amplified using mcrA gene specific primers. Bands of expected size when sequenced shared identities with the partial coding sequence of an uncultured methanogenic archaeon clone mcrA133 methyl CoM reductase.

Greenland Ice Sheet Project 2 (GISP2) ice core

Samples of GISP2 ice at 2954 m and just above (2953 m), and just below (2956 m) the depth at which excess methane had been found were used in this study. The surfaces of all of the ice core sections were decontaminated prior to melting, as described previously (Christner et al. 2005). Melted ice samples were filtered through 25 mm, 0.2 µm Supor membrane filters (Pall Corporation). DNA was extracted from the filters using a MoBio Power water DNA isolation kit. Two microlitres of the DNA sample was subjected to PCR amplification using primers 571F/1406R and 146F/1324R, followed by a second round of amplification with

A751F/UA1204R. Samples from each PCR reaction were evaluated by electrophoresis through 1.5% agarose gels and staining with ethidium bromide. DNA molecules of the expected length were obtained from samples at all three depths after nested amplification following first round with archaeon specific primers and from 2954 m depth after nested PCR following amplification with methanogen 16S rRNA gene-targeted primers. Interestingly, the sample at 2954 m depth had one of the largest methane spikes. Controls consisting of template-free reaction mixtures did not generate any amplicons using these PCR procedures.

Future work

The PCR products obtained will be cloned and sequenced as described earlier. The sequences obtained will be aligned and analyzed for phylogenetic relationships. In addition, the selective capture technique will be used to probe for methanogens in the GISP2 ice samples.

Poster presentation

Núñez, K.G., Antony, R., Doyle, S., Christner, B., Battista, J.R. 2012 "Selective capture: A Technology for Retrieving Specific DNA Sequences", 19th Annual International Meeting on Microbial Genomics, Lake Arrowhead, California.

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