The Lipman Log

April 2009

News from the Chair, Max Häggblom

Spring Greetings from the Department of Boisterous Biochemistry and Marvelous Microbes (our theme for this year's Ag Field Day/Rutgers Day. We have had an exciting Fall and Spring semester and this issue highlights some of our activities.

On November 5th, 2008, a re-enactment of the classic 1783 experiment by George Washington and Thomas Paine of their discovery of the nature of the fiery Will-O'-the-Wisp of marshes was performed by the Department. "It's a Gas!" was heard across the Millstone River in Rocky Hill NJ, at the site of the original experiment. The re-enactment was organized under the inspirational leadership of Dr. Doug Eveleigh, our department historian. Read more about this spectacular event on page 2.

On pages 4-6 you can read Dr. William Ward's reflections on the 2008 Nobel Price in Chemistry and about his work on green-fluorescent protein. Bill joined the Biochemistry and Microbiology Department in 1977, where he began his research program on the jellyfish *Aequorea* GFP. Bill played a key role in determining the structure of green-fluorescent protein and his work provided the underpinnings of our understanding of the GFP molecule. Since the 1990s Bill has developed and used GFP as a teaching tool for hands-on continuing professional education courses in protein purification.

We are seeing an expansion of our faculty and welcome Dr. Ning Zhang, the newest faculty member of our department. She has a joint appointment in Plant Biology & Pathology and Biochemistry & Microbiology. You can read more about her research on the population and evolutionary biology of fungi on p. 7. We are also completing our search for faculty hires in the areas of microbial physiology, biochemistry and genetics/bioinformatics. We thus expect an interesting year as we continue this development of our programs in microbiology and biochemistry.

The Microbiology Symposium is an established annual event (see page 3). Our keynote speaker was Dr. Jennie Hunter-Cevera, President, University of Maryland Biotechnology Institute, who earned her Ph.D. in Microbiology at Rutgers with Professors Doug Eveleigh and Hubert Lechevalier. The next Symposium is scheduled for January 2010.

The microbiology graduate students have founded a Student Chapter of the American Society of Microbiology at Rutgers, with membership open to any graduate or undergraduate student at Rutgers University. The G.H. Cook Biochemistry & Microbiology Club (for the Biochemistry and Microbiology undergraduate majors) and the Graduate Student Chapter of ASM hosted Dr. Douglas Beecher from the FBI for a visit to the Department and a seminar "Amerithrax" in March, (see page 11).

Finally, I wish to thank all the donors and supporters of the Department of Biochemistry and Microbiology. These donations provide special scholarships and travel awards for our students and enhance our departmental mission. One of our key goals is to obtain core endowment to support our research and educational programs, specifically in the form of graduate and undergraduate fellowships. We hope that you will continue to show your support for the department in the future.

INSIDE THIS ISSUE:

Presidential Microbiology: "It's a Gas!" The Rutgers Re-Enactment of the Washington/Paine Experiment from 1783

Pictured top to bottom: In boat: G. Swiatek, M. Häggblom, A. Isola and Z. Freedman.
Allison Isola and Max Häggblom
Ted Chase and Tamar Barkay.

"IT'S A GAS" - RE-ENACTMENT OF THE GENERAL GEORGE WASHINGTON & THOMAS PAINE EXPERIMENT OF THE MARSH WILL-O'-THE-WISP

A re-enactment of the classic experiment by General George Washington and Thomas Paine of their discovery of the nature of the fiery Will-O'-the-Wisp of marshes and rivers at the original site at the Millstone River, Rocky Hill, NJ was performed by the Department, last November 5th, 2008. Could the fiery Will-'O-the-Wisp be due to a bituminous substance such as turpentine or a gas? They probed the river mud while holding a flame above the river surface. Should the surface catch fire, it would be a bituminous substance. However a fiery flash was seen indicating the Will-'O-the-Wisp was a gas. Count Volta had performed the same experiment in 1776, but there are no indications that General Washington was reading the Italian scientific literature while directing the Revolutionary War. The Washington-Paine proof November 5th, 1783 was an independent discovery. Profs. Doug Eveleigh, Ted Chase and Craig Phelps were the inspirational leaders with the department working as a team to accomplish this re-enactment of what could be seen as the first significant American Science since the signing of the Treaty of Paris, September 3rd, 1783. The experiment is also noteworthy as the forerunner of "hands-on presidential science". The re-enactment was in association with the Delaware and Raritan Canal State Park, and "Princeton 1783" celebrations: the 225th anniversary of Princeton as the nation's capital when the Continental Congress met at Nassau Hall, The College of New Jersey. "*We hope that subsequent American presidents will maintain the keen interest in science expressed in this first major microbiological experiment of the young Republic*."

Principals included: Doug Eveleigh - senior historian; Diane Davis - costumes and 18th century cookies; Andy Marinucci - chief pyrotechnician; Gavin Swiatek both as George Washington and boat organizer; Peter Anderson and Craig Phelps - photographers.

Members of the re-enactment pictured L to R: Sharron Crane Hicks, Diane Davis, Doug Eveleigh, Caren Villano, Gavin Swiatek, Andy Marinucci, Allison Isola, Zac Freedman and Max Häggblom.

