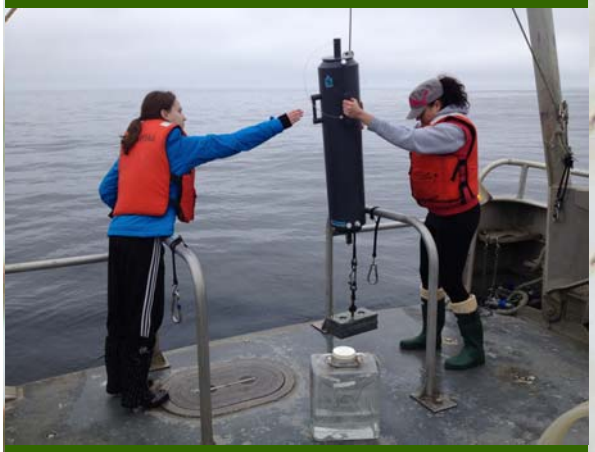
DEPARTMENT OF MICROBIOLOGY

December 2013

Research and Higher Education

OBSERVE

W O N D



DISCOVER

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Oregon State UNIVERSITY

FROM THE CHAIR

Greetings, Friends of Microbiology, from Nash Hall as we see 2013 coming to a close!

If you haven't visited the Corvallis campus and the Nash Hall vicinity recently, the surroundings will probably seem quite unfamiliar to you on your next visit. That's because we've had a heady few years with continuing growth in student numbers and consequent sustained faculty hiring and building on campus. After years in which the Space Committee had very few difficult or controversial decisions to make, we are now facing space shortages, especially of office space. We now wish we could remodel lab and office spaces to make their use more efficient for current times and the increasing impact of regulations that limit the usefulness of office space for students within labs. With more students, most courses have had to move to larger classrooms, and there has been a building boom across campus to address similar problems in all departments and colleges. In a couple of years, Nash Hall will be surrounded by three new buildings: the 2-year old Linus Pauling Science Center to the west and two buildings in the midst of construction on what was until recently a grassy field to the south: a major classroom building with seats for about 2000 students that will also be the home of the Honors College, and Austin Hall, the new home for the College of Business.

It was our pleasure this year to welcome a new Assistant Professor, **Tom Sharpton**. Tom, who describes his research on page ?, started his position in October. He was recruited through an unusual cluster hire targeted towards scientists with core competence in both biology and a quantitative science. Tom has 0.67 FTE with Microbiology and 0.33 FTE with Statistics. The three others hired through this initiative have joint positions with Biochemistry & Biophysics and Computer Science/Electrical Engineering, Mathematics and Integrative Biology (formerly Zoology), and Biomedical Sciences (VetMed) and Computer Science/Electrical Engineering. These new professors add to a very strong competency in bioinformatics and biologically oriented mathematical modeling and statistics at OSU and in Microbiology. These are increasingly important components of modern biological research.

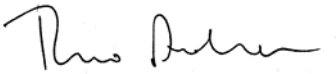
We have also extended a warm welcome this fall to the new Dean of the College of Science, **Sastry Pantula.** Dr. Pantula led the Department of Statistics at North Carolina State University for 8 years and recently was a division director at the National Science Foundation. He is working closely with Dan Arp, an OSU Distinguished Professor who took over as Dean of the College of Agricultural Sciences a bit over a year ago, in guiding the two colleges that include a few shared departments, such as Microbiology.

Several news releases this year have featured research based in the department: Assistant Professor **Becky Vega-Thurber** and studies on infectious and other causes of coral reef decline; Professor **Bruce Geller** and the use of antisense nucleic acid mimics as a new type of antibiotic; Professor **Stephen Giovannoni** and the discovery of abundant bacteriophages infecting the ubiquitous open ocean bacterium *Pelagibacter*, Postdoctoral Scholar **Tim Otten** discussing the expectation of intensification of toxic freshwater cyanobacterial blooms with increasing lake eutrophication. Our research is live and well!

This year, Associate Professor **Martin Schuster** is on sabbatical leave, visiting two laboratories in Germany to learn first-hand the underpinnings of mathematical modeling and its application to studying cell-cell interaction and gene expression circuits. He'll be applying that approach to his studies on quorum sensing and sociobiology in *Pseudomonas aeruginosa*. Martin was awarded a fellowship from the Alexander von Humboldt Foundation to partially support his sabbatical.

Professor **George Rohrmann** formally retired this summer after 36 years at OSU. His research on the insect-infecting baculoviruses, which are widely used as vectors for protein expression, has been internationally recognized for its scope and impact. He has published about 120 papers on baculoviruses between 1977 and 2013. George taught Virology in recent years and before that in the Molecular and Cellular Biology graduate program. We've been grateful for his contributions to OSU!

The following pages describe some of the research and teaching activities in the department. I trust you'll find some interesting reading during the coming holiday season, perhaps over an egg-nog or mulled wine in front of a warm fireplace. Happy, Healthy and Peaceful Holidays and New Year!

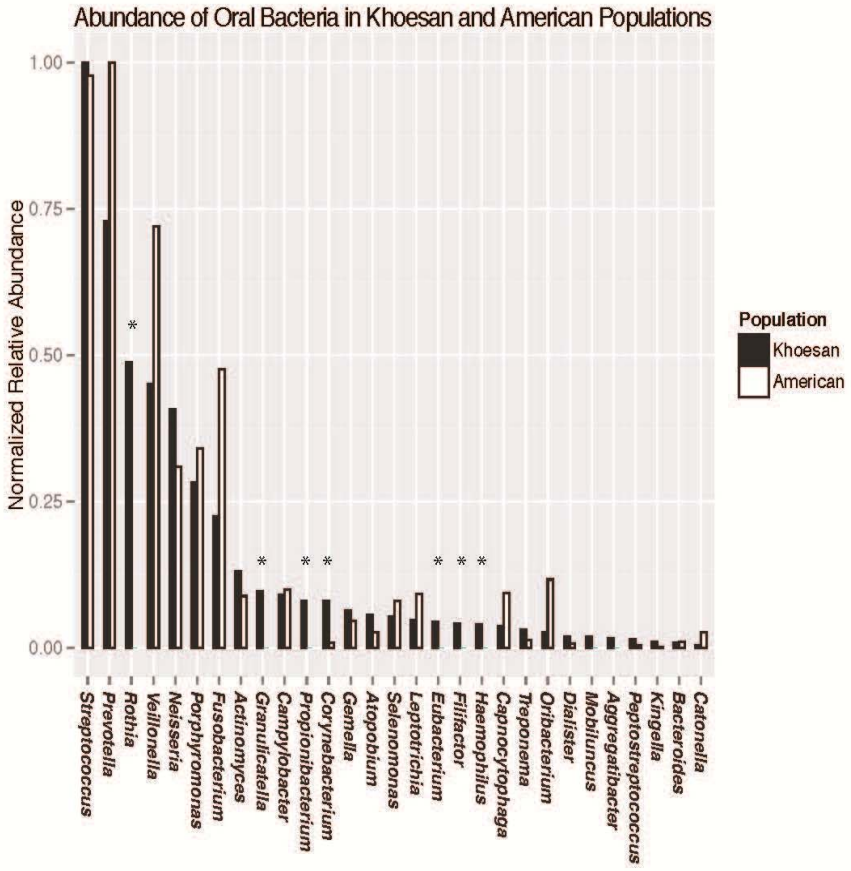


TOM SHARPTON LAB:

