JGI JOINT GENOME INSTITUTE
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Microbial and Plant Systems Modulated by Secondary Metabolites Meeting

May 2–4, 2016
Walnut Creek, California

Meeting Abstracts

BERKELEY LAB

U.S. DEPARTMENT OF ENERGY
Office of Science

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Agenda

Monday, May 2 (Walnut Creek Marriott)		
5:00 – 5:10 PM	***Opening Remarks***	*Host:* Susannah Tringe, JGI
5:10 – 6:00 PM	***Opening Key Note*: Niche adaptation of the *Arabidopsis* leaf microbiota**	Julia Vorholt, ETH Zurich
6:00 – 9:00 PM	**Poster Session and Reception** **Refreshments served*	

Tuesday, May 3 (JGI Room 149A)		
9:00 – 10:10 AM	***Overview of the JGI Capabilities*** **Will include morning break & working lunch*	*Chair:* Yasuo Yoshikuni, JGI
9:00 – 9:10 AM	**Welcome and Introduction** **Morning refreshments served*	Susannah Tringe, JGI
9:10 – 9:25 AM	**JGI Synthesis Science**	Yasuo Yoshikuni, JGI
9:25 – 9:40 AM	**JGI Synthesis Platform**	Sam Deutsch, JGI
9:40 – 9:55 AM	**IMG-ABC: A Resource for Biosynthetic Gene Cluster Discovery and Analysis**	Michalis Hadjithomas, JGI
9:55 – 10:10 AM	**JGI Metabolomics**	Trent Northen, JGI
10:10 – 10:30 AM	***Break***	
10:30 – 12:05 PM	**SESSION I: Science from the JGI User Communities**	
10:30 – 10:35 AM	**Introduction**	Yasuo Yoshikuni, JGI
10:35 – 11:05 AM	**Investigating Plant Terpene Metabolic Diversity for Pharmaceutical and Agricultural Applications**	Philipp Zerbe, University of California, Davis
11:05 – 11:35 AM	**Volatile Metabolites as Chemical Modulators of Plant-Microbe Interactions**	Dorothea Tholl, Virginia Tech University
11:35 – 12:05 PM	**Assessing the Role of Biotic and Abiotic Interactions in Determining the Community Composition of the Root Microbiome**	Omri Finkel, University of North Carolina, Chapel Hill
12:05 – 1:05 PM	**Group Discussion and Picture** **Working lunch served*	
1:05 – 2:40 PM	**SESSION II**	
1:05 – 1:10 PM	**Introduction**	Sam Deutsch, JGI
1:10 – 1:40 PM	**Chemical Discovery in the Microbial World**	Emily Balskus, Harvard

1:40 – 2:10 PM	**Biosynthetic Gene Clusters: Nature's Gift to Discovery and Application**	Bradley Moore, Scripps Institution of Oceanography
2:10 – 2:40 PM	**Metabolic Regulation of Community Behavior in *Pseudomonas aeruginosa***	Lars Dietrich, Columbia University
2:40 – 3:00 PM	***Break***	
3:00 – 5:00 PM	**Panel Discussion**	Kellye Eversole, Phytobiome Initiative Randy Verka, Novozymes Daniel Van der Lelie, FMC Steve Evans, Dow AgroScience Dilara Ally, Bayer CropScience Rich Broglie, DuPont Pioneer

Wednesday, May 4 (JGI Room 149A)

9:10 – 10:45 AM	**SESSION III**	
9:10 – 9:15 AM	**Introduction**	Elizabeth Sattely, Stanford University
9:15 – 9:45 AM	**Specialized Cell Types and the Rise of Chemical Diversity in Plants**	Mark Lange, Washington State University
9:45 – 10:15 AM	**Integrated Metabolomics for Triterpenoid Gene Discovery in the Model Legume *Medicago truncatula***	Lloyd Sumner, University of Missouri
10:15 – 10:45 AM	**Botanicals and Experimental Pharmacology in the Post-Genomic Era: New Directions**	Larry Walker, University of Mississippi National Center for Natural Products Research University
10:45 – 11:05 AM	***Break***	
11:05 – 12:40 PM	**SESSION IV**	
11:05 – 11:10 AM	**Introduction: Secondary Metabolites: Chemical Warfare in Plant-Pathogen-Antagonist Interactions**	David Weller, USDA
11:10 – 11:40 AM	**Rhizosphere Diversity of Fluorescent Pseudomonads and Cyclic Lipopeptides Correlates with Cocoyam *(Xanthosoma sagittifolium)* Resilience to the Pythium Root Rot Disease**	Monica Höfte, Ghent University
11:40 – 12:10 PM	**Crossfire: Diterpenoids in the Chemical Warfare Between Rice and a Bacterial Pathogen**	Reuben Peters, Iowa State University
12:10 – 12:40 PM	**Secondary Metabolites Have Primary Roles in the Lifestyle of the Soil Bacterium *Pseudomonas protegens***	Joyce Loper, USDA-ARS, Oregon State University
12:40 – 1:40 PM	****Group Discussion and Facility Tour*** **Working lunch served*	

1:40 – 3:15 PM	**SESSION V: Parts, Pathways and Performance: Discovery and Deployment of Plant Specialized Metabolic Pathways**	
1:40 – 1:45 PM	**Introduction: Parts, Pathways and Performance: Discovery and Deployment of Plant Specialized Metabolic Pathways**	Peter Facchini, University of Calgary
1:45 – 2:15 PM	**Harnessing Plant Metabolic Diversity**	Anne Osbourn, John Innes Centre, UK
2:15 – 2:45 PM	**How Do Plants Mediate Crosstalk Between Biochemical Pathways?**	Clint Chapple, Purdue University
2:45 – 3:15 PM	**Gene Centric Discovery of Plant Metabolic Pathways**	Elizabeth Sattely, Stanford University
3:15 – 3:30 PM	***Break***	
3:30 – 4:55 PM	**SESSION VI**	
3:30 – 3:35 PM	**Introduction**	Eoin Brodie, LBNL
3:35 – 4:05 PM	**TBD**	Gabriele Berg, Graz University of Technology
4:05 – 4:55 PM	**Closing Key Note: Mining Terrestrial and Aquatic Microbiomes for Novel Metabolites**	Jos Raaijmakers, NIOO Wageningen
4:55 – 5:00 PM	**Closing Remarks**	Axel Visel, JGI

*Note: AM and PM refreshments will be served after the meeting begins, while work is being performed. Attendance is required during these times.

Speaker Presentations

Abstracts alphabetical by speaker

Chemical Discovery in the Microbial World

Balskus, Emily (balskus@chemistry.harvard.edu)

Harvard.

Microorganisms like bacteria, archaea, and fungi have amazing chemical capabilities, performing challenging transformations and producing complex, biologically active molecules not easily accessed via any other means. However, we do not yet have an understanding of the enzymatic chemistry responsible for the full diversity of reactions performed by these organisms and we lack a complete understanding of the roles this chemistry plays in natural microbial habitats. Recent advances in DNA sequencing technologies have delivered a wealth of microbial genomes, providing an unprecedented opportunity to discover both enzymes that have the potential to change the way chemists make molecules, as well as metabolic activities that will transform our understanding of microbial and human biology. This talk will discuss my group's recent progress in deciphering the enzymatic chemistry responsible for the production of multiple bacterial metabolites, including scaffolds of unusual molecular architecture and disease-linked molecules made by the human gut microbiota.

Berg, Gabriele (gabriele.berg@tugraz.at)

Graz University of Technology.
Profession: Full Professor at Graz University of Technology, Institute of Environmental Biotechnology.

How Do Plants Mediate Crosstalk Between Biochemical Pathways?

Chapple Clint (chapple@purdue.edu)

Purdue University

The products of the phenylpropanoid pathway range from complex, insoluble polymers such as lignin and suberin, to soluble flavonoids and hydroxycinnamate esters, to volatile compounds used to attract pollinators. In addition to its important role in plant biology, lignin has a significant impact on the effectiveness of converting cell wall polysaccharides to ethanol or second-generation biofuels. For this reason, a great deal of research has focused on altering phenylpropanoid metabolism through mutation or RNAi-mediated down-regulation of genes encoding pathway enzymes. Although many of these manipulations lead to significant alterations in plant metabolism, defects at a number of biosynthetic steps lead to a common suite of pleiotropic phenotypes such as dwarfing and sterility, the severity of which is dependent on the strength of the metabolic restriction.