The Third Annual Mini-Symposium on Microbiology at Rutgers University: Cultivating Traditions, Current Strength, and Future Frontiers

Thursday, January 29, 2009

500-5:15 pm **Welcome: Robert Goodman**, Executive Dean of the School of Environmental and Biological Sciences

5:15-5:40 pm **Rutgers and Antibiotics**: **Joachim Messing,** Director Waksman Institute of Microbiology

5:40-6:00 pm **Presidential Microbiology: "It's a Gas!" The Rutgers reenactment of the Washington/Paine Experiment from 1783**
Doug Eveleigh, Biochemistry and Microbiology

6:00-7:00 pm **Keynote presentation: Microbiology: Past, Present and Fast Forward: Jennie Hunter-Cevera,** President, University of Maryland Biotechnology Institute **Introduction:** Doug Eveleigh

7:00 - 8:30 pm **Poster Session** Combined with a **Wine and Cheese Reception**

Friday, January 30th

8:00–9:00 am Coffee/Pastries

Fungi: fun, fuzzy friends - **Convener: Douglas Eveleigh**

9:00-9:30 am **Fungal interactions with radionuclides: Are fungi autotrophs?**
John Dighton, Rutgers Pinelands Field Station

9:30-10:00 am **Regulated antisense transcription controls expression of cell-type specific genes in yeast**
Andrew K. Vershon, Waksman Institute of Microbiology

10:00-10:30 am **Molecular phylogeny and array detection of *Fusarium solani* species complex**
Ning Zhang, Plant Biology and Pathology & Biochemistry and Microbiology

10:30-11:00 am Coffee break

Intracellular interactions: good and bad - **Convener: Tamar Barkay**

11:00-11:30 am **Membrane fluidity and acid stress modulate the activity and transcription of the *Listeria monocytogenes* F_oF_1ATPase**
Thomas Montville, Food Science

11:30 am -12:00 pm **Birth of a plastid: Organellogenesis in the thecate amoeba *Paulinella chromatophora***
Debashish Bhattacharya, Ecology, Evolution and Natural Resources and Marine and Coastal Sciences (July 2009)

12:00-12:30 pm **Genomic insights into pathogenicity mechanisms utilized by *Lysobacter enzymogenes*, an intracellular bacterial pathogen of lower eukaryotes**
Donald Y. Kobayashi, Plant Biology and Pathology

12:30-2:00 pm Lunch break combined with **Poster session**

Food, Drugs and Rock & Roll - **Convener: Max Häggblom**

2:00-2:30pm **Application of predictive models to food protection**
Donald W. Schaffner, Food Science

2:30-3:00 pm **New drugs against hepatitis C virus from pokeweed**
Rong Di and Nilgun E. Tumer, Biotechnology Center for Agriculture and the Environment

3:00-3:30 pm **Microbial mobilization of arsenic from black shale pyrite**
John Reinfelder, Environmental Sciences

3:30-4:00 pm **Poster Session** combined with Coffee Break

4:00-5:00 pm **Microbiology Graduate Program Discussion**

(Pictured: top to bottom): Jennie Hunter-Cevera; Organizers Max Häggblom, Tamar Barkay and Douglas Eveleigh; Costantino Vetriani; Poster Presenter; Joan Bennett, Ning Zhang and Charlie O'Brien.

OUR FACULTY

REFLECTIONS ON THE GFP NOBEL PRIZE

WILLIAM W. WARD

On October 8, 2008, the Nobel Prize for Chemistry was awarded to GREEN-FLUORESCENT PROTEIN researchers Osamu Shimomura, Marty Chalfie, and Roger Tsien. This is an account of the decades of research that preceded this award.

Fluorescence in the outer rim of the bioluminescent jellyfish, *Aequorea victoria,* had been noted as far back as 1848, but, green-fluorescent protein, the protein, was first studied in the very early 1970's by Harvard University scientists, Jim Morin and J. W. Hastings, and by John Wanpler and Milton Cormier of the University of Georgia. The Harvard group concentrated on the bioluminescent hydroid, *Obelia*, while the Georgia group studied the bioluminescent sea pansy, *Renilla reniformis.* Both groups recognized, as early as 1971, that the green fluorescent glow in these coelenterates came from a remarkable protein—one named "green-fluorescent protein" by Jim Morin. Very soon thereafter, in 1973, I joined the Cormier group, spending my next four years of postdoctoral research studying the physical properties of *Renilla* GFP. At about that same time, Frank Prendergast began working on structural properties of *Aequorea* GFP in the Mayo Clinic laboratory of John Blinks. Soon thereafter, Tony Campbell began his studies of photoproteins and GFP molecules at the Cardiff Medical School in Wales.

Dr. Blinks had spent a number of years in the 1960's, as had Princeton's Osamu Shimomura and Frank Johnson, isolating, from the jellyfish *Aequorea,* the calcium triggered, blue light-emitting, photoprotein, aequorin (a protein discovered by and named by Shimomura in 1962). In those years, Blinks concentrated on applications of aequorin as an intracellular calcium ion indicator, Shimomura focused his attention on the organic chemistry of aequorin and its tightly bound chromophore (a small molecule he later named coelenterazine), and Prendergast worked on the biochemistry of *Aequorea* GFP, becoming the first to purify and correctly characterize *Aequorea* GFP in 1978. Meanwhile, in Georgia, I was the first to purify and characterize *Renilla* GFP in 1979 (1). I showed that *Renilla* GFP, unlike *Aequorea* GFP, is an obligate dimer of identical 27 Kdal subunits. Most importantly, I demonstrated for the first time, in 1978, with a dual phototube luminometer, that *Renilla luciferase* forms an electrostatically stabilized complex, in dilute solution, with *Renilla* GFP (2). This complex produces green light (not blue light) by radiationless energy transfer from luciferase-bound luciferin. The GFP-luciferase complex is completely disrupted by the addition of sodium chloride to the reaction buffer, leading to blue light production. Furthermore, pure GFP molecules I painstakingly prepared from distantly related coelenterates, but ones having identical spectral characteristics, failed to generate green light under the same experimental conditions. This was the first unequivocal demonstration of radiationless energy transfer in the field of bioluminescence.