Thomas Sharpton joined the department as an Assistant Professor in October. Research in his lab is broadly directed towards ascertaining how human commensal microbiota and their genomic characteristics (*i.e.,* the human microbiome) relate to health. His laboratory specializes in the development and application of high-throughput computational and statistical tools that characterize microbiome biology, and investigates how microbiomes are distributed across space, time, and host physiology. The Sharpton lab aims to develop testable hypotheses about how humans and their microbiome interact, and strives to understand the evolutionary and ecological processes that influence community assembly, maintenance, and function within a host. Ultimately, this knowledge will be used to discover disease mechanisms, identify predictive and diagnostic biomarkers of disease, and develop tools to treat disease through manipulation of the microbiome. All of the data resources and software that his lab develops are freely available.

Current research in the Sharpton lab includes the development of bioinformatic tools that improve the analysis of microbiome function, investigations into the relationship between gut microbiome diversity and inflammatory bowel disease, and characterization of the composition of the oral microbiome associated with ancient human populations (*e.g.,* the Khoesan) to ascertain how humans and their microbiomes have coevolved. For example, we recently compared the Khoesan oral microbiome to the oral microbiome of healthy Americans and found that, while there are strong similarities in the microbiome composition between these two populations, the Khoesan harbor bacteria that are not detected among the Americans, often at high abundance. Many of these bacteria contribute to oral disease, suggesting that access to dental care may drive some of the interpopulation variation in oral microbiome structure. We are working with collaborators to explore how these differences translate across additional human populations and how they may relate to health differences between individuals.

Khoesan Bushman
Credit: Ian Beatty
http://en.wikipedia.org/wiki/Khoisan



Kidd and Sharpton, et. al. *Genome Biology* (In Review)

PETER BOTTOMLEY LAB:

MB448/548; 35 years and counting!

Well, here we are, December, 2013; 2014 will be my 35th year at OSU. Next spring I will teach Microbial Ecology for the 35th consecutive time. When I showed up in 1979 the Microbial Ecology class had not been taught for a couple of years and the chair, John Fryer, was anxious to have it taught again. During those first few years my classroom in Nash Hall was often full beyond its capacity (~50 seats) with folks sitting in temporary seats in the middle aisle and at the front of the class. Not that I was a "rockstar" teacher by any stretch of the imagination; it was more to do with microbial ecology emerging as the real deal, and I was the only show in town at that time. Grading was an awful grind as I persisted with the use of essay questions for both midterm and final regardless of enrollment. To further reinforce the idea that I (might) have serious mental issues, I have always graded my own tests without TA assistance. Ah… the good old days.. chalk talks at the blackboard, which predated my overly long use of transparencies on the overhead projector!! Just in case a few of you are wondering, I now use PowerPoints! The large enrollment bubble did burst and reality did set in at a more realistic number of about 15-20 students per year for the past decade.

The major challenges associated with teaching Microbial Ecology (then and now) involve dealing with the varying backgrounds of the students who take the class, and the fact that the class is a 400/500 split with the percentages of undergrads and grads fluctuating widely from year to year. In the early days of the class, in particular, there were some with unusual "backgrounds". Many students were older than I was at the time; I recall they came from various walks of life including a lumber mill sawyer, concrete guy, mushroom collectors, tree climbers, and back-to-nature hippies eking a living out in the Coast Range. They were enthusiastic… albeit somewhat under prepared.

It is interesting to reflect back and think about how the course content changed over the years. My first attempt at a curriculum was biased toward "soil microbiology". Historically, the class had been a service course for students from the College of Agricultural Sciences, and since the latter was paying half of my salary, I was "encouraged" to start with that emphasis. Also, of influence in the 70's and early 80's, was the widespread belief among many microbiologists that Microbial Ecology was really just another acronym for "Applied and Environmental Microbiology". As a consequence, early versions of the course emphasized applied/practical/problem solving aspects of Soil Microbiology. On the other hand, I was a bit of a "hard-ass" firmly believing that to appreciate Microbial Ecology, students needed to integrate their knowledge of the physical sciences of chemistry, physics, and math with the Microbiology. No wonder I had lots of "confused" people attending my office hours, and there were many who suffered during the long evening midterm!

When Dave Myrold was hired as a bona fide soil microbiologist by the Department of Soil Science in the mid 80's, I was able to deemphasize Soil Microbiology in my class. In addition, as the 1980's progressed into the 90's, microbial ecology really emerged as a bona fide sub discipline of microbiology. This came about because of the financial resources that were injected into microbial ecology research by the various funding agencies of federal governments throughout the world. This was driven by the disturbing fact that microbially generated products such as nitrogen oxides, methane, and reduced sulfur compounds were accumulating in the atmosphere of the Earth at unprecedented rates. In addition, there was increasing awareness that the subsurface of the Earth and that the groundwater resource was being polluted with fossil fuels and other man-made hydrocarbon products (remember how many of our local gas stations closed permanently during the 80's and 90's because of leaking underground storage tanks!). On a more restricted scale, the public became aware of serious subsurface pollution that was a legacy of the large-scale manufacture of atomic weapons and munitions during the "cold war". Not surprising, therefore, my approach to teaching Microbial Ecology morphed in response to the unprecedented growth of knowledge in microbial ecology that spread across different subspecialties of science, agriculture and engineering. Specific topics are continually being added to the course content, other topics are deemphasized, and others refitted and recycled.

I'll never forget a comment from Stan Gregory (Professor of Fisheries and Wildlife who taught Limnology in the adjacent classroom): …. Bottomley… what the &*%# do you know about ecology… you are just a small-minded microbiologist with a "pinhead's" perspective on life!. Partly due to Stan's ribbing, and partly due to my own perspective broadening over the years, I have tried to build a course that compares and contrasts the microbial ecology of different environments, and that treads a balance between paying attention to the details of microbial "minutiae" while also placing the microbe into context with global scale phenomena.

Over the past 3 years we have recruited several outstanding young microbiologists to our faculty. Many of their research interests can be defined as "microbial ecology." One new colleague, Kim Halsey, will co-teach Microbial Ecology with me in Spring 2014 and transition to full responsibility. Although I'm not sure how much longer I will stay around to participate in the class, regardless, it will be in good hands. It will be interesting to follow the "newbies" in future issues of the Newsletter to see how "Microbial Ecology" influences their own contributions to the teaching program. Best wishes to you all for the year of 2014.