Analysis of the reduced epidermal fluorescence4 (ref4) mutant has revealed that the REF4 protein play a role in the suppression of phenylpropanoid biosynthesis in wild-type plants. REF4 and its paralog, REF4-related 1 (RFR1), have recently been shown to be components of Mediator, a large multi-protein complex that facilitates interactions between DNA-bound transcription factors and RNA polymerase II. Mutants of *Arabidopsis* that lack REF4 and RFR1 hyperaccumulate phenylpropanoids and show little in the way of developmental changes. Surprisingly, ref4/rfr1 mutations mitigate the dwarf phenotype and sterility of the ref8 mutant of *Arabidopsis* which is defective in the early phenylpropanoid pathway enzyme p-coumaroyl shikimate 3-hydroxylase. Our data reveal that dwarfism in at least some phenylpropanoid pathway mutants is the result of a cascade of transcriptional mis-regulation that is dependent on Mediator. We have also recently found that crosstalk within the phenylpropanoid pathway and between the phenylpropanoid and glucosinolate pathways is also Mediator-dependent, suggesting that the complex may play a central role in coordinating metabolic pathway flux in plants.

A Scalable Refactoring Pipeline for the Production of Biomolecules

Deutsch, Sam (sdeutsch@lbl.gov)

DOE Joint Genome Institute.

Next-generation sequencing technologies have enabled single genomes as well as complex environmental samples (metagenomes) to be sequenced at an ever-increasing pace. Advanced bioinformatics analysis of the resulting genomics and transcriptomics data is revealing an unprecedented catalogue of proteins, and pathways that encode novel catalytic activities and a large diversity of novel compounds of potential value for environmental and biomedical applications.

Characterizing the products of predicted biosynthetic pathways remains challenging as (i) Clusters originate from diverse organisms many of which are difficult to culture and manipulate, (ii) Biosynthetic clusters are often under transcriptional or translational repression by unknown regulatory mechanisms, (iii) Biosynthetic clusters are often large and complex in terms of sequence composition, (iv) Molecule production may require the presence of unknown substrates or co-factors.

To characterize the products of cryptic biosynthetic clusters, improve the yield of biochemically-characterized pathways or produce novel molecules in heterologous hosts, we are developing tools for scalable design and construction of pathways specifically optimized for expression in a given host. Integrating strain construction to high-throughput genotyping technologies allows for data to be collected and analyzed such that design paradigms can be successively tuned to improve success rates. Multiple examples of how the pipeline is being implemented will be discussed.

Metabolic Regulation of Community Behavior in *Pseudomonas Aeruginosa*

Dietrich, Lars (ld2444@columbia.edu)

Columbia University.

Studies of signaling cascades can reveal important mechanisms driving multicellular development, but the models that emerge often lack critical links to environmental cues and metabolites. We study the effects of extra- and intracellular chemistry on biofilm morphogenesis in the pathogenic bacterium *Pseudomonas aeruginosa*, which produces oxidizing pigments called phenazines. While wild-type colonies are relatively smooth, phenazine-null mutant colonies are wrinkled. Initiation of wrinkling coincides with a maximally reduced intracellular redox state, suggesting that wrinkling is a mechanism for coping with electron acceptor limitation. Consistent with this, provision of nitrate renders phenazine-null colonies smooth. Mutational analyes and in situ expression profiling have revealed roles for PAS-domain and other redox-sensing regulatory proteins, as well as genes involved in motility and matrix production, in colony morphogenesis. To characterize endogenous electron acceptor production, we have developed a novel chip that serves as a growth support for biofilms and allows electrochemical detection and spatiotemporal resolution of phenazine production in situ. We are further developing this chip for detection of various redox-active metabolites. Through these diverse approaches, we are developing a broad picture of the mechanisms and metabolites that exert an integrated influence over redox homeostasis in P. aeruginosa biofilms.

Phytobiomes Roadmap and the International Alliance for Phytobiomes Research

Eversole, Kellye (eversole@eversoleassociates.com)[1,4]; Leach, Jan E.[2,4]; and Beattie, Gwyn[3,4]

[1]Eversole Associates, Bethesda, Maryland. [2]Colorado State University, Fort Collins, Colorado. [3]Iowa State University, Ames, Iowa. [4]International Alliance for Phytobiomes Research, Lee's Summit, Missouri.

To meet the global demands for food, feed, and fiber in 2050 and beyond, sustainable agricultural systems must improve considerably in the face of a steadily changing climate and increased biotic and abiotic stressors. These improvements will require a holistic, systems-level approach that integrates knowledge from a breadth of disciplines, including agronomy, pathology, physiology, genomics, genetics, breeding, physics, synthetic biology, and modeling. While genetic and genomic resources for plants have grown exponentially over the past ten years, we still have a limited understanding of genotype by environment by management (GxExM) interactions that determine agricultural productivity, quality, and the ability to withstand biotic and abiotic stressors. We need a better understanding of the interactions among plants, microorganisms, soils, and climate, in other words, a better understanding of the "phytobiome". There is a large diversity of phytobiomes, as each is unique to a region, a crop, a soil, etc. Recently, the Phytobiomes Roadmap for Research and Translation was released (www.phytobiomes.org) and the International Alliance for Phytobiomes Research, a new, public-private non-profit organization, is being established to implement the Roadmap. A brief overview of the Roadmap and the Alliance will be presented.

Parts, Pathways and Performance: Discovery and Deployment of Plant Specialized Metabolic Pathways

Facchini, Peter (pfacchin@ucalgary.ca)

University of Calgary.

Assessing the Role of Biotic and Abiotic Interactions in Determining the Community Composition of the Root Microbiome

Finkel, Omri M. (ofinkel@live.unc.edu), Gabriel Castrillo, Isai Salas González, Sur Herrera Paredes, Scott Yourstone, Meredith McDonald, Jeff Dangl

Biology Department, University of North Carolina at Chapel Hill, NC.

The structure and function of host associated microbial communities is affected by the host phenotype, but it can also drive it. Microorganisms are known to aid plants through processes like nutrient acquisition or protection from pathogens, but the complexity of microbial communities found in association with plants has limited our ability to draw general rules guiding the assembly and maintenance of a functionally robust microbiome.

We present here a suite of in-silico, in-vitro and in-plantae approaches to designing and testing synthetic communities. We are using these approaches in order to understand the relative contribution of a-biotic and biotic factors to determining the community composition of bacteria in and around plants. We are asking (a) what is the relative contribution of factors such as nutrient availability, salinity, pH and temperature to the assembly of bacterial communities in plants and (b) is there, along with the direct effect of the a-biotic conditions, an indirect effect on community composition, through modulation of plant exudates.

We aim to answer these questions using 200-member synthetic consortia of genome sequenced isolates obtained from A. thaliana roots. Plants are inoculated with these consortia under varying conditions. In parallel, in-vitro growth of all members of the community is measured in the presence of plant exudates produced under the same set of a-biotic conditions. Combining plant colonization patterns with in-vitro growth data and genomic features of the community members will provide us with a powerful tool to bridge the gap between observed patterns and mechanism.

Rhizosphere Diversity of Fluorescent Pseudomonads and Cyclic Lipopeptides Correlates with Cocoyam (*Xanthosoma sagittifolium*) Resilience to the Pythium Root Rot Disease

Höfte, Monica[1]; Feyisara Eyiwumi Olorunleke1; Niels Geudens[2]; Olumide Omoboye[1]; Lien Bertier[1]; Amayana Adiobo[3]; Joseph Onyeka[4]; Ayodeji Salami[5]; Davy Sinnaeve[2]; Jose C. Martins[2];

[1]Laboratory of Phytopathology, Ghent University, Coupure Links 653, B-9000, Belgium. [2]Department of Organic Chemistry, Ghent University, Krijgslaan 281, B-9000, Belgium [3]Institute for Agricultural Research for Development, Ekona, P.M.B 25, Buea, Cameroon [4]Plant Pathology Unit, National Root Crops Research Institute, PMB 7006 Umuahia, Abia State, Nigeria. [5]Department of Crop, Soil and Environmental Sciences, Ekiti State University, PMB 5363, Ekiti State, Nigeria.

