MCI Newsletter: 1st quarter 2010

Welcome to the first issue of the quarterly MCI Newsletter. We look forward to sharing initiative information, events, and milestones with you each quarter. Please feel free to send us your feedback and suggestions.

Message from the Director:

Happy New Year! It has now been about 5 months since the Microbial Communities Initiative research projects have started, and this is the Initiative's first newsletter.

Our most important activity has been activating 5 LDRD research projects in August. There was a lot of intense activity at the end of the 2009 fiscal year to get these in place and going on project work. All 5 continue work in 2010. In addition, we expect startup of a sixth project (Callister et al.), to augment the focus area on the use of "omics" technologies to answer ecological questions. We are in the process of soliciting ideas for a seventh project, based on input from our advisory committee. This project will integrate activities across focus areas, particularly by coupling the development of microfluidic and microanalytical technologies with biological experiments, driven by the needs for paramaterization and validation of a computational model that simulates the physical, chemical, and biological components within the microfluidic device.

We have also been occupied developing communications both within the initiative and with the outside world. We do have a website (http://www.pnl.gov/biology/research/mci/), that we will work on upgrading over the next two months. There is a link on the website to a series of YouTube videos from a short symposium that took place here on October 9, 2009, the day on which the new Biological Sciences Facility was dedicated. The symposium was entitled, "Microbial Communities: Stewards of the Biosphere." Many thanks to Professors Mary Lidstrom (U. Washington, Seattle) and Dave Ward (Montana State University) for giving excellent presentations on their research. For internal PNNL consumption (on the Initiative's Sharepoint site), I have started an MCI blog, "What's happening in microbial ecology?" that not only discusses initiative activities, but also interesting literature that has been published. Fred Brockman has put together a series of external seminar speakers to come to PNNL and discuss their research on microbial communities. And lastly, we have a series of brownbag lunch sessions planned. I gave the first of these in the fall, to discuss my view of "What is microbial community ecology?" Fred Brockman is organizing a series of four weekly discussions in which we will explore technological solutions to answer ecological problems in the "model" community that we will be investigating: cellulose decomposition in the compost piles produced by leaf cutter ants (see the article below regarding this system).

We have a mid-year review coming up in early March, and look forward to seeing the members of our Advisory Committee again for a thoughtful analysis of our directions and progress. This August, there is an important meeting relevant to the initiative in Seattle, the International Symposium for Microbial Ecology. LDRD project staff are working hard to generate results that can be presented at this biennial meeting, for which we expect more than 1500 attendees.

LDRD Projects for FY10:

"Community Diversity and Functional Redundancy of Cellulytic Microbial Communities in Soil Aggregates"- Vanessa Bailey and Lee Ann McCue

"Higher-Throughput, More Sensitive Stable Isotope Probing"- Helen Kreuzer

"Microscale Spectroscopic Analyses of Cellulose Degradation and Uptake by a Microbial Community"- Vanessa Bailey and Nancy Hess

"Multiscale Models for Microbial Communities"- Haluk Resat

"Oxygen Optode for Chemical Imaging in Microfluidic Microbial Models"- Jay Grate

"Proteomics Measurements of Functional Redundancy and Stability Testing of Cellulose Degrading Anaerobic Microbial Communities within Engineered Bioreactors" Stephen Callister, Brian Lamarche, Aaron Wright, Michael Wilkins

---

MCI Advisory Committee Members:

Birgitte Ahring, Washington State University-Tri-Cities
Gill Geesey, Montana State University-Bozeman
Kenneth Kemner, Argonne National Laboratory
Tom Schmidt, Michigan State University
Andrew Felmy, PNNL, EMSL
Blaine Metting, PNNL, FCSD
Ann Miracle, PNNL, EED

Upcoming Events:

- **MCI Brownbag four week series** will begin Friday, January 15th and end Friday, February 5th. All sessions of this series will be held on Fridays in the BSF Darwin room from 12:00 -1:30 pm. Watch for email and blog announcements about other future brown bag offerings

- **MCI Mid-Year Review Meetings** will be held March 9th- 10th, 2009 in the BSF Darwin Room.

- **ISME 13 "Stewards of a Changing Planet"**- International Symposium on Microbial Ecology will be held August 22-27th, 2010 at the Washington State Convention and Trade Center in Seattle, WA. ***Abstract submissions are due 3/5/10.*** http://www.isme-microbes.org/isme13

MCI Seminars (previous and future offerings):

- ***Tuesday, September 8th, 2009***- Dr. Michael Kuhl, University of Copenhagen. "Planar Optode Research"
- ***Friday, December 4th, 2009***- Dr. Doraiswami Ramkrishna, Purdue University. "The Metabolic Modeling Landscape"
- ***Thursday, February 4th, 2010***- Dr. Pieter Visscher, University of Connecticut. "Microbial Interactions in Saltern Microbial Mats"
- ***Monday-Tuesday, April 5th-6th, 2010***- Dr. Radhakrishnan Mahadevan, University of Toronto. "Integration of Genomic Information with Computational Modeling of Microbial Metabolism"
- ***TBD***- Dr. Cameron Currie, University of Wisconsin. "Recent Data from the Leaf-Cutter Ant -- Microbial Symbiosis"
- ***TBD***- Dr. Bruce Hungate, University of Northern Arizona. "Stable Isotope Research to Improve Understanding of Microbial Processes Important in Carbon Cycling"
- ***TBD***- BSD Frontiers Seminar Series presents Dr. Jill Banfield, University of California-Berkeley

