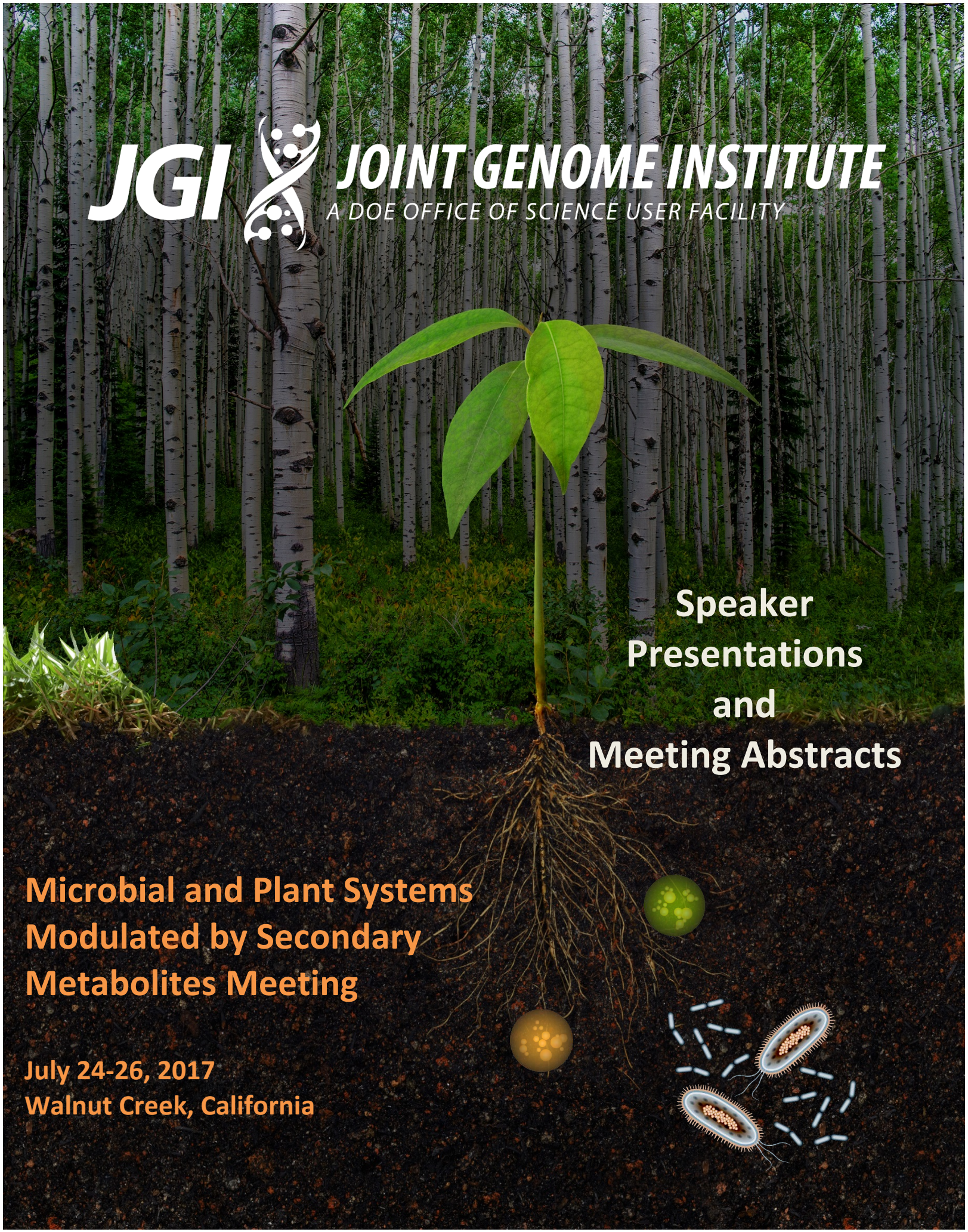
JGI
JOINT GENOME INSTITUTE
A DOE OFFICE OF SCIENCE USER FACILITY
Speaker
Presentations
and
Meeting Abstracts
Microbial and Plant Systems
Modulated by Secondary
Metabolites Meeting
July 24-26, 2017
Walnut Creek, California

All information current as of July 24, 2017

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JGI
Microbial and Plant Systems Modulated by Secondary Metabolites
2017 Conference

Agenda

July 24 to 26, 2017
Walnut Creek Marriott, Walnut Creek, CA
All functions will be held at the Walnut Creek Marriott.

