Annual Report for Period: 08/2002 - 08/2003
Submitted on: 07/25/2003
Principal Investigator: Ward, Bess B.
Award ID: 9981482
Organization: Princeton University
Title:
Collaborative Research: Biocomplexity of Aquatic Microbial Systems: Relating Diversity of Microorganisms to Ecosystem Function

Project Participants

Senior Personnel

Name: Ward, Bess
Worked for more than 160 Hours: Yes
Contribution to Project:

Name: Lane, Todd
Worked for more than 160 Hours: No
Contribution to Project:

Post-doc

Name: Taroncher-Oldenburg, Gaspar
Worked for more than 160 Hours: No
Contribution to Project:
In charge of genechip development

Name: Francis, Chris
Worked for more than 160 Hours: Yes
Contribution to Project:
In charge of nirK and nirS sequence libraries and database for chip development

Graduate Student

Name: O'Mullan, Gregory
Worked for more than 160 Hours: Yes
Contribution to Project:
Dissertation work will involve mesocosm experiments to investigate relationships between microbial diversity and ecosystem function with a focus on nitrification and nitrifying bacteria.

Undergraduate Student

Name: Griner, Erin
Worked for more than 160 Hours: No
Contribution to Project:
Senior Thesis involved optimization of hybridization conditions for the glass microarrays. She graduated in 2002 and is no longer affiliated with the project.

Technician, Programmer

Name: Schmitt, Danielle
Worked for more than 160 Hours: No
Contribution to Project:
General support technician, participates in field work. Danielle moved on to an other position in 2002 and is no longer working on the project.

Name: Nelson, Joshua
Worked for more than 160 Hours: Yes
Contribution to Project:
Josh has taken over most of the laboratory work with the microarrays, beginning in Nov 2002.

Other Participant

Research Experience for Undergraduates

Organizational Partners

University of Maryland Center for Environmental Sciences
Horn Point personnel are the lead on the biogeochemical process field work

University of California-Santa Cruz
Jon Zehr is doing the nifH gene work and developing membrane based macroarrays.

Texas A&M University Main Campus
George Jackson is our data analysis, synthesis and modeling expert

SUNY at Stony Brook

USGS, Reston VA
Dr. Mary Voytek, a USGS scientist, collaborates through a subcontract to Princeton University.

Renssalear Polytechnic Institute

Other Collaborators or Contacts

Mary Voytek at USGS, Reston, VA, is a subcontract on the project. She will be doing the amo gene work.

Activities and Findings

Research and Education Activities:
Sampling Program:

The regular sampling program in the Choptank River and Chesapaeake Bay has continued with 5 Choptank trips and 2 Chesapeake trips since Aug 2002. The Princeton component continues to take the lead in carrying out the sampling, while the Horn Point component has provided shorebased laboratory and small boat facilities and has coordinated the cruises. Participants from USGS (Voytek group) participated in some of the Choptank and Chesapeake expeditions and the UCSC component (Zehr group) participated in the April 2003 cruise.

On each Choptank fieldtrip, DNA samples (15 replicates of each) were collected by capsule filtration from each of four depths (two stations, two depths each). On the Chesapeake cruises, 9 DNA samples (15 replicates of each) were collected from three depths at each of three stations. A smaller number of replicates for RNA extraction were collected from the same set of stations. The DNA samples are always collected by the Princeton component and shipped by us to the other collaborators (California, New York and Virginia). Most of the samples are still being archived, but several analysis have been completed for a series April samples (see below).

Gene Microarray development:

The Princeton component has focused on characterizing the functional gene microarrays using glass slide technology. Hybridization sensitivity and resolution was optimized on a series of array designs with the goal of devising an array that can detect most versions of the target genes without cross reaction among probes. The criteria for optimal array performance were derived using the second generation microarray, which

was fabricated with 54 oligos derived from nitrite reductase (nirS) sequences obtained from a clone library from the Upper Choptank River plus 14 sequences from the published databases. The paper describing the results was published in AEM in January 2003 (Taroncher-Oldenburg et al.). Three sets of experiments comprise the essential results: 1) Pairwise comparisons with two labeled targets; 2) Multiple labeled targets in variable concentrations, 3) PCR amplified fragments from the field samples as unknown targets. The level of dissimilarity that allows two closely related sequences to be distinguished on the chips is 87 % (+ 3%). An additional parameter, the free energy of binding between probe and target, was also found to be predictive of hybridization behavior. Probe/target pairs with >56% mismatch/perfect match should hybridize. Using the competitive two color hybridization technique, we determined that a factor of two difference in concentration among multiple targets in complex mixtures can be detected. Signal was obtained from natural samples, and provided evidence in addition to the clone libraries (see below) that the denitrifier assemblages differ at different sites in the Choptank River.

