Welcome to the

5TH BI-OMP INVESTIGATOR’S
CONFERENCE
January 8 thru 9, 2004

Dear BI-OMP Workshop Participant:

Welcome to the 5th Biotechnology Investigations – Ocean Margin Program Conference sponsored by the US Department of Energy and hosted by the Industrial Biotechnology Program of the University of Puerto Rico at Mayagüez. For the past six years, our campus has been the location for the meeting of one of the BI-OMP team. Today, we are enthusiastic to share our tropical refuge with all BI-OMP workshop participants.

A tremendous amount of effort has been invested in preparing a program of great interest and one that will fulfill your expectations. Plenary activities, scientific sessions (oral and poster), and a moderated synthesis session to stimulate discussions and cooperation in biotechnology research are part of the goals to be met.

Aside from participation in the conference events, be sure to enjoy our beautiful island. Among our many cultural attractions, you can choose the surfing waves of the beaches of Rincon, the bioluminescent bay in Lajas or visit fine restaurants to delight in the local cuisine.

On behalf of our university administrators, personnel, faculty, students and our research team, Dr. James Tiedje (Michigan State University), Dr. Allan Devol (Washington University), and Jizhong Zhou (ORNL) it is a pleasure having you with us!

Cordially,

Arturo Massol, Ph.D.       Rosa Buxeda, Ph.D.
Program Chair            Program Co-Chair
Dear BIOMP researcher:

Welcome to the 2004 meeting of the U.S. Department of Energy’s Biotechnological Investigations-Ocean Margins Program! We are fortunate that the University of Puerto Rico-Mayaguez has graciously offered to host this year’s meeting. I am very grateful to Dr. Arturo Massol-Deya and his colleagues for organizing what promises to be a very exciting and important meeting.

BIOMP supports DOE’s mission in Climate Change Research. The program provides the fundamental understanding of the linkages between carbon and nitrogen cycles in ocean margins. Researchers are providing a mechanistic understanding of these cycles, using the tools of modern molecular biology. The data from the program will ultimately lead to improved conceptual and numerical models that will allow policy makers to determine safe levels of greenhouse gases for the Earth system.

BIOMP is also a partnership program dedicated to increasing the participation of under-represented groups in the scientific enterprise. By developing partnerships among universities, BIOMP has been highly successful in training a diverse group of young scientists to be the next generation of leaders in the field.

The annual Principal Investigator meetings are critical because they provide a forum for discussion of the latest research results in an open and informal setting. The meetings also serve an important role in enhancing existing collaborations and fostering new ones. I am confident that the 2004 meeting will generate the high level of scientific exchange and good fellowship that are a hallmark of the program.

Cordially,

Anna C. Palmisano, Ph.D.
BIOMP Program Manager
Climate Change Research Division
Biological and Environmental Research
U.S. Department of Energy
SPONSORS LIST
THURSDAY JANUARY 8, 2004

7:30 – 8:00 am   Transportation from the Hotel to UPRM

8:00 – 9:00 am   Registration & Continental Breakfast

9:00 – 9:45 am   Symposium Opening and Welcome:
   Dr. Rosa Buxeda, Industrial Biotechnology Program, UPR-M
   Dr. Arturo Massol, Department of Biology, UPR-M
   Dr. Jorge I. Vélez-Arocho, Chancellor, UPR-M
   Dr. Moisés Orengo Avilés, Dean, College of Arts & Sciences, UPR-M
   Dr. Fernando Bird-Picó, Director, R & D Center, UPR-M
   Dr. Lucy Williams, Director, Department of Biology, UPR-M
   Dr. Anna Palmisano, BI-OMP Program Director, DOE
   Dr. Jerry Elwood, Director of Climate Change Research, DOE

9:45 – 10:00 am  Break

10:00 – 11:00 am Opening Plenary Session
   Program Moderator: Dr. Lorenzo Saliceti-Piazza
   Dr. Frank Larimer, Keynote Speaker
   Group Leader, Genome Analysis and Systems Modeling
   Deputy Director, Center for Molecular and Cellular Systems
   Life Sciences Division, Oak Ridge National Laboratory

Oral Session I Program and Partnership Presentation (two presentations per project: one on research progress and one on partnering – for each time slot)

11:00 – 12:00 pm J.M. Tiedje, , J Zhou, A. Massol

12:00 – 1:00 pm  Lunch

1:00 – 5:20 pm   Oral Science Session I
   Program Moderator: Dr. Arturo Massol and Jizhong Zhou

1:00 – 2:00 pm   E. V. Armbrust, J. Cherrier, R. G. Keil

2:00 – 3:00 pm   J.H. Paul, A. Smith, F.R. Tabita

3:00 - 3:20 pm   Break
THURSDAY JANUARY 8, 2004

3:20 – 4:20 pm  L. Kerkhof, J. Corredor, G. Taghon, S. Seitzinger

4:20 – 5:20 pm  M. Frischer, Verity, Gilligan, Booth, Bronk

5:30 pm  Departure to Parguera

6:20 pm  Tour to the Marine UPRM Field Station, dinner, and tour to the Bioluminescence Bay at Parguera, Lajas PR (included)

9:00 pm  Transportation to the Hotel

FRIDAY, JANUARY 9, 2004

7:30 – 8:00 am  Transportation from the Hotel to UPRM

8:00 – 8:30 am  Continental Breakfast

8:30 – 10:15 am  Poster Science Session

Program Moderator:  Dr. James Tiedje & Veronica Gruntzig
Oral Session II – Program and Partnership Presentation

10:30 – 11:30 am  Wilkerson F.P, Takabayashi, M. Zehr J.P.

11:30 – 12:30 pm  Malmstrom R.R., Cottrell M., Kirchman D.L.

12:30 – 2:00 pm  Lunch

2:00 – 4:00 pm  Panel discussion

4:00 – 4:15 pm  Closure

4:15 pm  Transportation to the Hotel

BIOMP IS IN THE DOE CLIMATE CHANGE RESEARCH DIVISION. THE LONG TERM GOAL OF DIVISIONAL RESEARCH IS TO IMPROVE THE ABILITY TO SIMULATE AND ASSESS CLIMATE CHANGE. THE OCEAN CARBON CYCLE IS AN IMPORTANT TO THIS EFFORT BECAUSE OF ATMOSPHERIC CARBON DIOXIDE FORCING.

