- ✓ Introduction: **Cyd E. Hamilton, AAAS Fellow, Department of Energy (DOE) (10 min)**

Title: Symbiosis Conference Introduction

Summary: Motivation for conference, deliverable from conference, introduction to plenary speakers.

- ✓ Plenary Speaker: **John Ferrell, DOE, Bioenergy Technology Office (30 min)**

Title: The Future of Bioenergy Feedstock Production

Summary: The Bioenergy Technologies Office (BETO) at U.S. Department of Energy funds research, development, and demonstration to help develop sustainable, cost-competitive biofuels, bioproducts, and biopower. An important component of the program is biomass supply and logistics. Efforts include assessing biomass potential, enhancing production, and improving logistics. Meeting the future demands for bioenergy and bioproducts will require investments in integrated production, logistics, conversion, and deployment systems. BETO is working with various stakeholders on biomass accessibility, availability, and production issues, reducing costs for biomass as well as final product costs, and improving the quality of the feedstocks. Energy crop deployment and acceptance, crop yields, and sustainability are important feedstock production issues being addressed through multi-disciplinary approaches. Understanding and capitalizing on symbiotic relationships in enhancing production and biomass availability is another approach for consideration.

- ✓ Plenary Speaker: **Catherine Ronning, DOE, Office of Science (30 min)**

Title: Plant-Microbe Interactions: An Overview of Basic Research Supported by the DOE Office of Science

Summary: The DOE Office of Science, Office of Biological and Environmental Research (BER) supports a diverse portfolio of fundamental research to achieve a predictive systems-level understanding of complex biological systems to advance DOE missions in energy and the environment. Because of the critical role plant-microbe symbioses play in modulating mission-relevant events such as plant health and carbon cycling, BER is interested in understanding these very complex interactions at the molecular level and in relation to the ecosystem as a whole. An overview of BER-supported research in plant-microbe interactions will be presented, along with a vision of how these projects complement the overall program.

Session 1: Models and systems biology: Stable mutualisms – Moderator – Dr. James White, Jr

- ✓ Speaker: **James White, Rutgers University (30 min)**

Title: Enhancing plant growth and stress tolerance through use of fungi and bacteria that comprise plant microbiomes

Summary: Defensive and nutritional effects of fungi and bacteria in plant microbiomes will be discussed; and a strategy advocated for improving plant growth and stress tolerance through compositional manipulation of the population of microbes comprising crop microbiomes.

- ✓ Speaker: **Dale A Pelletier, Oak Ridge National Lab**

Title: Plant-microbe interactions in the Populus rhizosphere

Co-authors: Chris Schadt, Dave Weston, Jessy Labbe, Steve Brown, Mitch Doktycz

Summary: We have examined the phylogenetic diversity within the rhizosphere and endosphere microbial community of *Populus* by 16S pyrosequencing. Root endosphere and rhizosphere communities have been found to be composed of distinct assemblages of bacteria with largely non-overlapping OTU distributions. Within these distinct endophyte and rhizosphere habitats, community structure is also influenced by soil characteristics, geographic origin and/or plant genotype, while observed seasonal influences have been minimal. We have isolated over 2700 bacteria from these environments representing dominant community members *in situ.* Many of these isolates show distinct growth phenotypes with *Populus* and the consequences of microbial community assembly and abundance on host plant function is actively being investigated. This provides an ideal platform for investigating molecular interactions involved in establishing mutualistic microbial-*Populus* relationships.

- ✓ Speaker: **Jean-Michel Ané, University of Wisconsin**

Title: Understanding and engineering the molecular mechanisms controlling efficient symbioses between plants and microbes

Summary: Our primary research interest is understanding the establishment of mutualistic associations between plants and microbes, and the application of this knowledge to maximize the benefits of such associations in agriculture. Our first goal is to understand the genetic and molecular mechanisms controlling these associations. We focus on two types of mutualisms having significant impacts on terrestrial ecosystems and agriculture: rhizobia-legume associations and mycorrhizal associations. For this, we are working with various microbes (rhizobia, endo- and ecto-mycorrhizal fungi) and plant genetic models (*Medicago truncatula,* poplar, *Brachypodium distachyon,* and *Setaria viridis*). We are particularly interested in signals produced by symbiotic microbes, and signaling pathways allowing host plants to perceive and transduce these microbial signals. We are also transferring information from model systems to crops such as soybean, rice and maize. Our second goal is to understand the evolution of these mechanisms in order to identify the critical

Session 1: Models and systems biology: Stable mutualisms – Moderator – Dr. James White, Jr

innovations that allowed the evolution of such efficient associations between land plants and microbes. Our third goal is to use this knowledge about genetic mechanisms and their evolution to engineer more efficient associations between cereals and nitrogen-fixing bacteria in order to improve the sustainability of our agriculture for food, feed, and biofuel production. We will present how these ongoing activities may lead to short-term and long-term improvements on our utilization of mutualistic symbionts in bioenergy crop production.

