



Project Report: Delivery of Organic Materials to Planets

Focus Group Chair(s): Mitchell L. Sogin, David Des Marais

Executive Summary

The overarching objective of the Ecogenomics Focus group is to define the relationship between microbial diversity, complex gene-expression patterns, and biogeochemical processes that shape planetary environments. Using the hypersaline cyanobacterial mats of Guerrero Negro, Baja California Sur, Mexico as a field site, this interteam effort will characterize biogeochemical patterns in the microbial mats. Special emphasis will be placed on documenting trends with depth in the mat with respect to the chemical and light microenvironments of the microbiota, as well as their major products. These measurements will be coupled with molecular-based assessments of microbial population structures and DNA microarray measurements of gene expression. The gene-expression data will rigorously identify important biochemical processes that play key roles in the formation of and shifts in biogeochemical gradients in response to transient and periodic perturbations imposed by diel (24-hour) cycles. The ultimate objective is to establish models that can predict the behavior of these complex systems.

The five NAI teams currently participating in this project include Ames Research Center (ARC) (with links to University of Connecticut), Arizona State University (ASU), the University of Colorado (UC), the Marine Biological Laboratory (MBL), and the University of Washington (UW). ARC has taken the lead role in studies of biogeochemical measurements at the Guerrero Negro field site and the maintenance of a common data base – Science Organizer. UC and UW teams are using molecular cloning and sequencing-techniques?principally rRNA analyses (ribosomal RNA)?to study microbial diversity in the mat samples. ASU utilizes denaturing gradient gel electrophoresis (DGGE) to survey cyanobacterial populations in the mats. In a collateral project, the MBL is working on a high-throughput sequence-based technology to accelerate microbial population mapping surveys that are both more accurate and efficient than DGGE analyses or rRNA sequence studies. Both ARC and MBL are developing DNA microarrays for specific gene activities that may span multiple species (ARC) or for the entire genome of representative species of cyanobacteria.

During Year 4, the ARC team organized a field trip to the Guerrero Negro study site from 2–15 October. Major goals of the field trip included (1) establishing the microbial mat carbon and oxygen budgets for the fall season, (2) characterizing populations of sulfate-reducing bacteria, (3) measuring the lateral distributions of cyanobacterial populations within subtidal microbial mats, (4) conducting total microbial diversity surveys throughout the mat, and

(5) characterizing the biogeochemistry of sulfur in photosynthetic microbial mats.

In mats dominated by *Microcoleus* (subtidal) and *Lyngbya* (intertidal to supratidal) cyanobacteria, we observed the exchange of oxygen (O_2) and dissolved inorganic carbon (DIC) between mats and the overlying water, during diel (24-hr) cycles. Patterns of O_2 daytime release and nighttime uptake mirrored these DIC trends in both mat types. Nighttime DIC effluxes from *Microcoleus* mats were equivalent in the presence versus absence of O_2 , whereas nighttime effluxes from *Lyngbya* mats dropped markedly in the absence of O_2 . Thus, aerobic degradation of organic matter was more important in *Lyngbya* mats than in *Microcoleus* mats, perhaps because trapped O_2 bubbles persist only in *Lyngbya* mats at night and thus sustain populations of aerobes. Differences observed between *Microcoleus* versus *Lyngbya* mats forecast differences in their microbiota and in their patterns of gene expression. When exposed to a simulated Archean atmosphere with very low O_2 content, photosynthetic bacteria in these mats produce substantial amounts of hydrogen (H_2). Such a large- H_2 flux might have enhanced the global distribution and productivity of H_2 -consuming organisms, and also might have contributed significantly to oxidation of the oceans and atmosphere by means of H_2 escape to space. A model was constructed to simulate C, O, and sulfur (S) cycles, and also the growth of cyanobacteria and sulfur bacteria in a stratified hypersaline mat. The aim was to simulate microbial effects on the atmospheric chemistry of early Earth.

