Report on the Joint EU-US Workshop on *Microbial Community Dynamics: Cooperation and Competition*

St. Louis, Missouri November 4-7, 2012

A Workshop organized under the auspices of the European Commission-United States Task Force on Biotechnology Research

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WORKSHOP SUMMARY

Background

The European Commission (EC)-United States (US) Task Force on Biotechnology Research has a longstanding joint Working Group on Biotechnology for the Environment whose mission is to foster collaborations between researchers in the European Union (EU) and US in the field of environmental biotechnology. A special focus of the Working Group is to increase scientific interchange between early career scientists in the US and EU. Such interactions initiate a foundation of respect and trust needed to develop long-term collaborations.

In order to realize the full potential for the application of modern technologies to obtain a sustainable biosphere, it is vital to create conduits for knowledge exchange among scientists worldwide engaged in environmental microbial biotechnology research. Since its formation in 1994, the Working Group has organized many activities for early career scientists designed to promote this scientific exchange, including two week courses with hands-on research experience, intensive workshops of two or three days, and research scholar exchanges of one to six months. These interactions are focused on environmental problems that respect no international boundaries.

Rationale

Over the last few decades, it has become increasingly clear that the limited resources of the Earth have been consumed without a realistic plan for conservation, restoration or alternatives. The impact of this careless consumption on future generations is of some controversy as the predictions are based on imperfect data. Clearly, the first line of defense for our environment resides with the microbes that are abundant and essential for major cycles of elements on Earth.

The emerging field of environmental biotechnology is providing new insights into the roles of microbes in biogeochemical cycling, contaminant degradation or sequestration, climate change effects and bioenergy conversion. To develop innovative sustainable processes based on microbial activities, it is crucial that microbial interactions and interchanges be elucidated. It is now recognized that in nature, most bacteria grow as communities adsorbed onto surfaces where they 1) may have more resources, 2) can enter a "nutritional resting state" that may protect the cells from noxious chemicals, 3) have stability in a flowing system that could renew resources and 4) can readily participate in genetic exchange. The importance of these biotic and abiotic controls on microbial activity are evident but are poorly understood. For many years, it has been

acknowledged that physiological responses observed in pure culture studies in the laboratory do not readily extrapolate to the field. Concepts of intra- and inter-species cooperation or competition mechanisms are in their infancy as technologies to approach this complex web are only now being developed. Therefore, the foci of this workshop were to examine the interactions of microbes deduced from the application of meta-omics tools to laboratory-designed, or naturally occurring, communities and to be introduced to the computational tools necessary for handling the data.

Support

The importance and multi-disciplinary dynamics of microbial communities are reflected in the diversity of funding sources for the US participation in the workshop. Not only do microbial communities play a critical role in the Earth's geochemical cycles, but they also are important for alternative energy, mitigation of environmental contaminants, and stabilizing human health. US Federal agencies supporting the workshop were:

- Department of Energy
- Office of Naval Research
- National Institutes of Environmental Health Sciences[1]
- National Science Foundation

Private support was also obtained for social interactions via the following companies:

- Thermo Fisher Scientific
- Monsanto

University of Missouri entities sponsoring this activity were:

- Biochemistry Division
- College of Agriculture, Food and Natural Resources
- Off ice of the Provost through the Mizzou Advantage Program

WORKSHOP OUTLINE

Organization

The workshop was held in the U.S. in the center of downtown St. Louis, Missouri, at the Hilton St. Louis at the Ballpark Hotel that is quite near the symbolic Gateway Arch and was organized as follows:

1) Forty individuals (20 EU and 20 US scientists) were invited to participate in the workshop. Twenty were senior scientists with established international reputations in their research specialties. Each senior investigator sponsored an early career scientist to participate based on his/her potential for future research contributions.

