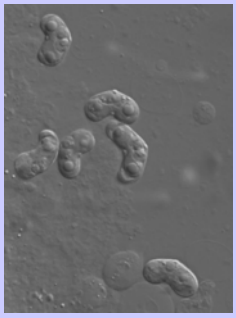
DEPARTMENT OF MICROBIOLOGY

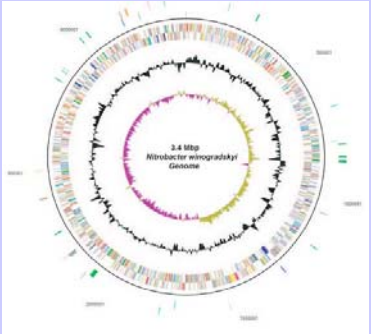
December 2010

OBSERVE



WONDER

DISCOVER



Research and Higher Education

Oregon State University OSU

FROM THE CHAIR

Season's Greetings to friends and colleagues whose lives have been touched in some way by OSU Microbiology. Microbiology continues to be a vibrant field of research with great relevance to society as evidenced by papers in almost every issue of *Science* and *Nature*. Students continue to be attracted to our major, and I was pleased to see that the success of students in obtaining employment or entering professional/graduate school after graduating these last two springs remained high despite the bleak economy and general unemployment. When I see the diversity of our upper level courses, the range of lab courses for gaining hands-on skills, and the involvement of undergraduates in cutting-edge research, I am grateful for the wisdom of our faculty and previous department heads in designing such a superb major and providing such great opportunities to students.

I am delighted to be able to strike a more optimistic note this year than in the recent past. Although declining state support for higher education remains a real problem, OSU is unexpectedly well positioned because of increased student enrolment. The increased income has been substantial because of success in recruiting students from outside Oregon, bringing out-of-state tuition dollars. OSU is projecting continued student growth beyond the current c. 24,000 and is currently embarking on the biggest faculty hiring initiative in many years (there are about 50 active tenure track searches). We will be interviewing for two positions in Microbiology in the next couple of months: the Environmental Microbiology position that we failed to fill two years ago and a Prokaryotic Systems Biology (physiology/biochemistry/genomics) position, both at the Assistant Professor level; we have around 150 applicants. OSU has over the last few months been able to make some limited investments in instructional and research equipment (from which we've benefited nicely), and expects to be able to support some faculty pay raises in coming years (scales remain below those at comparator universities). The current plan is for another 30 new faculty positions to be created in the coming year, and we will try our best to benefit from that additional opportunity. These changes will revitalize the department, whose age structure among research professors is decidedly "senior."

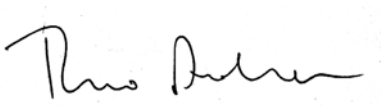
The disadvantages of the changes outlined above are that many at OSU, perhaps in particular support personnel, have been working under stressful conditions for extended periods. The positive changes have been superimposed on soul-searching reorganizations of business services and (in some cases) academic units. Discussions are still ongoing to clarify the organization of Microbiology, Biochemistry & Biophysics and Zoology into a School of Life Sciences within the College of Science (COS). We retain our connection with the College of Agricultural Sciences, although managerial actions now flow through COS.

Dick Morita

External funding will be as important as ever in augmenting university initiatives. Past students of Prof. Dick Morita recently mobilized to create a graduate scholarship endowment honoring Dick and his wife Toshi. I attended a wonderful party in October bringing together Dick and his family with past students who have now become benefactors. Alumna Joan Countryman Suit has also generously established a fund in support of graduate students this year. These gifts reflect support for graduate students as one of our greatest needs. Remodeling Nash Hall research labs is also on the wish-list of items that are not well supported by standard funding channels. Although the utilities in Nash Hall are being renovated (see notes by Cindy Fisher), the furniture and layout of our research space remains quite outdated.

On the following pages, you will find brief reports describing some of the activities in the department over the last year. I feel honored to serve the Department of Microbiology as Chair, and was recently honored by being named the Emile F. Pernot Distinguished Professor of Microbiology for the period 2010-15. Steve Giovannoni was the previous Pernot Professor (2005-10).

With best wishes for a happy and peaceful Holiday Season and 2011,



Theo W. Dreher, Ph.D.

DENNIS HRUBY LAB:

Smallpox: eradicated but not out of mind

Of all the poxviruses there is one that only infects humans - that is variola, known as smallpox. One-third of the world's population has died from smallpox through history. It spreads easily from one person to another and can even be spread from bed sheets, clothing and virus scabs. In its early stages, symptoms of the virus mimic that of the flu: high fever, severe body aches, headache and backache. The virus often goes undiagnosed, which makes it even more deadly. Often, by the time it is identified as smallpox - when the patient exhibits small, clear, fluid-filled lesions that follow a red, bumpy rash similar to chickenpox – it has already spread. It is most contagious during the first week of the infection and continues to be contagious until the scabs from the rash fall off.