Research is now underway on two new arrays. A chip containing a suite of amoA oligos has been constructed and we have completed initial experiments to verify whether it performs according to the criteria established using nirS. Aside from problems with target labeling efficiency, the amoA chip behaves comparably. The direct target labeling approach used until now has proven unreliable, and we have now initiated an indirect labeling method that achieves higher density of labeling and with similar efficiencies for Cy3 and Cy5. The new labeling method will be used in the next amoA experiments, which will use the competitive two color method to quantify amoA distribution and relative abundance along the Chesapeake Bay gradient. The USGS group has been an essential collaborator on this part of the project, supplying some of the sequences for the array.

While the competitive two color approach is the most widely used approach for array quantification, we have concluded that it is not optimal for field samples because there is no perfect control population. Therefore, we have instigated a new quantification method that uses internal standards on every probe dot, which is being optimized on the third generation nirS chip. That chip contains 40 nirS probes that were chosen using an algorithm developed by George Jackson (TAMU component). The algorithm selects the best subset of probes necessary to represent the entire database within the 87 % constraint. For our 623 sequences (the Chesapeake and Choptank sequences plus database sequences), the algorithm selected 111 probes, over half of which are predicted to hybridize with only one sequence in the database. The new array contains all the probes that are predicted to hybridize with 2 or more sequences. The arrays have been printed and hybridization experiments using the new labeling and quantification methods are underway.

Clone libraries and diversity:

Five clone libraries (from sediments at each of the Bay/River sampling stations) were sequenced for the nirS database that was used to design the third generation microarray (see above). Rarefaction curves for each library showed that none of them were saturated, but we decided to proceed with array experiments rather than sequence endlessly. The diversity data were interpreted in terms of measured fluxes (Cornwell, HPL) and a manuscript is in preparation (Francis et al., in prep). The nirS database is being used by George Jackson (TAMU) to explore the shape of the ôspeciesö abundance curve and for algorithm development (see above).

The same sediment samples were used to develop clone libraries for amoA. The diversity and environmental significance of the diversity distribution has been described in a manuscript (Francis et al., in press)

Database development:

The Princeton component took over management of the project webpage after the September 2001 meeting (http://geoweb.princeton.edu/research/biocomplexity/index.html). The TAMU component has set up a major website/database for the entire project and the other component groups are beginning to contribute data. The data from the published paper and all the sequences for the arrays are publicly available from the web page, as are data from most previous cruises.

Findings:

Described in Activities above

Training and Development:

The students and post docs are gaining experiment with cutting edge molecular biological techniques, from standard PCR and sequencing to microarray development and optimization. Several have also gained field experimence, working in shallow sediments and at sea. Erin Griner completed her senior thesis as part of the project and was a co - author on the firs publication about the microarray work.

Outreach Activities:

Journal Publications

Taroncher-Oldenburg, G, E. Griner, C. A. Francis and B. B. Ward., " Oligonucleotide microarray for the study of functional gene diversity of the nitrogen cycle in the environment", Applied and Environmental Microbiology, p. 1159, vol. 69, (2003). Published

Francis, C. A., G. D. OÆMullan and B. B. Ward., "Diversity of ammonia monooxygenase (amoA) genes across environmental gradients in Chesapeake Bay sediments", Geomicrobiology, p. , vol. 1, (2003). Accepted

Books or Other One-time Publications

Web/Internet Site

URL(s):

http://geoweb.princeton.edu/research/biocomplexity/index.html

Description:

This web site is maintained at Princeton University and describes the major components of the project. As our work is published, the site will be updated with our major findings.

Other Specific Products

Contributions

Contributions within Discipline:

While genome chips are becoming more common and are very powerful tools for both medical and environmental research, the gene chips that we are using are novel in that they contain many versions of the same gene, rather than a single version of each gene. Thus, the methods we are developing for sensitivity and specificity analysis face different and difficult challenges, but they will be applicable to other genes.

Contributions to Other Disciplines:

Contributions to Human Resource Development:

Post docs, graduate students and undergraduate students are involved in the research, and they will be trained and gain valuable laboratory experience thereby. They also learn to present their work in public forums, which prepares them for teaching and outreach in future careers.

Contributions to Resources for Research and Education:

Contributions Beyond Science and Engineering:

Special Requirements

Special reporting requirements: None

Change in Objectives or Scope: None

Unobligated funds: less than 20 percent of current funds

Animal, Human Subjects, Biohazards: None

Categories for which nothing is reported:

Activities and Findings: Any Outreach Activities

Any Book

Any Product

Contributions: To Any Other Disciplines

Contributions: To Any Resources for Research and Education

Contributions: To Any Beyond Science and Engineering