[1] : Funding for this conference was made possible (in part) by 1R13 ES022511-01 from the National Institute of Environmental Health Sciences. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

2) Oral presentations were given by each of the senior scientists that included a brief overview of his/her area of expertise, current results, and future directions or major questions yet to be addressed.
3) The twenty early career scientists presented their research results as posters that were displayed throughout the meeting.
4) A mentoring hour was arranged where early career scientists met with a senior scientist (who was not the sponsor) to discuss career plans and aspirations.
5) Members of the Working Group chaired the various sessions and lead discussions.
6) A banquet was held that allowed participants to discuss potential collaborative research funding.
7) A summary session lead by Jack Gilbert of Argonne National Laboratory and University of Chicago concluded the workshop. During this discussion, session Chairs summarized their topics. Two primary foci of this discussion were to identify future research perspectives and challenges as well as avenues for achieving transatlantic cooperation and collaboration in environmental biotechnology research.

Participants

ANNOTATED WORKSHOP PROGRAM

Day 1: Monday, November 5th

8:00 am *Welcome and Opening Remarks*
Robert Duncan, Vice Chancellor for Research, University of Missouri
Judy D. Wall (for R. Todd Anderson, US DOE, US Administrative Coordinator)
Herman Van Mellaert, EC Administrative Coordinator

The opening remarks recognized the positive historical track record of the Environmental Biotechnology Working Group and its focus on environmental issues facing both the US and EU. While the Working group has the goal of bringing current research closer to environmental application, it has also demonstrated a dedication to promoting the advancement of early career scientists. The fact that some of the former early career program participants are now members of the Working group was provided as a metric of the success of the Working Group.

Session 1 ***How diverse is diverse?***
Chaired by: Spiros Agathos, Université catholique de Louvain, Belgium

8:30 am **Jo Handelsman**, Yale University
"Molecular and functional diversity of environmental microbial communities"
9:00 am **Christoph Tebbe**, Institute for Biodiversity, Braunscheweig, Germany
"Bed and breakfast – how soil organic matter drives bacterial communities"
9:30 am **Howard Ochman,** Yale University
"Genomic analysis of diversity within bacterial communities"

The major theme of this session was the vast diversity of microbial communities and the molecular techniques used to profile and analyze this diversity. Microbial communities have

been traditionally profiled by targeting the 16S rRNA gene. In this session Dr. Handelsman highlighted that differences in molecular methodologies targeting the 16S rRNA can influence resulting data.

Session 2 ***Microbes in the light and the dark***
Chaired by: Judy D. Wall, University of Missouri

10:30 am **Richard Bardgett**, Lancaster University, Lancaster, UK
"Linking plants, soil microbes, and ecosystem nutrient cycles"

11:00 am **Angela Sessitsch**, Austrian Institute of Technology, Seibersdorf, Austria
"Diversity, community dynamics and functional characteristics of plant endophytes"

11:30 am **Vittorio Venturi,** Intl Center for Genetic Eng. and Biotechnol., Trieste, Italy
"Setting up models to study bacterial interspecies competition and cooperation as well as interkingdom signaling in plant associated bacteria"

12:00 pm **Raina M. Maier**, University of Arizona
"Making a living while starving in the dark: microbes in Kartchner Caverns"

Microbial communities present in special environments, soils, plants, as well as caves, were discussed in this session. With soil possessing 2.7 X more carbon than the atmosphere, it is imperative to understand how increased temperatures affect soil microbial activity. Increased microbial activity clearly increases CO_2 release to the atmosphere and in turn plant photosynthesis. This primary production will, in turn, increase the bioavailability of carbon in the soil through root exudates, thus creating a carbon cycle feedback loop. Therefore, vegetation changes will impact soil carbon dynamics opening the possibility that plant management might mitigate future CO_2 release.