WALT REAM LAB:

Agrobacterium effector proteins bind host transcription factors that stimulate plant transformation and suppress host defenses

This year the Ream laboratory collaborated with Stan Gelvin's laboratory at Purdue University to complete a study of *Agrobacterium rhizogenes* effector proteins that stimulate plant transformation. Wei Wei, a second year PhD student, and Dr. Maciej Maselko, a postdoctoral fellow, did the work at Oregon State, joined by Drs. Yaling Wang and Lan-Ying Lee at Purdue. OSU undergraduates Daniel Barrack and Katie Zimmerman assisted. A grant from the National Science Foundation funded the research in both laboratories. Wei Wei received support from the Wei Foundation (no family relation) and Maciej received a fellowship from the USDA National Institute for Food & Agriculture (NIFA) program. A brief description of their work follows.

Agrobacterium tumefaciens and *A. rhizogenes* transfer plasmid genes and virulence proteins into plant cells. Transferred DNA (T-DNA) is inherited and expressed in plants, causing crown gall or hairy root disease. Disarmed *A. tumefaciens* is widely used to create transgenic plants for research and biotechnology. DNA transfer from *A. tumefaciens* into plants resembles plasmid conjugation: a type IV secretion system (T4SS) exports single-stranded T-DNA (T-strands) and proteins from the bacteria. One exported protein, VirE2, coats T-strands and may mediate their nuclear import. Despite the importance of VirE2 for gene transfer, some strains of *A. rhizogenes* lack VirE2 but still transfer T-DNA efficiently.

The *GALLS* gene from *A. rhizogenes* substitutes functionally for *virE2*, but GALLS proteins do not resemble VirE2. GALLS protein is made in two forms: full-length (GALLS-FL) and a more abundant C-terminal domain (GALLS-CT) translated from an internal in-frame start codon. GALLS-FL is essential for transformation of all hosts, whereas GALLS-CT is not essential but stimulates gene transfer to many plant species, including *Arabidopsis thaliana.* Both GALLS proteins have protein-binding domains and T4SS signals. GALLS-FL also has ATPase/strand transferase and nuclear localization signal (NLS) motifs. GALLS-FL enters the nucleus and binds VirD2, a pilot protein attached to the 5-prime ends of T-strands. The putative strand transferase of GALLS-FL may pull T-strands into the nucleus.

GALLS-CT stimulates an early step in gene transfer, apparently by binding host transcription factors that suppress host defenses. Overexpression of these transcription factors in transgenic *A. thaliana* increases expression of known repressors of plant defenses (*WRKY38*, *WRKY62*, and *NIMIN1*). Overexpression of *WRKY38* in *A. thaliana* makes plants more susceptible to *Agrobacterium*-mediated transformation (Fig. 1).

Efficient delivery of single-copy transgenes is desirable for plant science and biotechnology. GALLS is superior to VirE2 for plant transformation. Using otherwise isogenic strains, GALLS-mediated transformation of *A. thaliana* yielded ~30% single-copy transgenes and ~6-fold more transgenic plants than did VirE2, which gave <10% single copy events.

Improvements in plant transformation are crucial to advance our understanding of plant biology and to improve crops to feed growing populations and overcome challenges posed by climate change. *Agrobacterium*-mediated gene transfer is the preferred means to create transgenic crops, which are planted on >100 million acres and save US farmers ~$2 billion annually while reducing pesticide use by >46 million pounds. The findings from our research will help us better understand how plants respond to the GALLS effector proteins transferred by *A. rhizogenes* into plant cells during infection. We have identified genes that make plants more susceptible to transformation, which may improve transformation of recalcitrant crop species.

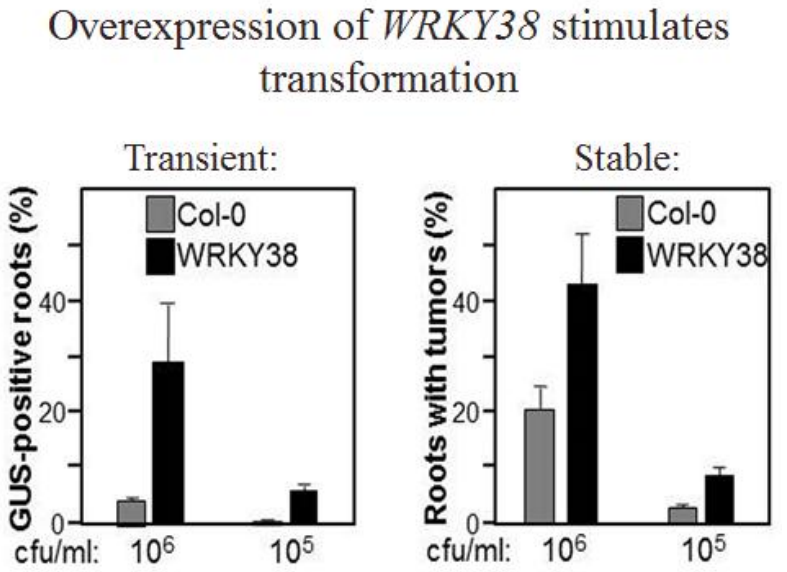


Figure 1. Overexpression of *WRKY38* in transgenic *Arabidopsis thaliana* stimulates transient (left panel) **and stable** (right panel) **transformation.** Roots from wild-type Col-0 (gray bars) and *WRKY38*-transgenic plants (black bars) inoculated with *A. tumefaciens.* Cfu/ml indicates inoculum concentration.

KIMBERLY HALSEY LAB:

Growth strategies of some of the most important microbes on the planet

Over the past decade, DNA sequencing and remote sensing technologies have developed at a tremendously fast rate, and the explosion of data generated from these platforms gives high resolution spatial and temporal views of ocean plankton communities. Phytoplankton are tiny single-celled plants in the ocean that are responsible for nearly half of the photosynthesis on the planet. (For an eight year view of global phytoplankton distributions, check out this link: http://oceancolor.gsfc.nasa.gov/SeaWiFS/HTML/SeaWiFS.BiosphereAnimation.html)

One of the main projects in our lab is to understand the different growth strategies used by phytoplankton species that cause the observable variations in their distributions and rates of productivity. Phytoplankton primary productivity initiates the marine carbon cycle, nutrient recycling by heterotrophic respiration, and carbon sequestration to the deep sea. In a manuscript published this year in *New Phytologist*, we demonstrated that two very unrelated phytoplankton species use nearly identical strategies of photosynthetic energy utilization when growing under a wide range of different nitrogen availabilities (about 70% of the global oceans are nitrogen limited with concentrations <1 μM). To study these growth strategies, we tracked the flow of carbon and energy through multiple metabolic pathways that terminate with net carbon accumulation (cell growth). When growing under extreme nitrogen stress, both a large green algae (*Dunaliella tertiolecta*) and a diatom (*Thalassiosira weissflogii*) allocate the majority of their fixed carbon to very short-lived carbon forms. In contrast, cells growing in more nitrogen rich environments allocate the bulk of their fixed carbon to long-lived carbon forms, including polysaccharides and lipids. Furthermore, across all nitrogen concentrations and for both species, the amount of carbon available for consumption by higher trophic levels is only 30% of the total energy harvested.