A KEY QUESTION IS: HOW CAN WE USE RESULTS FROM BIOMP TO IMPROVE OUR CONCEPTUAL AND NUMERICAL MODELS OF THE OCEAN CARBON CYCLE? ONE MEMBER FROM EACH BIOMP TEAM WILL SERVE AS A PANELIST TO LEAD THE GROUP DISCUSSION.
Dr. Frank Larimer
Keynote Speaker

Group Leader, Genome Analysis and Systems Modeling
Deputy Director, Center for Molecular and Cellular Systems
Life Sciences Division, Oak Ridge National Laboratory
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Diatoms are the most common component of phytoplankton blooms in coastal waters and are responsible for as much as 75% of the annual primary productivity in these environments. Because of their dominance under nitrate-rich conditions and their high sinking rates, carbon models commonly assume that diatom production is equal to the new production that can be exported from surface waters. And yet, it is well known that under different environmental conditions, diatoms can and do release up to 50% of their fixed carbon and nitrogen, which is then available for microbial recycling within the euphotic zone. Diatom photorespiration is a physiological response to environmental conditions commonly associated with blooms (e.g., high light) and is known to result in the release of organic compounds into seawater. The balance between diatom photosynthesis (carbon fixation) and photorespiration (carbon/nitrogen release) will influence the amount of fixed organic matter exported from the upper ocean and will impact the efficiency of the biological pump that draws CO2 from the atmosphere to deeper waters. The goal of our BIOMP collaboration is to understand the interplay between environmental conditions, diatom photorespiration-induced release of organic compounds, and the utilization of these compounds by bacteria. We monitor the impact of the environment on diatom photorespiration in two ways - by following transcription of key photorespiration genes and by determining the suite of organic compounds released under a given set of conditions. The focus thus far has been the consequences of changes in light intensity. We have found that the photorespiration-associated gene that encodes glycine decarboxylase (gdc) is regulated in a light intensity-dependent manner consistent with the premise that the extent of photorespiration within a cell is also light dependent. Cells shifted to higher light intensities release a significant amount of dissolved organic carbon and nitrogen. Moreover, the dissolved organic nitrogen compounds are released in an amount directly proportional to inorganic NO3 uptake. The photorespiration-specific compound, glycolate, is released only when cells are shifted between the most extreme light conditions suggesting that release of this compound is tightly regulated. We recently expanded the environmental conditions examined to include temperature, nitrogen concentration and nitrogen source in addition to light intensity. We have also increased the number of genes monitored to include pathways that interact with photorespiration such as nitrogen metabolism and carbon fixation, as well as additional genes required for photorespiration. The response of bacteria to diatom-derived compounds is also determined in two ways. First, we measure bacterial uptake of specific DOM constituents, as well as bulk low and high molecular weight organic matter, that are released by diatoms under different environmental conditions. The response of bacterial communities to released DOM has important implications for the flow of carbon in coastal systems. Second, we use a molecular approach that focuses on bacterial utilization of glycolate since we know that in the ocean, this compound is produced specifically by phytoplankton. Our molecular target is the gene that encodes glycolate oxidase since it is required for glycolate utilization. Regardless of whether laboratory isolates or environmental clone libraries are examined, we find that only certain bacteria within a given clade possess this gene indicating that only a subset of bacteria can utilize glycolate. We have compared overall bacterial diversity (16s rDNA) with the diversity of bacteria that can utilize glycolate (glcD) in both oligotrophic and eutrophic environments. We find that glcD diversity varies more dramatically between different eutrophic sites than between different oligotrophic sites suggesting that glycolate and thus diatom respiration may play a role in structuring bacterial communities.
REGULATION OF CARBON FIXATION BY NITROGEN IN COASTAL PLUME ENVIRONMENTS

Paul, J.H.¹, Murugesan, R.², Wawrik, B.¹, Smith, A.³, Tabita, F.R.², Smith, T.E.³ and John, D.¹

¹University of South Florida, ²The Ohio State University, and ³Howard University

Understanding the fate of anthropogenic CO₂ in the oceans is a key mission for the US Department of Energy. The Mississippi River Plume may play a vital role in carbon fixation in the Gulf of Mexico and the North American Carbon Budget. Nitrate and phosphate-rich, fresh water from anthropogenic activities floats on the oligotrophic Gulf of Mexico water, in intimate contact with atmospheric CO₂. The overall goal of the proposed research is to understand the control of CO₂ fixation by nitrogen in picoplankton in culture and in our model macronutrient enrichment laboratory, the Mississippi River Plume.

The following progress has been made on this project:

1. Although the Mississippi River Plume only accounted for 2.75% of the surface area of the oligotrophic Gulf of Mexico, carbon fixation by this plume was estimated to be 41% and 13% of the surface and total water column productivity, respectively, in the Gulf of Mexico in July 2001. Community genetic analysis indicated that the plume phytoplankton community could be divided between coastal and far offshore diatom communities and an intermediate Synechococcus-dominated community.

²¹N uptake studies indicated that recycled ammonium was the major source of nitrogen for phytoplankton growth, even though nitrate concentrations greatly exceeded ammonium concentrations at all stations. 2) A bacterial artificial chromosome (BAC) library containing 3200 clones made from DNA obtained from the Prochlorococcus-dominated depth from the Gulf of Mexico in July 2001 yielded 10 clones hybridizing to the Form IA rbcL gene probe. These are being further characterized and hopefully sequenced with assistance from DOE. 3) We are studying the influence of nutrient stress, namely nitrogen and iron levels, on rbcL expression in marine picocyanobacteria. This has led to the finding that the transcriptional regulators NtcA and GlnB have varying abilities to influence rbcL transcription, and this is dependent on the nitrogen status of the cells. 4. Finally we have discovered that a short anti-sense ntcA mRNA is transcribed from the ntcA gene in response to differing nitrogen regimes in Synechococcus WH7803 and related strains. The anti-sense transcript of ntcA was found to be ca. 380 nucleotides long and was maximally transcribed in the presence of elevated levels of ammonia. The regulation of this transcript is opposite that of the sense transcript, which is repressed by combined nitrogen. This antisense RNA may play an important role in the regulation of nitrogen metabolism in marine picocyanobacteria. Partnership activities continue with Howard University. Four manuscripts have been submitted for publication describing these results during this annual period, with others in draft form.

L. Kerkhof, J. Corredor, G. Taghon, S. Seitzinger

This BIOMP project initially focused on measuring nitrous oxide gene expression and half-life in marine sediments. The research was predicated on obtaining intact nosZ mRNA from natural samples and has proven more problematic than anticipated. Efforts have been re-directed toward completing the characterization of samples obtained during the GRIST experiment. Our objective was to identify active microorganisms in water column and/or sediment samples using a combination of intact ribosome fingerprinting and bromo-deoxyuridine (BrDU) incorporation into newly-synthesized DNA. For water column samples, there was good agreement between results from both rRNA and BrDU fingerprints. Amplification of 16S genes by RT-PCR identified members of the Proteobacteria, the Planctomyces, the CFB group, the Gram positive, and the Cyanobacterial groups. The 18S rRNA gene analysis by RT-PCR was consistent with measurements of expressed RUBISCO genes with members of the Bacillariophyceae, the Dictyochophyceae, the Cryptophyceae, and the Haptophyceae being detected. Additionally, 18S genes from Flagellates, Ciliates, and zooplankton could be characterized from intact rRNA collected during the experiment. Finally, incubations with sediment slurries and intact sediments in experimental flumes demonstrated the ability of sand associated bacteria to take up BrDU in short incubations. Differences between the active populations in slurries and intact sediments were readily apparent. This
research will aid in elucidating the linkages between the structure and function of the active members of the microbial community and carbon and nitrogen cycling in coastal environments.

