

MCI Newsletter: 2nd quarter 2010

MCI Newsletter, Issue 2, June 2010

Message from the Director:

Welcome to the second MCI newsletter. The initiative has continued to develop its plans over the first part of 2010. This has included the start of two new LDRD projects and the expected start of a third one this June. Stephen Callister has written a short article for this newsletter on the project that he and his colleagues have initiated to apply activity-based protein profiling to answer a community ecology question – functional redundancy of microbial communities. This represents an example of how MCI is applying the technological capabilities of PNNL to address significant ecological questions that could be applied to a broad variety of natural and engineered environments. The application of proteomics to microbial communities is less mature than the use of nucleic acid sequence analysis. A significant limitation has been the need for appropriate genome sequences to interpret proteome profiles. The activity-based approach may provide a means to make proteome analysis more independent from extensive metagenome analysis in order to proceed with functional analysis of complex microbial communities.

MCI is also looking forward to a significant assembly of microbial ecologists in the Pacific Northwest in August. The International Society for Microbial Ecology (ISME) is holding its 13th Symposium in Seattle from August 22-27. Jim Fredrickson and Allan Konopka are the chair and co-chair of the Local Organizing Committee and have worked extensively with the ISME Executive Council to develop the scientific program in collaboration with a number of Pacific Northwest and West Coast microbial ecologists. We believe the meeting has developed an excellent scientific program, with a variety of topics that are of significant interest to MCI. Approximately 2100 abstracts were submitted, with a very high representation of scientists from Europe, a very good number from Asia and a substantial number of U.S. scientists. Jim and I are currently working on a possible late-breaking session that would cover the microbial ecology of the oil spill in the Gulf of Mexico – this would cover reflections on what research upon earlier spills had shown as well as some current data that are just now being collected in the Gulf.

We are also looking forward to our first annual review of the initiative in late July. All of the scientists feel that we have been putting together the technical pieces to address the scientific questions raised by MCI, and the group has worked quite effectively together to integrate disparate activities to the extent that makes scientific sense. It will be exciting to see these efforts mesh in the coming months.

New LDRD Projects Initiated in FY10 (in addition to 5 projects continuing from FY09):

Two new projects have been initiated in calendar year 2010, with a third project to begin in June.

- "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
- "Proof-of-Principle Demonstration of Fluorescence Labeling of Cellulose and Microscopic Fluorescence Imaging of Cellulose Degradation". Fred Brockman, Jay Grate, Marvin Warner, Dehong Hu, and Galya Orr. This was designed as a 6 month project that will be incorporated into the third new project.
- The third project will be an integration of microfluidic model experimentation, multimodal imaging, and modeling. Several of the technologies initiated in other MCI projects will be combined in this integrative project.

S&T Highlights:

When your project hits a major milestone, discovery, or other 'nugget', please draft a brief highlight and send it to Allan Konopka. There is no specific format but include a technical description, why the item is important (Scientific American level), who should care, and an image/graphic. The LDRD Office is periodically asked for material about PNNL's LDRD portfolio. Highlights will serve as an 'index card' or starting point; if/when the LDRD office uses it, they will get back to the PI and/or Initiative leadership to tailor the highlight to the request the LDRD office has received.

Annual Project Updates:

PI's will need to complete an annual report and make scope revisions to their proposal in the mid-August to September 1st timeframe.

Upcoming Events:

- **MCI Annual Review Meetings** will be held Wednesday and Thursday July 28th – 29th in the BSF Darwin Room. *Additional details found in article below.*
- **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. <http://www.isme-microbes.org/isme13>. There have been 4 abstracts submitted from MCI projects to this conference (see list below).

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, April 19th, 2010***- Dr. Bruce Hungate, University of Northern Arizona. “From the Globe to the Cell and Back: Microbial Biogeochemistry in a Changing World”
 - ***Tuesday, June 8th, 2010***- Dr. Cameron Currie, University of Wisconsin-Madison/Great Lakes Bioenergy Research Center. “Leaf Cutter Ant Microbial Community”
 - ***Fall 2010 (date TBD)***- Dr. Radhakrishnan Mahadevan, University of Toronto
-

Presentations:

Callister SJ, MJ Wilkins, AT Wright, BL LaMarche, BK Ahring, MS Lipton, and A Konopka. 2010. “Proteomics Measurements of Functional Redundancy and Stability Testing of Cellulose Degrading Microbial Communities within Engineered Bioreactors.” Abstract submitted to International Symposium on Microbial Ecology- ISME 13, Seattle, WA. PNNL-SA-71886

Hess NJ, SJ Fansler, D Hu, and VL Bailey. 2010. "Discrimination of Microorganisms using Surface-Enhanced Raman Spectroscopy." Abstract submitted to International Symposium on Microbial Ecology- ISME 13, Seattle, WA. PNNL-SA-71793.