We used 16S rRNA gene-based methods to assess and compare cyanobacterial community structures from an environmentally homogeneous hypersaline pond and an environmentally heterogeneous intertidal area at the Guerrero Negro site. Previous observations have shown that the comparative use of three cultivation-independent methods of community structure quantification, based on 16S rRNA genes, carotenoids, and morphotypes, respectively, showed that when dealing with large data sets, all yield similar trends. Cyanobacterial diversity and spatial scale heterogeneity was studied by DNA extraction from the microbial mats, specific amplification of the 16S rRNA gene using cyanobacterial specific primers, and allelic separation of polymerase chain reaction (PCR) product DNA using denaturing-gradient gel electrophoresis (DGGE). Our results show that environmental gradients influence cyanobacterial community structures. The hypersaline pond is a stable environment with no significant spatial variation in environmental parameters. Cyanobacterial diversity in these permanently submerged mats remained virtually constant over all spatial scales sampled, with only minimal differences seen at the millimeter scale. Although the absolute diversity was comparable to that in the hypersaline pond, spatial heterogeneity was clearly detected in the intertidal area along a desiccation gradient.

Using molecular survey methods in which ribosomal RNA (rRNA) genes are obtained directly from natural environmental DNA by Polymerase Chain reaction, we find that cyanobacteria, although conspicuously present in these mats, are a relatively less numerous component of the microbial community. The generally more abundant organisms (as identified by their rRNA genes) are representatives of the "Green Nonsulfur" phylogenetic division of bacteria.

The studies so far have discovered several hundred novel species of microorganisms.

In order to determine the scale of variability among a broader array of microorganism in addition to the cyanobacteria, core samples were taken across a range of scales (millimeter to kilometer) along a horizontal transect. Bulk community DNA was extracted from these cores and analyzed using terminal restriction fragment analysis (T-RFLP). This technique relies on DNA sequence differences, which result from evolutionary divergence among groups, to assess microbial diversity. Results showed similar microbial populations across all spatial scales; however, the relative abundance of community members varied across samples. Determining the spatial scale of microbial diversity is essential to our understanding of population variation in these complex microbial communities and is vital to informing our ongoing sampling efforts in understanding this environment.

Focus group meetings were held during the AbSciCon02 meeting in April 2002 at Ames Research Center, as well as by video conferencing. Several of the principal investigators participated in a NASA Life Sciences sponsored workshop, "Outcomes of Genome-Genome Interactions" at the Jonson Center in Woods Hole.

Focus Group Description & Activities

The Marine Biological Laboratory. DNA from *M. chthonoplastes* strain PC7420 was provided for construction of genomic libraries. DNA was cloned into pzero vector with an average insert size of 650–800 bp (base pairs). High-quality single-primer sequences (10,241) were obtained. Because the cultures were not axenic, we only identified 3,370 sequences that produced BLAST scores with probability values of $1e-04$ or better against cyanobacterial entries in GenBank. Using Pfam, 1,876 we identified significant bit scores > 30 with approximately 575 unique domains. The BLAST taxonomic breakdown of the sequences is as follows: 46.82% hit to a cyanobacteria (36% to *Nostoc* and 9% to *Synechococcus*), and the major contaminants were alpha proteobacteria (9.27% *Mesorhizobium*, 5.59% *Sinorhizobium*). Based on comparisons with limited data from other *Microcoleus* strains, the genomic sequence conservation for *Microcoleus* strains is not as high as predicted. We will construct DNA microarrays using amplicons from clones that correspond to reliable Pfam and BLAST hits against cyanobacterial genes (~1,000). These will be used to study gene expression for *Microcoleus* under differing conditions in the laboratory. The application of this technology to field samples will follow.

We have also developed a rapid means for studying microbial population structures based upon sampling rRNA sequence diversity that is patterned after SAGE (Serial analysis of gene expression). With SAR, we can sample at the sequence level, short species-specific regions of amplified rRNAs genes from many microorganisms in a single reaction. With SAR, the PCR products from orthologous hypervariable regions in rRNA genes are ligated together to form large concatemers. A single DNA sequencing reaction of a cloned concatemer can describe as many as 20–30 orthologous hypervariable

regions represented in a population of nucleic acid molecules. In this way, samples loaded onto a 96-channel capillary sequencing machine can provide information about thousands of microorganisms in an analyzed sample.