Session 3 ***Microbial Communication***
Chaired by: Philippe Corvini, University of Applied Sciences, Northwestern Switzerland

1:30 pm **Josephine Chandler**, University of Washington, Seattle (Dr. Greenberg's lab)
"Acyl-homoserine lactone-dependent eavesdropping and interspecies competition"

2:00 pm **Roberto Kolter**, Harvard Medical School
"Chemical ecology of interspecies interactions"

2:30 pm **Claudia Schmidt-Dannert,** University of Minnesota
"Engineering synthetic microbial communities for biotechnology

Integral components of a microbial community are signaling and communication between its members. Discussed were the use of quorum sensing and incentives for cells to cooperate in communities. Our current knowledge regarding chemicals synthesized by bacteria during interspecies interactions is dramatically limited. The versatility of microbial metabolism increases the likelihood of success with synthetic microbial communities designed to produce desired products. Traditional genetic engineering has focused on genetically modifying individual cells to express complex pathways for synthesis of products for biotechnology. A

new approach is to bioengineer relationships and communication between different microorganisms that do not normally encounter one another for the synthesis of a diverse range of high value compounds.

Day 2: Tuesday, November 6th

Session 4 ***The niche chosen or prisoners dilemma?***
Chaired by: Barbara Methe, J. Craig Venter Institute

8:30 am **Barth Smets,** Technical University of Denmark, Lyngby, Denmark
"Spatially structured autotrophic nitrogen removing communities: Competition and cooperation"

9:00 am **Daniele Daffonchio,** University of Milan, Milano, Italy
"Arthopod-microbe symbiosis and the microbial diversity principles"

9:30 am **Jens Aamand,** Geological Survey of Denmark and Greenland, Copenhagen, Denmark
"Introduction of specific pesticide-degrading bacteria into waterworks sand filters – a technology for remediation of pesticide-polluted drinking water"

While microbial communities are dynamic and complex, members have their specific niche. Whether this niche is chosen or necessitated for mere survival depends on the environment. The presence of anthropogenic contaminants create specialized environments that select for microbial communities that tolerate or interact to remove contaminants.

In special cases, the community may include an interaction with a host. Bacterial associations with insects can be parasitic or mutualistic and may play a role in the nutrition, the physiology, or the reproduction of the host insect.

Session 5 ***Phage and plasmids in diversification***
Chaired by: Kelly Bender, Southern Illinois University

10:30 am **Sallie W. (Penny) Chisholm,** Massachusetts Institute of Technology
"What Prochlorococcus and its phage have been trying to teach us about microbial ecology and evolution"

11:00 am **Martha Clokie,** University of Leicester, Leicester, United Kingdom
"Impact of bacteriophages on bacterial physiology, population structure and long-term evolution: insights from bacteriophage genomes reveal novel mechanisms of bacterial manipulation"

11:30 am **Kornelia Smalla,** Inst. for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany
"Contribution of plasmids to bacterial adaptation and diversification"

Sometimes overlooked components of microbial communities are the bacteriophage and plasmids that can play key roles in determining the structure and niche as well as the genetic potential of particular members. Plasmid transfer within communities can also be a significant reservoir for metabolic diversity and host niche adaptation. Plasmid acquisition can allow bacteria to respond quickly to environmental challenges. These responses can include resistance

to metals or antibiotics as well as the ability to degrade contaminants. While phage can also transfer genes horizontally, bacteriophage infection has temporal, spatial, and seasonal effects on community dynamics as host lysis releases nutrients for other populations. Bacterial viruses are the most abundant entities on earth and are capable of wiping out entire microbial niches.

Session 6 ***Intimate microbiomes***
Chaired by: Balbina Nogales Fernández, University of the Balearic Island, Palma

1:00 pm **Eric Alm,** Massachusetts Institute of Technology
"The human microbiome in health and disease"
1:30 pm **Andrew Goodman,** Yale University
"Genetic approaches for characterizing community dynamics in the human gut microbiota"

With the evolution of high-throughput sequencing techniques and the discovery that microorganisms play a pivotal role in maintaining human health, understanding the community dynamics of the diverse human microbiome is imperative.