**MOLECULAR APPROACHES FOR IN SITU STUDIES OF NITRATE UTILIZATION BY MARINE BACTERIA: PROGRAM SUMMARY**

Marc E. Frischer

Skidaway Institute of Oceanography, Savannah, Georgia

Traditionally, the importance of inorganic nitrogen (N) for the nutrition and growth of marine phytoplankton has been recognized, while inorganic N utilization by bacteria has historically received less attention. However, accumulating evidence suggests that bacteria compete with phytoplankton for nitrate (NO$_3^-$) and that heterotrophic bacteria may have a profound effect on the flux of N, and therefore carbon (C), in ocean margins. Although it is technically difficult to differentiate between bacterial and phytoplankton N uptake using conventional biogeochemical techniques, the application of molecular gene based approaches to this problem is yielding important new insights. Understanding the controls and rates of NO$_3^-$ assimilation by ocean bacteria is critical because assimilation of N by bacteria that does not result in new phytoplankton growth and CO$_2$ uptake, represent mechanisms that may increase atmospheric CO$_2$ levels and enhance global warming.

Since 1997, with the support of the DOE Biotechnology Investigations – Ocean Margins Program, we have developed molecular tools (PCR and RT-PCR primer sets) that allow us to selectively isolate, characterize, and study the diversity and genetic expression (mRNA) of the structural gene responsible for the assimilation of NO$_3^-$ by heterotrophic bacteria (nasA). To date, our studies have revealed that bacteria capable of assimilating NO$_3^-$ are ubiquitous in marine waters and that the expression of nasA can be regulated by a variety of different variables including the concentration of NH$_4^+$, NO$_3^-$, and the quality of available dissolved organic matter.

Continuation of this project through 2007 will focus on the development of molecular tools for studying the role of heterotrophic bacterial utilization of NO$_3^-$ and the use of these tools in conjunction with new molecular methods (T-RFLP and quantitative PCR) to investigate the factors regulating bacterial NO$_3^-$ utilization in diverse ocean margin environments. New components of this project include the development of flow cytometer based methodologies that will provide the ability to sort individual cells with the identification of their ability to incorporate NO$_3^-$, new molecular methods to study urea uptake, and the implementation of an existing Ocean Carbon Model to explore the global significance of NO$_3^-$ uptake by marine bacteria. Completion of this study will provide new molecular methods suitable for use in the field to address questions relative to the utilization of NO$_3^-$ by bacteria and the underlying mechanisms that control NO$_3^-$ flux into bacteria.

In addition, this project will expand the relationship between the Skidaway Institute of Oceanography (SkIO) and Savannah State University (SSU) and build new research/education partnerships between the SkIO and a second teaching institution, Roanoke College. The project also depends on active collaboration with the Virginia Institute of Marine Sciences (VIMS) and the University of Georgia (UGA). Specifically, this project will vigorously support the undergraduate marine science bachelors program and the implementation of a new graduate program in marine science at Savannah State University. The project will provide support for up to 10 undergraduate and 4 graduate student research assistantships. There is no doubt that if this program is successful, it will serve as an important and sustainable mechanism to support future research in marine biotechnology and to increase the number of under represented scientists, particularly African Americans, in the field of marine sciences.
We have been investigating the molecular and biogeochemical bases for coupling between carbon and nitrogen cycles within phytoplankton communities found in coastal upwelling and the GRIST (LEO site) ecosystems for our BiOMP project funded by the Department of Energy. Our trilateral BiOMP partnership has involved researchers at all levels (three PIs, four postdocs and two MSc graduate students) from San Francisco State University, the University of California at Santa Cruz and Princeton University. It also supported summer internships for two undergraduate students at Romberg Tiburon Center, SFSU.

The coupling between nitrogen and carbon assimilation by phytoplankton in ocean margin ecosystems helps determine the carbon export that can result via the biological pump. Our approaches for studying the functional genes involved in N assimilation by phytoplankton are two-fold; investigation of nucleotide sequence diversity within and among target taxonomic components of phytoplankton communities; and quantification of such gene expression and regulation in these target taxa. We have been investigating the genes of key N assimilation enzymes; nitrate and ammonium transporters (Nat and Amt), assimilatory nitrate reductase (NR), and glutamine synthetase (GS). Nat and Amt transport nitrate and NH$_4^+$ from the environment to the cytoplasm where nitrate is reduced to NH$_4^+$ by the rate-limiting enzyme NR. Glutamine synthetase, GS, then assimilates the NH$_4^+$, for incorporation into amino acids via glutamate.

Universal eukaryotic and group specific PCR primers for nar and hnat (high affinity nat) genes in diatoms and green algae have been used to characterize the phytoplankton communities in Monterey Bay and New Jersey (the GRIST experiment at the LEO site); see poster by Allen et al. The gene responsible for GS in cyanobacteria, glnA, as well as narB have been isolated and characterized from cultures and environmental DNA samples from the Pacific Ocean, Monterey Bay, and the Chesapeake Bay. Four groups of glnA sequences were obtained after amplification by RT-PCR from total RNA extracted from the GRIST experiment at the LEO site during a diel cycle. Sequence diversity for glnA differed between sampling times, with the greatest diversity at 16:00 and the lowest diversity (primarily one sequence type) at 7:04. Phylogenies based on glnA and narB sequences resolve Synechococcus, Synechocystis, filamentous and heterocystous cyanobacteria group collected from these environmental samples into clusters of the corresponding types of cyanobacteria from the databases. In contrast to cyanobacteria, diatoms have at least two, if not three isoforms of GS presumably due to their history of endosymbiotic events. Genome sequencing project of a diatom, Thalassiosira pseudonana, at Joint Genome Institute revealed that this diatom has genes for GSI, GS II and GSIII isoforms. Our analysis of total RNA extracted from T. pseudonana grown in N-enriched media showed that apparently functional polyadenylated transcripts for all three gln types are produced in this diatom species. Reverse transcription quantitative PCR (RT-QPCR) assays have been designed to measure transcription of GSI (glnA), II (glnII), and III (glnN) genes for T. pseudonana and glnII for two other diatoms, Skeletonema costatum and Chaetoceros debilis. This method was used to monitor the differential gene expression of GS isoforms in diatoms grown in different N regimes; see poster by Barada et al. Our preliminary results indicate that glnII is induced by external N whereas glnN (GSIII) is constitutively expressed. Further experiments are underway to quantify gene expression of GS and RuBisCO (the primary enzyme of the Calvin cycle) in other diatom species grown under different N and light regimes to study the coupling between C and N cycles at the gene and enzyme levels.
CONTRIBUTION OF SAR11 BACTERIA TO C AND N CYCLING IN THE NORTH ATLANTIC OCEAN