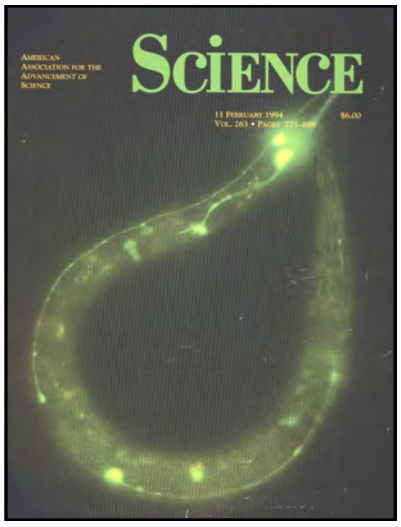
Kazuo Hori, in the Cormier lab, was first to synthesize an active luciferin (coelenterazine) molecule. Later, Russ Hart, another synthetic organic chemist who joined the Cormier lab in 1975, would show that dark variants of luciferin could transfer excitation energy quite efficiently to *Renilla* GFP—the final proof of radiationless energy transfer. As a controversy had arisen about the nature of the bound chromophore of photoproteins, I performed crucial experiments to show that the here-to-fore unidentified choromophores in the coelenterate photoproteins, aequorin, mnemiopsin, and berovin, are all identical to the *Renilla luciferin* Dr. Hori had characterized by chemical synthesis.

In 1977, I joined the Biochemistry and Microbiology Department at Rutgers University, Cook College, as an Assistant Professor, where I began an intensive research program on *Aequorea* GFP. This research, like that of Dr. Shimomura, required mounting a major jellyfish collecting program nearly 3000 miles from New Jersey, at the University of Washington's marine lab, Friday Harbor Laboratories in the San Juan Archipelago. Along with as many as 8 colleagues and students, I made this trip for 17 summers, hand-collecting and hand-dissecting as many as 100,000 jellyfish in 3 weeks time each summer. Every summer, Dr. Dennis Willows, FHL director for most of that time, graciously accommodated our conspicuous, sloppy crew of jellyfish collectors. He must have realized more value in what we were doing than we, ourselves recognized. After reducing the collected material to a manageable volume at FHL and transporting the precious material on dry ice back to Rutgers, we then spent 6-person months each year purifying the GFP to homogeneity.

We accomplished much in the years that followed. Our group was the first to demonstrate (3) the spectroscopic identity of *Renilla* and *Aequorea* GFP in the denatured state, providing a convenient means to calculate molar extinction coefficients for all future GFP's based on the very careful work I had done with *Renilla* GFP in the Cormier lab. Those calculations remain, to this day, the absolute standard for determining molar extinction coefficients and fluorescent quantum yields for all genetic variants of *Aequorea* GFP. Breaking with the Nobel Prize winning experiments of Christian Anfinsen, we showed that *Aequorea* GFP can be reversibly denatured, but only if removed from the denaturing agent very rapidly (Anfinsen had stressed SLOW removal on thermodynamic and kinetic grounds).

We needed to remove the denaturant quickly, as two otherwise fully buried cysteine residues in the protein become slowly oxidized when the protein is denatured, thus preventing proper refolding. While working on this problem, we discovered that the GFP chromophore, previously thought to be "rock stable," is quite easily (and reversibly) perturbed upon dimer formation and upon exposure to organic solvents and extremes of pH.

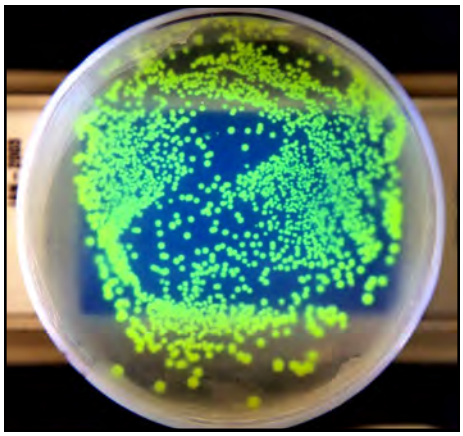
Our discovery that *Aequorea* GFP forms reversible dimers at high protein concentration provided a new, and highly revealing "tail is wagging the dog," (or, as I like to put it, "your Camelions are mating") interpretation for some of the FRET-based (fluorescence resonanance energy transfer) assays already being marketed by Aurora Biosciences, notably the calcium-sensing Camelion. If blue-emitting and yellow-emitting forms of GFP (on opposite ends of a FRET construct) are able to self-associate (head to tail with another FRET construct) in the absence of calcium, as we clearly demonstrated they would (4), the calcium-free background of the Camelion assay would rise, making data interpretations much more difficult. This problem was later corrected by mutagenizing the contact interface of the dimeric pair, but only because we raised the issue in public. We were first to obtain *Aequorea* GFP crystals that diffract X-rays, and along with Doug Prasher, V.K. Eckenrode, Prendergast and Cormier were first to complete the primary sequence of *Aequorea,* GFP in 1992 (5). One year later, we became the first to identify, correctly, the chromogenic hexapeptide of GFP (6). Our lab was the first to show an oxygen requirement for the "greening" of recombinant GFP and, in collaboration with others, we were the first to synthesize GFP in a cell-free system. In another collaborative effort, we were first to demonstrate the safety of GFP in rat-feeding experiments (7). Our lab collaborated in the 1994 blockbuster cover story in Science that showed the first successful cloning of GFP , a paper, with 3400 citations at last count, and one considered to be among the 10 most cited papers in all of biotechnology (8). As I had purified *Aequorea* GFP to homogeneity and as I possessed the most convincing spectral data from native *Aequorea* GFP, I was in the best position to validate, for the Science paper, the identity of the native and recombinant proteins.