These results have a number of interesting ramifications. First, despite being evolutionarily distinct, the common strategies for carbon and energy partitioning suggests that principles of energy economy may operate across a broad range of phototrophs. In fact, a study also published in *New Phytologist* showed a nearly identical partitioning strategy is used by trees (maples) in a temperate forest, although the turnover times of the short and long-lived carbon pools are, of course, very different. Second, as the oceans warm, they are predicted to become more stratified, making the surface layer where phytoplankton are photosynthetically active increasingly nutrient limited. Our results suggest that under these nutrient-poor conditions phytoplankton carbon composition will become less energy rich. There is strong evidence that as phytoplankton become more nutrient limited they will be of lower food quality for higher trophic levels, thereby impacting food-web dynamics.

Phytoplankton are also an attractive system for bioenergy and bio-product production because their growth draws down atmospheric CO_2, has minimal impacts on fresh water resources, and does not compete for agricultural land. Phytoplankton can also be manipulated genetically, and altering their growth environment can yield a wide range of carbon end-products, including oils for biofuels, bioenergy precursor molecules, as well as vitamins and proteins. The research described above forms a strong basis for understanding how cells adjust their growth processes to maximize bio-product accumulation. We are pursuing research to collect gene and protein expression data and evaluate the corresponding metabolic process that can be optimally tuned for bio-product accumulation.

Joining the lab this year are Dr. Bethan Jones (back left), a post-doctoral researcher with expertise in proteomics and coccolithophore physiology; Nerissa Fisher (front right), a Masters student in Microbiology with a passion for phytoplankton; and Rachel Tullsen (back right), undergraduate Microbiology student. I'm front left.

A couple of other students participated in lab research this year, including Janet Berkstresser who graduated with a B.S. in Microbiology, and Jake Dittrich. Jake graduated with a B.S. from the Honors College in BioResource Research. His thesis described his research on *Synechococcus* WH8102. We are currently following up on some of his interesting findings for this cyanobacterium.

To read more:

Halsey, K.H., R.T. O'Malley, J.R. Graff, A.J. Milligan, and M.J. Behrenfeld. 2013. A common partitioning strategy for photosynthetic products in evolutionarily distinct phytoplankton species. *New Phytologist* **198**:1030.

MAHFUZ SARKER LAB:

Clostridium spores: formation, germination and food poisoning

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 transition to growing vegetative cells, while inducing germination would yield spores that have lost their resistance properties, thus have become more sensitive to inactivation by milder treatments. A brief summary of recent projects follows.

1) Molecular mechanism of spore germination: Previous studies have indicated that the germinant receptor (GR) proteins encoded by the bicistronic *gerKA-KC* operon are required for normal germination of *C. perfringens* spores. We now have studied the individual roles of these GR proteins by analyzing the germination of strains carrying mutations in their genes. Conclusions from this work include the following: 1) *gerKC* mutant spores germinated more slowly in response to a range of inducers (KCl, L-asparagine, NaPi, a 1:1 chelate of Ca^{2+} and dipicolinic acid); 2) the germination defects in mutant *gerKC* spores were largely restored by expressing the wild-type *gerKA-gerKC* operon in trans; and 3) GerKC is located in the spore's inner membrane, with ~ 250 molecules/spore. GerKC is thus the main GR protein required for nutrient and non-nutrient induced germination of spores of *C. perfringens* food poisoning isolates.

2) Spore inactivation strategies: The contamination of *C. perfringens* spores on food contact surfaces poses a serious concern to the food industry due to their high resistance to various preservation methods controlling food-borne pathogens. We aimed to develop a strategy to inactivate *C. perfringens* spores on stainless steel (SS) surfaces by inducing spore germination and killing of germinated spores with commonly used disinfectants. The mixture of L-asparagine and KCl (AK) at 40 °C was effective in inducing spore germination for all tested *C. perfringens* food poisoning (FP) and non-food-borne (NFB) isolates. Prior AK-induced germination enhanced the inactivation of spores by treatment with disinfectants for FP strain SM101 but not NFB strain NB16. This was true in spore suspensions and for spores adherent to SS chips. Consequently, the incorporation of an AK-induced germination step prior to decontamination of SS chips with disinfectants significantly ($p < 0.05$) inactivated the spores of FP isolates. The inclusion of a germination step should thus provide a practical and effective strategy to inactivate *C. perfringens* spores adherent to food contact surfaces.

3) Molecular mechanism of spore formation: In collaboration with the laboratory of Dr. Paredes-Sabja (former PhD graduate from Sarker Lab, currently Assistant Professor at Universidad Andrés Bello, Santiago, Chile) the Sarker lab has studied the mechanism of *Clostridium difficile* spore formation. The exosporium cysteine-rich protein (CdeC) is expressed under sporulation conditions and localized to the *C. difficile* spore. Through the construction of a Δ*cdeC* isogenic knockout mutant derivative of *C. difficile* strain R20291, we demonstrated that: 1) the distinctive nap layer is largely missing in Δ*cdeC* spores; 2) CdeC is localized in the exosporium-like layer and is accessible to IgGs; 3) Δ*cdeC* spores were more sensitive to lysozyme, ethanol, and heat treatment than wild-type spores; and 4) Δ*cdeC* spores adhered at higher levels than wild-type spores to intestinal epithelium cell lines (i.e., HT-29 and Caco-2 cells). Collectively, these results indicate that CdeC is essential for exosporium morphogenesis and the correct assembly of the spore coat of *C. difficile*.

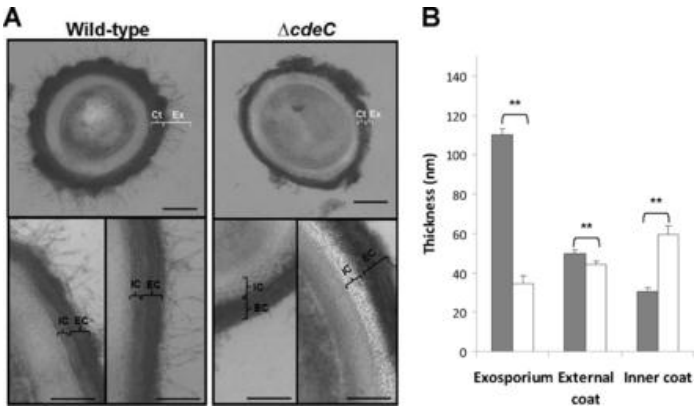


Fig. 1. *C. difficile* Δ*cdeC* spores have a defective exosporium layer. (A) Thin sections of *C. difficile* wild-type and Δ*cdeC* spores. (B) The thicknesses of the exosporium and outer and inner coat layers of wild-type (gray bars) and Δ*cdeC* (white bars) spores.