Ames Research Center. In mats dominated by *Microcoleus* (subtidal) and *Lyngbya* (intertidal to supratidal) cyanobacteria, the exchange of O₂ and dissolved inorganic C (DIC) was observed between mats and the overlying water during diel (24-hr) cycles. Patterns of O₂ daytime release and nighttime uptake mirrored these DIC trends in both mat types. Nighttime DIC effluxes from *Microcoleus* mats were equivalent in the presence versus absence of O₂, whereas nighttime DIC effluxes from *Lyngbya* mats dropped markedly in the absence of O₂. Thus, aerobic degradation of organic matter was more important in *Lyngbya* mats than in *Microcoleus* mats, perhaps because trapped O₂ bubbles persist only in *Lyngbya* mats at night and thus sustain populations of aerobes. In both mat types, effluxes of H₂, CH₄ and short-chain fatty acids were much greater at night in the absence of O₂. Differences observed between *Microcoleus* versus *Lyngbya* mats forecast differences in their microbiota and in their patterns of gene expression.

The primary role of photosynthetic members in mats is to extract reducing power (electrons) from water and to use it to “fix” CO₂ into organic carbon (biomolecules). However, when exposed to a simulated Archaean atmosphere with very low O₂ content, these organisms divert a substantial fraction of the captured reducing power to produce H₂. Globally, this source of H₂ could have outstripped geologic sources by 2 to 4 orders of magnitude. Such a large-H₂ flux might have enhanced the global distribution and productivity of H₂-consuming organisms, and also might have contributed significantly to oxidation of the oceans and atmosphere by means of H₂ escape to space.

A model was constructed to simulate C, O, and S cycles, and also the growth of cyanobacteria and sulfur bacteria in a stratified hypersaline mat. The aim was to simulate microbial effects on the atmospheric chemistry of early Earth. The model was constructed in a Stella™ environment.

University of Colorado. Ecogenomics related activities in the Pace laboratory are focused mainly on a molecular analysis of the microbial constituents of hypersaline microbial mats, mainly at Guerrero Negro, Baja California. The goal of these studies is to understand the organismal makeup of these communities and how the individual kinds of organisms contribute to the support of this remarkable concentration of biomass. The results contribute to our knowledge of the diversity of life in extreme environments. Although substantial effort has been invested in the study of chemical aspects of the Guerrero Negro system, relatively little is known about the organisms that compose these communities.

Most previous studies of the microbial biology of the Guerrero Negro and other hypersaline microbial mats have relied on direct microscopy or on development of cultures of microbes for laboratory studies. However, microscopy detects only morphologically conspicuous organisms, and not many microbes are culturable with standard techniques. Consequently, we are using molecular survey methods in which ribosomal RNA (rRNA) genes are

obtained directly from natural environmental DNA by polymerase chain reaction (PCR) and molecular cloning techniques. The studies of Guerrero Negro mats have only begun, but already they promise to revolutionize our view of the makeup of such communities. Specifically, previous conclusions based on microscopy and culture have focused on cyanobacterial photosynthesis as the main source of primary productivity (conversion of carbon dioxide into biomass). We find, however, that cyanobacteria, although conspicuously present in these mats, are only one component, and generally a minor component, of the numerically dominant organisms. The generally more abundant organisms (rRNA genes) are representatives of the “Green Nonsulfur” phylogenetic division of bacteria. This was an unexpected result that changes fundamentally the way that the community needs to be modeled. The studies so far have discovered and molecularly described several hundred novel species of microorganisms.

Arizona State University. We used 16S rRNA gene-based methods to assess and compare cyanobacterial community structures from an environmentally homogeneous hypersaline pond and an environmentally heterogeneous intertidal area at the Guerrero Negro site. Previous observations have shown that the comparative use of three cultivation-independent methods of community structure quantification, based on 16S rRNA genes, carotenoids, and morphotypes, respectively, showed that, when dealing with large data sets, all yield similar trends. Cyanobacterial diversity and spatial scale heterogeneity was studied by DNA extraction from the microbial mats, specific amplification of the 16S rRNA gene using cyanobacterial specific primers, and allelic separation of PCR product DNA using denaturing-gradient gel electrophoresis (DGGE). Our results show that environmental gradients influence cyanobacterial community structures. The hypersaline pond is a stable environment with no significant spatial variation in environmental parameters. Cyanobacterial diversity in these permanently submerged mats remained virtually constant over all spatial scales sampled, with only minimal differences seen at the millimeter scale. Although the absolute diversity was comparable to that in the hypersaline pond, spatial heterogeneity was clearly detected in the inter-tidal area along a desiccation gradient.

