A novel method for measuring microbial respiration at very low temperatures. J. S. Bowman[1] and J. W. Deming[2], [1]University of Washington School of Oceanography, Box 357940, Seattle, WA 98195-7940, bowmanjs@u.washington.edu, [2] University of Washington School of Oceanography, Box 357940, Seattle, WA 98195-7940, jdeming@u.washington.edu.

The extent of Earth's cryosphere and the preponderance of cold extraterrestrial environments have made the study of cold-adapted (psychrophilic and psychrotolerant) microorganisms a priority. Determining the lower temperature limit of growth and activity for terrestrial microorganisms will allow a more informed discussion regarding the presence of life on Mars, Europa, Enceladus, and other bodies of astrobiological interest. Recent studies have demonstrated microbial growth (cellular reproduction) to –12°C [1, 2], microbial DNA production (using ^{3}H-thymidine) to –15°C [3], and microbial biosynthetic activity ($^{14}CO_2$ uptake) to –20°C [4]. Microbial respiration has been measured to –20°C in Arctic sea ice brines, using a stain that fluoresces when reduced during aerobic respiration [5], and for Arctic permafrost microorganisms by measuring CO_2 production from radiolabelled substrates [4]. Lower temperature values for various measures of microbial activity have also been reported in the literature, from below –20 to –80°C (and lower), but the activity has been characterized as transient and likely linked to energy storage for later recovery under clement conditions [4, 6, 7]. Largely missing from the literature is a non-invasive method to measure microbial activity in undisturbed frozen habitats.

To advance this field of inquiry, we are developing a new method for measuring the respiration rates of microbes, in culture and *in situ,* based on the fractionation of stable carbon isotopes as an indicator of respiration. To validate the approach, we grew the model psychrophilic gammaproteobacterium, *Colwellia psychrerythraea* strain 34H, in the medium "marine broth 2216" which contains isotopically light glucose derived from C3 sugar beets. Air containing isotopically heavier CO_2 was periodically subsampled from the culture-vial headspace and the stable C isotopes analyzed by gas chromatography isotope ratio mass spectrometry (GC-IRMS). Changes to the $\delta^{13}C$ value of CO_2 contained in the headspace over time were attributed to the bacterial respiration rate. With measurements of initial CO_2 concentration, cell count, and headspace volume, the respiration rate per cell can also be calculated. Using this stable carbon isotope fractionation analysis of respiration (SCIFAR), we measured bacterial respiration from 2°C to –25°C (the lowest temperature tested to date). At –25°C the respiration rate was estimated to be 5.5×10^{-14} g C day^{-1} (n = 3, SD = 4.3×10^{-14}) for 8 mL cultures after a 20-day incubation period. This result represents a lowering by 5°C of the temperature limit of metabolic activity for *C. psychrerythraea* strain 34H [6].

Since the natural pool of organic carbon available for chemoorganotrophic metabolism is derived from photosynthesis, it is isotopically light compared to the carbon dioxide in Earth's atmosphere. We are taking advantage of this fact to apply SCIFAR beyond cultures of *C. psychrerythraea* strain 34H to undisturbed natural microbial communities respiring on their native substrates in subzero habitats under *in situ* conditions. Our initial efforts are focused on applying SCIFAR to the measurement of microbial respiration in sea ice and its surficial crystalline structures known as frost flowers (in winter 2010; work supported by NSF). These structures, recently shown to contain a high abundance of bacteria as a function of the high salt content (unfrozen brine) of frost flowers [8], are exposed to extreme air temperatures of Arctic winter that fall below the current lower temperature limits for microbial growth and sustained activity. As such they represent new and largely untested habitat analogs for very cold extraterrestrial environments.

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