LaMarche BL, SJ Callister, AR Shah, AT Wright, MJ Wilkins, ME Monroe, KL Crowell, GA Anderson, and RD Smith. 2010. "A mathematical approach for reference selection in high throughput proteomics." Abstract submitted to American Society for Mass Spectrometry, Salt Lake City, UT. PNNL-SA-70704.

McCue LA, VL Bailey, SJ Fansler and AE Konopka. 2010. “Community Diversity in Individual Soil Aggregates.” Abstract submitted to International Symposium on Microbial Ecology- ISME 13, Seattle, WA. PNNL-SA-71780.

Moran JJ, MK Newburn, ML Alexander and HW Kreuzer-Martin. 2010. “Spatially Resolved Stable Isotope Analysis at the Microbial Community Level.” Abstract submitted to International Symposium on Microbial Ecology- ISME 13, Seattle, WA. PNNL-SA-71778.

Proposals:

Fred Brockman and Jay Grate. EMSL User Proposal: “Microscopic Fluorescence Imaging of Cellulose Degradation in Microfluidic Devices”. This proposal seeks instrument time and expertise of staff to operate the specialized fluorescence microscopes in EMSL.

Liz Alexander. EMSL Partner Proposal (letter of intent stage) “Scientific Partnership Proposal for Developing Laser Ablation Isotope Imaging as a New EMSL Capability”

*MCI Feature Article:
Stephen Callister, LDRD Co-PI*

Research activities began in January 2010 for the 6th LDRD funded by the MCI, which focuses on extending “omics” capabilities to answer ecological questions. This feature article presents a brief overview of this LDRD titled, “Proteomics Measurements of Functional Redundancy and Stability Testing of Cellulose Degrading Anaerobic Microbial Communities within Engineered Bioreactors”.

In nature, an important service provided by microbial communities is the breakdown of cellulosic biomass, comprised mainly of cellulose, hemicellulose, and lignin. The combination of different lignocellulose degrading enzymes generates synergism for efficient degradation, in part because the initial byproducts of cellulose degradation can inhibit the activity of some enzymes, requiring the activity of other enzymes to reduce the concentrations of inhibiting substrates. Evolutionary processes also lead to families of related enzymes with slightly different substrate ranges and catalytic activities. Thus, the stability of lignocellulose degradation may result from the presence of multiple enzymes, originating from different microbial populations residing in different microniches within a sample or environment, capable of carrying out the same function (functional redundancy).

In ecology, the concept of functional redundancy is hypothesized to explain why biological diversity positively correlates to ecosystem stability (McCann, 1998). This hypothesis has been coined by ecologists as the “Insurance Hypothesis” (McCann, 1998). In microbial ecology, testing of the insurance hypothesis is challenged by our ability to measure microbial diversity, but also by our ability to ascribe the functional roles of this diversity at the population level and “omics” level. Genomic sequencing technology is proving useful for characterizing microbial diversity, and bioinformatics tools are, to some extent, able to predict the functional roles of proteins identified from the computerized translation of genome sequences; thus, providing an in-silico means for studying functional redundancy.

An alternative is the use of activity-based protein profiling (ABPP) (Cravatt, Wright et al. 2008), which is an emergent technology in the field of proteomics. ABPP involves the design, synthesis, and testing of chemical probes that bind irreversibly to the active site of a class of enzymes present in a complex proteome. An ABPP probe includes a reactive group (analog of enzyme substrate) to target the active site of an enzyme, a chemical specificity moiety that directs the probe toward a particular enzyme family, and a reporter tag for providing feedback on probe-labeled enzymes. Once tagged, proteins in a complex mixture can be enriched and analyzed by mass spectrometry. In conjunction with mass spectrometry, ABPP provides information on expressed and active protein targets, and can potentially provide a direct readout of functional redundancy without the need for developing antibodies, or being limited to studies of genetic potential using metagenome sequencing or PCR based amplification of targeted genes. At minimum, ABPP is a different type of readout that will allow new insights into the basis of functional redundancy.

