STATEMENT OF WORK

MICROBIOLOGY IN DEEP-CORAL HABITATS
2008-2011

Objectives:

1) identifying and characterizing the microbial communities associated with live *Lophelia pertusa* at multiple sites in the Gulf of Mexico
2) comparing the microbial communities associated with live *Lophelia* to those associated with dead *Lophelia* and surrounding sediments
3) determining if *Lophelia*-associated mobile fauna are acting as vectors and transporting specific bacteria between coral colonies
4) identifying and characterizing the microbial communities associated with other deep-sea coral species (e.g. *Madrepora, Enallopsammia*) in conjunction with the population genetics task

Proposal Synopsis:

The microbial ecology of deep-sea coral habitats will be investigated. Specially-designed sampling gear will be used to collect samples of deep-sea corals and sediments for both culture-based and molecular analyses. These methods will also be employed to screen coral-associated mobile fauna for specific bacterial types to determine if the fauna are acting as vectors (either of disease or as natural dispersal agents for commensal coral-associated bacteria). The information generated by this research will be disseminated in the peer-reviewed literature as well as reports.

Introduction and Background

This work will expand previous studies of *Lophelia*-associated microbial ecology from two sites in the Gulf of Mexico (GOM) and allow for comparisons to be made involving coral-associated bacterial community composition on different coral colonies, between coral species, and between geographic sites in the GOM.

Results from previous work (Lophelia I Project) revealed a diverse and unique microbial community associated with *L. pertusa*. Although only two sites in the northern GOM were sampled, dramatic differences in the coral-associated bacterial communities were evident between them. This microbial spatial variability seemed to track similar results from the coral genetics studies. Shallow-water studies have found that: 1) coral-associated bacterial communities are species-specific, e.g., the communities vary by coral species not geographic location (Rohwer et al. 2002), and 2) coral-associated bacterial communities shift in response to coral disease or stress (Klaus et al. 2005; Pantos et al. 2003). This potentially links the observed microbial differences in *Lophelia* to external factors such as temperature variability or internal factors such as genetic susceptibility. Obtaining data from other sites in the GOM will clarify this issue and also provide for the first time a perspective on large scale variability of the *Lophelia* microbial community. It will also be useful to examine the microbial diversity of other deep-sea corals (e.g., *Madrepora oculata, Enallopsammia profunda*, gorgonians) for comparative purposes.

Previous research has also revealed the presence of an apparently *Lophelia*-specific bacterium (designated VKLP1) that is related to seep-clam symbionts. *Lophelia* colonies at one site (VK826) were dominated by novel *Firmicutes*, most similar to bacteria commonly characterized as pathogens. It is possible that coral-associated mobile fauna are acting as transport vectors for some of these dominant members of the coral microbial community (Sussman et al. 2003); that is, ferrying bacteria from coral to coral, or between seep communities and corals. We propose to sample the microbial communities of other taxa closely associated with corals, especially species that contact the corals. Candidate species will be selected from among those being sampled for community genetics (e.g., galatheid crabs, corallivorous snails, conger eels). Microbial and genetics sampling will be closely coordinated to share species and address mutual hypotheses of variability on coral mounds.

Purpose and Scope of Study

The purpose of the study is to identify and characterize deep-sea coral-associated microbes in an effort to understand their roles in deep-coral habitats (e.g., base of the food web, vectors of disease, biologically significant coral commensals/symbionts, etc.). This information is key to an overall understanding of these deep-coral habitats which is necessary to appropriately manage these resources.

Study Elements

1. Personnel: USGS Research Microbiologist, Dr. Christina A. Kellogg will participate in and coordinate the design and construction of sampling gear, sample collection, laboratory analyses, data analyses, and writing of reports and journal articles. Michael Gray, microbiological technician, will assist with the laboratory experiments, data analyses, and writing.
2. Facilities: Major facilities and equipment will be provided by USGS, including office space, laboratory space, computers, and field vehicles.
3. Travel Expenses: Funds will be provided for travel to participate in research cruises and to present findings at stakeholder, national, and international meetings.
4. Science products: DNA sequence data generated from bacterial clone libraries will be deposited in GenBank (online genetic database). Reports will be produced as required by MMS. Journal articles based on molecular and culture data will be written.

Methods

Four to five submersible/ROV cruises are planned during the first three years of the study with sampling areas in deep-sea coral habitats ranging from the central GOM through the West Florida Slope (300-800 m). Two or three sites (e.g., VK 826, Gulf Penn or similar, W. FL slope) will be selected for focused research, and most of the microbiology data would be collected from these intensive study sites. The USGS–St. Petersburg is equipped to perform microscopy, culturing, DNA isolation, amplification (PCR), electrophoresis, and analysis of DNA sequences using several established methodologies. *Lophelia* samples will be collected with specialized sampling gear that allows the addition of fixative to half of the samples (preserving *in situ* microbial diversity). Live samples will be immediately cultured on appropriate marine agar.

Preserved samples will have total microbial community DNA extracted using the MoBio PowerSoil DNA extraction kit. Clone libraries will be constructed from coral samples, sediments, and mobile fauna; screened, and sequenced to identify the associated microorganisms.

Products

Peer reviewed publications will be prioritized, but technical reports, oral presentations (at MMS ITMs, the 11th International Coral Reef Symposium, the 4th International Symposium on Deepsea Corals, and potentially other conferences), and other products will also be prepared. Products will be produced and released with the knowledge of MMS and in coordination with "Lophelia II" PIs. DNA sequence data will be deposited in the public database GenBank.

References

Klaus, J.S., J. Frias-Lopez, G.T. Bonheyo, J.M. Heikoop and B.W. Fouke. 2005. Bacterial communities inhabiting the healthy tissues of two Caribbean reef corals: interspecific and spatial variation. Coral Reefs 24: 129-137.

Pantos, O. and coauthors. 2003. The bacterial ecology of a plague-like disease affecting the Caribbean coral *Montastrea annularis*. Environmental Microbiology 5(5): 370-382.

Rohwer, F., V. Seguritan, F. Azam and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. Mar. Ecol. Prog. Ser. 243: 1-10.

Sussman, M., Y. Loya, M. Fine and E. Rosenberg. 2003. The marine fireworm *Hermodice carunculata* is a winter reservoir and spring-summer vector for the coral-bleaching pathogen *Vibrio shiloi*. Environmental Microbiology 5(4): 250-55.