One of the great successes of modern public health programs has been the elimination of circulating smallpox. However, a current concern is that known stocks preserved in the US and Russia, or virus recreated by genetic engineering, could be accidentally released or come into the hands of bioterrorists. There is no approved drug to treat or prevent smallpox or any other orthopoxvirus. And while there is a vaccine for smallpox it can have severe complications in certain individuals and mass immunization of the general population is no longer recommended. Since many countries stopped vaccination for smallpox several years ago, the majority of the population around the world now lacks immunity. The complex logistics of rapid emergency vaccination in response to a smallpox release, and the likelihood that a portion of the population would refuse or would be unsuitable for vaccination, increase the need for an effective smallpox therapeutic.

Smallpox is one of the most deadly of bioterrorist agents. It can be deliberately and easily spread by aerosol because of the stability of the virus stays in a vaporized form. The genome of the virus is in the public domain, and someone with a basic degree in biology has the rudimentary skills to reverse engineer the virus from other commonly found orthopoxviruses, such as camelpox. Most troubling, it is widely believed that not all known stores of the virus have been accounted for and the virus could potentially be in unfriendly hands.

Just one case of smallpox would be a national emergency. Measures to stop the spread would need to be taken immediately. It can be aerosolized and easily released in heavy traffic flow areas like stadiums, airports, and transit hubs. Smallpox is easy to grow and can potentially be engineered; virus containment is lacking. Quarantine measures are unlikely to be effective, vaccination is problematical, and post-exposure prophylaxis options are limited. Smallpox is asymptomatic for up to 14 days, during which time a terrorist could spread the disease quietly and broadly.

Although smallpox has been eradicated, research continues at two high security laboratories at the U.S.A. and Russia. As the World Health Assembly prepares to covene in May of 2011 to again consider destruction of existing virus stocks and cessation of current research, the Advisory Committee for Variola Virus Research met in Geneva this November to consider this question. Dr. Dennis Hruby, OSU Professor of Microbiology, presented to the Committee and participated in the deliberations. Dr. Hruby continues to play a central role in designing and implementing our nation's biodefense program.

MB 420/520 MICROBIAL DIVERSITY UPDATED:

MB 420/520, a class that has been taught for a number of years, underwent a name change last year to Microbial Genomes, Biogeochemistry, and Diversity. The change reflects updated course content, designed to teach students about how new advances in DNA sequencing and bioinformatics are changing our view of the microbial world. Students learn about the strategies used to quantify microbial diversity and the challenges faced in arriving at a bacterial "species concept." Students discover that the growing field of microbial genomics has provided support to the rRNA based tree of life, but has also revealed a whole host of new questions, for example: What are the approximately 30% of unknown genes in every microbial genome coding for? Can microbiologists identify a "core genome?" The course is a mixture of lectures and in-class activities, where class participation is strongly encouraged.

Steve Giovannoni has taught the course for several years, but Stephanie Yarwood (Instructor) joined as a co-instructor last fall to help redesign the course and position it from its biannual offering to being taught every year with the aim of attracting some non-Microbiology majors. Recommendations (the course was "different from any other course they had taken") have already resulted in increased enrollment.

BRUCE GELLER AND MANOJ PASTEY LABS:

Bird-flu vaccine

The Geller and Pastey (Biomedical Sciences, College of Veterinary Medicine) labs are collaborating on a project to make a vaccine for avian influenza. The US poultry industry produces 43 billion pounds of meat and 90 billion eggs per year, and accounts for $20 billion in annual revenue. Commercial chickens and turkeys regularly become infected with influenza virus and have to be destroyed, which can be economically devastating to the owners of the infected flock. Infected birds are highly contagious, and the disease rapidly spreads throughout the flock. In addition, some strains of avian influenza virus have acquired the ability to infect and kill humans, which poses a serious threat to human health. There is no vaccine for avian influenza currently available in the US.

Our labs have genetically engineered two different non-pathogenic bacteria (*Lactococcus lactis* and *Streptococcus gordonii*) to produce highly conserved peptides from two of the proteins on the outside surface of influenza virus. Chickens have been vaccinated with the engineered strains, and some have developed a humoral and/or cellular immune response to the specific influenza peptides. Our preliminary results have shown that at least one of the vaccines that expresses a peptide from the hemagglutinin protein of the virus protected birds from a lethal challenge. Further trials are in progress to boost the immune responses using adjuvants such as cholera toxin B subunit. These preliminary results are encouraging, and suggest that the engineered bacteria could provide an economically viable way to vaccinate US commercial poultry against avian influenza.