Rex R. Malmstrom¹, Matthew T. Cottrell¹, and David L. Kirchman¹
College of Marine Studies, University of Delaware, Lewes, DE 19958

Current models of C and N cycles are very simplistic in depicting the interactions between microbes and the largest pool of organic C and N in the oceans, dissolved organic material (DOM). Bacteria are usually represented as a single compartment, even though we know that marine bacterial communities are incredibly diverse. The general goal of this project is to determine which DOM components are used by the dominant bacterial groups found in marine environments. The main approach we use to examine this problem combines identification of bacteria with fluorescence in situ hybridization (FISH) and detection of DOM uptake by microautoradiography (Micro-FISH). The Micro-FISH approach was used to examine the uptake of DOM by one of the most abundant bacterial groups in the ocean, SAR11. We found that the abundance of bacteria in the SAR11 clade varied from nearly undetectable levels to as much as 40% of total prokaryotic abundance. The average cell size of SAR11 bacteria was as big or bigger than the average prokaryote in the Gulf of Maine, Sargasso Sea and Atlantic coastal waters. Furthermore, these bacteria appear to be highly active in DOM uptake, since more than half of SAR11 assimilated free amino acids and phytoplankton osmolyte dimethylsulfoniopropionate (DMSP). Because of this activity and their high abundance, SAR11 bacteria accounted for about 50% of dissolved amino acid and 33% of dissolved DMSP assimilation in the surface waters we sampled. These data indicate that the SAR11 clade is highly active and plays a significant role in C and N cycling in the ocean.
MOLECULAR REGULATION OF CARBON FIXATION BY NITROGEN IN SYNECHOCOCUS sp. STRAIN PCC 7002

Rajaram Murugesan and F.R.Tabita  (From the Paulsmith/Tabita Consortium)  
Department of Microbiology, The Ohio State University, Columbus, OH 43210

Previous studies indicated that nitrogen-depleted culture of Synechococcus sp. strain WH7803 and Synechoccus sp. strain PCC 7002 retained considerable metabolic potential even after fairly long periods of starvation. Strain WH7803 is a phycoerythrin-containing organism found in temperate coastal waters and strain PCC7002, originally isolated from Puerto Rican waters, is representative of marine cyanobacteria found in warmer coastal and brackish waters. We have initiated studies to probe the molecular basis for the ability of marine cyanobacteria to maintain their metabolic potential in the face of nutrient shortages. These fluctuations in ambient nitrogen levels are typical of the marine environment with the laboratory studies performed in conjunction with field studies. Because strain 7002 is well described biochemically and physiologically, has the fastest doubling time of any known cyanobacterium, possesses a genome that is now sequenced, and is an organism that has the best developed genetic system of arguably any cyanobacterium, and certainly any marine cyanobacterium, we have focused on strain 7002 for these studies, with the thought that these findings might then be applied to other environmentally significant marine cyanobacteria.

Current studies indicate that the product of the ntcA gene (NtcA) has an indirect effect on carbon assimilation and the genes involved in the carbon concentrating mechanism (ccm operon) of strain 7002. There is an NtcA binding site upstream from the ccmM gene. The product of the ccmM gene plays an important role in carboxysome assembly and inorganic carbon transport within the cell. Carboxysomes are proteinaceous polyhedral bodies that contain most of the intracellular Rubisco of cyanobacteria. Because of the location of this NtcA binding site, we hypothesized that under nitrogen limiting conditions the transcriptional regulator NtcA binds at the region upstream of ccmM, near the transcription start site, and blocks the transcription of ccmM. This hypothesis was experimentally proven by Electrophoretic Mobility Shift (EMS) assays and Northern blot analysis. To do these experiments, we first had to purify the NtcA protein. Thus, the Synechococcus PCC 7002 ntcA gene was amplified and cloned and then placed under the control of a phage T7 promoter in expression vector pET28a; this plasmid was used to transform Escherichia coli strain BL21 so that high levels of recombinant strain 7002 NtcA protein were synthesized. The recombinant NtcA protein was then purified. This purified preparation was subsequently used for EMS experiments. A probe containing the NtcA binding site (GTA-N8-TAC) upstream from the ccmM gene of strain 7002 was then amplified and labeled with [32P]-CTP. NtcA binding activity was detected as a single shifted band and was sensitive to both boiling and proteinase K treatment. No binding activity was detected in binding assay mixtures lacking the NtcA protein or when assays were performed with an equivalent amount of extract from E.coli BL21 containing parent vector pET28a lacking the ntcA insert. Further controls indicated a specific interaction; competition studies with a 100-fold excess of unlabeled probe DNA resulted in loss of the shifted signal while additions of a 200-fold excess of non specific DNA (salmon sperm DNA) had no effect. With respect to nitrogen metabolism, very low binding efficiency was noted in assay mixtures containing 10 mM glutamine or 10 mM ammonia, suggesting that high intracellular nitrogen reserves have an effect on NtcA-mediated control of cccM gene expression in vivo. Thus, experiments were subsequently designed to address this issue directly. It was found that dramatic differences in ccmM gene expression resulted when cells were incubated in nitrogen limiting medium compared to nitrogen replete growth medium; e.g., high levels were found in media containing excess nitrate or ammonium. There was no difference in rbcL transcript levels under either nitrogen excess or limitation after 24 h incubation. Previously, we showed that expression of the signal transducer glnB was also affected by nitrogen nutrition. In the current study, we studied the effect of carbon on glnB expression. GlnB transcript levels
were 57% higher when cells were incubated in the presence of ammonia and aerated with low CO$_2$ (air levels) compared with high CO$_2$ (2% CO$_2$ in air). Under conditions of nitrogen limitation and at low CO$_2$ concentrations a more significant differences in $glnB$ transcription was noted. Since the ntcA levels remained constant while the $glnB$ transcript levels showed dramatic fluctuations at different levels of carbon, it did not appear that $glnB$ transcription was regulated by ntcA transcript levels as in fresh water cyanobacteria. Rather it appears that $glnB$ expression is under control of the relative levels of nitrogen and carbon found within the cells; there also is no NtcA binding site upstream from $glnB$ in strain 7002. There are, however, NtcA binding sites in upstream regions of genes that appear to be important for transport of nitrogen into the cell; i.e., the nitrate reductase (narB) and nitrate permease (nrtP) genes. Thus, we hypothesize that NtcA may affect expression of narB and nrtP genes under nitrogen limiting conditions. Certainly, NtcA has a distinct effect on $ccmM$ transcription. It would appear then that NtcA regulates nitrogen and carbon metabolism indirectly by regulating genes involved in nitrogen and carbon transport.