Cocoyam (*Xanthosoma sagittifolium*), is an important carbohydrate staple cultivated in Nigeria and Cameroon. Its production is impaired by the Cocoyam Root Rot Disease (CRRD), attributed to the Oomycete pathogen Pythium myriotylum, which can cause yield losses of up to 90% on cocoyam fields. In Cameroon, volcanic andosol soil types show resilience to CRRD, while in Nigeria, all soils examined so

far are conducive to the disease. This study aimed at deciphering the taxonomic and metabolic diversity of fluorescent Pseudomonas spp. associated with the cocoyam rhizosphere in soils of Cameroon and Nigeria and to substantiate a possible link between microbial diversity and disease resilience. A large collection of Pseudomonas strains was isolated from the rhizosphere of cocoyam plants in various farms located in Cameroon and Nigeria. Phylogenetic relationships of these strains were established using multi locus sequence analysis and cyclic lipopeptides (CLP) were characterized using HPLC/MS and NMR. Isolates obtained from suppressive soils clustered in the *P. fluorescens* complex and P. putida group, while for conducive soils, isolates obtained predominantly belonged to the P. putida and P. aeruginosa group. HPLC/MS and NMR analysis showed that diversity in CLPs was highest within the *P. fluorescens* complex. Moreover, within the P. putida group, CLP diversity was higher for isolates from disease resilient soils than for isolates from conducive soils. Some CLP types only occurred in specific taxonomic subgroups, while others were dispersed, hinting at horizontal gene transfer of the corresponding biosynthetic gene clusters. Thus, CRRD resilient soils possess a significantly higher representation of Pseudomonas strains belonging to the *P. fluorescens* complex and a higher diversity of CLPs compared to conducive soils.

Specialized Cell Types and the Rise of Chemical Diversity in Plants

Lange, Bernd Markus (lange-m@wsu.edu)

Institute of Biological Chemistry and M.J. Murdock Metabolomics Laboratory, Washington State University.

As non-mobile organisms, plants face many biotic and abiotic challenges. Specialized metabolites play important roles in coping with these stresses. Plants have evolved secretory cells types and tissues to synthesize and store bioactive specialized metabolites. In this presentation, I will provide a brief overview of the evoluation of secretory structures and will emphasize the importance of building spectral resources to capture the distinctive specialized metabolites accumulated therein. I will also highlight how integrative approaches have been used to unravel the structural genes and enzymes but also the regulatory machinery associated with specialized metabolite biosynthesis in plant secretory structures.

Secondary Metabolites Have Primary Roles in the Lifestyle of the Soil Bacterium *Pseudomonas protegens*

Loper, Joyce E. [1,4]; Qing Yan[1]; Benjamin Philmus[2]; Barbara J. Taylor[3]; Brenda T. Shaffer[4];

[1]Department of Botany and Plant Pathology. [2]Department of Pharmaceutical Sciences. [3]Department of Integrated Biology, Oregon State University. [4]USDA-ARS, Corvallis, Oregon, USA.

Bacteria can be both highly communicative and competitive in natural habitats and secondary metabolites have key roles in both of these processes. Our studies focus on the soil bacterium *Pseudomonas protegens* Pf-5, which produces seven known secondary metabolites (pyoluteorin, pyrrolnitrin, the lipopeptide orfamide A, derivatives of rhizoxin, 2,4-diacetylphloroglucinol, hydrogen cyanide, and toxoflavin), many of which were discovered via genomics-enabled approaches. Pf-5 was first described for its capacity to colonize seed surfaces to suppress plant diseases caused by seed-infecting fungi and oomycetes due to the production of secondary metabolites with antibiotic activity. More recently, we have demonstrated a role for secondary metabolites in many other aspects of the bacterium's lifestyle, including its toxicity to insects. At least two of Pf-5's secondary

metabolites, pyoluteorin and 2,4-diacetylphloroglucinol (DAPG), function in intracellular and intercellular communication, both as autoinducers of their own production. Phloroglucinol, an intermediate in DAPG biosynthesis, also serves as an intercellular chemical messenger that is required for pyoluteorin production and the transcription of pyoluteorin biosynthesis genes. Positive regulation of the pyoluteorin biosynthesis gene pltL by the linked transcriptional regulator PltR requires phloroglucinol and is abolished by a mutation in pltM, a gene in the pyoluteorin gene cluster that encodes a putative halogenase. A pltM mutant of Pf-5 does not produce pyoluteorin or express pltL even in the presence of phloroglucinol. Purified PltM converts phloroglucinol into chloro-1,3,5-trihydroxybenzene and 2-chloro-1,3,5-trihydroxybenzene, two chlorinated derivatives of phloroglucinol that induce pltL expression in a pltM deficient strain. Our data show that coordination between the DAPG and pyoluteorin pathways is mediated by novel signaling molecules synthesized from components of both pathways: phloroglucinol and PltM. The large spectrum of secondary metabolites produced by P. protegens Pf-5 have key roles in its varied lifestyle, as weapons or shields mediating competitive interactions, and as chemical messengers facilitating communication between bacterial cells.

Biosynthetic Gene Clusters: Nature's Gift to Discovery and Application

Moore, Bradley S.

University of California at San Diego.

Microbes typically cluster genes encoding the biosynthesis of secondary metabolites together with regulatory, resistance, and transport elements in so-called biosynthetic gene clusters (BGCs). This convenient genomic clustering has experimentally facilitated the remarkable explosion of research into their discovery and application in the life science and biomedical fields. This presentation will discuss new methods from the author's laboratory to link BGCs to small molecules as a multifaceted enabling technology to drive natural product discovery, pharmacology, medicinal biochemistry, mechanistic enzymology, chemical ecology, synthetic biology, and even chemical synthesis.

Harnessing Plant Metabolic Diversity

Osbourn, Anne (anne.osbourn@jic.ac.uk)

Plants produce a wealth of natural products that are valuable as industrial or pharmaceutical products. For example, plant-derived drugs represent >5% of the total pharmaceutical industry with sales revenues of £18 billion. The growing reliance on chemicals from plants is driving demand for green, environmentally friendly and sustainable feedstocks across industrial sectors in order to enable us to reduce our dependence on products derived from chemical refineries. Importantly, many of the natural products that are produced by plants are structurally complex and beyond the reach of chemical synthesis. These compounds are commonly extracted from plant material either growing in the wild or in cultivation. Availability is limited by difficulties in accessing and cultivating source species, low yield and problems of purification. The scale of the economic opportunity for improving the supply of high value products from plants is therefore enormous.

The vast majority of the natural product diversity encoded by plant genomes remains as yet untapped. The explosion in available plant genome sequence data coupled with affordable DNA synthesis and new DNA assembly technologies now offer unprecedented opportunities to harness the full breadth of plant natural product diversity and generate novel molecules in foreign hosts using synthetic biology approaches. The recent discovery that genes for the synthesis of different kinds of natural products are organised in biosynthetic gene clusters in plant genomes is now opening up opportunities for systematic mining for new pathways and chemistries. The production of plant and plant-inspired molecules in heterologous plant and microbial expression systems will enable the development of rational strategies to produce known and new-to-nature chemicals that are tailored for particular applications. This presentation will focus on our work on triterpene engineering using synthetic biology approaches.

Crossfire: Diterpenoids in the Chemical Warfare Between Rice and a Bacterial Pathogen

Peters, Reuben J. (rjpeters@iastate.edu)

Roy J. Carver Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, IA 50011.