We are developing ABPP probes and supporting bioinformatics capabilities to measure functional redundancy and characterize the stability (to the extent possible) of cellulose degradation in microbial communities affected by environmental perturbation. We are also using the perturbation response archetype as a means to affect change and understand stability in a bacterial community is a potentially important management practice of biological processes.

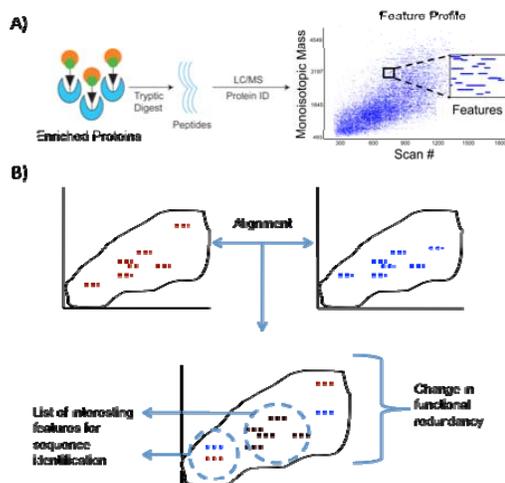
Currently, a suite of ABPP designs for glycoside hydrolase enzymes probes specific to cellulose, and synthetase enzymes probes specific to nitrogen assimilation have been completed. The synthesis steps for the first of six glycoside hydrolase probes have been worked out, and a prototype synthesized. The synthesis of this first probe represents significant progress in ABPP development because of the challenge of synthetically modifying carbohydrate substrates, such as those associated with this prototype. Initial testing on purified enzymes, purchased commercially, shows broad active-site specificity to an array of glycosidase hydrolases, including celliobiase from *Aspergillus niger*, β -glucanase from *Trichoderma longibrachiatum*, and β -galactosidase from *Aspergillus oryzae*. Almost all commercially available purified enzymes involved in cellulose degradation originate from fungi. However, the testing of this prototype on bacteria having sequenced genomes is beginning. These bacteria include *Cytophaga hutchinsonii*, *Clotridium cellulolyticum*, and ant colony refuse pile strains recently isolated by researchers at the University of Wisconsin.

In addition, data analysis tools are being developed to help analyze mass spectrometry measurements made on the enriched proteins. One such tool, MultiAlign, aligns multiple high-resolution, high mass measurement accuracy mass spectrometry datasets. These datasets generate a 2-dimensional profile of mass and liquid chromatography normalized elution time features, illustrated in the Figure. Most features represent peptides. All datasets of feature profiles are aligned to a baseline dataset, which poses the question of which dataset to select as baseline? The number of datasets to be generated from future planned experiments makes the systematic comparison of all datasets computationally inefficient and not feasible. The use of fractal geometry to calculate how “space filling” a dataset is represents a possible solution to this question. Ultimately, overcoming this baseline selection challenge will help to accurately align multiple feature profiles, which has a potential broader application to environmental proteomics research. Here, proteomics datasets generated from temporal or spatial environmental field experiments can be aligned and features of significance identified for targeted tandem mass spectrometry analysis and *de novo* peptide sequencing. In this context, proteomics analyses could be conducted without the initial requirement of a metagenome sequence.

In the near future, the described proteomics and bioinformatics capabilities will be applied to cellulose degrading engineered bioreactors operated by the Bioproducts, Sciences, and Engineering Laboratory located on the WSU Tri-cities campus (Richland, WA). The microbial communities within each reactor will be perturbed and cellulose degradation monitored in an attempt to correlate the stability of this important biological function with changes in functional redundancy of enzymes associated with cellulose degradation.

Cravatt, B. F., A. T. Wright, et al. (2008). "Activity-based protein profiling: From enzyme chemistry." *Annual Review of Biochemistry* **77**: 383-414.

McCann, K. S. (2000). "The diversity-stability debate." *Nature* **405**: 228-233.



A) Enriched proteins utilizing ABPP technology will be analyzed by high-resolution, high mass measurement accuracy liquid chromatography mass spectrometry resulting in profiles of peptide features.

B) Bioinformatics capabilities are being developed to better align profiles of peptide features obtained from dynamic experiments to be conducted on cellulose degrading bioreactors. Aligned feature profiles will potentially be used to calculate a functional redundancy metric and identify interesting features for further targeted proteomics analysis.

MCI Advisory Committee Members:

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

Don't forget to check out the MCI Blog "What's Happening in Microbial Ecology" for relevant news articles, announcements, and other information.

<https://pnlweb.pnl.gov/projects/MCI/AllanMusings/default.aspx>