**ROLE OF PHOSPHOENOLPYRUVATE CARBOXYLASE IN THE REGULATION OF CARBON FIXATION BY NITROGEN IN COASTAL PLUME ENVIRONMENTS**

Thomas E. Smith, Marguerite W. Coomes, and Aubrey Smith

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Included among the goals of the Biotechnological Investigations – Ocean Margins Program (BI-OMP) are: (1) to make a determination of “the mechanisms and processes that control the dynamics of nitrogen fixation or denitrification in coastal waters... [and a definition of] the coupling and/or decoupling of carbon and nitrogen cycles in coastal environments...” and (2) to develop a collaborative partnership between institutions with a strong tradition of research in marine science and an institution that has traditionally served groups underrepresented in the sciences. The collaborative interactions with John Paul’s group at the University of South Florida and my laboratory group at Howard University serve that function and Aubrey Smith’s progress towards fulfillment of requirements for the Ph.D. degree suggest that research interest in this area of DOE’s concern might be advanced.

BI-OMP emphasized the importance of the use of modern molecular biological tools to examine genes and gene expression to investigate the mechanisms by which carbon and nitrogen cycles might possibly interact with each other and be co-regulated. Specific enzymes mentioned (and by inference genes that code for them) included ribulose bisphosphate carboxylase (RubisCO), nitrogenase, and nitrate reductase. Phosphoenolpyruvate carboxylase (PEPCase) is another enzyme involved in CO$_2$ fixation. It is essentially ubiquitous in prokaryotic cells where it serves an anaplerotic role. In plants and in some photosynthetic bacteria, it might also function as a source of photosynthetic carbon fixation. A product of PEPCase action is a C-4 dicarboxylic $\alpha$-keto acid that has direct access to nitrogen fixation through transaminase reactions. In addition, its product feeds directly into the tricarboxylic acid (TCA) cycle that leads to production of $\alpha$-ketoglutarate, a precursor of glutamate and glutamine. PEPCase of some photosynthetic organisms exists in more than one isoform and in some cases, the activity of the enzyme is regulated by the nitrogen-containing amino acids mentioned above and also by three-carbon compounds that could be derived from products of RubisCO. In other cases, mostly plants, enhanced synthesis of PEPCase occurs as a function of inorganic phosphate availability and its catalytic activity is regulated by covalent modification. Thus, many mechanisms exist for the interaction of PEPCase in both carbon and nitrogen metabolism, not all of which involve new synthesis of the enzyme. Therefore, defining the role of PEPCase may not be possible by looking only at changes in cellular levels of the mRNA responsible for its synthesis. This discussion will focus on regulatory properties of PEPCases and their possible implication in the mechanism of interactions between carbon and nitrogen metabolism. Since little is known about the regulatory mechanisms of the PEPCase of *Synechococcus*, literature data about this enzyme from other sources will be used. The gene for PEPCase of *Synechococcus* PCC 7002 has been isolated and cloned recently in our laboratory. Once
the enzyme has been expressed and purified, regulatory properties will be evaluated in the context of determining the role this enzyme may play in bridging the gap between carbon and nitrogen metabolism in Synechococcus and possibly other marine organisms.

**ISOLATION AND MOLECULAR CHARACTERIZATION OF DENITRIFYING BACTERIA FROM COASTAL SEDIMENTS OF THE PACIFIC NORTHWEST**

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Denitrifying bacteria play a major role in marine sediment nitrogen balance. Slow-growth enrichment microcosms, clone libraries and in situ Reverse Transcription PCR (RT-PCR) for the nirS gene were used to characterize denitrifying bacteria from marine sediments. The enrichment microcosms were amended with nitrate and DMSO as the sole added carbon source. Two sets of microcosms were prepared in this manner and incubated at 24°C and 4°C in the dark for more than 6 months. After gas production was observed, samples were drawn, serial diluted in a seawater buffer, and plated in nitrate agar (pH 7.6). Approximately 80 strains were isolated and subsequently inoculated in nitrate broth. An inverted Durham tube was used to test for nitrate reduction and gas production. Out of the 80 isolates, 22 were positive for both nitrate reduction and gas production. Genomic DNA was extracted from each Puget Sound denitrifying culture and from six well-characterized denitrifying strain provided by the Center for Microbial Ecology (Michigan State University). ARDRA and 16S rDNA sequence analysis were performed to compare and establish similarities between the Puget Sound and control cultures. Cluster analysis showed differences of microcosm isolates from the reference strains. The isolated cultures at 25°C and 4°C grouped in different clusters. The partial rDNA gene sequence of representative denitrifying isolates was obtained. A phylogenetic analysis was performed by using the PHYLIP® interface program of the Ribosomal Database Project II (RDP II). Results were consistent with ARDRA, even though three of the isolates were more closely related to P. stutzeri (I-60, I-62A and I-80) than previously thought. Most of the isolates were considerably different from any of the reference strains, as 5 of 10 sequences were identified as previously uncultured bacteria. In addition, in situ reverse transcription PCR (RT-PCR) method has been optimized to study the expression of the nirS gene in denitrifying bacteria. Pure cultures of reference denitrifying isolates from marine sediments were used to optimize the in situ RT-PCR protocol. We performed cell fixation after visible gas production was observed. In situ RT-PCR was performed after cell fixation and enzymatic permeabilization. The nirS 1F and nirS 6R primers were used for the amplification of cDNA and subsequently fluorescent in situ hybridization (FISH) was done to increase the detection specificity of the amplification product. Only active denitrifying cells were detected by this approach using fluorescence microscopy.

**SIZE-FRACTIONATED NITROGEN UPTAKE IN NORWEGIAN MESOCOSM EXPERIMENTS**

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Three mesocosms (11 m²) were set up in fjord waters offshore from the Marine Biological Field Station in Bergen, Norway. Two mesocosms received inorganic nutrients (16 M nitrate, 1 M phosphate) initially with 10% of those concentrations added daily thereafter. A third mesocosm that did not receive nutrient additions was used as a control. Ambient nutrient and chlorophyll concentrations and inorganic (ammonium and nitrate) and organic (urea and amino acids) uptake rates were measured over the course of 31 days. During the first 12 days, chlorophyll concentrations dropped rapidly. During the first 20 days of nutrient-amended mesocosms the chlorophyll concentrations continued to increase (5–30ug/l) due to a bloom of Phaeocystis. During the first 5 days, ammonium concentrations dropped rapidly. During the first 20 days concentrations of nitrate and
nitrite steadily declined to below detection limits. In contrast, dissolved organic nitrogen concentrations remained constant in all mesocosms during the first 20 days but then increased; the increase was greater in the mesocosms that received nutrient additions. At the peak of the bloom, net uptake rates of nitrate were greater than ammonium in the greater than 8 m fraction but there was little difference in the 0.2 – 0.8 m size class.