To read more:

Banawas S, Paredes-Sabja D, Korza G, Li Y, Hao B, Setlow P, Sarker MR. (2013). The *Clostridium perfringens* germinant receptor protein GerKC is located in the spore inner membrane and is crucial for spore germination. *J Bacteriol.* **195:**5084-5091.

Udompijitkul P, Alnoman M, Paredes-Sabjaa D, Sarker MR. (2013). Inactivation strategy for *Clostridium perfringens* spores adhered to food contact surfaces. *Food Microbiol.* **34:**328-336.

Barra-Carrasco J, Olguín-Araneda V, Plaza-Garrido A, Miranda-Cárdenas C, Cofré-Araneda G, Pizarro-Guajardo M, Sarker MR, Paredes-Sabja D. (2013). The *Clostridium difficile* exosporium cysteine (CdeC)-rich protein is required for exosporium morpho genesis and coat assembly. *J Bacteriol.* **195:**3863-3875.

UNDERGRADUATE MICROBIOLOGY PROGRAM:

Senior Instructor Dr. Linda Bruslind and Instructor Dr. Tasha Biesinger

Greetings from the Undergraduate Teaching and Advising sector of the Department of Microbiology! We have seen continued increase in the Microbiology undergraduate student population over the past year with increasing numbers of incoming freshman, transfer students and OSU students who change their majors to Microbiology. Most of our Microbiology courses are filled to capacity and we are actively working on increasing course and laboratory seats to accommodate the continued departmental growth. All of these are excellent indicators of the quality education, positive environment and marketability of the degree offered by the Department of Microbiology.

With all of the growth in the department, it should come as no surprise that some serious efforts are underway to increase course access. We have made significant inroads with developing Ecampus (online) courses to offer students alternatives. Online teaching has become a significant sector of education delivered at most universities, with some entire degree programs offered online at OSU (including Fisheries & Wildlife Sciences and German). Introduction to Microbiology (MB 230) and General Microbiology (MB 302) have been offered as Ecampus courses for several terms now and work is ongoing to develop online laboratory exercises to supplement the two required on-campus MB 230 laboratory days. Additionally, Dr. Sascha Hallett has been working diligently preparing materials for our first Ecampus General Parasitology (MB 480) course to be offered during Spring 2014. All of our faculty members are working on increasing capacity and improving quality of our courses across the board.

Departmental efforts to increase our course offerings to the larger OSU undergraduate student community have included: the development of a Mechanisms of Infectious Diseases course by Dr. Michael Kent; re-implementing Diseases and Society (MB 330) on the Corvallis campus thanks to the efforts of Dr. Jan Spitsbergen; and offering Emerging Infectious Diseases and Epidemics (MB/BI 385) on a more consistent schedule. Each of these courses will help fulfill baccalaureate core and/or writing intensive course requirements for students from various academic colleges at Oregon State University.

Thanks to the tremendous efforts of previous and current faculty members, the Microbiology Program is flourishing and we hope to see continued excellence in our undergraduate courses, laboratories and research!

Necropsies are fun, but what is more fun than a necropsy? This year in the Pathogenic Microbes Laboratory, we decided to give the students some experiential learning with some of the more common personal protective equipment (PPE) that may be required when working with pathogens. To this end, students donned disposable gowns, face masks and safety glasses, and double gloved while performing an organism necropsy to isolate the pathogen. Students quickly discovered some of the discomfort of wearing PPE such as fogging eyewear, condensation within the facemask and less breathability from the disposable gowns. But, more importantly, the discomfort was a continuous reminder to the students of the presence of a pathogen and why thinking about where the pathogen is, where it isn't and how easily it can be transferred to tools, clothes and other surfaces is just as important as the task of collecting the pathogen from tissues. So, what is more fun than a necropsy? A necropsy under some BSL3 conditions!

Conferences and Microbiology Student Association: Broadened Horizons

This year, Dr. Bruslind and Dr. Biesinger attended the American Society for Microbiology Conference for Undergraduate Educators (ASMCue), held in Aurora, Colorado in May. The trip was made possible by a grant from the OSU L.L. Stewart Faculty Development Fund.

The ASM CUE is a forum specifically designed for those individuals engaged in instruction of microbiology at the undergraduate level. It provides a unique opportunity for colleagues from across the country to interact and collaborate, sharing best practices in the arena of undergraduate teaching of microbiology. There are seminars presented by experienced educators, activities, discussion of resources, and a chance to network with colleagues. Linda and Tasha were particularly interested in information presented on "flipping the classroom," a technique with rapidly expanding popularity where students are assigned specific activities outside the classroom to learn the concepts, freeing up class time to engage the students in more interactive assignments or project-based learning. Both Linda and Tasha followed up by trying some of the techniques in their summer courses and were pleased by some initial success. Both have plans for further implementation in future courses, with the idea of improving the microbiology course offerings.

In November, Linda and Tasha led a group of 38 Microbiology majors to attend the weekend Northwest ASM Branch annual meeting at the University of Washington in Seattle. Jessica Tran, current President of the Microbiology Student Association, writes about her experience:

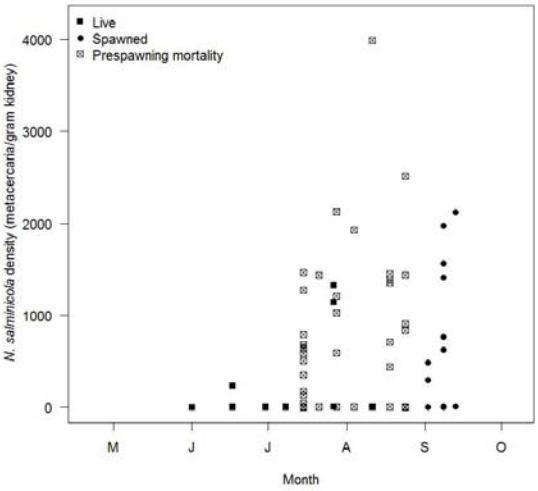
As an undergraduate, listening to a microbiology presentation can be overwhelming. I remember last year, sitting at the NW ASM branch meeting my eyes glazed over as I futilely attempted to process the concepts and vernacular. But this year, everything I had learned from the micro classes and labs came together and I had that light bulb moment. It was exciting like having a smartphone and finally understanding how to utilize it. One memorable presentation was Dr. David Suskind's regarding his work with the microbiome and inflammatory bowel disease. He focused a lot on immunological concepts and I could see the students who had taken MB416 mentally high fiving themselves because everything was so clear.

