MEASURING THE EFFECT OF ORGANIC IRON LIGANDS ON MARINE MICROBIAL COMMUNITY STRUCTURE AND ACTIVITY

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I. BACKGROUND

The purpose of this proposal is to conduct ship-based incubation experiments of microbial communities collected aboard the R/V Kilo Moana off of Hawai’i during the 3-day C-MORE cruise scheduled for September 18-21, 2009. These experiments will consist of collecting water samples under trace metal clean conditions and performing incubations of native microbial communities with additions of iron and naturally occurring organic iron-binding ligands. Using a combination of trace metal techniques to sample and determine the extent of microbial iron uptake and of meta-transcriptomic analysis to monitor changes in gene expression of the microbial community, we will explore the hypothesis that complexation with organic ligands makes iron more accessible to the marine microbial community.

a). Iron in the marine environment

Despite its prevalence in the earth’s crust, iron remains scarce within marine habitats largely due to its low solubility as well as its rapid uptake and utilization by phytoplankton species. Iron concentrations in many regions of the ocean are extremely low, ranging from picomolar to nanomolar values. Iron fertilization experiments have identified iron limitation of ocean productivity in various open-ocean habitats, including the Equatorial Pacific, Subarctic Pacific, and the Southern Ocean, as well as in coastal systems off the coasts of California and Peru. As a co-factor of key enzymes, for example those controlling nitrogen fixation and photosynthesis, iron availability limits key biochemical reactions that drive the carbon, nitrogen, and phosphorous biogeochemical cycles.

Over 99% of total iron in seawater is complexed with organic ligands of unknown origin. Previous studies have suggested that these organic ligands are of biological origin and may be marine siderophores similar to those identified in terrestrial ecosystems. It has been argued that complexation with organic ligands may allow iron to be more accessible to the marine microbial community. Recent work by Dan Repeta at the Woods Hole Oceanographic Institution has allowed for the concentration and collection of naturally occurring organic iron-binding ligands from open ocean water using the hydrophobic resin XAD16. These resins are later extracted and the ligands obtained can be analyzed in order to determine the conditional stability constants and total iron concentration.

b). Meta-transcriptomics

Recent advances in gene sequencing technologies have allowed for an increased understanding of the diversity and function of marine microbes. Large metagenomic surveys of sequence data obtained by the direct sequencing of environmental DNA have described a large number of previously unknown genes and protein families, highlighting the large unknown microbial diversity and functional potential in the ocean. More recently, it has become possible to track short-term changes in gene expression of entire microbial communities in situ, as demonstrated on samples from Hawaii Ocean Time Series (HOT) station ALOHA. This has opened new
avenues for experimental meta-transcriptomics, in which the change in gene expression of an entire microbial assemblage is explored as a result of experimental manipulation.

A similar experiment to the one proposed was performed on the BLOOMER cruise in a collaboration between members Ed DeLong’s and Dan Repeta’s labs. In this experiment, the change in gene expression of the microbial community was monitored in response to amendments of dissolved organic matter (DOM) to surface seawater. Initial analysis of the meta-transcriptomic data has revealed interesting results, notably the shift to a microbial community that is not only dominated by Prochlorococcus and Pelagibacter, but also by Alteromonadales. The field of meta-transcriptomics is in its infancy, but we are well positioned to benefit from the DeLong’s lab leading expertise in the field.

II. APPROACH

In this collaborative project, we propose to follow changes in the marine microbial population's community structure and in its gene expression profile in response to different iron treatment additions. One treatment will consist of naturally occurring organic ligands extracted from XAD16 resin. This treatment will allow us determine whether these compounds make iron more available as a micronutrient for the microbial community. Taking advantage of opportunities provided by C-MORE, we propose to perform this research aboard the R/V Kilo Moana in Hawai’i, during the 3-day C-MORE cruise scheduled for September 18-21, 2009. Our microbial community water will be collected from the surface using a trace metal free pump system operated off the ship deck. The incubation experiments will be performed using the flow-through incubators on the deck of the vessel. All the sample handling will be done under trace metal clean conditions, including the construction of a trace metal clean bubble around a portable laminar flow hood. Training and help from E. Bertrand is essential for the success of this project.

The following figure summarizes the incubation treatments and sampling strategy (note that treatments will be run in triplicates instead of duplicates):
At the relevant time points, DNA and RNA samples will be collected to follow changes in the microbial community's gene expression profile, with special focus on identifying specific transporters that may recognize naturally-occurring organic ligands. Chlorophyll and flow cytometry measurements will shed light on the effects of the treatments on the phototrophic microbial community. Samples for total, dissolved, and particulate iron will allow us to determine the extent of iron uptake in the different treatments as well as the success of the trace metal clean procedures. It is important to mention that all these analyses will be performed by the four participants in this project.

III. JUSTIFICATION

The proposed research project addresses four of C-MORE’s education goals listed for as priorities for EDventures funding: it bridges research and education, it fosters partnerships, is based at C-MORE partner institutions, and is innovative and may lead to external funding.

This experiment provides an opportunity for E. Bertrand, a senior graduate student in Mak Saito’s lab with proven experience working on trace metals (Bertrand et al., Limnol. Oceanogr., 2007), to train three possible participants in the C-MORE 2010 cruise. Such training will allow for similar experiments merging meta-transcriptomics with trace metal techniques to take place during this cruise. Additionally, these techniques will assist graduate students L.-A. Ventouras, a member of E. DeLong’s lab, and K. Munson, a member of D. Repeta’s lab, in trace metal clean techniques that will be used as they progress in their graduate research.

This experiment is designed and the analyses will be carried out by students at the Massachusetts Institute of Technology and the Woods Hole Oceanographic Institution. The experiment will be performed on a C-MORE cruise led by scientists from the University of Hawai’i.

The proposed research combines aspects of research funded by the laboratories of Ed DeLong, Dan Repeta, and Mak Saito in an innovative way, the results of which may guide the design of experiments performed on multiple cruises planned for 2010.

IV. ITEMIZED BUDGET

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TOTAL BUDGET REQUESTED $11,060