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STEPHEN GIOVANNONI LAB:

First investigation of the microbiology of the deepest layer of ocean crust reveals hydrocarbon oxidizing bacteria

Former COAS graduate student Olivia Mason, Microbiology Department Professor Stephen Giovannoni, and COAS geologist Martin Fisk attracted attention in the news when they reported finding hydrocarbon oxidizing bacteria in gabbro, the deepest layer of ocean crust. The work, published in PLoS One, was part of Olivia's PhD thesis. The discovery was significant because in previous work the team had found very different bacteria in basalt rocks that form the upper layer of ocean crust. The microbial community in the deep gabbro rocks resembled communities from oil formations and methane deposits, raising questions about the source of the deep hydrocarbons. That answer came from other scientists, who reported that chemical reactions between carbon dioxide, water and rocks are producing hydrocarbons abiotically (without life) in the vicinity. This work was made possible by the Integrated Ocean Drilling Program (IODP), which drilled for four months to reach 4,600 feet in the Atlantic Massif, a seamount located near the middle of the Atlantic Ocean. Since leaving OSU with her degree Olivia did postdoctoral research at Cal Tech and then moved to Lawrence Berkeley National Laboratory, where she and her husband, John, had their first child, Sophia, and Olivia began working on oil degrading bacteria. Her work came in a full circle when she was sent to the Gulf of Mexico to study the Deepwater Horizon oil spill, and subsequently published one of the most prominent microbiological papers about that event in the journal *Science*.

Olivia Mason

To read more: 1. Mason OU, Nakagawa T, Rosner M, Van Nostrand JD, Zhou J, Maruyama A, Fisk MR, Giovannoni SJ. (2010) First investigation of the microbiology of the deepest layer of ocean crust. *PLoS One.* 5:e15399. 2. Hazen TC, et al. and Mason OU. (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* 330:204-8.

MICROBIOLOGY STUDENT ASSOCIATION (MSA)

Linda Bruslind (Senior Instructor), MSA mentor

As a student club designed to promote the field of microbiology and connections between microbiology majors though education, extension activities, and community, MSA is an important part of many Microbiology students' OSU experience. As faculty mentor for this wonderful club, I get the chance to interact with a great group of students on many different levels during the year.

In May I accompanied 14 undergraduate Microbiology majors, all active members of MSA, to the American Society for Microbiology (ASM) national conference in San Diego for 4 days. This was an incredible opportunity for the students to hear about the most current research in microbiology, as well as to meet prospective employers and renowned researchers in the field. This trip and similar ones in past years have been partially supported by funds generously donated by alumna Sheila van Zandt.

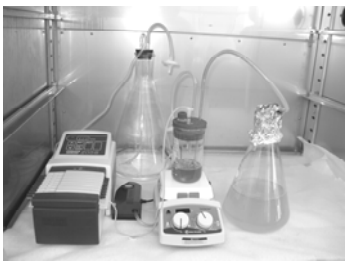
In addition to hearing keynote speakers talk about novel drug development, attending poster sessions, and meeting with industrial representatives, the students and I had several opportunities to enjoy each other's company. We attended the opening night ceremonies, with free food and entertainment. We all took the train to have dinner at the Gordon Biersch Brewery, where we met the Brew Master and received a personal tour of the brewhouse operations. We attended a San Diego Padres baseball game one evening, after eating at a local Irish restaurant. While we saved money by squeezing 3-4 to a hotel room, the students were all good sports about the close quarters! Students talked about the trip for weeks afterwards, discussing talks they had attended, things they had learned, and people they had met.

MARTIN SCHUSTER LAB:

Social life of bacteria

We work in the area of bacterial quorum sensing. We are trying to understand how and why bacteria cooperate and communicate to perform important behaviors such as virulence and biofilm formation. A highlight for the lab this year was the attendance of the *ISME* meeting, the biennial conference of the International Society for Microbial Ecology, in Seattle in August. With about 2,000 attendants, it is one of the largest microbiology conferences. Four graduate students, Rashmi Gupta, Jessica Huie, Brett Mellbye, and Cara Wilder, presented their work, interacted with scientists from all over the world, and explored Seattle in their free time. The conference offered a great venue for us to expand on ecological aspects of quorum sensing, an area of research that has primarily been approached from a molecular genetic perspective. Inspired by all this, we submitted Cara Wilder's most recent study on cooperation and cheating in bacteria (yes, even bacteria cheat!) to the society's new journal, decision pending…

Jessica Huie, Brett Mellbye, Martin Schuster Cara Wilder, Rashmi Gupta



Recent papers from the Schuster lab: 1. Wilder CN, Allada G, Schuster M. (2009) Instantaneous within-patient diversity of *Pseudomonas aeruginosa* quorum-sensing populations from cystic fibrosis lung infections. *Infect Immun.* 77:5631-9. 2. Gilbert KB, Kim TH, Gupta R, Greenberg EP, Schuster M. (2009) Global position analysis of the *Pseudomonas aeruginosa* quorum-sensing transcription factor LasR. *Mol Microbiol.* 73:1072-85. 3. Gupta R, Gobble TR, Schuster M. (2009) GidA posttranscriptionally regulates rhl quorum sensing in *Pseudomonas aeruginosa. J Bacteriol.* 191:5785-92.