Rice (Oryza sativa) is an important crop plant whose susceptibility to disease can critically impact food security. Among the means by which rice resists microbial pathogens is the production of diterpenoid phytoalexins, although the importance of such natural products biosynthesis is not fully understood. My group has taken a systematic approach towards elucidating both the underlying metabolic network and the physiological relevance of the resulting diterpenoids. Our work on diterpenoid metabolism has been based on the extensive sequence information available for rice (i.e., functional genomics), and includes the discovery of relevant biosynthetic gene clusters, as well as development of a synthetic biology approach in which we reconstitute rice metabolic pathways in E. coli, relying on the use of synthetic genes for functional incorporation of cytochrome P450 mono-oxygenases. Based on our comprehensive map for the early steps mediated by diterpene synthase in rice metabolism, we also are now taking a reverse genetic approach towards elucidating the biological function of the resulting diterpenoid natural products, including the application of genome editing technology. Conversely, we have found that the important bacterial rice leaf streak pathogen Xanthomonas oryzae pv. oryzicola produces the diterpenoid phytohormone gibberellin as a virulence factor. Our most recent results from investigation of this diterpenoid crossfire in the chemical warfare between rice plants and X. oryzae will be discussed.

Mining Terrestrial and Aquatic Microbiomes for Novel Metabolites

Raaijmakers, Jos, J. (Raaijmakers@nioo.knaw.nl)

NIOO Wageningen.

Gene Centric Discovery of Plant Metabolic Pathways

Sattely, Elizabeth (sattely@stanford.edu)

Stanford.

Humans have become extraordinarily reliant on plants and plant-derived molecules for food, medicine, and energy. However, remarkably little is known about how plants perform the chemistry responsible for making these molecules. New plant genome sequences and synthetic biology tools have opened the door to three transformative research areas: 1) Identifying and exploiting the enzymes responsible for synthesizing known plant-derived chemicals, and 2) discovering new molecules from plants, and 3) developing new strategies for sustainably enhancing plant fitness. This talk will describe efforts in my lab to use a combination of biochemistry, synthetic biology, bioinformatics, transcriptomics, and metabolomics to accelerate the discovery and engineering of plant metabolism. We use both a candidate gene approach to uncover novel pathways and new molecules, as well as a candidate molecule approach for targeted elucidation of metabolic enzymes. Our vision is to build metabolic pathways from newly discovered enzyme catalysts that can enhance human health, plant health, and the production of sustainable chemicals.

Integrated Metabolomics for Triterpenoid Gene Discovery in the Model Legume *Medicago truncatula*

Sumner, Lloyd W. ; Bonnie Watson; Vered Tzin; Daniel Wherritt; Dong Sik Yang; John H. Snyder; David V. Huhman; Stacy Allen; Yuhong Tang; Derek Nedveck; John Stanton-Geddes; Peter Tiffin; Nevin Young

Triterpene saponins are structurally diverse secondary metabolites found in many plant families, including the Leguminosae. They possess a broad spectrum of bioactivities ranging from allelopathy and anticancer activities to antifungal, antibacterial, anti-insect and anti-nutritive properties. In spite of their functional importance, the biosynthetic pathways for saponins remain largely uncharacterized. We are using an integrated metabolomics, correlated gene expression profiling and genome wide association studies (GWAS) for the discovery, prioritization, and characterization of novel saponin biosynthetic genes in the model legume *Medicago truncatula* which is known to accumulate a large variety of differentially glycosylated saponins. This presentation will describe the integrated technologies and approaches used. This will include GC-MS and UHPLC-MS for comparative metabolomics, and it will also include a sophisticated UHPLC-MS-SPE-NMR approach that we have developed for higher-throughput metabolite identification. Large-scale and confident metabolite identification is the number one grand challenge faced by the Metabolomics Community. The presentation will also provide multiple examples of novel gene discoveries related to saponin biosynthetic cytochrome P450s and glycosyl transferases.

Volatile Metabolites as Chemical Modulators of Plant-Microbe Interactions

Tholl, Dorothea (tholl@vt.edu)

Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, USA.

Odor is the most frequent and chemically diverse carrier of biological information. Plants employ volatile molecules in both plant-plant and inter-kingdom interactions. Molecular based studies of the model plant *Arabidopsis thaliana* exemplify biosynthetic plasticity and roles of volatiles in plant immunity and anti-herbivore defense above and below ground. Moreover, volatile compounds have been recognized for their effects on plant-colonizing microbial communities. In this context, we investigate the function of volatile terpenoids as host-specific chemo-selective factors in the rhizosphere of switchgrass (Panicum virgatum L.). In an ongoing functional genomics study, we elucidate the volatile terpenoid metabolism in switchgrass roots to establish a mutant based approach for analyzing the multifunctional role of common root volatiles in the nutrient/defense balance of the rhizosphere that affects microbial community composition and consequently plant performance and resilience.

Niche Adaptation of the *Arabidopsis* Leaf Microbiota

Vorholt, Julia A. (jvorholt@ethz.ch)

Institute of Microbiology, ETH Zurich.

The aerial parts of the plants, which are dominated by leaves, represent one of the largest terrestrial habitats for microorganisms. This habitat, called the phyllosphere, is occupied by a diverse community of microorganisms, which is important for plant health and growth. A predominance of Proteobacteria, Actinobacteria and Bacteroidetes living in the phyllosphere of numerous plants has been revealed, while metagenomics and metaproteomics approaches gave insights into the general bacterial adaptation strategies to the phyllosphere. We used complementary in situ metabolomics approaches such as imaging high-resolution mass spectrometry to identify *Arabidopsis* leaf surface compounds and their possible involvement in the epiphytic lifestyle by relative changes in compound pools. Specific alterations in the phylloplane metabolite composition could be detected as a function of bacterial colonization highlighting the power of environmental metabolomics to aid in elucidating the molecular basis underlying plant–epiphyte interactions. Recently, we conducted large-scale experiments to isolate *Arabidopsis* leaf bacteria as pure cultures. Individual plants as well as individual leaves were sampled at different European sites to determine their core leaf community and to establish a reference strain collection using flow cytometry and dilution series plating. After identifying approximately 3,000 isolates using a high-throughput DNA sequencing-based method we selected representative strains belonging to 52 genera of the major phyllosphere phyla covering the majority of the culture-independent taxonomic diversity. Draft genomes of 232 selected isolates were generated. Recolonization experiments using synthetic communities in a gnotobiotic model system showed reproducible colonization patterns and represents a valuable starting point to identify mechanisms of community formation and function.

Vorholt 2012 Nature Rev Microbiology 10:828-40; Bodenhausen et al. 2014 PloS Genetics 10:e1004283; Bai et al. 2015 Nature 528:364-9; Ryffel et al. 2016 ISME J 10, 632–643

Botanicals and Experimental Pharmacology in the Post-Genomic Era: New Directions

Walker, Larry (lwalker@olemiss.edu)[1,2]; David Pasco[1,2]; Pier Paolo Claudio[1,2]; Melissa Jacob[1]; and Ikhlas Khan[1,2]

[1]National Center for Natural Products Research. [2]Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, MS 38677.

The modern discipline of experimental pharmacology was largely based on observation of the effects of plant-derived metabolites – e.g., the toxicities of alkaloids. Our constructs of the sympathetic nervous system, neuromuscular transmission, pain pathways and cardiac contractile mechanisms, among many others, were developed in this way. In the 20th century, the advances in testing flourished and the "age of synthetic chemistry" exploded, the majority of our modern pharmaceuticals were still natural product-derived or inspired.

The post-genomic era has brought new insights into intracellular signaling pathways, cell-cell communications, disease targets, systems pharmacology, with more innovative screening tools and genetic manipulation of targets, and with a growing number of sophisticated animal models for human disease. These afford new horizons for utilization of natural products, both as experimental tools, and as new therapeutics. Natural products are emerging as important tools for understanding complex cellular signaling pathways; the talk will consider approaches to natural product discovery at the National Center for Natural Products Research, especially aimed at metabolites that modify cellular signaling and transport pathways important in cell proliferation, survival, and chemotherapy resistance.

Secondary Metabolites: Chemical Warfare in Plant-Pathogen-Antagonist Interactions

Weller, David (david.weller@ars.usda.gov)

USDA.

Investigating Plant Terpene Metabolic Diversity for Pharmaceutical and Agricultural Applications

Zerbe, Philipp (pzerbe@ucdavis.edu)

University of California-Davis, Department of Plant Biology, Davis, CA 95616, USA.

