CARBON HIDE AND SEEK: TRACKING CARBON EXCHANGE AMONG INDIVIDUAL METHANOGENS

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Introduction: Within the Euryarchaeota are organisms that fix carbon through a pathway quite possibly more ancient than all other forms of metabolism. This process, known as methanogenesis, may offer us a glimpse of metabolic life strategies potentially used by extraterrestrial life. These organisms are anaerobic and capable of growth in harsh subsurface ecosystems; it has been proposed that similar microorganisms could survive on Mars, Titan, and other planetary bodies. On Earth, methanogens play an interesting role in carbon cycling in marine environments such as the 30,000 year old Lost City, a hydrothermal vent field near the Mid-Atlantic Ridge.

This ecosystem is of particular astrobiological interest because it presents an exciting example of early Earth conditions. These chimneys differ from typical black smoker vents because they are driven by serpentinization reactions, rather than magmatic activity. The serpentinization reactions create high pH fluids that mix with surrounding cold seawater to form calcium carbonate chimneys. The methanogens form a biofilm inside the chimneys and are thought to transform carbon dioxide and hydrogen into methane for bacteria outside the chimneys to oxidize. A new species, belonging to the *Methanosarcinales* clade, was recently characterized from the hydrothermal vents in Lost City. An unusually high genetic and morphological variability within the single *Methanosarcinales* species that forms the biofilms has been previously documented [1]. However, it is unclear how these differences relate to methane production and nutrient cycling.

In general, little is known about microbial metabolism of carbon within the Lost City ecosystem. A previous study detected methanogenic activity in Lost City chimneys through incubation of recently collected samples with ^{13}C-labeled carbon dioxide [1]. However, only bulk measurements of activity were measured and could not be linked with genomic data. Raman spectroscopy offers analysis of single cells in environmental samples before DNA extraction and sequencing. This technology is effective for bacterial identification [2], and recent research has shown the technology is particularly effective in detecting assimilation of ^{13}C-labeled glucose into biomass [3]. Therefore, one could incubate an environmental sample with ^{13}C-labeled molecules and track the labeled carbon into the biomass of a single cell prior to sequencing of DNA from the same single cell. With this approach, carbon flow between species can be observed when an environmental sample is exposed to a primary source of ^{13}C.

Coupling the spectral data with microscopy and the genomics of a single cell will reveal the species which incorporated the labeled carbon, which macromolecule (lipid, nucleic acid, or carbohydrate) the carbon was incorporated into, the morphology of the cell, , and a list of potential genes associated with metabolism of the ^{13}C. This data will be used to test the following hypotheses about carbon cycling within the Lost City biofilm communities.

Hypotheses:

1. Within a single species, there will be variability among cells with respect to their metabolic capabilities and these physiological differences will correspond to subtle or major genomic differences.
2. Within a single species, cells with similar genomic characteristics will correspond to cells with similar morphological characteristics.
3. Transfer of carbon between methanogens and bacteria inhabiting the colder, exterior zones of the chimneys will be mediated by one bacterial species, perhaps an aerobic methanotroph, that is dependent on carbon processed by the methanogens.

Methods: When fresh enviromental samples from the Lost City carbonate chimneys become available (to be determined), they will be incubated in a chamber containing ^{13}C-labeled carbon dioxide. After the sample is allowed to incubate for a period of time, cells that incorporated the ^{13}C-labeled carbon will be identified with a microscope equipped with a Raman laser. These cells will be selected for single-cell genome sequencing. In the meantime, these methods will be tested with previously cultivated strains of methanogens.

Developing this spectral method to research methanogens allows for observation of carbon cycling in an astrobiologically-relevant ecosystem at single-cell resolution. Studying the specific roles microbes play in nutrient cycling allows deeper understanding of the critical cycles for life on Earth, which ultimately increases our understanding of what nutrient cycles may look like on other plantary bodies.

Key words: Raman spectroscopy, methanogenesis, Archaea, carbon cycling, serpentinization

References:

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