More recently, I have applied the method of three-phase partitioning as an elegant way to purify recombinant GFP, widening the scope of this method to include many other proteins in the form of a 2008-patented protein mini-prep kit. Also patented (in 2008) and licensed exclusively to our 2003-incorporated biotech company, Brighter Ideas, Inc. www.brighterideasinc.com is our unique protease and protease inhibitor assay called GFP-on-a-String (GoaS). The GoaS assay has the capability to fingerprint, in a multichannel HTS format, the exact substrate specificity of a given (or unknown) protease in comparison with an extensive database.

The above are several of our more recent commercial applications of GFP (the protein) as opposed to gfp (the gene), making our group unique in respect to how we have applied GFP commercially. However, almost 20 years ago, we began using GFP as a spectacular teaching tool in biochemistry and biotechnology hands-on laboratory instruction for biotech and biopharm professionals needing training in protein purification www.rci.rutgers.edu/~crebb/protein.html . More than 1500 scientists from around the world have now taken our 5 ½-day hands-on course in protein purification, learning not only about protein purification, but becoming exposed, perhaps for the first time, to the wonders of GFP.

In addition to those I have already acknowledged as pioneers in GFP research, others throughout the decades of the 1980's and 1990's, including Cormier, Shimomura, Prendergast, Campbell, K. Ward, G. Phillips, F. Tsuji, Hart, and Prasher all made significant contributions to our understanding of the structure and function of GFP. But, by 1993, Doug Prasher, with assistance from V.K. Eckenrode, W.W. Ward, F.G. Prendergast, M.J. Cormier, C.W. Cody, and W.M. Westler made two of the most crucial advances with GFP. Together we deduced the entire primary structure of *Aequorea* GFP (5) and the correct structure of the chromogenic hexapeptide (6). In, particular, Doug Prasher cloned the gene. Prasher's cloned gene failed to produce a fluorescent product as it contained, at one end, twenty additional amino acids that interfered with the proper folding of the protein. Having lost his funding and, therefore, unable to fix the small error in this gene, Doug gave the gene to Marty Chalfie and Roger Tsien who capitalized brilliantly on the applications that followed the repair of Prasher's gene.



The "magic" of GFP is that the cyclic tripeptide, that is the fluorescent chromophore of GFP, is part of the primary sequence of the protein (Fig.4) . Isolated from the protein, as we had shown in the 1970's, this chromophore is non-fluorescent. But, sequestered within the can-shaped barrel of the protein (a structure determined by George Phillips), that same chromophore becomes brilliantly fluorescent. So, unlike other chromoproteins (like hemoglobin) that need multiple genes, (one or more genes to make the protein and many more to make the chromophore), GFP needs only one gene. Remarkably, that gene, transfected into and then expressed in other cells, tissues, organs, and organisms, almost invariably expresses its brilliant fluorescence. The, GFP is now widely used as a marker for gene induction and countless other cellular and physiological processes.

At present, nearly 20,000 research papers have been written about GFP, virtually all about how GFP is utilized to solve basic cellular, biochemical, and biomedical questions (9). An additional 20,000 research papers cite one or more of these original works.

There is an even bigger take home message that should be communicated through the GFP story. That message is to use the GFP story to uncover, for every reader of this article and every decision maker in our society, what really drives scientific inquiry. I would argue that the driving force for most scientific inquiry is the aesthetic appreciation science brings to the investigator. In particular, research on bioluminescence epitomizes this scientific thirst for the art and the aesthetics of science.

More than 5 decades ago, as a high school freshman, I took the Kuder Preference Test, a binary choice test that evaluates the sorts of activities a young student appreciates and might gravitate toward in a career choice. My top two categories came out "SCIENTIFIC" and "ARTISTIC." I did not doubt the scientific, but I wondered, then, about the artistic. Now having lived most of my life as a scientist studying bioluminescence, I know that Kuder was telling me the truth. I am strongly motivated by the beauty and artistry of bioluminescence. In fact, many of my activities from elementary grades through college were highly artistic (constructing dozens of flour and salt relief maps, designing and building intricate working models of Roman weapons pictured in my Latin II text book, spending countless hours doing nearly perfect biology drawings, and volunteering to draw the Acropolis for a quiz in a college Humanities course, only to misidentify at quiz time 2 of the 10 buildings and statues I had so carefully drawn. At age 45 I discovered community theatre and I have now performed in 8 or 10 musicals including 4 Gilbert and Sullivan operettas, having met my current wife doing Pirates of Penzance. I must admit, that I will never come close to matching Tony Campbell's fabulous Welsh voice. I am now writing humorous satires—2 published and 5 on the way, including one on higher education published by CCB Publishing,

"Hey, Doc! Does Speling Count?" available on Amazon.com and Barnes & Noble web sites. I have even turned real jellyfish into a work of art www.marineimpressions.net.