Terpenoids form the largest and most diverse class of plant specialized metabolites with wide-ranging functions in plant development and ecological interactions. This chemical diversity also provides a rich and underutilized resource for crop improvement and various bioproducts, most notably pharmaceuticals and biofuels. However, a broader industrial application of plant-derived terpenoids remains limited by their narrow taxonomic distribution, low abundance and complex diversity in nature. To deepen our knowledge of specialized terpenoid metabolism and accelerate the development of

biotechnology applications, we established deep transcriptome resources for more than a dozen plant species that produce terpenoid metabolites of established or potential economic importance. We developed an efficient gene discovery platform, integrating metabolite and transcript profiling with functional enzyme characterization through co-expression in microbial and plant-based platforms that enable effective enzyme cross-validation. This approach revealed numerous novel terpene synthases and cytochrome P450 monooxygenases as key enzymes in terpenoid metabolism, including novel enzyme activities with potential use for drug discovery or improved crop resilience. Across several species, we identified multi-enzyme terpene synthase families that form part of dynamic modular pathways, where catalytically distinct enzymes can function in different combinations to enhance metabolic diversity. Following nature's lead, we develop proof-of-concept yeast and plant production platforms for diterpenoids through combinatorial expression of functionally distinct terpenoid pathway genes

Poster Presentations

Posters alphabetical by first author

Identification of Enzymes Involved in Peridinin Biosynthesis

Andersen-Ranberg, Johan (joar@berkeley.edu)[1]; Johan A-Ranberg[1]; Krishna K. Niyogi[1,2]

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Peridinin is a carotenoid found in marine dinoflagellate. The photosystems of dinoflagellates posses a soluble light-harvesting-complex with a high carotenoid to protein ratio, called the peridinin-chlorophyll protein (PCP).

The biosynthesis of peridinin requires an acetylation, the elimination of three carbons and oxidations on the central alkene chain of neoxanthin. To identify putative genes involved in neoxanthin metabolism, RNAseq data from the well-studied dinoflagellate species Symbiodinium minutum are being mined for carotenoid-related biosynthesis genes. Candidate genes for peridinin biosynthesis, is subsequently functionally tested in the Nicotiana benthamiana.

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A Transposon-Based Approach for Dissecting Actinomycete Interactions at the Genetic Level

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Actinomycete bacteria produce secondary metabolites from which many clinically important antibiotics have been derived. Although secondary metabolites have been well studied for their applications in medicine, little is known about the roles they play in nature. Novel secondary metabolites are produced by actinomycetes during interspecies interactions, but the molecular basis for the induction of these secondary metabolites is not known. We are building an S. coelicolor transposon library with the goal of performing a genetic screen to identify genes involved in regulatory networks that govern secondary metabolite induction during interspecies interactions. Determining the molecular mechanisms underlying the induction of secondary metabolism during interactions may

provide new approaches to finding novel bioactive secondary metabolites and give us insight into their function in microbial communities.

Genome-Wide Identification of Bacterial Plant Colonization Genes

Cole, Benjamin

DOE Joint Genome Institute.

Plant-associated bacteria affect diverse aspects of plant growth and health. A thorough understanding of how these microbes colonize their hosts is critical for targeted bioengineering, but the molecular mechanisms underlying colonization remain incompletely understood. To systematically investigate genes involved in root colonization in vivo, we employed a transposon-mediated genome-wide mutagenesis strategy (Bar-Seq) to study colonization of *Arabidopsis thaliana* roots by a Pseudomonas fluorescens strain known to colonize roots and stimulate defense against leaf pathogens. We identified 340 *P. fluorescens* genes whose inactivation diminished or enhanced the ability of the strain to colonize A. thaliania. Some mutations affected functions known to be required for root colonization, such as motility and carbohydrate metabolism, whereas many encode proteins with hitherto unknown functions or with predicted functions unrelated to plant association. To further characterize these genes, we assayed the same mutagenesis library under 88 in vitro conditions, linking many of the genes required for root colonization to defined functions. Our results demonstrate that saturation mutagenesis via Bar-Seq can inform high-resolution maps of complex bacterial functions important to plant ecosystems. The substantial set of plant root colonization genes identified through this screen offers a starting point for targeted improvement of the colonization capabilities of beneficial microbes.

J-RNA Spacer (JRS) Driven Tunable Polycisrtonic Expression

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Joint Bioenergy Institute.

Metabolic engineering requires multistep enzymes expressed in a spatial and temporal manner. A major challenge facing in the field is to control the enzyme expressions with limited promoter options. In order to optimize the ability to engineer the metabolic pathway, we plan to build a library of optimized RNA spacers (JRS) to drive protein expressions in a polycistronic manner. In our preliminary results, several JRS candidates can differentially express the bicistronic genes of interests (GOIs). We further investigated the effects of the first cassette (A) and the second cassette (B) to the expression of both GOIs. The results suggested the detailed cassette may have interactive effects of controlling the expressions. Moreover, we also tried to investigate the potential domains that close to the translational start sites (ATG) close to the second GOI to better understand the constructions of these JRS.

Discovery and Characterization of Refactored Modular Phenazine Pathways

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DOE Joint Genome Institute.

Characterization of the rapidly expanding biosynthetic pathways is limited by incomplete computational predictions and access to reliable pathway expression platforms. Additionally, investigations of the bioactivity of heterologously expressed natural products can rarely be accomplished in a plant associated expression host. We have developed a unique pathway discovery workflow that incorporates the identification of unique pathway modules involved in intermediate production and expresses each module independently in a Pseudomonas expression host that can be deployed for biocontrol of fungal phytopathogens. Using the JGI-IMG-ABC (Atlas of Biosynthetic gene Clusters) we identified over 300 phenazine pathways. Among these pathways we found unique pathway modules that cluster according to compound intermediate. We refactored and expressed a selection of these new phenazine pathway modules and have discovered new phenazines with bioactivity against fungal phytopathogens.

Mining Rhizobacteria-Induced Metabolome Reprogramming in Plants

Etalo, Desalegn (D.Etalo@nioo.knaw.nl)

NIOO-KNAW.

Several plant-associated microorganisms induce beneficial responses in plants such as growth promotion and tolerance to biotic and abiotic stress. To date, the plant metabolic changes associated with these phenotypic responses remain largely unknown. Here we investigated major plant metabolome changes induced in different plant species by various rhizobacterial genera. Substantial alterations of the plant metabolome were observed, many of which were dependent on the rhizobacterial strain, plant species, plant cultivar or plant tissue. Our results suggest that rhizobacteria-mediated metabolic reprogramming may boost the level of high value natural products (HVNP). Moreover, major regulatory genes associated with HVNP that are targeted by rhizobacterial traits can be identified and used for crop improvement programs as well as in synthetic biology.

Co-Option of Bacterial Quorum Sensing for Interkingdom Signaling

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University of Washington.

Some plant-associated bacteria sense their host by detecting an unknown plant signal recognized by a LuxR-subfamily of transcription factors. We describe a regulator we call PipR, in the Populus root endophyte Pseudomonas sp. GM79. The genes flanking pipR code for proteins annotated as peptidases and an ABC-type peptide transporter. PipR activates one of its linked peptidase gene (pip) in response to plant leaf macerates. GM79 peptidase mutants show increased PipR activation of pip. Plant-macerate induction of pip was lost in ABC-type transporter-defective mutants. The involvement of a putative

peptide transporter in the PipR system suggested that the plant signal might be a peptide. In fact protein hydrolysates and a tripeptide can serve in place of plant macerates to activate pip in a PipR-dependent manner. Understanding these plant-responsive LuxR homologs and their plant signals is important as they occur in dozens of bacteria associated with economically important crops.

Regulation of Aflatoxin Biosynthesis and Branched-Chain Amino Acids Metabolism in *Aspergillus flavus* by 2-phenylethanol Reveal Biocontrol Mechanism of *Pichia anomala*

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USDA-ARS-WRRC.