Monday, July 24

4 to 5 PM	**Opening Keynote Presentation** Speaker: Jo Handelsman, Wisconsin Institute for Discovery Convergence of model systems and the natural world
5 to 6 PM	**Rapid Fire Talks** Poster session attendees
6 to 9 PM	**Opening Reception and Poster Session** Host: Yasuo Yoshikuni

Tuesday, July 25

9 to 9:10 AM	**Welcome & Introduction** Axel Visel
9:10 to 9:40 AM	**JGI Synthetic Biology** Yasuo Yoshikuni
9:40 to 10:10 AM	**JGI Metabolomics** Trent Northen
10:10 to 10:30 AM	**Break**
10:30 to 11 AM	**Caroline Roper, University of California, Riverside** Plant-associated microbiomes and the effects on disease outcomes
11 to 11:30 AM	**Ronnie de Jonge, Utrecht University, Utrecht** Understanding the role of the root microbiome and plant health
11:30 to noon	**Matthew Traxler, University of California, Berkeley** Specialized metabolism in root nodule communities

Noon to 12:15 PM	**Natalia Damasceno, Center of Molecular Biology and Genetic Engineering** Mapping the colonization of a synthetic microbial community inoculum from sugarcane microbiome in maize and soybean plants (short talk)
12:15 to 12:30 PM	**Katherine Murphy, University of California, Davis** Root-associated microbiome of a diterpene-deficient maize mutant (short talk)
12:30 to 1:30 PM	**Working Lunch**
1:30 to 2 PM	**Alisa Huffaker, University of California, San Diego** Understanding the role of specialized metabolic networks mediating biotic interactions in maize through integrated multidisciplinary approaches
2 to 2:30 PM	**Jie Luo, Huazhong Agriculteral University** Dissection of metabolic and phenotypic traits in major crops
2:30 to 3 PM	**Gary Siuzdak, The Scripps Research Institute** Metabolomics activity screening
3 to 3:15 PM	**Candice Swift, University of California, Santa Barbara** Deciphering the role of fungal secondary metabolites within anaerobic microbial communities (short talk)
3:15 to 3 PM	**Heino Heyman, Pacific Northwest National Lab** Metabolomics-guided isolation of significant biological plant, soil, and microbial secondary metabolites (short talk)
3 to 3:30 PM	**Break**
4 to 4:20 PM	**Victoria Knight-Connoni, Indigo Agriculture** Harnessing nature to help farmers sustainably feed the planet
4:20 to 4:40 PM	**Jill Paulik, AgBiome** Mining the plant microbiome for novel agricultural pest control solutions
4:40 to 5 PM	**Johan Kers, Ginkgo Bioworks** Strategies to design custom microbes for multiple markets using a foundry model
5 to 5:20 PM	**Barry Bochner, Biolog** Optimizing conditions that induce or repress toxin production using Phenotype MicroArrays
5:20 to 5:40 PM	**Marci Surpin, Valent Biosciences** Plant growth regulators and plant growth-promoting microbes for crop enhancement

Wednesday, July 26

9 to 9:30 AM	**Nancy Keller, University of Wisconsin, Madison** Omics approaches to deorphanize fungal natural products
9:30 to 10 AM	**Sophien Kamoun, The Sainbury Laboratory** Membrane trafficking at the host-pathogen interface
10 to 10:30 AM	**Gillian Turgeon, Cornell University** Comparative phylogenomics identifies fungal secondary metabolites allied with virulence to plant hosts
10:30 to 11 AM	**Break**
11 to 11:30 AM	**Huimin Zhao, University of Illinois, Urbana-Champaign** Discovery and engineering of novel natural products via synthetic biology
11:30 AM to noon	**Sarah O'Connor, John Innes Centre** Harnessing the chemistry and biology of plant metabolism
Noon to 1:15 PM	**Working Lunch – tour at the JGI (limited to 30 people)**
1:15 to 1:45 PM	**David Mead, Varigen Biosciences** Metagenomic and synthetic biology approaches for natural product discovery
1:45 to 2:15 PM	**Yi Tang, University of California, Los Angeles** Genome mining for new herbicides
2:15 to 2:45 PM	**Eriko Takano, The University of Manchester** Harnessing synthetic biology for the production of fine and specialty chemicals
2:45 to 3 PM	**Break**
3 to 3:30 PM	**Michael Smanski, University of Minnesota** Redesigning the genetics of secondary metabolism in *Streptomyces*
3 to 4 PM	**Wenjun Zhang, Lawrence Berkeley National Laboratory** Discovery of novel signaling secondary metabolites from *Clostridium*
4 to 5 PM	**Closing Keynote Presentation** Speaker: Gerald Tuskan, Oak Ridge National Lab Interkingdom signaling: a *Populus* case study
5 PM	**End of Secondary Metabolite Conference**