So, I would argue that the love of art and aesthetics drives good science and that good science gives rise to good practical applications. It is not important what the scientific project is, so long as it is approached with passion accompanied by good observational skills. Virtually every major scientific discovery comes about purely by accident within basic research projects not foreseen to have practical applications. Such is the case with bioluminescence and GFP—such is the case with most other scientific discoveries.

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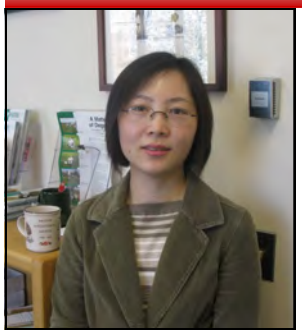
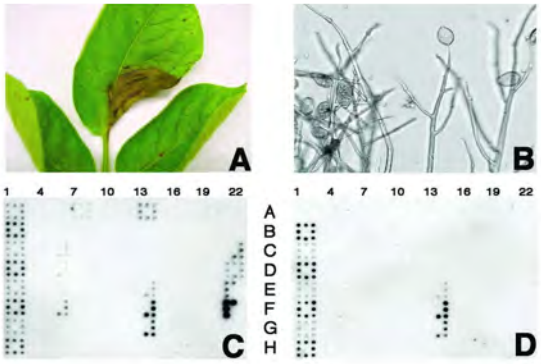
Our Faculty continued:



Ning Zhang joined the Department in January 2009, with a joint appointment in Plant Biology & Pathology and Biochemistry & Microbiology. Her research interests are in population and evolutionary biology of fungi, and the molecular detection of fungal pathogens

I have two primary research interests: population and evolutionary biology of fungi and molecular detection of important plant pathogenetic fungi of turfgrasses. The basic questions we try to address include (1) Why is there so much genetic variation within populations? (2) What are the evolutionary forces that maintain variation in populations? (3) Are there associations between genetic variation and virulence of plant pathogens? (4) How did the plant diseases originate and evolve? (5) What's the phylogenetic relationship between turfgrass pathogens and fungi in other habitats? etc. New pathogens are emerging all the time and many known pathogens are fast evolving due to dramatic climate change, host switching, and frequent transportation by humans. In addition to studying the evolution of fungi, we are interested in developing molecular tools for rapid pathogen detection and identification, such as Pathochip (DNA array technique) and real-time PCR. The application of the new tools will facilitate disease management, plant breeding, and the study of microbial community.



Current personnel in the Zhang lab:
Sanjana Kirloskar (January 2009-present), undergraduate student, Biotechnology Major.
Sirrika Samuels (February 2009-present), undergraduate student, Animal Science Major.
Suli Hu (February 2009-present), part-time lab technician.

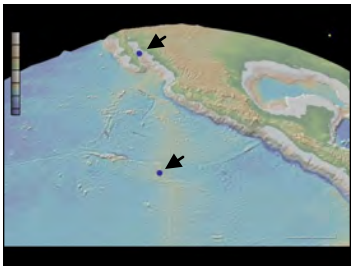
GRANTS AND PROJECTS

Costantino Vetriani (P.I.) and **Elisabetta Bini** (Co-P.I.) were awarded a 3-year $374,000 grant from NSF-MCB: Transcriptional analysis of the deep-sea vent *Epsilonproteobacterium*, *Caminibacter mediatlanticus*, in response to different growth conditions.

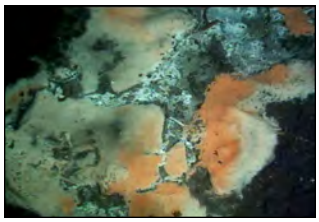
Elisabetta Bini received a SEBS Pre-Tenure Faculty Career Development Award (Nov 2008-June 2009): "Effects of metal ions exposure on regulation of oxidative stress pathways and development of genetic tools for its analysis". She was also awarded a grant of the New Jersey Water Resources Research Institute ($30,000): "Antibiotic pollution of aquatic habitats and impact on the development of environmental pools of resistance in natural microbial communities".

Tamar Barkay was awarded The MERCTIC: Mercury biogeochemistry in the high Arctic − A Marie Curie International Incoming Fellowship.

Max Häggblom is the PI on a new 3-year project "Assessing the Potential for Anaerobic Microbial Dechlorination of PCDD/Fs in River Kymijoki Sediments" funded by the Nessling Foundation in Finland. The work will mainly be conducted at the University of Helsinki, Department of Ecological and Environmental Sciences in collaboration with the Häggblom lab at Rutgers. The grant supports a Ph.D. student at the University of Helsinki co-supervised by Dr. Häggblom.



Costantino Vetriani and **Melitza Crespo-Medina** participated in an oceanographic expedition aboard the Research Vessel Atlantis in October/November 2008. They used the Deep-Submergence Vehicle Alvin to explore and sample the deep-sea vents located in the Gulf of California and on the East Pacific Rise at 9°N (See blue circles on map for diving sites).



Extensive orange and yellow microbial mats dominated by *Beggiatoa* spp. cover sufide-rich sediments in proximity of deep-sea hydrothermal vents at the bottom of the Gulf of California (depth: 2,000 m).

IN PRINT

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Ph.D. & MS. THESES: 2008/2009

Hisako Masuda - Identification and characterization of monooxygenase enzymes involved in 1,4-dioxane degradation in *Pseudonocardia* sp. strain ENV478, *Mycobacterium* sp. strain ENV421, and *Nocardia* sp. strain ENV425. Graduate Program in Microbiology and Molecular Genetics, Dissertation Director: Gerben Zylstra.