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Recently we conducted a simulated upwelling study to investigate the effect of inorganic nutrient fertilization on bacterial, phytoplankton, and protist activity and food web structure in a coastal Norwegian fjord system. These studies were conducted at the Bergen National Mesocosm Facility operated by the University of Bergen from February 28 thru April 1, 2003 in floating, circulating, 11 m³ mesocosms. As a component of this study we investigated the response of the heterotrophic bacterial community capable of assimilating nitrate by characterizing diversity of the heterotrophic bacteria-specific assimilatory nitrate reductase (nasA). Fertilization of mesocosms with NO₃⁻ (16 µM daily) and PO₄³⁻ (1 µM daily) induced a bloom of the haptophytic algae Phaeocystis pouchiti over the 32 day study period. DNA was extracted from water samples (0.2-0.8 m fraction) collected every third day from two fertilized mesocosms (16 M NO₃⁻ and 1 M PO₄³⁻) and from one receiving no nutrients. The diversity of nasA genes was determined by terminal restriction length polymorphism (T-RFLP) analysis using a previously developed nested PCR primer set. For a given sample, fluorescently-labeled nasA PCR product was digested separately with HhaI, MboI, and RsaI (three replicates each) and separated using an automated sequencer. Based on differences in T-RFLP profile patterns, nasA amplicons from selected samples were cloned and sequenced. Sequence analysis revealed that nasA types present over the course of the study encompassed three phylogenetically distinct clades, each of which could be clearly differentiated by digestion with the restriction enzyme MboI. For a given sample, the relative distribution of the clades within a library was similar to their proportion in a T-RFLP profile. Changes in the relative proportion of the three nasA clades occurred over the course of the P. pouchiti bloom. It will be determined whether a correlation exists between shifts in nasA clade distribution and the myriad of environmental parameters concurrently measured including NO₃⁻ uptake rates, NO₃⁻ concentration, and bacterial productivity. Data gathered from this study provides the foundation necessary for the design of Q-PCR primers to determine how nasA transcript levels change during the development of the bloom.

INORGANIC AND ORGANIC NITROGEN UTILIZATION OVER TWO DIEL PERIODS AT LEO-15

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The Geochemical Rate/RNA Integration Study (GRIST) sought to correlate important biogeochemical flux rates with measurements of gene expression and mRNA abundance to demonstrate the application of molecular approaches to assess the presence and magnitude of a suite of biogeochemical processes. In this component of the GRIST study, we characterized ambient nutrient concentrations and measured uptake rates for dissolved inorganic nitrogen (DIN: ammonium, nitrate and nitrite) and dissolved organic nitrogen (DON: urea and dissolved free amino acids) at the Long-Term Ecosystem Observatory (LEO-15) on the New Jersey continental shelf. During two diel sampling periods, concentrations and rates were measured every three to five hours in both surface and bottom waters using wet chemical and ¹⁵N tracer techniques, respectively.
Results indicate a highly stratified water column with large differences in nutrient availability between surface and bottom waters. Furthermore, preliminary data indicate that concentration and uptake trends were similar for both diel periods; DON contributed from 75-90% of the total nitrogen uptake in the surface water, whereas DIN comprised approximately 70% of the uptake in the bottom water. Temporal trends over the two diel periods suggest that DON uptake as a percentage of the total nitrogen utilized increased with time during each diel, while DIN uptake was highest during the morning hours. These results indicate that the plankton community on the continental shelf relies heavily on DON, particularly urea, to meet its nutritional needs.

Testing the functionality of novel nirs genes from marine sediments

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Molecular techniques have led to the retrieval from nature of an enormous number of novel gene sequences which might play an important role in the chemistry of the biosphere. However, the functionality of these sequences has generally not been tested and therefore their in situ function remains questionable. This is also true for denitrification genes, like the nirS gene coding for the heme cd1 nitrite reductase. In previous studies, three clusters of nirS clones which did not include any nirS from a cultured denitrifier were detected in sediment samples from the Pacific Northwest (Braker et al., 2000, AEM 66: 2096-2104). These novel sequences could correspond to novel denitrifiers, however their functionality is unknown. We are therefore applying a complementation assay in order to test the ability of these sequences to restore the denitrification pathway in a NirS- mutant. The previously retrieved nirS clones represent only part of the nirS gene sequence. To obtain a complete sequence, total genomic DNA was extracted from Puget Sound sediment samples and partially digested with restriction enzymes. Fragments twice the size of the nirS gene were cloned into the broad host range vector pSUP104. The mutant strain Pseudomonas stutzeri MK202, which lacks the heme cd1 nitrite reductase, was used to test the ability of the cloned fragments to complement the defect in nitrite reduction. Although individual steps were successful, the length of the protocol with loss of genetic material in each step prevented the recovery of a complemented mutant. Therefore, reverse PCR with specific nirS primers is being applied to first retrieve a complete novel nirS gene from environmental samples, which can then be used to test its ability to complement the NirS- mutant.

A pipeline/repository for denitrification sequences.

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Although only a limited number of complete denitrification gene sequences from cultured denitrifiers are publicly available, the number of environmental sequences retrieved through cloning experiments is constantly increasing. These environmental sequences often form clusters independent from any cultured denitrifier and therefore represent interesting targets to be further studied. We will be analyzing denitrification pathway gene sequences, both to understand their natural relationships, and to design specific primers and probes to the various denitrifying gene clusters. To help with analysis, we are designing a functional gene pipeline and repository. This pipeline/repository will help us rapidly search for gene sequences of interest from the public databases and to use these sequences for primer/probe development. In addition to sequence data, the repository will maintain annotation data and allow the user to organize the sequences by taxonomy, environmental source, and reference information.

We have constructed a demonstration harvesting and analysis pipeline. This pipeline starts with a small number of known sequences for a given gene. These sequences are translated, and the resulting protein sequences aligned with clustalW (Chenna et al., 2003). These aligned sequences are then used to train a hidden Markov model (HMM) (Eddy, 1998) and the model used to search the NCBI non-redundant (NR) protein library. Additional models for common motifs in the target gene, for example cytochrome C and heme D motifs in the nirS gene, are also used to search the NR database and the results from all searches collated and sorted by HMM score.
cutoff score for the gene of interest is determined by inspection, the high-scoring protein sequences are aligned to the HMM. The source gene (DNA) sequences are then obtained and used to 'back translate' the protein alignment to obtain the DNA alignment. This pipeline has been tested for the nirS gene, among others. We are now designing a repository to store these data, and tools to allow easy selection of results by organism name, environmental source, and published reference. These tools will link to analysis tools such as PHYLIP (1993) and to primer/probe design software.