Day 3: Wednesday, November 7th

Session 7 ***Future research perspectives and challenges***

8:00 am **Jack Gilbert,** Argonne National Laboratory & University of Chicago
"Modeling the microbial world: the Earth Microbiome Project"
8:45 am **Highlights and Open Discussion-** Jack Gilbert, Facilitator

A fitting end to the community dynamics workshop was an overview of the ambitious Earth Microbiome Project. This multi-disciplinary project represents an ecological study of the world's microbial diversity and the processes that drive these patterns. An overall goal is to characterize the Earth by environmental parameters and obtain biomes from these biogeochemically distinct niches by analyzing omic data from 200,000 samples. A major strength associated with this project is the immediate, free, and open access to massive collections of data.

The overall discussion provided an opportunity for the attendees to summarize areas of importance such as inter- and intra- species signaling, effects of ecosystem specific pressures, and bacteriophage control of microbial communities. It was acknowledged that the field has undergone an intensive descriptive and characterization phase. A question remains about the sufficiency and value of currently collected data. Will scientists be able to utilize these data sets for modeling and predictions? With large metagenomic and metatranscriptomic data sets being collected to analyze the phylogenetic and functional diversity of communities, metabolic predictions are limited by the plethora of genes annotated as ORFs with unknown function. How do scientists determine the function of these genes?

This discussion opportunity also highlighted additional areas where advancement is necessary. One of the major issues facing our planet will be how to use environmental and microbial community data to promote CO_2 sequestration.

Another suggestion by the group was to focus on hypothesis based research versus characterizing samples, especially since physiology and mechanistic experiments must be coupled to the ecological work. As for the EU-US aspect, many of the attendees believed there would be great value in collaborating as multi-disciplinary teams. More avenues of funding between the US and EU are first necessary. These types of collaborations have the potential of high success, especially with teleconferencing and media technology advancing. Some specific points addressed by the attendees are listed below.

Current Challenges:

- The attendees admitted that better temporal and spatial descriptions of systems are needed before community dynamic predictions can be made.
- A plethora of meta-data sets have been generated, but what are the standards for analysis and are the data comparable due to differential sample handling?
- A major challenge is integrating and sharing a wide range of data types (meta-, geochemical, chemical, temporal, spatial, etc). More databases such as the Earth Microbiome Project need to be initiated.
- Long-term field sites also need to be preserved and promoted.
- Gene annotation is limited.

Future Research Areas:

- Some issues that demand attention are the metabolome and abiotic chemical reactions that occur in communities. What role do these molecules play during microbial interactions?
- Mechanisms to scale up current community experiments to translate to the field need to be focused on. One possibility is to focus on engineered microbial communities so they become scalable, manipulable, and predictable to validate the derived models.
- A potential funding area should promote the characterization of hypothetical genes and improved gene annotation. This is especially prudent now that advanced genetic and protein techniques are available.

Mentoring Hour

A highlight of the workshop was the opportunity for young scientists to discuss future career goals with senior scientists. Feedback from both the mentors and mentees was very positive. With pairs of mentors and mentees from different sides of the Atlantic, the mentors were surprised to be the ones benefiting by learning about differences in scientific and academic career paths in the US versus the EU.

Feedback from Attendees

The workshop received very positive feedback from the attendees. Below are some of the responses.

Early Career Scientists:

- 'I thought it would be nice to let you know that I am now writing a paper with another one of the young scientists - a collaboration that was formed during and after the

workshop!'

- It was an absolute honor to participate in the EU US Environmental Biotechnology Workshop last week in St. Louis. It was a fantastic meeting and I was so pleased to learn about the research ongoing in diverse labs around the world and to get to know so many new people.
- Thank you very much for the invitation to participate in the wonderful workshop and I definitely hope that our paths cross again in the future. If there is any way in which I could be helpful in organizing future meetings, courses or workshops, I would be delighted to hear from you. Thank you again for everything!'

Established Scientists:

- 'I just want to thank you for organizing the workshop last week. There were so many interesting talks - I really enjoyed being there. I am now back at work, where unfortunately a lot of administration has accumulated. I hope I can keep the inspiration from the workshop alive for a long time.'
- 'I wish to warmly thank you for organizing the workshop bringing together a very high profile group of people – It was very useful for me getting to know several people in this growing field of research.'