1,4-Dioxane is widely used as an organic solvent and it has become a great concern as a ground water contaminant throughout United States and elsewhere. It has been shown to have carcinogenic properties, however, due to its physical characteristics, traditional remediation technologies are not effective. Biodegradation has attracted attention as better alternative for its remediation. Biodegradation of 1,4-dioxane in three soil bacteria capable of degrading 1,4-dioxane via cometabolic process was examined in this study. A combination of two techniques, protein expression profile analysis and genome-wide screening of candidate genes by PCR using sets of degenerate primers, was utilized to identify the enzymes possibly involved in the initial step of 1,4-dioxane degradation.

Karen Pesce - Genetic and functional analysis of polycyclic aromatic hydrocarbon degradation by *Comamonas testosteroni* GZ38 and GZ39. Graduate Program in Microbiology and Molecular Genetics, Dissertation Director: Gerben Zylstra.

Polycyclic aromatic hydrocarbons (PAHs) are toxic pollutants that are present in a variety of environments. Some bacterial species have the ability to metabolize these compounds and use them as energy sources for growth. *Comamonas testosteroni* GZ38 and *Acidovorax* sp. GZ39 were isolated from the Passaic River, NJ, based on their ability to grow on phenanthrene. These isolates are unusual in that they can grow on phenanthrene but not on naphthalene. The genes that code for phenanthrene degradation in GZ38 and GZ39 were identified. The DNA sequence of the genes is unique with respect to other sequences in the GenBank database with a single match to a putative phenanthrene degradation gene cluster from the phenanthrene degrader Alcaligenes faecalis AFK2 (GenBank accession number AB024945). BLAST matches to the putative genes in both operons suggest that phenanthrene degradation proceeds through phthalate. Although the phenanthrene degradation genes of GZ38, GZ39, and AFK2 are nearly identical, this is the first report of functional information relating to these genes.

Marie Montes - Substrate specificity of a unique monooxygenase from *Burkholderia xenovorans* LB400. Graduate Program in Microbiology and Molecular Genetics, Dissertation Director: Gerben Zylstra.

Whole genome sequences have been key elements in discovering new genes and in predicting protein function. The complete genome of *Burkholderia xenovorans* LB400 has been sequenced and published. We have identified an LB400 gene encoding a protein that is similar to the *p*-cymene monooxygenase from *Pseudomonas putida* F1. The *P. putida* F1 enzymeconsists of a hydroxylase component (CymA1) and a reductase component (CymA2) responsible for the conversion of *p*-cymene to *p*-cumic alcohol through addition of one atom of molecular oxygen to the methyl group. In order to analyze the *B.* xenovorans LB400 monooxygenase enzyme in more detail, we cloned the cognate genes into the expression vector pQE.30 and performed biotransformation assays to determine the substrate specificity of the CymA1A2-like enzyme. Our results demonstrate that the LB400 enzyme enzymatically hydroxylates *p*-cymene to the product alpha, alpha 4-trimethylbenzenemethanol. This result is unusual in that the monooxygenation reaction occurs at the isopropyl group of p-cymene, at the carbon adjacent to the ring, and not at the single methyl group *para* to the isopropyl group as would be expected from a CymA1A2-like enzyme. We tested for the ability to oxidize substrates related to *p*-cymene. The enzyme shows activity toward a variety of aromatic compounds and in some cases the enzyme shows some preference for compounds substituted at the *para* position.

Ruyang Han - Phthalate biodegradation: comparative gene organization, regulation, and environmental biodiversity. Graduate Program in Microbiology and Molecular Genetics, Dissertation Director: Gerben Zylstra.

The three phthalate isomers are widely found in the environment due to their extensive use in the manufacture of plastics. Many microorganisms have been isolated for their ability to degrade phthalate isomers. In this study, we focused on nine different phthalate degrading bacterial strains which were isolated from Passaic River sediment and belong to different genera (*Comamonas*, *Pseudomonas*, *Acinetobacter*, and *Arthrobacter*). Our work aims to identify the presence and divergence of the phthalate,isophthalate and terephthalate degradative genes in the nine strains isolated from the same sediment sample. The *oph*, *iph*, and/or *tph* genes in *Comamonas testosteroni* strains (YZW-B, -E, and -F) and *Pseudomonas* strains (YZW-A and -G) were determined by PCR and inverse PCR. Sequence analyses indicate that phthalate, isophthalate and terephthalate degrading bacterial isolates at the same location are not simply clones of each other and that the genes identified are linked specifically to these bacterial strains.

Sean Bugle - An intergrated biomarker approach for assessing exposure and effects of endocrine disruptors and other contaminants in Killfish (*Fundulus heteroclitus*) from the New York– New Jersey Harbor Estuary. Graduate Program in Environmental Sciences (Toxicology track). M.S. Thesis Director: Keith Cooper.