**The Importance of Photorespiration to Carbon and Nitrogen Metabolism in Diatoms**

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Our BIOMP collaboration seeks to understand the role photorespiration plays in the cycling of carbon and nitrogen between diatoms and bacteria in the upper ocean. Diatoms are commonly associated with export production, mediating the flux of carbon and nitrogen out of the mixed layer. However, when diatoms photorespire they produce several organic compounds that may be leaked to the surrounding seawater and utilized by marine bacteria, providing a mechanism for remineralization. Therefore, understanding the environmental factors that elicit photorespiration in diatoms is central to our research goals. We have developed a molecular marker for assessing the occurrence of photorespiration in the centric diatom *Thalassiosira weissflogii*. This marker is the gene encoding the T-protein of the enzyme glycine decarboxylase (GDC), a central enzyme in the photorespiratory pathway. Using this marker, we have successfully demonstrated the importance of light in stimulating photorespiration and the release of the photorespiratory-specific compound glycolate.

Our current work focuses on using the sequences of genes in the photorespiratory pathway, carbon fixation and nitrogen utilization to follow the dynamics of carbon and nitrogen cycling within the diatom cell under conditions expected to enhance photorespiration. We have designed primers for quantitative PCR to measure the transcript levels of these genes. The targeted genes include glutamine synthetase and nitrate reductase (nitrogen utilization), sedoheptulose 1,7-bisphosphatase and carbonic anhydrase (carbon fixation) and glycine decarboxylase T-protein and phosphoglycolate phosphatase (photorespiration). The experimental conditions include extremes of temperature, light, and nitrogen source. These conditions are all thought to influence the dynamics of carbon and nitrogen metabolism during photorespiration. These experiments will allow us to examine key steps in the metabolic pathways associated with photorespiration and determine interactions under different environmental conditions. Ultimately, the results of these experiments will allow us to generate hypotheses about how photorespiration may influence carbon and nitrogen cycling in the field. Future directions include developing degenerate primers to amplify target genes from field populations of diatoms.

**Development of Oligonucleotide Microarray for Studying Microbial Diversity at Gulf of Mexico**

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Microarray technology currently enables parallel, high-throughput gene expression profiling of thousands of genes simultaneously. Microarrays also promise to be an excellent technology for understanding microbial community dynamics for similar reasons as those stated above. Because there are many diverse laboratories doing molecular microbial ecology, comprehensive cDNA functional gene arrays (FGA) are hard to construct for microbial community analysis. Oligonucleotide microarrays may be a better way to approach studying microbial communities in the environments. Oligo-microarrays can be easily constructed from sequence databases, have greater specificity and increased sensitivity for target DNA than cDNA arrays. However, oligo-microarrays have not been extensively studied for microbial community analyses. We developed a 50 mer oligonucleotide microarray, for studying microbial genes involved in carbon, nitrogen, sulfur and phosphorus cycling. Because of the current sensitivity limits of glass slide based microarrays and also that the range of even-labeling efficiency for genomic DNA is usually from 300 pg to 1000 ng, detecting genes from low biomass environments such as deep ocean sediments is challenging. The genomic DNA detection limit for our combined techniques was 5 ng from pure cultures, and was quantitative range in the range 25 ng to 1000 ng. We applied these techniques to the study of ocean sediments collected from five stations in the Gulf of Mexico in November 2003 at depths of 62 m, 221m, 510m, 1035m, and 1870m. The microarray results show abundance of nifH, nifD, nirS, nirK and dsr genes. While better computational algorithms are need for comprehensive probe design, the results from our functional gene array already show that oligo-microarray technology is a very promising tool for understanding microbial C, N, S and P cycling genes in marine sediments and potentially other microbial communities.

O3- uptake in ocean margin ecosystems is important because it sets an upper limit to biomass yield at higher trophic levels and supports new production that, over large geographic and temporal scales, is related to carbon export from the system via the biological pump. Molecular approaches targeted to functional genes involved in nitrate assimilation by phytoplankton enable the investigation of nitrate assimilation gene diversity and expression in specific phytoplankton taxa. The detection of functional genes encoding high affinity nitrate transporters (hNat) and assimilatory nitrate reductases (NR) from pure cultures of marine phytoplankton were reported previously in our laboratory. Using these sequences, we developed universal eukaryotic NR primers and group specific primers targeting hNat genes in diatoms and green algae. Seasonal and geographical patterns in marine phytoplankton NR and hNat genes were investigated in Pacific (Monterey Bay) and Atlantic (LEO site) populations. Diatom NR genes were detected in Monterey Bay samples and NR genes from a range of diverse eukaryotes were detected in samples from the diel studies at the LEO site. Diatom hNat genes were detected at both sites but green algal hNat genes were detected only in Monterey Bay samples. T-RFLP analysis and phylogenetic analysis of cloned hNat genes from the two sites indicated the presence of geographically distinct phytoplankton communities. Seasonal changes in hNat gene diversity were detected in Monterey Bay samples. Changes in marine phytoplankton community structure were also observed in hNat genes at the LEO site, which was sampled over two consecutive days. Thus, nitrate reductase and nitrate transporter genes can be targeted to determine the variation of functional guilds assimilating nitrate in the marine environment. Variability in gene expression, as well as community structure, can be studied with more tools developed here.
**EFFECT OF SALT ON THE COMMUNITY STRUCTURE OF MARINE HETEROTROPHIC BACTERIA**

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Proteobacteria and Cytophaga-like bacteria are the most abundant aquatic heterotrophic bacteria, and they play an integral role in all aquatic systems. Previous research shows that beta-proteobacteria are the most abundant type of proteobacteria in freshwater systems, while alpha-proteobacteria are the most abundant in seawater. This experiment tested the hypothesis that salinity is the main reason for this phenomenon. It was hypothesized that the abundance of beta-proteobacteria would decrease with the addition of salts, while the abundance of alpha-proteobacteria would increase with the addition of salts. Diffusion chambers containing freshwater were placed in carboys with seawater so that the freshwater bacterial communities were exposed to seawater conditions. Similarly, marine communities were exposed to freshwater conditions. The experiment was allowed to run for 72 hours. Changes in bacterial abundance and community composition were monitored with DAPI-stained direct counts and fluorescence in situ hybridization (FISH) respectively. The abundance of beta-proteobacteria decreased with the addition of salts, though the hypothesized increase in the abundance of alpha-proteobacteria was not observed. These data suggest that salt can affect some bacterial groups in estuaries, but the composition of estuarine communities does not appear to be controlled by salinity alone.