Pichia anomala WRL-076 is a biocontrol yeast which has been shown to inhibit growth and aflatoxin production of *A. flavus*. Using the SPME-GC/MS analysis we identified that the volatile, 2-phenylethanol (2-PE) produced by this yeast and demonstrated that the compound inhibited aflatoxin production. We further characterized the temporal transcriptome response of *A. flavus* to 2-PE. The aflatoxin gene cluster were significantly decreased during the first 48 h treatment. Gene Ontology (GO) analyses showed that GO terms related to metabolism of propionate and branched-chain amino acids were significantly enriched in the down-regulated gene group. Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the down-regulated genes were those involved in valine, leucine and isoleucine degradation, propanoate metabolism, and tryptophan metabolism. These degradation and metabolic pathways most likely are required for aflatoxin biosynthesis by providing building blocks and energy.

Investigating a Novel Acytransferase in Legume: *Rhizobium* Symbiosis

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Legumes are particularly interesting for their ability to form symbiotic relationships with nitrogen-fixing *rhizobium* bacteria in their rhizosphere. The establishment of symbiosis involves intricate chemical communication between plants and bacteria. However, this important process is far from being fully understood. Recent transcriptomic profiling of the model legume *Medicago truncatula* revealed that several hundred genes were differentially expressed during nodulation, implicating their potential roles in symbiosis. Within this dataset, we identified a BAHD acyltransferase(HCTL) in *M. truncatula*, which is upregulated during nodule formation. Preliminary phylogenetic analysis and protein structural modeling suggest that this acyltransferase evolved from HCT, but likely neofunctionalized to serve alternative metabolic functions unique to legumes. We further characterize this HCTL and hypothesize that *M. truncatula* use it to communicate with rhizobia via bacterial quorum sensing.

Pleiotropic and Epistatic Network-Based Discovery of Plant Functions Involved in Microbial Interactions

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ORNL.

Biological organisms are complex systems that are composed of pleiotropic functional networks of interacting molecules and macro-molecules. Complex phenotypes are the result of orchestrated, hierarchal, heterogeneous collections of expressed genomic variants regulated by and related to biotic and abiotic signals. However, the effects of these variants are the result of historic selective pressure, and, as such, their co-occurrence can be seen as genome-wide associations in a number of different manners. We are using data derived from the re-sequenced genomes from over 1000 alternate Populus trichocarpa genotypes in combination with transcriptomics, metabolomics and phenomics data across this population in order to better understand the molecular interactions involved in plant-microbe interfaces. The resulting integrated networks, are proving to be a powerful approach to determine the pleiotropic and epistatic relationships responsible for plant-microbiome associations.

Powdery Mildew-Induced Host DNA Endoploidy Fuels Fungal Proliferation

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University of California, Berkeley, PMB.

Powdery mildew fungi are obligate biotrophs that alter plant cellular architecture and metabolism to acquire their nutrients from the plant, while limiting plant defense. Using site-specific analyses, we found that the powdery mildew Golovinomyces orontii induces endoreduplication (~16-fold elevated DNA content) in *Arabidopsis* mesophyll cells underlying the fungal feeding structure concomitant with fungal proliferation (PNAS 107:460-5). Increased endoploidy has long been associated with enhanced metabolism and we found the extent of fungal proliferation correlates with DNA ploidy levels (MPMI 26:537-45). Using RNAseq, genetic, biochemical, and metabolite analyses we have now identified specific ploidy-dependent alterations in host primary metabolism used to support fungal reproduction. Together, this study provides a mechanistic underpinning that links enhanced ploidy to elevated host metabolic capacity and fungal reproductive output.

Extending the Red Queen Hypothesis to the Management of Weed Populations: Can products of Co-Evolving Interactions Be Harnessed for Weed Control?

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Co-evolving interactions between the plant and its deleterious microbiota are often referred to as an arms race or the Red Queen scenario. These continuous offensive and defensive interactions can yield a rhizosphere enriched in metagenomes containing the blueprints for a vast array of natural products. We aim to demonstrate that soil microbial communities co-evolving with their plant hosts serve as genetic reservoirs for biosynthetically produced allelopathic compounds. We are using both cultivation-dependent and cultivation-independent isolation techniques to uncover biosynthetic gene clusters in weed suppressive soils. Activity-based screening of soil metagenomic libraries will allow us to isolate small molecules produced in vector-host expression systems containing the large-insert DNA fragments extracted from the target weed rhizospheres, while isolation techniques involving in vivo cultivation within weed rhizospheres enable the use of whole genome bacterial analysis.

Indole Antibiotic Biosynthesis in Edible Plants

Klein, Andrew

Stanford University.

Cruciferous vegetables (Brassicaceae family) produce a bouquet of sulfur-containing molecules that restrict pathogens. Knowledge of the enzymes that assemble these natural compounds — termed the biosynthetic pathway — is crucial to understand their biological functions. Here I describe three studies that discover and characterize enzymes involved in plant specialized metabolism. These investigations employ a combination of mass spectrometry-based metabolite profiling, gene expression analysis, comparative genomics, and enzyme biochemistry. The studies include: (i) uncovering a branch point for phytoalexin biosynthesis in *Arabidopsis*; (ii) identifying the first enzymes in the brassinin pathway from Brassica rapa; and (iii) revealing the entire pathway of Brassica phytoalexin biosynthesis. The biochemical and genetic basis of phytoalexin biosynthesis elucidated here provides insights into the evolution of the complex chemical communication among plants and their associated microbiota.

Using Shotgun Metagenomics to Investigate Natural Products from Uncultured Bacteria in Marine Invertebrates

Kwan, Jason

University of Wisconsin-Madison.

It is estimated that only ~0.1% of environmental microbes have ever been cultured in the laboratory, meaning that the biosynthetic potential of the remaining ~99.9% is largely unexplored except through culture-independent sequencing (metagenomics). We used a custom bioinformatics pipeline to examine two marine sponges with high microbial diversity (both Hippospongia lachne, collected from Florida, U.S.A), and one sponge with low microbial diversity (unidentified species, collected from Florida, U.S.A). This yielded up to ~100 high quality genomes per metagenome, many of which were found to belong to unknown phyla. AntiSMASH analysis revealed that secondary metabolite pathways were widespread amongst genomes in H. lachne. In future studies we will use RNAseq aligned to pre-assembled genomes to probe the behaviour of individual bacteria within sponge microbiomes, with the aim of determining the ecological function of natural products.

Evolution Meets Bioorganic Chemistry: Phylogenetics-Guided Structure Function Analysis of Defensive ACYLSUGAR ACYLTRANS-FERASES in Solanaceae

Last, Robert

Michigan State University.

Glandular trichomes on the surface of tomato stems and leaves produce biotic stress-protective acylsugars. We have elucidated the metabolic network that produces these compounds in cultivated tomato and wild relatives. Relatives of tomato produce diverse acylsugars and we have used a phylogeny-guided structure-function analysis to identify amino acid residues that control variation in enzymatic activities in these species. In vitro reconstruction of the metabolic network allows us to evaluate the impact of these variant enzymes on the types of acylsugars produced. This work paves the way for reconstruction of these defensive molecules in microbes and plants that do not normally make acylsugars.

Selected Publications:
1. Schilmiller, A.L., A.L. Charbonneau and R.L. Last. 2012. Proc. Natl. Acad. U.S.A. 109:16377-16382.
2. Fan, P., A.M. Miller, A.L. Schilmiller, X. Liu, I. Ofner, A.D. Jones, D. Zamir and R.L. Last. Proc. Natl. Acad. USA, 113 (2) E239-E248. doi/10.1073/pnas.1517930113

Detergent Free Isolation of The Dhurrin Pathway: A Novel Approach for Metabolon Isolation and Pathway Discovery

Laursen, Tomas

JBEI.

Plant pathway discovery often constitutes a bottleneck in synthetic biology based approaches to produce structurally complex flavors, spices, colorants, sweeteners and pharmaceuticals in heterologous hosts. A plethora of such commercially lucrative products are synthesized by enzymes from the cytochrome P450 family anchored to the endoplasmic reticulum membrane system. Many of these pathways assemble into transient enzyme complexes, metabolons, facilitating efficient substrate channeling. The dhurrin pathway of *Sorghum bicolor* involves four enzymes CYP79A1, CYP71E1, POR and UGT85B1, which assemble into metabolons at the surface of the endoplasmic reticulum. The competence of a styrene maleic acid (SMA) copolymer to carve out discrete lipid particles (SMALPs) from biological membranes was adopted to extract the metabolon harboring the entire membrane bound dhurrin pathway directly from microsomal fractions of *Sorghum bicolor*.