A multi-tiered approach was used to evaluate fish population health by examining a suite of biomarkers in killifish inhabiting Newark Bay and a reference population in Tuckerton, NJ. Biomarkers investigated included classical endpoints (histopathology, morphometrics, gonad maturation), hepatic mRNA expression (CYP1A, metallothionein, vitellogenin I), ovarian aromatase mRNA expression, hepatic protein levels (CYP1A and vitellogenin I) and chemical exposure analyses (bile PAHs). Newark Bay fish had significantly higher levels of bile PAHs compared to reference fish. Females had significantly higher concentrations of naphthalene, pyrene and benzo[a] pyrene. Males had significantly higher concentrations of pyrene and higher concentrations of naphthalene and benzo[a] pyrene. Histological lesions of the liver and pancreas were similar to reference fish. Newark Bay fish had significantly higher expression of hepatic CYP1A for both males and females and CYP1A protein levels for both males and females. Endocrine disruption in male gonads is demonstrated by a decreased gonad weight, altered testis development and up-regulated aromatase expression, which indicates exposure to endocrine active compounds. Killifish are being exposed to high amounts of PAHs and aryl hydrocarbon agonists and that reproductive health of killifish in the NY-NJ Harbor Estuary is impaired. Impaired reproductive health is possibly due to disruption of steroid signaling by aryl hydrocarbon receptor-estrogen receptor crosstalk leading to decreased vitellogenin production.

OUR DEPARTMENT

Several undergraduate students working on research projects in the Department received Aresty Awards for 2008-2009: **Puja Patel** (Bini Lab) **Jennifer McConnell** (Häggblom Lab), **Francis Ortega** (Haggblom Lab), **Amy Dimeco** (White lab), and **Nicholas Sawyer** (Kahn lab).

Tamar Barkay, presented a paper "Reduction of mercury in saturated subsurface sediments and its potential to mobilize mercury in its elemental form" at the DOE-ERSP Annual PI meeting, Lansdowne, VA, April 20-24, 2009.

Peter Kahn presented a paper on March 24 entitled, "*Perennial Plants for Sustainable Production of Food and Bioenergy and for Restoration of Damaged Land: A Proposal,"* at the Oxford Round Table on Public Policy in Oxford, England. This is a small meeting of 35 or so invited participants. The paper is a collaboration with a group from the Plant Sciences Department: Thomas Molnar, Gengyun Zhang, Joseph Heckman, Bruce Clarke, James White, and C. Reed Funk, who is Professor Emeritus. A draft of the paper was sent to all participants before the meeting to promote discussion after the presentation. Revisions based on the discussions will lead to submission to the Round Table's peer review organization.

Rutgers Magazine, Winter 2009, Noble Help on the Nobel Prize, in Insights/ Discoveries Section, Contributed by **William W. Ward**.

Science Advisory Board (Fall 2008), **William W. Ward.** Galileo Could Not Be Reached For Comment, in Perspectives Section www.scienceboard.net.

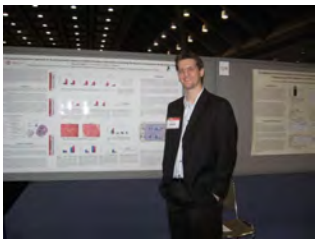
Science Advisory Board (Fall 2008), **William W. Ward.** The Nobel Prize for Green Fluorescent Protein (GFP) Researchers, In Perspectives Section. Science Advisory Board (Fall 2008), The Ward Group at Rutgers University Finds New Ways to Use GFP in Biomedical Diagnostics, 5-p. Personal Profile.'

Elisabetta Bini presented the invited seminar: "Archaeal interactions with copper and genetic response at extreme temperatures" at the Microbiology and Molecular Biology Seminar Series, University of Nebraska, Lincoln, NE. February 27, 2009.

2009 Paul A. Stellhorn New Jersey History Award: The Paul A. Stellhorn New Jersey History Award is sponsored by the New Jersey Historical Commission (Department of State), the New Jersey Studies Academic Alliance, the New Jersey Council for History Education, the Friends of the Rutgers University Libraries, and the New Jersey Caucus of the Mid-Atlantic Regional Archives Conference.

The award acknowledges an outstanding undergraduate paper on an aspect of New Jersey's history. Ms. Amisha Patel received the award for her paper, *"The Science of George Washington Honed in New Jersey,"* which was nominated by **Douglas Eveleigh**, Fenton Professor of Applied Microbiology, Department of Biochemistry and Microbiology, School of Environmental and Biological Sciences, Rutgers University. The award was announced during the annual New Jersey History Issues Convention in Trenton on Friday, March 20. Amisha Patel (received a B. S. degree in Biological Sciences from Rutgers Cook College in 2008). She is employed by Osteotech, Inc., of Eatontown, New Jersey. Working with **Dr. Eveleigh** Amisha brought together diverse Washington's scientific interests: during the Revolutionary War he successfully used invisible ink for secret military communiques, he sanctioned attack of British vessels by America's first submarine, *The Turtle*, at Boston Harbor besides the use of drifting mines in Philadelphia Harbor. Washington's 1780 decision of mandatory variolation of the Army against smallpox has been cited as perhaps his most significant military decision. In agriculture, Washington was the scientific gentleman farmer: he evaluated over 60 different crops, was an early planter to switch from tobacco to wheat, designed his own plough, used crop rotation, and addressed soil erosion. He operated perhaps the largest rye whiskey distillery in the country and his porter recipe continues to receive accolades. Thus Amisha illustrated how our first President, George Washington, merits greater recognition as a remarkable Enlightenment Philosopher (scientist).

This year's Stellhorn Award competition produced a record fourteen submissions on behalf of their students by professors at Kean, Rider, Rutgers, Seton Hall, William Paterson, and Monmouth Universities, and the College of Saint Elizabeth. The Stellhorn Award includes a plaque, a monetary prize plus State -wide press recognition. Congratulations Amisha!