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

Convergence of model systems and the natural world

Jo Handelsman (jo.handelsman@wisc.edu)

Wisconsin Institute for Discovery, Madison, WI, USA.

Secondary metabolites have proved critical to microbial communication, competition, and behavior in binary interactions in the laboratory. The roles of these compounds in more complex microbial interactions—particularly in multispecies communities—remains murky. The challenge in studying these interactions in natural systems, such as the rhizosphere and soil, lies in the complexity of these microbial communities, many of which contain thousands of species and are subject to continual and often unpredictable changes.

The monumental growth of biology over the last century is predicated on several model organisms that have provided powerful tools for unmasking the secrets of molecular, cellular, and organismal processes. Likewise, models that simplify study of microbial communities could catapult microbial ecology to a new level of understanding. To address the need for such a model, we designed a community containing three members isolated from the rhizospheres of field-grown soybean plants. Community members were chosen from among a group of "*Bacillus cereus* hitchhikers," which are bacteria that co-isolate with *B. cereus* from soybean roots. From a large collection of hitchhikers we chose two isolates, *Pseudomonas koreensis* and *Flavobacterium johnsoniae*, which when combined with *B. cereus* represent the three major phyla of the rhizosphere.

This model community revealed interactions that were not evident in binary interactions. The community members produce several secondary metabolites that affect other members differently when community members are present in different combinations. Moreover, the community forms robust biofilms that are not observed with any of the three individually or in pairs. Genome sequences and genetic analysis have the potential to reveal the rules that govern establishment and robustness of this model community.

Plant-associated microbiomes and the effects on disease outcomes

Caroline Roper (caroline.roper@ucr.edu)

University of California, Riverside, CA, USA.

Pierce's disease (PD) of grapevine, caused by the xylem-limited bacterium *Xylella fastidiosa* (Xf), is a major threat to the grapevine industry. In vineyards that are under high PD pressure, there are interesting examples of vines exhibiting either no symptoms or very mild PD symptoms (disease-escaped). These differences are likely not attributed to the genetics of the plant because all vines in a vineyard are clonal. We hypothesize that the microorganisms inhabiting the xylem in these disease-escaped vines are inhibitory to Xf and subsequently reduce disease severity, due to their shared ecological niche. We have characterized the microbial communities residing in PD-infected vines and compared them to disease-escaped vines and identified a subset of beneficial organisms that are antagonistic to Xf. We envision harnessing these microbes to construct a beneficial synthetic phytobiome that can be deployed into grapevines during the nursery propagation process. We are also

currently assessing the secondary metabolite profiles of endophytic grapevine microbes and are developing some of these discoveries into PD mitigation tools.

Understanding the role of the root microbiome and plant health

Ronnie de Jonge (r.dejonge@uu.nl)

Utrecht University Institute of Environmental Biology, Utrecht, Netherlands.

The interaction between genotype-dependent root exudation and the rhizosphere microbiome composition and activity will be discussed.

Specialized metabolism in root nodule communities

Matthew Traxler (mtrax@berkeley.edu)

University of California, Berkeley, CA, USA.

This talk will describe our efforts to explore specialized metabolism in microbial communities that inhabit root nodules of legume plants. We hope to develop the root nodule as a model system for studying an ecologically relevant microbial community. This will include metabolomic and metagenomic characterization of fava bean root nodules.