- ✓ Speaker: **Michael Grisham, USDA-ARS**

Title: Potential of diazotrophic, endophytic bacteria associated with sugarcane for energycane production
Co-Authors: Anna H. Hale, Paul M. White
Summary: Crosses between sugarcane and wild species of *Saccharum* and other closely related genera are made to introgress new genes from the wild species into sugarcane. Characteristics of the progeny from these crosses may include increased biomass and the ability to be grown in a broader geographical range than sugarcane. Progeny from these wide genetic crosses have been selected for their potential as feedstock for the production of bioenergy and, as such, have been referred to as energycane. As part of an objective to reduce production costs of sugarcane and energycane, seven genera of diazotrophic, endophytic bacteria were identified among 85 isolates recovered from sugarcane. Experiments are being conducted to determine the potential of these bacteria to lower nitrogen rates among varieties of sugarcane and energycane. The bacterial isolates are also being tested for the ability to reduce the effect of systemic pathogens.

- ✓ Speaker: **Dan Moebus-Clune, Cornell University**

Preliminary Title: Managing multi-symbiont complexes

Summary: Managing symbioses, natural systems, multi-partner symbiotic systems, interactions more complex than simple trade models, C and N movement between components of a multi-host (grass and legume), multi-symbiont (*Glomus* and *Rhizobium*) complex, managed to improve proportional reliance on biological N fixation.

- ✓ Speaker**: Heike Bücking, South Dakota State University**

Title: Arbuscular mycorrhizal interactions – an important trait for biomass production of bioenergy crops?

Summary: The arbuscular mycorrhizal symbiosis is arguably the most important symbiosis on Earth and plays an essential role in the nutrient uptake of the majority of land plants. The extraradical mycelium of the fungus acts as an extension of the root system and increases the uptake of P and N, and arbuscular mycorrhizal fungi have an effect on many other important plant traits that control biomass production. Despite their clearly documented effects on yield and nutrient efficiency of many crops, the impact of arbuscular mycorrhizal fungi on biomass production

of bioenergy crops has not extensively been studied. The presentation will highlight some of these effects, and will discuss some of the research gaps that need to be filled to reach the maximum potential of these interactions for bioenergy crop production.

- ✓ Speaker: **Gerald Tuskan, ORNL**

Title: Fungal diversity within the Populus rhizoshpere and endosphere

Co-authors: Jessy Labbe, Pascale Frey-Klett, Aurelie Deveau, Dale Pelletier, David Weston, Gregory Bonito, Rytas Vilgalys

Summary: Fungal isolates from both the rhizosphere and endosphere of *Populus* have been collected from multiple field campaigns via direct isolation from soil and root samples as well as from trap plant experiments. To date, we have over 1500 alternate isolates in our collection. We have re-inoculated many of these isolates back onto *Populus* to characterize their effects on growth and performance across 3 *Populus* species. In addition, we have initiated a series of experiments to characterize the combinatorial effects of bacteria on fungal growth and performance during *Populus* colonization. Specifically, we looked at the bacterial effect on *Laccaria* growth and mycelium morphology (branching and density) and the expression level of seven target genes in *L. bicolor* shown as regulated by the MHB strain BBc6R8. Four MHB strains showed high helper effects and one significantly improving *P. deltoides* colonization by *Laccaria*. The *Pseudomonas fluorescens* strain GM41 has a significant effect on the growth of the mycelium and root colonization (ANOVA, $p \geq 0.01$). To test the importance of these functions, we constructed random miniTn5 mutant library: 4,032 mutants in BBc6R8 and 5 in GM41. The *P. fluorescens* BBc6R8 mutant *Δflgk* significantly impacted *Laccaria* growth (ANOVA, $p \geq 0.01$).

- ✓ Speaker: **Hilary Mayton, Rutgers University**

Title: Long-term sustainable production of biomass through biodiversity research trials and plant breeding

Summary: The presentation will focus on research conducted with warm season perennial grass mixtures for yield on marginal and prime land in the northeast region of the USA and an overview of plant breeding for improved resistance of Switchgrass to disease in the northeast.

Session 2: Agency perspectives, policy, and sustainability – Moderator – Dr. James White

- ✓ Speaker: **Bryce Stokes, CNJV, LLC (30min)**

Title: Importance of Biomass Production and Supply

Summary: An important requirement for a new bioeconomy is to have sufficient quantities of biomass feedstocks at low-cost and high quality. DOE's BETO is working aggressively to meet this requirement. This presentation discusses these aspects of biomass production and some of the supply implications. Land constraints, low crop yields, heterogeneous biomass characteristics and

high costs are barriers to full deployment of biomass-derived products, but an integrated approach to providing biomass can still provide an opportunity for the U.S. to provide over a billion dry tons of biomass annually at competitive costs that meet or exceed conversion specifications.