---

Publications/Presentations:

Konopka, A. 2009. "What is microbial community ecology?" The ISME Journal 3(11): 1223-1230. doi:10.1038/ismej.2009.88

MCI Feature Article:
Fred Brockman, MCI Assistant Lead

Ecology and characterization of leaf-cutter ant microbial communities

At the first review of the MCI held in June 2009, the Advisory Review Committee asked that we identify a model microbial community. We have had a range of discussions on this and decided to pursue a *synthetic community composed of select bacteria derived from the dump (compost pile) of the leaf-cutter ant.* Key criteria for selecting a model microbial community were a cellulose-degrading community composed of aerobic/facultatively anaerobic bacteria that had been well characterized at the isolate and community levels. A body of extant data will minimize time and resources spent by MCI on community characterization, and maximize our ability to develop and integrate advanced technologies.

This feature article is a brief description of the ecology of the leaf-cutter ant microbial communities, and the status of available information on these communities from the Curry lab at the University of Wisconsin. Cameron Curry receives funding from the Great Lakes Bioenergy Research Center (GLBRC), which is funded by the DOE Genomics Science (formerly Genomics: GTL) program.

Ecology of the fungus garden. Leaf-cutter ants (genera *Atta* and *Acromyrex*) are one of the most dominant herbivores in New World tropical ecosystems. A mature colony of *Atta* has up to 8 million workers and occupies an underground volume of 20 m^3 or more. In contrast to many other insects that break down plant material in their guts, leaf-cutter ants

harvest leaves and bring them underground to cultivate a 'fungus garden'. The fungus catabolize organic carbon in leaves and the ants use the fungus as their food source. In this ant-fungus mutualism, leaf-cutter ants break down plant antifungal barriers and provide optimum growing conditions for the fungus by aerating the leaf piles to maintain proper gas exchange, temperature, and humidity. In turn, the fungus breaks down plant anti-insect toxins. The fungus garden is enabled in large part by a second mutualism: that between the leaf-cutter ants and *Pseudonocardia* (an Actinobacteria). The *Pseudonocardia* grow on patches of the surfaces of the ants, are inoculated onto the leaves by ants tending the garden, and produce antibiotics to help defend the garden from parasites that attack the cultivated fungus. A third mutualism exists between the ants and symbiotic nitrogen-fixing bacteria (mainly *Klebsiella* and *Pantoea*): these bacteria convert nitrogen gas into reduced forms of nitrogen that increase the nitrogen content of the fungal garden, and provide approximately 50% of the nitrogen found in the ant biomass. Other microbial interactions have also been identified in the ant-fungus-bacterial community.

Ecology of the dump. Ants constantly remove partially-degraded leaf cuttings from the bottom of the garden, bring them back to the surface, and drop them from a branch of a bush or tree into a conical mound called a dump. When deposited, these partially-degraded leaf cuttings retain approximately half of their original cellulose content. The dump has a much higher (and more favorable) nitrogen to carbon ratio than forest litter because of bacterial nitrogen fixation in the fungus garden. The same dump is used year after year so that the lower, older annual layers become consolidated and humus-like, similar to the lower levels of a backyard compost pile. The lower levels of the dump reach moderately thermophilic temperatures, and are thought to become saturated (or nearly so) in the rainy season.

Microbial characterization of the fungus garden (the Curry lab has submitted a paper to Science). Bacterial metagenomic and 16S-rRNA gene sequences indicate that gamma proteobacteria (in particular, the enteric bacteria *Klebsiella* and *Pantoea)* dominate. Physiological tests show *Klebsiella*, *Pantoea*, and *Enterobacter* isolates have the highest cellulytic activity. *Klebsiella* and *Azospirillum* isolates have the highest nitrogen-fixing activity. Comparisons with other metagenomes show the carbohydrate utilizing gene profile in the garden bacterial community is most closely related to that of the bovine rumen, showing evolutionary convergence at the metabolic level. Draft genomes have also been obtained for the *Klebsiella* and *Pantoea* (as well as other) isolates.

Microbial characterization of the dump (recent data from the Curry lab). Fungal biomass is lower in the dump than in the fungus garden. A bacterial metagenome composited from the upper 50% of the dump material is completed, and is in the early stage of analysis. It appears the bacterial community is more diverse in the dump than in the garden and is dominated by members of the gram-positive divisions Bacteriodes and Actinobacteria, and beta proteobacteria. As such, the dump community is similar to compost piles, and is more similar (compared to the garden community) to the community found in soils. Bacterial metagenome sequencing on the lower 50% of the dump community will occur in 2010. Genome sequences are currently available from three microcrystalline cellulose-degrading dump isolates: they are novel genera related to *Cellulomonas*, *Microbacterium*, and *Brevibacterium*. The Curry lab has many dozens of isolates from the dumps, and they continue to screen isolates for cellulytic activity and use of cellulose-derived metabolites. Genome sequencing of additional dump isolates is likely in the future. In addition, Mary Lipton (a collaborator in the GLBRC) is characterizing the metaproteome of both the upper and lower dump samples, as well as select cellulose-degrading isolates.

The above information was assembled from personal communications with the Curry lab, and from the following publications and references.

Symbiotic nitrogen fixation in the fungus gardens of leaf-cutter ants. A.A. Pinto-Thomas et al., *Science* 326:1120-1123 (2009).

Experimental evidence of a tripartite mutualism: bacteria protect ant gardens from specialized parasites. C.R. Currie et al., *Oikos* 101:91-102 (2003).