Beyond the intellectual stimulation, the conference was rewarding because it was a great opportunity to meet others. In general, students within the department don't meet each other until they finish the general science classes. More often, graduation is just around the corner when this occurs. But this conference changed all that. From the van to the hotel lobby, I met so many students I never knew were micro majors. Besides this, the trip was a great glimpse into the future. After the opening ceremony, a group of students and I went out with the keynote speaker, Dr. Joseph Petrosino, and were able to hear about his experiences, including how he was deported from Kazakhstan. It's so easy to watch a research presentation and become intimidated – as students, we often forget that researchers are just regular people who survived the onslaught of work and uncertainty we're currently dredging through. It was nice to know that we'll make it to our destination someday and thrive, possibly even undertaking (hopefully!) a nurturing role to future generations.

MICHAEL KENT LAB:

Investigations of prespawning mortality in Chinook salmon, a multidisciplinary study

All adult Pacific salmon species, except Steelhead, die after spawning in the fall. Adult salmon dying prematurely in rivers before spawning, referred to prespawning mortality (PSM) is a widespread and often serious concern. PSM will likely cause population declines in a specific run when it exceeds 50%. For the last 5 years we have been investigating the causes of PSM in Chinook salmon in the Willamette River, in which PSM in many years has been 60-90%. Nowadays, research is usually a multi-disciplinary effort involving groups of scientists with different areas of expertise, often from different units or institutions. This approach is exemplified in our study funded by the Army Corps of Engineering: Michael Kent is a parasitologist and fish disease expert, and leads the pathology aspects of the study; the research team is led by Carl Schreck, a fish physiologist with the USGS Co-op Unit situated in Nash Hall and professor in the Dept. of Fisheries and Wildlife; James Peterson and Michael Colvin (Dept. of Fisheries and Wildlife) are experts in mathematics and modeling, and provide evaluations of our complex data set; and Brian Dolan (Dept. of Biomedical Sciences) leads our immunology studies. Fisheries biologists Cameron Sharpe (Oregon Department of Fish and Wildlife) and Chris Claudill (University of Idaho) are also important collaborators. Together we are investigating correlations with pathogens, histological changes, and temporal progression of diseases through the summer before spawning, with climate and hydrological parameters. The ultimate goal of the project is to determine the factors influencing PSM and to develop strategies that fisheries managers can use to reduce this problem.



We have evaluated the prevalence and abundance of pathogens and associated lesions in about 500 adult salmon collected from the Willamette River system over the last several years. The studied fish fall into the following categories: early run, midsummer healthy, PSM mortalities collected from the river, and post-spawned fish collected from the river or Willamette Hatchery. Fish were sampled from both the lower and upstream portions of the watershed. Some adult salmon were also collected in the summer and held until spawning at an OSU facility that has excellent water quality (cool and pathogen free). Some overall trends are emerging as follows: 1) PSM fish have heavier pathogen burdens than healthy fish collected in the summer, 2) the pathogen profiles of the PSM fish are more consistent with fish that have survived to spawn in the fall, and which are naturally destined to die a short time after spawning, and 3) fish held in captivity on cool, pathogen free water have lower PSM levels than fish from the river. The fish held in these conditions at OSU that died before spawning almost always exhibited severe bacterial kidney disease or furunculosis, both of which are common in hatchery-reared fish and can be controlled with antibiotics. Holding adult fish in cooler, pathogen-free water provides an alternative for fisheries managers, particularly in years when high PSM is predicted (e.g., when fish return earlier than usual or summer temperatures are higher). We are now developing models to provide more precise predictions to guide management for reducing PSM by various holding strategies.

In addition to documenting well-recognized pathogens of salmon, we have made some new discoveries. For example, we are seeing a difference in the pattern of development of *Ceratomyxa shasta* (Myxozoa) in adult salmon compared to juvenile salmon. This myxozoan parasite is well known as a severe pathogen in parr and smolt salmon in the Pacific Northwest. In young salmon, the parasite completes its development and sporulates within a few weeks after infection in warm summer temperatures. We consistently see large numbers of presporogonic stages in about half the adult salmon fish that survive to spawn in the fall. Interestingly, we have seen few spores in these fish, even though they were exposed and presumably infected for months before spawning. In a recent experiment, we observed that the presporogonic forms continue to develop in fish after death, with increases in spore counts in fish ranging from 1.5 to 8 times after holding the intestines at 17^{o}C (62.5^{o}F) for 7 days after death. Researchers in the Department of Microbiology have been studying *C. shasta* for decades. This research was first led by Dr. John Fryer (a long-serving Department Head) and his student, J.E. Sanders, and a large research program is continuing in the Jerri Bartholomew laboratory. Sanders and Fryer reported in 1970 that *C. shasta* was an important cause of PSM in spring Chinook adults in the Willamette River, because considerably more spores were observed in PSM carcasses collected from the river than in fresh adult fish. Our findings do not contradict this conclusion, but provide another explanation for the high numbers of spores seen in dead fish (i.e., the parasites continue to produce spores after fish death). This is a remarkable finding in that the accepted paradigm for myxozoan parasites has been that development ceases after death, and that only fully-formed spores are viable at this time.

Further reading:

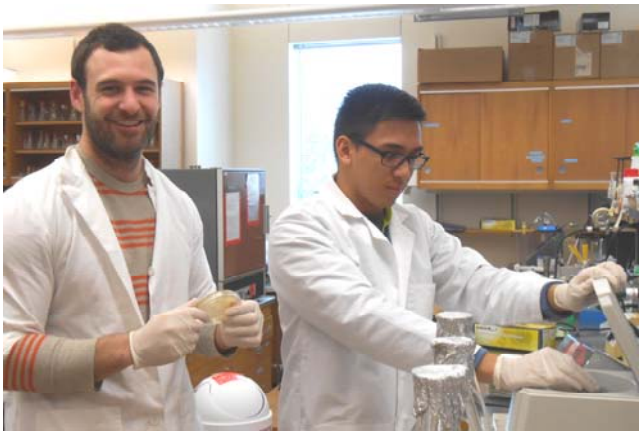
Kent, M.L., Benda, S., St-Hilarie, S., Schreck, C, B. (2013). Sensitivity and specificity of histology for diagnoses of four common pathogens and detection of non-target pathogens in adult Chinook salmon (*Oncorhynchus tshawytscha*) in freshwater. *J. Vet. Diagn. Invest.* **25:** 341-351.

JANINE TREMPY LAB:

New food sensations may be coming your way

After 11 years of service to the College of Science as an Associate Dean, I happily returned, full time, to my position as a professor in the Department of Microbiology. Fortunately, I was able to maintain my teaching and research programs during those years in administration. My research program, capably and enthusiastically managed by my Senior Scientist, Karen Dierksen, has generated a number of patented technologies.