MODERNIZATION OF 40-YEAR-OLD NASH HALL

Cindy Fisher

A year in construction

Nash Hall is now a year into the energy efficiency project to replace the 1970s era heating, ventilation and air conditioning system in the building. Beginning in January 2010, Anderson Construction has taken over a floor of Nash Hall every three months to install the new HVAC mechanical system and ductwork. The project completion date is December 2011. Additional improvements to Nash were made possible by the lowered cost of labor and materials due to the depressed economy. In all, new chemical fume hoods, lighting in the labs and offices, new interior paint, new windows and blinds were improvements added onto the original scope of HVAC project. No improvements or upgrades have been made to Nash since it was built in 1970, so the impact of all the changes that have taken place in the newly renovated spaces is amazing to see.

Before and after pictures Nash hallway

Moving, moving, moving

The occupants of Nash Hall purged, cleaned and packed up their labs and offices in preparation for their move to their temporary spaces, some willingly and some grudgingly. Every spare office, room and laboratory that was available in the building has been occupied by our displaced colleagues through this past year of construction. The moving days and the deadlines have been nerve racking at times for the occupants. No one has been spared the ordeal but we all have survived the experience thus far. The old joke of who's on first and who's on second has taken on a whole new meaning here at Nash.

Energy efficiency in the 21st century

The new HVAC design for the building has variable speed fans and a split system that differentiates between laboratories and offices. This allows the temperature in research labs to remain at a steady 72°F, twenty four hours a day, year in and year out. Offices, halls and other non-laboratory rooms are on motion sensors that activate lighting and temperature thermostatic controls when the area is occupied. The sensors shut off lighting and lower temperatures once the area is vacated. No hands needed!

\Before and after pictures of a lab — new lighting has made a huge difference

New chemical fume hood

Emergency generator for Nash Hall

After 40 years, Nash Hall finally has an emergency generator that will power up sensitive equipment and ultra cold freezers during power outages that occur from time to time in the northwest. The generator will be shared with the new Linus Pauling science building that is being built in the Nash parking lot. Nash and Linus Pauling are on different power grids so it will be unlikely that both buildings will need to use the generator at the same time. The generator is programmed to divide the power between the two buildings in the case of a catastrophic outage that takes out power to both buildings. The generator is located on the NW corner of the Nash patio and will be used for the first time in December for a scheduled construction power outage.

New generator

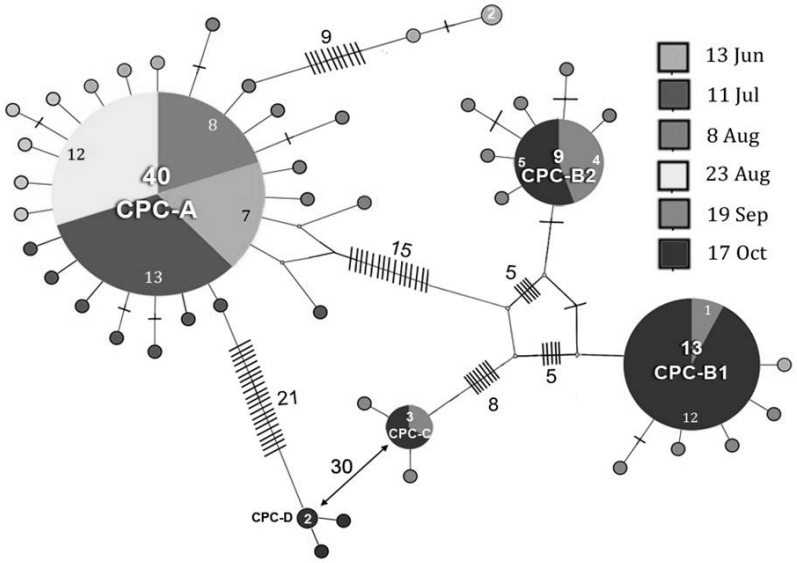
THEO DREHER LAB:

Toxic cyanobacteria in Pacific Northwest lakes and reservoirs

The Dreher laboratory is conducting studies on cyanobacterial (blue-green algal) blooms that are occurring with increasing frequency and intensity around the world. Our focus is on blooms that occur, mostly during the summer and fall, in Oregon and neighboring states. One goal is to modernize the monitoring of these blooms, which currently depends on microscopic observation, by using DNA-based identification. We would like to implement such methods in building a database of bloom occurrences in freshwater bodies (lakes, reservoirs, slow-moving streams) in Oregon. These efforts involve partnerships with governmental agencies such as US Forest Service, US Army Corps of Engineers and the Oregon Department of Human Services. There is high concern about the potential toxicity of blooms that impact recreational sites and drinking water; *Microcystis* and other genera can be associated with hepatotoxins, and *Anabaena* can be associated with neurotoxins. Dog deaths in Oregon and northern California over the last two years were probably associated with the latter neurotoxins. We conduct annual workshops at OSU to familiarize agency employees with these increasingly prevalent blooms.

Another goal of our research is to genetically identify the population structure of a bloom, revealing the precise identity of species and strains present. Such analyses can be used to track population changes over time and relationships between sites, providing insight into bloom development and decline. We recently published such a study, revealing a transition from predominantly toxigenic to non-toxigenic strains of *Microcystis* in Copco Reservoir on the Klamath River in northern California. The limited genotypes of *Microcystis* present in Copco allowed classification into discrete groups that could be a useful target for monitoring aimed at understanding bloom dynamics, which are poorly understood beyond knowing that excess nutrients are the major drivers.

To read more: 1. Bozarth CS, Schwartz AD, Shepardson JW, Colwell FS, Dreher TW. (2010) Population turnover in a *Microcystis* bloom results in predominantly nontoxigenic variants late in the season. *Appl Environ Microbiol.* 76:5207-13.



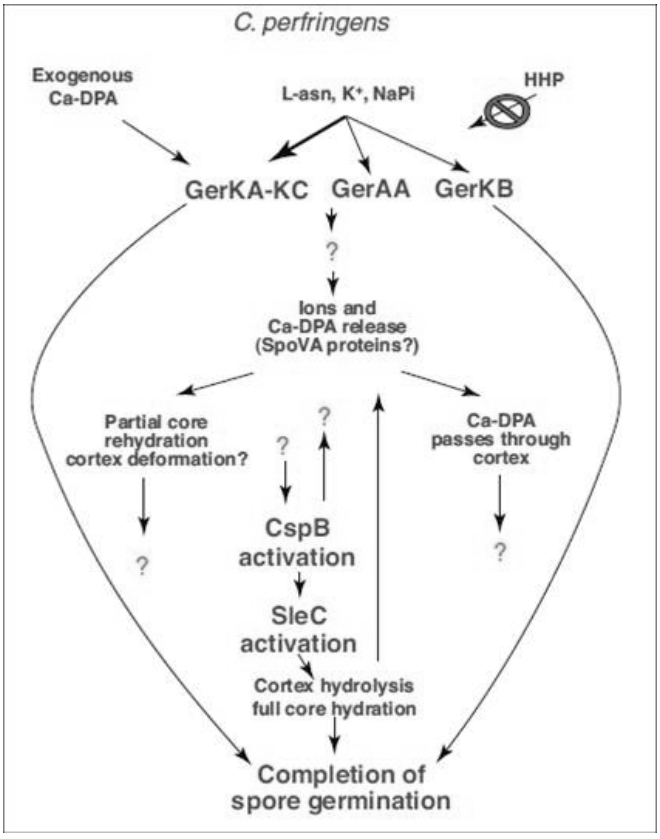
MAHFUZ SARKER LAB:

Mahfuz Sarker

A graduate student with about 20 peer-reviewed papers

Clostridium species are important anaerobic, Gram-positive, spore-forming, enteric bacterial pathogens. *Clostridium* dormant spores are highly resistant to heat and other environmental insults, and can survive for long periods in the environment. Once conditions are favorable, these spores undergo germination, an irreversible process by which a dormant spore is transformed into a metabolically active cell. These *Clostridium* cells then produce toxins and cause disease in humans and animals. One approach to develop efficient therapies against clostridial diseases is to block or induce spore germination. Blocking spore germination would block the resumption of growing vegetative cells, while inducing germination would yield spores that have lost their resistance properties to conventional sanitization treatments applied in the food industry and in clinical settings, and thus becoming more sensitive to inactivation by milder treatments. Furthermore, a detailed understanding of spore-host interactions is also likely to yield novel insights on the development of alternative therapies for clostridial infections.