**INVESTIGATING THE EFFECTS OF MARINE HETEROTROPHIC BACTERIAL ASSIMILATION OF NITRATE ON CARBON FATE IN OCEAN CARBON MODELS**

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Recent investigations, especially those of previous BI-OMP projects, have revised our appreciation of the potential importance of NO₃⁻ as an N source for marine heterotrophic bacteria. It is our underlying premise that these revisions at small scales may have large consequences at the global scale. Existing ocean carbon models (OCM’s) provide a framework for exploring and estimating the large scale ramifications of a significant fraction of NO₃⁻ being incorporated into CO₂-respiring bacterioplankton. For example, recent models published by Anderson and Williams (1998), Spitz et al. (2001), and Walsh et al. (2001) each include microbial components and processes that could be modified to explicitly consider the competition between autotrophs and heterotrophic bacteria for NO₃⁻. Our principle modeling objective is to test the hypothesis that bacterial assimilation of NO₃⁻ affects estimates of C fixation, sinking, or respiration. To address this hypothesis, we plan to synthesize existing data from our BI-OMP project to produce a modified microbial sub-model that includes assimilation of NO₃⁻. The revised sub-model will be incorporated into an existing carbon model of a Southern Ocean ecosystem model (Walsh et al. 2001). Through running different scenarios, we will compare how including assimilation of NO₃⁻ will affect the model’s estimates of C fixation, sinking, or respiration. The ecological implications of our results at both the local and global scale will be discussed.
The oceanic carbon cycle is tightly linked to global climate, therefore understanding it is essential in order to predict effects of extensive phenomenon, e.g. global warming. In marine systems nitrogen is an important limiting nutrient and is closely linked to atmospheric-ocean carbon flux via the biological pump whereby phytoplankton assimilate both nitrogen and carbon that is exported to the deep ocean. This mechanism is particularly important in ocean margins where coastal diatoms play a major role. The goal of our project is to understand the mechanistic basis of phytoplankton competition for inorganic nitrogen sources at the molecular level and its relevance to carbon flux. Ammonium assimilation occurs by the action of glutamine synthetase. Three forms of the enzyme have been found in the diatom, Thalassiosira pseudonana: GSI, GSII, and GSIII. This experiment utilizes quantitative reverse transcription polymerase chain reaction (QRT-PCR) techniques to quantify expressed mRNA of GS isoforms under different environmental nitrogen regimes. It was also possible to extrapolate the starting concentration of messenger RNA. With different nitrogen regimes (nitrate alone, ammonium alone or both together), the diatoms grew and depleted the media of nutrients, confirming activity by glutamine synthetase. The differential transcription of the three GS isoforms shown by the QRT-PCR was dependent on the availability of different forms of nitrogen. Transcription of GSII was induced especially in nitrate containing treatments whereas GSIII was constitutively expressed. Transcription levels of GSI were below detection at most sampling times. These results suggest that GS isoforms are regulated differently in response to external N sources. Determination of GS isoform active sites along the N assimilation pathways would compliment our results in understanding how GS isoforms are used differentially.

Abstract. Fusions to reporters genes such as lacZ and phoA,, have been an important tool to perform genetic and physiological analysis at transcriptional and translational levels. Another important reporter gene with applications also in environmental microbiology and biotechnology is the luciferase (lux) gene. We seek to isolate bioluminescent bacteria, from different marine ecosystems in Puerto Rico, searching for possible novel contaminant biosensors. After isolating bioluminescent bacteria from diverse aquatic ecosystems we microbiologically and molecularly characterized the organisms to determine whether there were any new species present and to test their potential use as genetic tools and bio-indicators. General microbiological techniques are used for initial isolation and screening of the isolates. The bioluminescence candidats were grown on Luminescent agar (LA) and characterized molecularly using domain specific (16S rDNA) PCR amplification, followed by restriction enzyme analysis. A total of eight gram-negative bacteria with rods and vibrio morphologies were isolated, also showing variable bioluminescence intensity emission. The 16S rDNA amplified genes have been sent to be sequenced at a Molecular Resource Facility for further in silico analysis. Experiments are in progress to perform genetic
comparisons between the lux genes DNA sequence, and operon organization in the bioluminescent isolates, and also determine their potential as tools for the detection of specific water contaminants.

ACCESSING THE CULTIVABLE AND UNCULTIVABLE MICROBIAL FLORA FROM SEVERAL HABITATS IN PUERTO RICO USING MOLECULAR ANALYSIS AND FUNCTIONAL GENOMIC APPROACHES

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The soil is one of the most valuable and useful resource on earth and also one of the least understood habitats. Physiological and chemical factors have limited the discovery of new organisms in diverse ecosystems. The last decades of research and the limiting tools available for culturing the soil micro flora demonstrated that the cultivable microorganism from soil, represent less than 1% of the total population. The other uncultivable 99% of the microorganisms could be seen as an opportunity for the discovery of new groups of microbes. The project seeks to develop environmental libraries (metagenomic and rDNA) from soil samples from different environments such as forest and crops in Puerto Rico, searching for new metabolic activities (functional genomics), and to analyze the microbial community present. Total DNA was isolated from soil samples using in situ and ex situ approaches. Domain specific primers were used to amplify the rDNA, and genetic engineering techniques were used to generate and evaluate the metagenomic and rDNA environmental libraries. Total genomic DNA was extracted from forest, crop land, and ripariane vegetation habitats. The preliminary rDNA amplification analysis of all the samples demonstrated not only bacterial, but archaeal 16S rDNA. To confirm this, genomic libraries from all the soil samples were generated, and some of the candidates have been sent to be sequenced for further in silico analysis. An initial metagenomic library of one of the soil samples has also been developed, and screening for metabolic activity is in progress.

COMPUTATIONAL STUDIES OF SIRNA SPECIFICITY

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RNA interference (RNAi) is a powerful method of post-transcriptional gene silencing that is conserved from C. elegans to humans. It is induced by small interfering RNAs (siRNA), which are double-stranded RNA molecules around 19-21 nucleotides long that are perfectly complementary to their target transcripts. However, two sets of experiments monitoring global transcriptional changes after RNAi treatment yielded contradictory evidence regarding the specificity of siRNA targeting. One set of data implies that global transcriptional changes depend on the gene targeted by RNAi, and are siRNA independent, while the second set suggests that observed alterations in transcript level are dependent on the siRNA employed. To address this predicament, we used computational methods to study how siRNA selection criteria may influence the resulting expression profile. Specifically, we analyzed the melting temperature difference (ΔTm) between the intended target RNA and possible cross-hybridizing transcripts for each of the siRNA used in the global transcription monitoring experiments. Although not all possibilities have been explored yet, our studies suggest that the selection of an optimal Δ for a given siRNA is not related to the specificity of the resulting transcriptional profile. One possibility currently under consideration is the cell-specific response to RNAi and its relationship to interferon pathway stimulation. The outcome of such studies of siRNAs specificity will be vital for evaluating the accuracy of conclusions drawn from RNAi experiments, for development of RNAi as a therapeutic tool, and for computational prediction of endogenous siRNAs and their targets.
PHOTOS TAKEN IN PUERTO RICO BY: JUAN D. JEANNOT