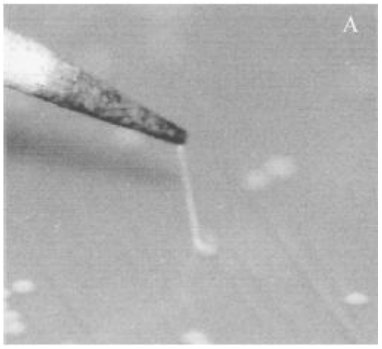
Our attention has moved from basic research and proof of concepts to technology transfer activities in recent years. We isolated and characterized a natural lactococcal isolate, *Lactococcus lactis* ssp. *cremoris* Ropy 352, from the strain collection of mixed cultures of Bill Sandine (Bill was a Professor in Microbiology between 1960 and 1996, and his dairy-related patents have provided welcome income to the department). The Ropy 352 isolate produces a unique biopolymer (encoded by a unique combination of genes) that thickens milk, lactose-free milk, coconut milk, rice milk, as well as Slim Fast™ and Ensure™. The thickening characteristics imparted by the Ropy 352 bacterial strain add a desirable smooth and creamy property to products designed for human consumption.



Trempy lab, Collin Thompson and Roberto Garcia

The high molecular weight Ropy 352 biopolymer is an exopolysaccharide composed of sugar residues that do not appear to have pyrogenic properties and is produced by an organism with GRAS (Generally Regarded As Safe) status; therefore, this biopolymer can be used in food as a stabilizer and in liquids as a natural thickener. The market for polymers, such as the Ropy 352 biopolymer, has an expected worth of $7 billion dollars by 2018 through uses in the food, petroleum, biomedical and pharmaceutical industries. Additionally, the lactococcal strain producing the Ropy 352 biopolymer has the potential to add probiotic characteristics to fermented food products. Recent research links biopolymer-producing probiotic organisms to health benefits. The global demand for probiotic products in 2011 was $27.9 billion with an expected increase to $44.9 billion by 2018.

Currently, Oregon State University's Technology Transfer Office is negotiating with one of the largest ingredient companies in the world to transfer this technology to the private sector for use in products consumed by humans. Roberto Garcia, first an undergraduate and now a graduate student in my program, has extended upon the early work on Ropy 352, done by my former students, Karen Dierksen, Eric Knoshaug and Anthony Covington, and has also characterized other natural biopolymer-producing lactococcal isolates. In addition, Roberto is applying natural genetic processes to equip thermophilic GRAS organisms with the ability to produce the Ropy 352 biopolymer for their use in fermented food products produced at higher temperatures. Talented students, such as Roberto, lead to great outcomes in research programs!!! I have been very fortunate to be a member of a department that attracts great students!!!



Ropy biopolymer produced by Lactococcus lactis ssp. cremoris Ropy 352 cultured on whey medium.

Un-inoculated 2% Milk.

2% milk inoculated with

Lactococcus lactis ssp. cremoris Ropy 352.

RYAN MUELLER LAB:

Research in the Mueller Lab is focused on examining metabolic interactions between microbial populations that live in aquatic environments by addressing questions related to resource utilization patterns in ocean microbial food webs and predator-prey interactions between microbial eukaryotes and bacteria. In support of the former goal, Sam Bryson (Graduate Student), Ryan Mueller (Assistant Professor), and Xavier Mayali (OSU Courtesy Professor & Lawrence Livermore National Laboratory Research Scientist) participated in a cruise led by the Monterey Bay Aquarium Research Institute (MBARI) this past year as part of a Gordon and Betty Moore Foundation sponsored research project. The samples collected during this trip are now beginning to be analyzed with the goal of defining functional guilds (interacting organisms) within the natural microbial communities found in coastal seawater. This work will involve a combination of metagenomics, stable isotope labeling, and newly developed mass spectrometry techniques to track resource utilization by microbial populations and incorporation into RNAs and proteins. Pilot experiments performed by Sam this year have proven successful in validating this approach by showing significant stable isotope enrichment into numerous newly synthesized peptides (SIP signal) from distinct populations of active seawater bacteria growing on labeled amino acids in water collected from the Oregon coast (Figure 1). The lab is eagerly anticipating the results of the new, fully elaborated experiments performed at MBARI, which aim to define resource utilization patterns across whole communities using a wide range of substrates.

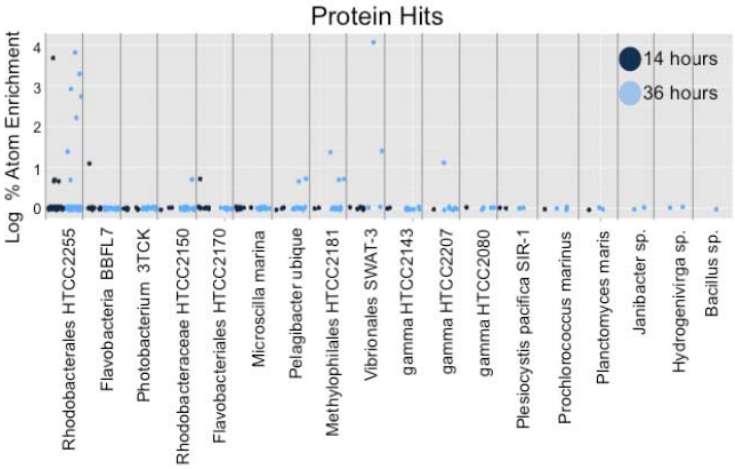


Figure 1. Significant incorporation of SIP label into detected peptides originating from multiple bacterial populations (see *Rhodobacteriales* HTCC2255 as best example).

Sam Bryson, Ryan Mueller, and Francisco Chavez aboard the R/V Rachel Carson collecting seawater off the coast of Monterey, CA (Oct, 2013).

In addition to developing these lines of research during Ryan Mueller's first year at OSU, he has also been working with Walt Ream to modify the content of the Molecular Microbiology Lab (MB 311), a required course in the Microbiology undergraduate curriculum. New protocols, which include the use of the Illumina MiSeq sequencing platform at OSU, have been introduced into the course with the goal of familiarizing students with next-generation sequencing technology and the associated data analysis approaches used in contemporary environmental microbiology studies (i.e., performing community diversity studies using 16S ribosomal DNA amplicons and whole genome sequencing of environmental isolates).

For more information on Proteomics-SIP approach being developed for this research see: Pan, C., *et al.*, (2011). Quantitative Tracking of Isotope Flows in Proteomes of Microbial Communities. *Molecular & Cellular Proteomics* **10:**M110.006049

BRUCE GELLER LAB:

Antibiotic resistance stimulates research on a new class of antibiotics

Margo Kaller, Daravuth Cheam, Bruce Geller

Antibiotic resistance has increased dramatically worldwide and poses a major threat to public health. Healthcare providers, research scientists and patients are now preparing for a post-antibiotic era, where antibiotics are no longer effective. One of the many factors that complicates the situation is that the number of new antibiotics and the number in the developmental pipeline has dwindled over the past 2 decades. If we are to gain the upper hand in this fight, new strategies of discovering antibiotics must be found.