Research in *Clostridium* species is very challenging, exciting, and highly rewarding. These attributes allow students in my lab to establish an excellent student-mentor relationship. Currently I have 1 MS, 3 PhD and 1 postdoctoral fellow from different countries such as USA, Saudi Arabia, Thailand and Chile. A clear example of how exciting and rewarding *Clostridium* research can be in my lab is the case of Dr. Daniel Paredes-Sabja. He joined my lab in 2004 to pursue his PhD studies. After successful completion of his PhD degree, he stayed as a post-doctoral fellow from July, 2009. During his stay in my lab, he established a tightly woven environment with other students in the lab. He pursued research on many new important research avenues such as, i) molecular mechanisms of spore germination; ii) developing spore inactivation strategies; and more recently iii) molecular mechanism of spore-host interactions. His excitement for research has led him to publish 19 papers in peer reviewed journals and 2 book chapters. In addition, he has 3 manuscripts currently under review and 4 under preparation.



Dr. Paredes-Sabja's intensive training from my lab has allowed him to obtain a competitive faculty position (tenure-track Assistant Professor) at the Universidad Andres Bello, in his native country, Chile. During his stay in my lab, he understood the urgency for research in the *Clostridium* field and thus he has planned to continue research on molecular pathogenesis of *Clostridium* species and establish a strong long-term collaboration with my lab.

To read more: 1. Paredes-Sabja D, Setlow P, Sarker MR. (2010) Germination of spores of *Bacillales* and *Clostridiales* species: mechanisms and proteins involved. *Trends Microbiol.* Nov 26. [Epub ahead of print].

2. Paredes-Sabja D, Udompijitkul P, Sarker MR. (2009) Inorganic phosphate and sodium ions are cogerminants for spores of *Clostridium perfringens* type A food poisoning-related isolates. *Appl Environ Microbiol.* 75:6299-305.

3. Paredes-Sabja D, Setlow P, Sarker MR. (2009). The protease CspB is essential for initiation of cortex hydrolysis and dipicolinic acid (DPS) release during germination of spore of *Clostridium perfringens* type A food poisoning isolates. *Microbiology* 155:3464-72.

JERRI BARTHOLOMEW LAB:

Graduate Student Emily Nebergall:

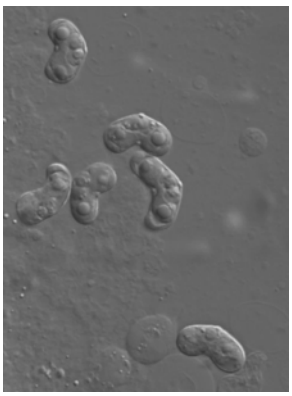
I completed my undergraduate research at the College of William & Mary in Virginia, studying a streptococcal pathogen of striped bass from the Chesapeake Bay. *Streptococcus parauberis* is a poorly understood emerging pathogen of fish; when I began my research, there was no genomic sequence available for this species in GenBank. Furthermore, *S. parauberis* had not previously been seen in fish in North America. I uncovered the presence of virulence factor genes using degenerate primers designed with gene sequence from other closely related streptococcal pathogens. At the Virginia Institute of Marine Science, I conducted a challenge study using a zebrafish model to compare Chesapeake Bay *S. parauberis* isolates to pathogenic and non-pathogenic reference strains from overseas. This fall, I joined the OSU Department of Microbiology as a Master's student.

My graduate research at OSU focuses on the ecology of disease resistance in endangered frog species, such as the chiricahua leopard frog, *Rana chiricahuensis*. Amphibians carry a distinct and diverse bacterial community on their skin, and some members of that community secrete anti-fungal metabolites. When anti-fungal bacterial species are present at high enough levels, they can confer resistance to the fungal pathogen *Batrachochytrium dendrobatidis (Bd),* the causative agent of Chytridiomycosis. *Bd* is an emerging pathogen that has caused population declines and extinctions in over 200 amphibian species. Efforts to combat this disease may in the future involve "bioaugmentation" of amphibian skin with anti-fungal bacteria naturally found at less than inhibitory levels on most individuals in a population. Because disruption of normal microflora is associated with disease, bioaugmentation should be undertaken with a comprehensive understanding of the interactions between amphibians and bacterial symbionts. To optimize bioaugmentation strategies for multiple frog species, I will isolate commensal skin bacteria from wild individuals, and test those isolates *in vitro* to identify anti-fungal strains. I will also test the inhibitory effect of those strains *in vivo* by challenging frogs against *Bd* with and without prior bioaugmentation treatments. Funding for this work is through the US Geological Survey, as this project is a component of the recovery plan that seeks to improve the status of threatened *Rana chiricahuensis* frogs from Arizona and New Mexico.

Thesis title: Diversity of anti-chytrid bacteria present in amphibian skin communities.