University of Washington. We participated in two ecogenomics EMERG group field trips in 2001 (June and October) to the Guerrero Negro evaporation ponds. This collaborative project involves geochemical and molecular biological analysis of the microbial mat populations in these ponds. These mat populations are visually homogeneous over kilometers of extent and display steep geochemical gradients (e.g., light, oxygen) with depth. Core samples of microbial mats were frozen and returned for molecular analysis. DNA was subsequently extracted from these samples for use in two main projects investigating microbial diversity in the samples.

In the first project, we are measuring the distribution and diversity of bacteria in these microbial mat samples. We used standard PCR techniques to amplify the conserved bacterial 16S rRNA gene. We are assessing bacterial diversity using T-RFLP, a rapid method for determining total community structure and composition. We have assessed diversity over two scales: horizontally, at varying spatial scales over a kilometer distance, and vertically, using cores

sliced at submillimetric scales with depth. Results indicate that bacterial communities are remarkably stable across even large spatial scales (~1 km); however variation at even fine scale (cm) was detected. Significant variation in community structure was also observed with depth.

In the second project, we are investigating a specific group of microorganisms, the sulfate-respiring prokaryotes. High levels of activity for this group have been measured in this site. In collaboration with Woods Hole MBL, we are determining the DNA sequence diversity of genes specific to this group with depth in the microbial mat samples.

Highlights

- When exposed to a simulated Archaean atmosphere with very low O₂ content, these photosynthetic microbial mats produce substantial amounts of H₂. Such a large H₂ flux might have enhanced the global distribution and productivity of H₂-consuming organisms (ARC)
- Hypersaline microbial mats display high levels of bacterial diversity. Community diversity profiles are similar over hundreds of meters of spatial distribution (UW).
- We have developed a high-throughput technique that allows us to sample sequences amplified from natural populations of microorganisms that is at least an order of magnitude less expensive than more traditional rRNA sequencing strategies. (MBL)
- Cyanobacterial diversity in permanently submerged mats remained virtually constant over all spatial scales sampled (millimeter to kilometer), with only minimal differences seen at the millimeter scale. Although the absolute diversity in the intertidal area along a desiccation gradient was comparable to that in the submerged mats in the hypersaline pond, greater spatial heterogeneity was clearly detected in the intertidal areas. (ASU)

Roadmap Objectives

- [**Objective No. 4: Genomic Clues to Evolution**](#)
- [**Objective No. 5: Linking Planetary Biological Evolution**](#)
- [**Objective No. 6: Microbial Ecology**](#)
- [**Objective No. 12: Effects of Climate Geology on Habitability**](#)
- [**Objective No. 13: Extrasolar Biomarkers**](#)
- [**Objective No. 14: Ecosystem Response to Rapid Environmental Change**](#)

Mission Involvement

Although this work is not directly related to a specific NASA mission, it does provide information that will be important in the search for extraterrestrial life. The field studies carried out by the Ecogenomics Focus Group will help us to understand the range of conditions that were present on early Earth. We seek

to understand how microbial ecosystems affected the early atmosphere and the biological processes that left traces of early life in ancient sedimentary rocks. It is clear that the discovery of life on other solar system bodies would most likely be microbial. These studies allow us to design life–detection experiments and to interpret geological studies of samples returned to Earth.

Field Expeditions

Field Trip Name: Ecogenomics field trip to Baja California, Mexico	
Start Date: 10/02/2001	End Date: 10/15/2001
Continent: North America	Country: Mexico
State/Province: Baja California Sur	Nearest City/Town: Guerrero Negro
Latitude: 27 degrees 40 minutes N	Longitude: 113 degrees 55 minutes W
Name of site(cave, mine, e.g.): Exportadora de Sal, S. A. salt works	Keywords: microbial ecology, microbial mats, biosignature gases, ecogenomics, cyanobacteria
Description of Work: Microbial ecological studies of the populations, processes and products of marine hypersaline microbial mats.	
Members Involved: Participants included members of the Ames, Arizona State, Washington and Colorado NAI teams: B. Bebout, S. Carpenter, R. Castenholz, D. Des Marais, J. Dillon, E. Fleming, F. Garcia–Pichel, T. Hoehler, M. Huerta–Diaz, R. Ley, M. Hogan, S. Miller, T. Norris, E. Omoregie, M. Rothrock, J. Spear, K. Turk, Jesse Dillon, David Stahl.	