- ✓ Speaker: **Mei Chuansheng, Institute for Advanced Learning and Research**

Title: Developing a Low Input and Sustainable Switchgrass Feedstock Production System Harnessing Beneficial Bacterial Endophytes

Summary: Switchgrass is a promising feedstock crop for US energy sustainability. Improvement of poor establishment, biomass yields, and stress tolerance can be improved via harnessing of beneficial bacterial endophytes documented to enhance Switchgrass performance under poor growth conditions. The goal is to develop a low input and sustainable feedstock production system for marginal lands that will not compete with food crop production. Molecular mechanisms under which beneficial bacterial endophytes promote plant growth are identified. In addition, the environmental effects of bioenergy production involving endophyte applications are explored specific to changes in required nutrition availability to the host plant and symbiotum effects on soil carbon sequestration.

Session 3: Commercialization: Barriers, strategies to scale-up and effectively distribute – Moderator – Dr. Cyd E. Hamilton

- ✓ Speaker: **Gary Harmon, formerly Cornell University (30 min)**

Title: *Endophytic plant symbionts that enhance plant growth and productivity and that assist bioremediation*

Summary: Endophytic plant symbionts can markedly assist production of biomass for energy production. Some organisms, such as highly selected strains of *Trichoderma*, already are being widely used for growth and yield enhancement of agricultural crops. They improve resistance to disease, abiotic stresses including soil contaminants, increase N use efficiency in plants, enhance root development and, very importantly increase plant photosynthetic efficiency. Highly effective strains were selected using genomic approaches that illustrated systemic changes in plant gene expression. They are broadly effective on both bioenergy and agricultural crops and can be delivered in a variety of methods. They are readily commercially available and provide long-term benefits to plant productivity because they colonize and grow with roots.

- ✓ Speaker: **Geoffrey von Maltzahn, Principal, VentureLabs**

Title: Experiences in the emerging industrial space of microbiome biotechnology

Summary: Fueled by exponential trends in our ability to interrogate microbial ecosystems, insights into natural microbiota have the potential to impact multiple industries and provide fundamentally new solutions to challenges in clean energy, global health, materials, and

sustainability. The realization of this potential will be driven by highly adaptive and inventive teams with diverse expertise, common goals, and the ability to swiftly navigate scientific, technological,

market, and legal complexities—areas where I believe innovative startups can excel. This presentation will highlight experiences at the interface of biology, microecology, and entrepreneurship, including examples from the founding of two VentureLabs startups, focused respectively on pioneering a new class of therapeutics around the biology of the human microbiome and on augmenting plant microbiome functions to increase crop yield and stress resilience.

LIST OF ATTENDEES

Ibrahim Aikawa, Engineer, University of Wolverhampton, UK
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Vanessa Bailey, Senior Research Scientist, Pacific Northwest National Laboratory
Dr. Bailey's research focuses on the microbial ecology of soils and other terrestrial ecosystems. Related to this are efforts to understand the carbon cycling processes of microbial communities. Her research is conducted both in the field, where landscape processes are observed and soils sampled, as well as in the lab where studies occur in controlled microcosms. Traditional, microbial, and molecular biological tools are all used in this rese
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David Benham, **Energy Program Specialist, General Services Administration**
Interested in renewable and sustainable energy technologies with strong interest in algae
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David Culley, Senior Research Scientist, Pacific Northwest National Laboratory
Research interests: Biochemical and biological conversion of cellulosics to advanced biofuels; genome, metabolic, and genetic exploration of filamentous fungi; evolved associations between photosynthetic organisms and heterotrophic fungi.
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James Hannasch, **Senior Project manager, AZDEMA AZARNG CFMO**

Steve Lewis, Vice President, Innovation, POET
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Lead role in development of innovative processes for starch to ethanol, new product development, integrated technology designs.

Christine McKiernan, VP Engineering, Bioferm Energy Systems
Bioferm Energy Systems is a company of Viessmann Group. Product range foci are heating and climate control, anaerobic digestion-dry fermentation systems.
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Manan Parikh, Biomass Research Associate, American Council on Renewables (ACORE)
Manan Parikh is a biomass policy and engineering research associate with the American Council On Renewable Energy (ACORE). He is focused on applying the data obtained from scientific breakthroughs with first and second generation biofuels to the policies that affect the growth of this industry; effectively serving as a liaison to those who are at a disconnect from the innovators. His past work is extensively research-based, holding biophysics and biochemistry associate positions at institutions such as UNC-Chapel Hill and the National Naval Medical Center, respectively. He holds a B.S. in Biochemistry from The University of Texas at Austin, and recently completed a professional certification program from Stanford University in Energy Innovation and Emerging Technologies. He is a member of the Clean Energy Leadership Institute in Washington, DC.
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Reda Wahb, Engineer, Egypt
Research interests: Seawater desalination, seawater and waste water treatment, with interests in renewable energy and greenhouse agriculture production.
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Peter Woodbury, Senior Research Associate, Dept of Crop and Soil Sci, Cornell University
Research foci include ecological effects of stressors such as global change, tropospheric ozone, and land use change on soil, air & water quality, and GHGe in agricultural and forest ecosystems. Development of spatially explicit models to understand various ecosystems at multiple scales and utilize a variety of model to quantify agricultural sustainability and forest ecosystems including management and policy.
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