Sean Bugle (Cooper Lab) was awarded a Graduate Student Grant for 2009-2010 from The New Jersey Water Resources Research Institute. The grant was for $5000 and is titled *"Development of two in vivo fish bioassays to study the anti-estrogenic action of polycyclic aromatic hydrocarbons and to evaluate endocrine activity in NJ wastewater effluents".*

Zachary Freedman (Barkay Lab) and **Isabel Horna Gray** (Häggblom Lab) are the recipients of the 2008 Robert S. and Eileen A. Robison Scholarship Award for Excellence in Graduate Studies. The Award was established in 2003 and is supported by the Robison family. The scholarship is awarded to graduate students who have demonstrated competence and accomplishment in their academic and research program while at Rutgers University, have shown an active participation in or a leadership role in the activities of the department, college, university or community, and are motivated to help and improve the human condition at this time and upon graduation.

Microbiologists at the International Microbiology Congress, Rio de Janeiro, 1950. (l to r) Unknown, Norberto Palleroni, Unknown, Professor Santos Soriano, Sir Alexander Fleming, Dr. Alfredo Sordell and Dr. Molina.

Dr. Norberto Palleroni was featured in the recent Microbiology Today (Feb. 2009. p.32) of the British Society for General Microbiology, in an issue focused on the last half century of antibiotics. Norberto with his Buenos Aires colleagues met with Sir Alexander Fleming (Nobel Prize laureate for the co-discovery of penicillin), at the International Microbiology Congress, Rio de Janeiro, 1950. In Norberto's commentary on the photograph he recalls the meeting which also illustrates the winding path that one's career can take. Professor Fleming asked "*What are you doing in your laboratory, young man ?" I simply said "I am interested in yeast genetics". Fleming looked at me for a minute or two, and his answer clearly painted the great man as a humble admirer of life's diversity. "I must confess to you that I do not know anything about yeast or about genetics*". And thus we talked about the beauty of the region and so forth.

However, as regards career paths, Norberto went on to be a pioneer in using molecular biological approaches to the identification of prokaryotes, became the world's foremost authority of *Pseudomonas* (see Bergey's Manual), and also specifically addressed screening for antibiotics from members of the motile *Actinoplanes* genus. He is a true microbiologist and a descendant of the famed Delft Microbiology Microbial Family Tree through his mentor Professor Santos Soriano (Robertson, L. The Delft School of Microbiology - a look at the family tree, 1996). Norberto has been honored with ASM's Bergey Medal, 1995, and having a genus (*Palleronia marisminoris*) and three species (*Pseudomonas palleroniana*, *Hydrogenophaga palleroni,* and *Actinoplanes palleroni*) named in his honor. Others similarly honored at Rutgers can be surmised from the following list: *Streptomyces fradii, S. halstedii, S. lipmanii, Penicillium waksmani, Microbispora* (syn. *Waksmania*) and *Lipomyces starkeyi* . (The Editor requests further examples.) To end on a whimsical note, Norberto's description of the new *Pseudomonas saccharophila* was initially rejected as lacking criteria regarding pathogenicity. His careful response of "not known to be pathogenic to whales and sweet potatoes" and "production of indole on egg nog agar not reported" was deemed useful and resulted in publication (J. Bact. 89:264, 1965).

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G.H. Cook Biochemistry & Microbiology Club Student Chapter and the Graduate Student Chapter of ASM—Rutgers University

Seminar: AMERITHRAX

DR. DOUGLAS BEECHER
Hazardous Materials Response Unit,
FBI Laboratories, Quantico, VA

Students: Jen, Suman, Allison, Francis and Dr. Beecher

Dr. Doug Beecher (Ph. D 90', MacMillian Lab) presented the seminar "AMERITHRAX: One FBI scientist's account of forensic microbiology and investigating the 2001 anthrax attacks" on March 26, 2009. Dr. Beecher has done extensive research on the structure and function of bacterial toxins. In 2000, he joined the FBI Hazardous Materials Response Unit (HMRU) in one of only two microbiologist positions in the bureau at that time. He has contributed to a variety of investigations involving biological threat agents and has developed and applied novel methods for microbiological sampling at crime scenes, including methods used to identify key evidence in the 2001 anthrax attack investigation. His seminar described HMRU operations, the forensic application of microbiological techniques at crime scenes, and personal experiences and observations from criminal investigations, most notably the domestic anthrax attacks of 2001.

Continued: *Our Department:*

American Society for Microbiology Student Chapter

Student Chapter of the ASM: The microbiology graduate students have founded a Student Chapter of the American Society of Microbiology at Rutgers. Membership in this chapter is open to any graduate or undergraduate student at Rutgers University. This year the ASM Student Chapter will be cooperating with the Theobald Smith Society to develop the agenda for the Meeting in Miniature, and will resume student presentations in addition to poster presentations.

The goals of this organization are to promote overall interest and undergraduate interest in microbiology at Rutgers, to improve the quality of the graduate program at Rutgers, provide aid for microbiology students, and to promote student involvement in local, state and national ASM meetings.

For more information or to join please contact **Jessica McCormick**, President at jmccormi@eden.rutgers.edu.

We are updating our contact list - please email me any changes in address or email
E-mail: maguire@aesop.rutgers.edu

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Department of Biochemistry and Microbiology
School of Environmental and Biological Sciences
Lipman Hall—76 Lipman Drive
New Brunswick NJ 08901-8525
732.932.9763

Editors: Max M. Häggblom, Douglas E. Eveleigh and Kathy Maguire