Graduate Student Matthew Stinson:

Fish and fish diseases are how I would describe my career as a biologist. It was only natural that I came to Oregon State University to pursue my master's degree in Microbiology to learn about the pathogens associated with fish in the wild and in hatcheries. My studies have mostly focused on *Ceratomyxa shasta* (*Cs*), a myxozoan parasite that is found only in the Pacific Northwest. A survey of adult salmon has shown that there are fish species-specific strains of *Cs* and this finding has important management implications. Studies that I conducted in the nearby Willamette and Deschutes Rivers revealed that there is seasonal variation in the different *Cs* strains, which is likely linked to when adult salmon return from the ocean to their natal freshwaters. Dams that segregate rivers have restricted the movement of salmon and isolated certain strains of the parasite. My studies identified salmon species that were resistant to *Cs*, and revealed that a little known strain of *Cs* infected a broad range of salmon species. Other projects I have been involved with explore whether hatchery and wild fish differ in their susceptibility to viral, bacterial and parasite challenges.



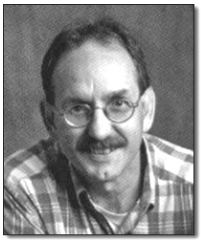
C.s. myxospores

Severely infected rainbow trout

Funding for my research is provided by the Oregon Department of Fish & Wildlife and PacifiCorp. I have presented my research results at Oregon Chapter American Fisheries Society, Oregon Parasitologists, Western Fish Disease Workshop, Pacific Northwest Fish Health Planning Committee, and Klamath River Fish Health Workshops. I plan to defend my master's thesis in 2011 with aspirations to continue working with fish diseases for the state of Oregon. You can learn more about my research at: http://microbiology.science.oregonstate.edu/barthol_lab_stinson.

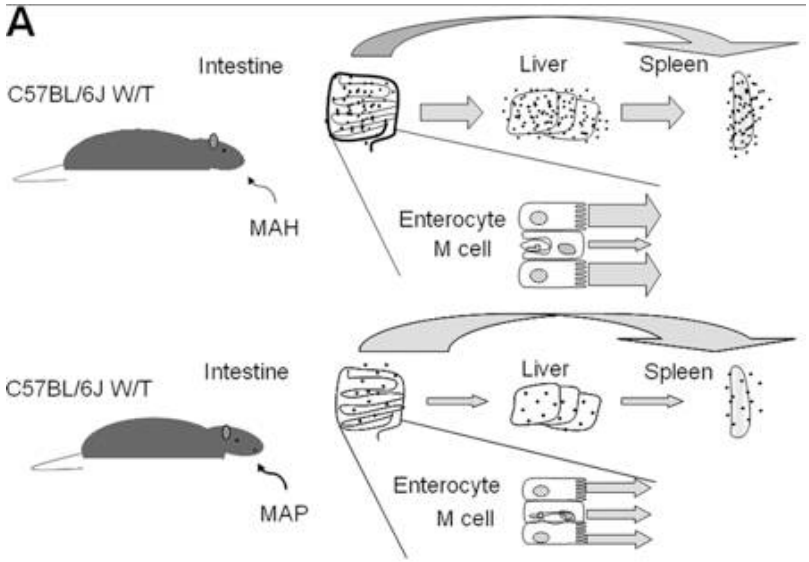
LUIZ BERMUDEZ LAB:

Johne's Disease impacts the dairy industry



Johne's disease (JD) is a significant economic problem for farms in the United States and Oregon in particular. The production loss for any dairy herd seems to be related to the JD prevalence and the average age or lactation status of the herd. JD incurs substantial veterinary costs associated with the treating the resultant diarrhea. As there is no effective cure, it is important to recognize JD early to reduce the amount of treatment and risk of antibiotic residues in milk or meat. Sixty-six percent of dairy herds may have at least one JD infected cow and 42% of herds may have 2 infected JD cows nationwide, while in California the percentage of herds with JD is as high as 90%. JD is caused by *Mycobacterium paratuberculosis* that is acquired by ingestion at early age. The cumulative lost productivity costs to the U.S. dairy industry from JD are estimated at $200-$250 million annually.

Based on the knowledge that *M. paratuberculosis* can be acquired from a number of environmental sources, we designed studies to investigate the efficiency of the pathogen, obtained from a variety of sites, to cause infection of bovine intestinal epithelial cells. The results, suggested that bacteria present in milk or feces had several-fold more ability to infect intestinal cells than bacteria obtained from other sources. As a second step, we screened for *M. paratuberculosis* genes associated with infection of bovine intestinal cells. We identified a number of genes, and further proved the role of those genes in a mouse model for intestinal infection. Using several of the discovered genes, we determined at the molecular level how *M. paratuberculosis* interacts with the bovine intestinal epithelium. Then, using the knowledge that by incubating the bacterium in milk resulted in much higher efficiency of infection, we looked for genes that were expressed when the bacterium is incubated in milk. As a result of this experiment, we discovered a chief virulence regulator in the bacteria, and several surface proteins that are under the influence of the regulator. Next, we created mutants unable to express each of these proteins on their surface, and discovered that these proteins are associated with the binding of *M. paratuberculosis* to the intestinal epithelium. In their absence, bacteria are no longer able to infect epithelial mucosal cells. In collaboration with a USDA laboratory in Iowa, we are now producing antibodies against these specific *M. paratuberculosis* surface proteins to determine in an animal model if antibodies to these receptors can prevent infection by blocking bacterial binding to the intestinal epithelium. If the strategy works, we will be able to vaccinate adult cows and stimulate the production of protective antibodies in the milk. Together with hygienic measures, this should have a major impact on JD disease. This indirect vaccination approach is necessary because of the difficulty in obtaining systemic protection against mycobacterial infection by vaccines.