Staphylococcus aureus Shifts Towards Commensalism in Response to Corynebacterium Species

Lemon, Katherine

Forsyth Institute.

Staphylococcus aureus is an important human pathogen that asymptomatically colonizes ~25% of humans as part of the nostril and skin microbiota. *S. aureus* utilizes regulatory pathways including the quorum sensing accessory gene regulator (agr) system to transition between commensal and pathogenic states. We found that *S. aureus* responds to growth with *Corynebacterium striatum*, a common skin commensal, with decreased expression of agr-induced virulence genes and increased expression of agr-repressed genes encoding surface-associated proteins. Among the *S. aureus* genes with increased expression are a number reportedly expressed during nasal colonization. In a murine abscess model, *S. aureus* populations shrank during coinfection with *C. striatum*, whereas *C. striatum* numbers expanded. These data support a model whereby *S. aureus* responds to commensal *Corynebacterium* spp. by limiting virulence and shifting to a commensal state.

Microbial Small Molecules in Plant Infection and Biocontrol

Li, Bo

University of North Carolina Chapel Hill.

Plant pathogens cause billions of dollars of crop damage worldwide annually. These bacteria produce many phytotoxins and antimicrobial molecules, which allow them to survive and flourish in competitive environments. While genomics-guided efforts have yielded success in assembly-line enzyme pathways such as non-ribosomal peptides and polyketides, short and non-canonical pathways have been mostly overlooked. Here, we developed a dual approach that combines heterologous expression with in vitro enzyme activity screening, which enabled us to elucidate the chemical structure of a structurally and functionally distinct phytotoxin. The distribution of this three-gene pathway was also demonstrated in a variety of phytopathogens. This work represents a key step towards understanding the collection of small-molecule arsenals that P. syringae produces. Our work also shows the importance of amide ligases as a rapidly growing class of enzymes that are employed in secondary metabolism.

Exploring the Molecular Players Involved in the Sequestration, Detoxification, and Storage of Simple Phenolics

Liu, Chang-Jun

Brookhaven National Laboratory.

Phenylpropanoids represent a vast variety of aromatic metabolites important for plant growth and development, and plant-environmental interactions. Whereas their biosyntheses are relatively clear, how those diverse metabolites are modified and sequestrated within the cells remains enigmatic. To explore the molecular basis of the intra- and inter-cellular translocation of the simple phenolics, we fed

an array of simple phenolics to the cultured *Arabidopsis* seedlings, and subsequently conducted RNA-seq analyses. The transcriptomic study revealed that the expression of 132 genes, including a subset of genes encoding UDP-glucose: glucosyltansferases, and membrane transporters/transport proteins, was up-regulated in the coniferyl alcohol-fed seedlings, compared to the un-fed controls. This study provides a rich source for further exploring the molecular players involved in the processes of phenolics' biotransformation and cellular translocation, and in the plant-chemical stress responses.

Secretion of Arabinogalactan Proteins and Glycolipids is Required for Establishing a Functional Plant-Microbial Root Symbiosis in *Medicago truncatula*

Moore, William

Joint BioEnergy Institute.

Plant-microbial symbiosis depends on bi-directional signaling cascades that lead to the development of specialized plant membranes that act as an interface between plant cells and their associated microbial liaisons. Here we report the discovery of several arabinogalactan protein encoding genes in *M. truncatula* that are specifically expressed during symbiosis with either AMF or *Sinorhizobium meliloti*. Functional studies using RNAi-mediated knockdown of specific AGPs result in loss of arbuscule formation during AM symbiosis and a putative cell death response in nodule cells harboring S. meliloti. We have also identified a glycosyltransferase enzyme by co-expression that partially recapitulates the AGP phenotypes. Preliminary data suggest that this GT is involved in sphingolipid biosynthesis, which is likely a key component of the interface. Our data indicate that secretion of glycoproteins and glycolipids at the plant-microbial interface is imperative for a functional symbiosis.

The Functional Specificity of a Rhizosphere Microbiome

Mueller, Henry

Graz University of Technology, Environmental Biotechnology.

The rhizosphere is one of the most active microhabitats, which is particular important for plant nutrition and health. The common rhizosphere concept describes an enrichment of specific microbial taxa in comparison to soil, but modern technologies enable much deeper insights and expand our understanding of the functional being of the rhizosphere microbiome and plant–microbe interactions. It has been shown that microbes attracted by nutrients in combination with plant-specific secondary metabolites. Here we report new insights into the rhizosphere by a metagenomics approach. By analyzing metagenomes of rhizosphere microbiomes of two different plant species (lettuce, oilseed rape), we identified genetic elements that are particularly enriched in the rhizospheres (e.g. signaling factors, resistance mechanisms, transport mechanisms in response to plant's secondary metabolites) and putatively represent functional key features of rhizosphere competence and plant-microbe interactions.

Down the Rabbit Hole: Who Produces Fischerellin A and How?

Philmus, Benjamin

Oregon State University.

Bacteria live in complex communities in the natural world and constantly interact and respond to the presence of secondary metabolites (aka natural products) produced by other microorganisms. Cyanobacteria are a particularly good model system as they live in close proximity to other bacteria that reside and feed on the exopolysaccaride sheath secreted by cyanobacteria. Many bioactive secondary metabolites (e.g. having anti-cancer, anti-bacterial, anti-fungal) have been isolated from unialgal, xenic cyanobacterial cultures (one cyanobacteria with multiple closely associated bacteria). However, the true producer of these natural products and their role in inter-cellular communication and maintenance of the bacterial biodiversity remains underexplored.

Here we begin looking at the biosynthetic producer of fischerellin A, a photosystem II inhibitor isolated from a xenic culture of *Fischerella muscicola*. Metagenomic sequencing shows that the xenic culture has the capacity to produce many more natural products then have been observed in extracts. A biosynthetic gene cluster for fischerellin A was not easily identifiable suggesting that it is produced in an unexpected way. We outline our strategy for identifying the gene cluster for fischerellin A biosynthesis using heterologous expression.

A Quorum Sensing System in a Dominant Freshwater Methane-Oxidizing Bacterium Regulates the Expression of a Novel Nonribosomal Peptide Synthetase

Puri, Aaron

University of Washington.

Many bacterial metabolic niches are underexplored resources for novel biosynthetic diversity. Aerobic methanotrophic bacteria use methane as their sole source of carbon and energy and serve as a major sink for the potent greenhouse gas methane in freshwater ecosystems. We have identified a quorum sensing system in the genome of *Methylobacter tundripaludum*, a dominant methane-oxidizer in Lake Washington (Seattle, WA, USA). We have characterized both the signal produced and genes regulated by quorum sensing in this bacterium, and have discovered this system regulates the expression of a novel nonribosomal peptide synthetase biosynthetic gene cluster. We are currently working to identify the function and product of this gene cluster in an effort to better understand the mechanisms and roles of intra- and interspecies interactions using specialized metabolites in this important bacterial community.

A Synthetic Microbial Community System to Understand the Ecological Underpinnings of Microbial Exometabolite Interactions

Shade, Ashley

Michigan State University.

The Shade Lab investigates the eco-evolutionary dynamics of microbial communities in environmental and laboratory systems. One of our major research objectives is to understand how interactions among microbial members influence emergent community robustness to perturbation. We have developed a laboratory-scale synthetic community system to ask how microbial interactions are facilitated by exometabolites, and how those exometabolite interactions change over time and in response to stressors. We are using this system to query microbial interactions in environmental microbial systems, including plant-associated and soil-associated microbial communities.

A Robust Gene Stacking Method Utilizing Yeast Assembly for Plant Synthetic Biology

Shih, Patrick

JBEI/ Lawrence Berkeley National Laboratory.