PPMOs (peptide-conjugated phosphorodiamidate morpholino oligomers) are synthetic compounds that mimic the structure of DNA. Each PPMO has a unique sequence of bases that binds a specific mRNA in the bacterial cell. When a PPMO binds to an essential mRNA, it kills the bacterial cell. The dramatic increase in sequenced microbial genomes now enables us to develop genus- and gene-specific antimicrobials targeted to many bacteria.

Acinetobacter are Gram-negative bacteria that can be opportunistic human pathogens. *A. baumannii* and *A. lwoffii* are the two most common species associated with human disease, and can typically cause pneumonia, bacteremias, urinary tract infections and wound infections. Importantly, these pathogens are frequently resistant to multiple antibiotics. This can make treatment of these infections particularly challenging. There are increasing reports of these pathogens being pan-resistant to all available antibiotics.

We recently reported in the *Journal of Infectious Diseases* that PPMOs are potent antibacterial compounds against *A. lwoffii* and *A. baumannii*, both in vitro and in a mouse model of pneumonia. These results are significant for several reasons: First, this was the first time PPMOs have been used to treat pneumonia. Second, this was the first time antibacterial PPMOs have been administered to the lung, which more closely resembles how we envision human therapy for pneumonia. Third, this was only the second time PPMOs have been shown to be effective against multidrug-resistant bacteria, the first being our earlier report on PPMOs against *Burkholderia cepacia* complex. Finally, this was the first time an antibacterial PPMO was shown to be effective when initial treatment was delayed after infection. Delayed treatment is a more stringent test of efficacy than administering the PPMO immediately upon infection, and more closely resembles how PPMOs would be used for human therapy.

We think PPMOs have advantages over conventional antibiotics in fighting the development of resistance. Perhaps most importantly, PPMOs are species-specific. If resistance to PPMOs were to arise by spontaneous mutations in the target sequence, there would be no advantage to other genera of bacteria that might acquire the mutant gene by horizontal transmission. If resistance were to arise to a specific sequence of bases in a PPMO, the PPMO could be re-designed and directed to a different gene. A more practical avenue for clinical development of antibacterial PPMOs may be to start with a mixture of two or three PPMOs, each targeted to a different essential gene within one bacterial species. This would make it practically impossible for resistance to arise through spontaneous mutations in targeted genes. Another advantage of species-specific antibiotics is that they would not kill beneficial bacteria that are required for good health.

Targeting bacteria in a species-specific manner is a strategy that could be applied to a variety of infections where there is time to determine the cause of the infection. An example is ventilator-associated pneumonia. Another example would be chronic lung infections such as those associated with cystic fibrosis or COPD patients, whose lungs can remain colonized with a few known pathogens. There are many other settings and pathogens for which targeted therapy make sense. It is clear that new approaches are urgently needed to combat the double crisis of increasing drug resistance and decreasing antibiotic development. We hope that antisense platforms could be one innovative strategy for dealing with these problems.

To read more:

Geller BL, Marshall-Batty K, Schnell FJ, McKnight MM, Iversen PL, Greenberg DE. (2013). Gene-silencing antisense oligomers inhibit *Acinetobacter* growth in vitro and in vivo. *J Infect Dis* **208:**1553-1560

Greenberg DE, Marshall-Batty KR, Brinster LR, Zarember KA, Shaw PA, Mellbye BL, Iversen PL, Holland SM, Geller BL. (2010). Antisense phosphorodiamidate morpholino oligomers targeted to an essential gene inhibit *Burkholderia cepacia* complex. *J Infect Dis* **201:**1822-1830

JERRI BARTHOLOMEW LAB:

.This year has seen many changes in the Bartholomew lab. Three students graduated, starting with Luciano Chiaramonte, who defended his MS thesis on Climate Warming Effects on the Life Cycle of the Parasite *Ceratomyxa shasta* in Salmon, followed by Emily Nebergall who completed an MS degree on a study of the Ecology and Applications of Cutaneous Mechanisms of Resistance to Amphibian Chytridiomycosis, and Robert (Adam) Ray who defended his PhD thesis Modeling Abiotic Influences on Disease Dynamics for the Complex Life Cycle of *Ceratomyxa shasta*.

In summer we were joined by Darrelle Fiorito, a high school science teacher from Central Linn High School, Halsey OR, and the recipient of an M.J. Murdock Charitable Trust award. The primary goal of the Murdock Charitable Trust program is to provide high school teachers with opportunities to work at the cutting edge of science, which in return revitalizes their teaching, and helps them develop new inquiry-based teaching strategies. OSU only received two of these grants in 2013! Darrelle worked with postdoctoral scholar Julie Alexander on aspects of our ongoing salmon parasite research in the Willamette River. Here's what she had to say about it: "This program has allowed me to see the intricacies of scientific research and then communicate to students how technology and science are utilized in research that can have real world applications. In the Pacific Northwest, we really care about salmon, and I got to be part of a research team that is investigating disease - an important factor affecting salmon survival. As an educator, I strongly encourage my students to explore careers in science based fields and this partnership has allowed me to show my students a local and real example where we are using scientific methods to solve problems." The award stipend covers two summers and we are excited to continue the partnership in 2014.

This was also a year for international travel and visitors. Jerri Bartholomew spent December 2012 in the laboratory of Drs. Edson Adriano and Antônio Maia at the Federal University of São Paulo, Brazil. In March she spent several weeks at Hauzhong Agricultural University, Wuhan, China, visiting the laboratory of Dr. Gu Zemao. Drs. Zemao and Isa Zhai Yanhua (PostDoc) then joined us in June. They will stay with us for a year to learn more about our myxozoan research.

Other lab visitors this year included Beth Okamura, Prof at the Natural History Museum London, who shared her latest research on bryozoan-hosted myxozoans**.** Arik Diamant, Head of the National Center for Mariculture, Eilat, Israel, spent three months of his sabbatical with us, commencing in April. Arik helped collect aquatic annelids and dissect a range of native and non-native fishes, including an invasive carp infested with *Myxobolus* and a rockfish that harbored a curious copepod. María Alonso Naveiro, a PhD student at the Instituto de Acuicultura de Torre la Sal – CSIC also joined us for summer to focus on genetic characterization myxozoans. And Gema Alama Bermejo (pictured with Charlene Hurst), a postdoctoral researcher at the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic, joined us in August to work on parasite proteases and transcriptomics; she will be with us for over a year.

Department of Microbiology
Oregon State University
220 Nash Hall
Corvallis, OR 97331-3804

Campus Construction: Nash Hall is the brick building in the foreground. Top picture (before construction) has the soccer field to the right. Bottom picture shows a half completed Austin Hall, the future home of the College of Business. To the left of Austin Hall is construction on a new Classroom Building that will house much needed classroom space.

The front cover is a photomicrograph of a Gloeotrichia colony.
It is a nitrogen-fixing, bloom forming freshwater cyanobacterium.