To read more: 1. Alonso-Hearn M, Eckstein T, Sommer S, Bermudez LE. (2010) A *Mycobacterium avium subsp. paratuberculosis* LuxR regulates cell envelope and virulence. *Innate Immunity* 16:235 2. Bermudez LE, Sommer S, Petrofsky M, Barletta R. (2010) Peyer's patch deficient mouse indicates that *Mycobacterium paratuberculosis* crosses the intestinal mucosa by both M cells and enterocyte routes. *Infect Immun* 78:3570.

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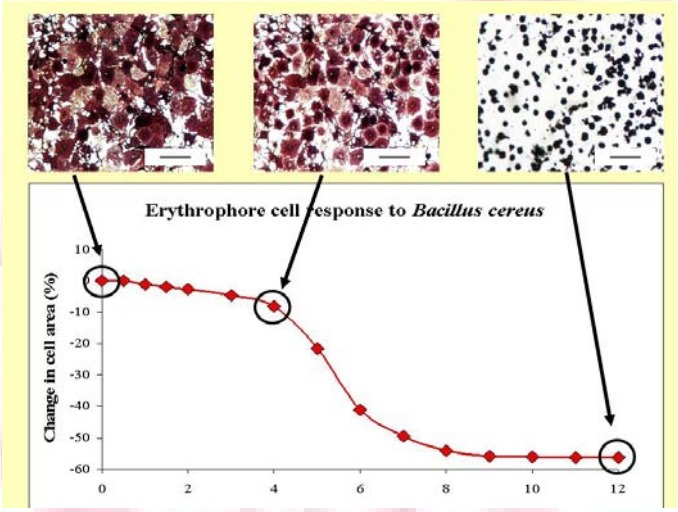
JANINE TREMPY LAB:

Fish cells as microbial biosensors

The Trempy research program analyzes the response of fish pigment cells, called chromatophores, to bacterial and chemical toxicity. Chromatophore cells redistribute their pigment organelles in a characteristic pattern upon exposure to toxic substances. Pigment redistribution within the chromatophore cell can be easily assessed, both visually and mathematically. Earlier models for testing the hypothesis that chromatophores could be used as cell based biodetectors for assessment of toxicity focused on the use of chromatophores from the brilliantly colored *Betta splendens* (Siamese fighting fish). This program made the discovery that chromatophores from *B. splendens* could rapidly detect (in less than 10 minutes) bacterial toxicity and contamination induced by certain food-associated pathogenic bacteria (*Salmonella enteritidis, Bacillus cereus, Clostridium botulinum).* We extended these studies to other fish species, selecting *Oncorhynchus tshawytscha* (Chinook salmon) as a source of chromatophores to compare chromatophores from fish species inhabiting two different ecological niches. We discovered that chromatophores from *O. tshawytscha* and *B. splendens* share a similar response to pathogenic bacteria and chemical toxicants (such as mercury and arsenic), suggesting this response to toxicity is biologically conserved. Chromatophore cells from both fish species have great potential for use as cell based biodetectors to rapidly assess toxicity. Chromatophore based biodetectors assess the toxicity and viability of a contaminating agent rather than just its present or absence. Instead of responding to the presence of a conserved nucleic acid or antibody structure, chromatophore based biodetectors relay a signal based on the toxic behavior of a particular biological or chemical agent. This allows for a much more realistic appraisal of the impact of the contaminating bacteria or chemicals. Recently graduated Ph.D. students from the Trempy research program, Stephanie Dukovcic and Janine Hutchison, were the enthusiastic drivers of this successful research effort. They both experienced success upon graduation by rapidly finding jobs and having their research published.



To read more: 1. Dukovcic SR, Hutchison JR, Trempy JE. (2010) Potential of the melanophore pigment response for detection of bacterial toxicity. *Appl Environ Microbiol.* 76:8243-6. 2. Dukovcic SR, Hutchison JR, Trempy JE. (2010) Conservation of the chromatophore pigment response. *J Appl Toxicol.* 30:574-81.