We present a strategy utilizing in vivo yeast homologous recombination to assemble multiple gene cassettes to facilitate plant metabolic engineering, which we have named jSTACK. In doing so, we have also generated a library of DNA parts consisting of promoters, genes, and terminators that will be publicly available as a resource to the plant synthetic biology community. We demonstrate how this method can facilitate pathway engineering of molecules of pharmaceutical interest, production of potential biofuels, and shuffling of disease resistance traits between crop species. In vivo homologous recombination has been leveraged for the large-scale DNA assembly of synthetic chromosomes and genomes in microbes. Likewise, our approach extends this technology to plants, providing a powerful alternative to conventional approaches for stacking traits and genes to address many impending agricultural challenges.

Discovery and Transfer of Novel Pathways for Phosphate Solubilization

Shulse, Christine

DOE Joint Genome Institute.

High yield agricultural plant growth is currently dependent on costly and environmentally damaging phosphate fertilizers. One approach to alleviating this dependency is to develop bacterial strains that can convert existing phosphorus sources in the soil to soluble forms available for plant uptake. Past attempts at developing such strains have been hindered by incomplete knowledge of the genes required for phosphorus solubilization, and failure of bacterial strains to survive in the plant root environment. To address these challenges, we are using genome wide mutagenesis of phosphate solubilizing bacteria to discover novel genes and pathways underlying solubilization of phosphorus sources, as

well as bioinformatics approaches to identify homologs of known phosphatesolubilization genes from microbial genomes and environmental metagenomes in the Integrated Microbial Genomes database. We have used synthetic biology approaches to synthesize these novel genes and transfer them to the plantassociated bacterium Pseudomonas fluorescens WCS417r. This research has the potential to result in significantly improved understanding of the genetics of phosphorus solubilization; novel biological tools for studying the interactions between plants, microbes and nutrients in the environment; and a first step in the development of alternative approaches to sustainable phosphorus use in agriculture.

Re-Wiring the Genetics of Natural Product Biosynthesis in Streptomyces

Smanski, Michael

University of Minnesota.

We propose a systems-level approach to quickly characterize the regulatory networks controlling secondary metabolism and learn how to manipulate them for the discovery of new natural products. The overarching hypothesis is that genome-wide overexpression or inactivation of the few hundred transcriptional regulators encoded in a typical Streptomyces genome will yield many mutants that overproduce new natural products. The major research outcome of this proposal will be new methods for increasing the throughput of mutagenesis and chemical characterization that allow for natural products discovery at a scale not previously possible. This will allow natural products discovery from a genome scale instead of a single-compound basis.

TARgeting Self-Resistance for Genomics-Based Discovery of Novel Antibiotics

Tang, Xiaoyu

Scripps Institution of Oceanography, UCSD.

Recent genome sequencing efforts have led to the rapid accumulation of orphan secondary metabolic biosynthesis gene clusters (BGCs) in public databases. This has given rise to significant challenges in the field of natural product genome mining. Here we introduce a novel genome mining strategy that by correlating putative antibiotic resistance genes with orphan BGCs, we can predict the biological function of pathway specific small molecules before they have been revealed in a process we call target-directed genome mining. Two orphan gene clusters from two Actinobacteria were selected using this approach. The metabolites produced were identified through direct cloning and heterologous expression and represent a group of previously known bacterial fatty acid inhibitors of unknown gene origin. This finding is the first demonstration of a target-directed genome mining strategy for discovery of antibiotic producing BGCs without a priori knowledge of the molecule synthesized.

Thomas, Michael

University of Wisconsin-Madison.

A Combination of Comparative Genomics and Laesi-Ms Facilitates the Discovery of Amino Acid Analogs Produced by Plant-Associated Bacteria

Trippe, Kristin

USDA Agricultural Research Service

Non-proteinogenic amino acids (NPAA) mediate many processes in host-microbe and microbe-microbe interactions. 4-formylaminooxyvinylglycine (FVG) arrests the germination of grasses and inhibits the growth of the *Erwinia amylovora*. In *P. fluorescens* WH6, a 13-kb gene cluster is required for FVG production. Homologous clusters are present in other strains distributed throughout the Pseudomonas genus. We sequenced the genomes of seven strains and analyzed other available genomes for the presence of FVG genes. To test the ability of strains to produce FVG, we developed a high-throughput method utilizing LAESI-MS. Analysis of gene context suggests that the cluster was introduced multiple times within the Pseudomonas lineage. In addition, clusters containing a subset of the FVG genes were identified in a broad range of bacteria and may produce novel metabolites. This work has broadened our understanding of NPAA, which have importance in mediating host-microbe and microbe-microbe interactions.

Constructing a Defined Microbiome to Study Plant Growth Promotion

Vogel, John

DOE Joint Genome Institute.

Plant growth promoting microbes hold tremendous potential to greatly increase crop yields by stimulating plant growth and preventing disease. A major limitation to the use of beneficial microbes is their unpredictable performance in the field. Thus, fundamental knowledge about all aspects of the interaction between plants, beneficial microbes and the microbiome is needed to realize the tremendous potential of beneficial microbes. The extreme complexity of the soil microbiome is a major impediment to acquiring this knowledge. Conversely, studying the interaction between a plant and a single microbe is of limited value for predicting field performance. We are establishing an experimental system of moderate complexity that will allow us to reproducibly study the interaction between a model plant, Brachypodium distachyon, and a defined microbiome of intermediate complexity. A summary of our progress and preliminary results will be presented.

Mechanisms and Adapttion of the *Arabidopsis thaliana* Root Microbiota in Iron Mobilization and Uptake from Iron-Limiting Environments

Voges, Mathias

Stanford University.

The root microbiota may assist plants in overcoming poor nutrient availability in low quality soils. However, there is more left to discover regarding the extent to which the root microbiota improves root uptake of the third most limiting nutrient in agriculture: iron. In collaboration with the Schulze-Lefert lab, we have identified root-associated bacteria that promote the growth and development of A. thaliana in conditions simulating low iron availability in vitro. These strains will be employed to investigate the mechanics facilitating plant iron acquisition by its microbiota. By applying reverse genetics and metabolomics, I investigate the role of A. thaliana secondary metabolism in mediating plant-microbe signaling in the rhizosphere. In parallel, we are investigating the adaptation of the A. thaliana root microbiota to iron-limiting conditions using gnotobiotic systems.

Antitermination of Secondary Metabolite Gene Clusters

Winkler, Wade

The University of Maryland.

In this study, we analyzed the phylogenetic distribution of the transcription elongation factor, NusG. We found the core NusG protein, present in all bacteria, as well as a subgroup containing RfaH protein, which acts as to prevent Rho-termination across operons that encode outer membrane components. However, we also discovered a third subgroup, which we named LoaP. Genes encoding LoaP are oftentimes positioned within or adjacent to pathways for specialized metabolites. We established Bacillus amyloliquefaciens LoaP as a paradigm for this protein subgroup, and showed that it promotes readthrough of intrinsic terminators located within two different antibiotic biosynthesis gene clusters. Both of these antibiotics have been implicated in plant-protective activities; therefore, the newly identified protein controls an important regulon of secondary metabolite genes. These data introduce a subgroup of NusG proteins as a new class of regulatory proteins for secondary metabolite pathways.

Coexpression Networks Connect Genes to Secondary Metabolic Pathways in Plants

Wisecaver, Jen

Vanderbilt University.

Plants have a vast array of ecologically specialized metabolic pathways. Functional and comparative genomic analyses have identified the types of genes that encode these specialized pathways, but connecting specific genes to specific pathways is challenging and time consuming. We harnessed the power of thousands of independent gene expression data sets to identify modules of coexpressed genes in plants. Modules were enriched in specialized metabolic functions and recover known

pathways including those for the production of terpenes, indoles, and glucosinolates. We found that putative gene clusters that have been identified based on enzyme annotation alone are comprised of genes that are not coexpressed, making it unlikely that putative clusters correspond to functional biochemical pathways. Our analyses identify dozens of uncharacterized coexpressed modules containing genes that code for biosynthetic enzymes such as terpene synthases and cytochrome P450s whose products await discovery.

Codon Usage is Involved in the Regulation of Antibiotic Production in *Pseudomonas protegens* Pf-5

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