Mapping the colonization of a synthetic microbial community inoculum from sugarcane microbiome in maize and soybean plants

Natália Damasceno (nbdbio@gmail.com)

Center for Molecular Biology and Genetic Engineering, UNICAMP, Campinas, Brazil, and Department of Genetics and Evolution, UNICAMP, Campinas, Brazil.

Plants live in association with a highly complex community of bacteria and fungi groups. Recent advances in microbial studies have shown that host genotypes influence the diversity, structure, and composition of plant microbiomes. However, the genetic and molecular mechanisms involved in plant-microbe communication that are responsible for establishing the microbial community in plant tissues remain unknown. Unraveling the traits involved in microbe-host interaction during microbiome assemblage is an imperative step towards building biotechnological tools to transfer microbes and their beneficial functions to economically relevant plants. In this work, we sought to explore the genetic and molecular mechanisms involved in cross-species microbial compatibility by studying the colonization of two important crop plants from distinct physiological groups, maize and soybean. Our main strategy involves transferring a bacterial community to a new C_4 plant and to a C_3 plant to evaluate if the bacterial members of this community are capable of colonizing both plants with contrasting physiological profiles. First, we designed a synthetic consortium of microorganisms composed of the most abundant bacterial groups from sugarcane, a C_4 bioenergy crop. Secondly, we established a platform for inoculation assays using maize, another C_4 crop, as a plant model. Maize seeds are germinated and planted in sterile conditions and are inoculated with the synthetic community. With this platform, we can evaluate the effect of the presence of the bacterial community in plant growth and also test different nutrient solution concentrations or stress conditions. The presence of the synthetic community improves plant growth even under low nutrient availability. We also tested whether this

community would be able to colonize the plant tissues. By using culture-independent techniques to profile inoculated and non-inoculated plants, we show that only a part of the synthetic community robustly colonizes the maize plants. This data indicates that even among phylogenetically close plants there might exist traits underplaying host colonization by specific microbial groups. Additionally, plants inoculated with the synthetic microbial community had their total fresh and dry biomass on average up to three times increased when compared with uninoculated plants. We sought to investigate if the synthetic community is also able to colonize soybean plants with a similar pattern and a beneficial plant response. Dual RNA-seq of plant and microbial community will reveal genes and pathways involved in the establishment of the microbial community and its beneficial functions.

Root-associated microbiome of a diterpene-deficient maize mutant

Katherine Murphy (kmmurphy@ucdavis.edu)

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

Plants deploy specialized metabolites to communicate with other organisms and cope with environmental challenges. This includes interactions with microbial communities, in which plants exchange sugars for available nutrients as well as protection against environmental stressors. However, the molecular mechanisms by which a plant recruits its particular microbial community and the role of specialized metabolites in this communication are poorly understood. Here, we report that maize root diterpenes, a group of specialized metabolites with versatile functions in stress resilience, influence rhizosphere bacterial communities. In addition to the previously described kauralexin metabolites with key roles in maize pathogen and environmental stress resistance, we recently elucidated a novel group of maize-specialized diterpenes, termed dolabralexins, which also show activity against biotic and abiotic stress. Distinct from the gibberellin biosynthesis pathway, both kauralexins and dolabralexins are synthesized via the copalyl diphosphate synthase ZmAn2 before branching into separate pathways. The an2 (anther ear 2) maize mutant is deficient in forming both kauralexin and dolabralexin metabolites, and exhibits enhanced stress susceptibility. Using 16S rRNA sequencing, we determined the bacterial community compositions of the an2 mutant compared to its wild type sibling. Under well-watered conditions, distinct bacterial communities and diversities were observed between mutant and wild type plants, whereas the microbiome compositions became indistinguishable under drought conditions. These findings suggest that diterpenes play an important role in shaping the rhizosphere microbiome, while alternate mechanisms may be dominant under drought stress.

Understanding the role of specialized metabolic networks mediating biotic interactions in maize through integrated multidisciplinary approaches

Alisa Huffaker (ahuffaker@ucsd.edu)

University of California, San Diego, CA, USA.

All plants biosynthesize complex blends of specialized metabolites that enable and shape community interactions with other organisms. Planted on over 140,000 square miles of arable US farmland and genetically tractable, maize (*Zea mays*) is an excellent Poaceous model for studying effects of chemical diversity on biotic interactions. Focusing on root and crown tissues of maize, we use metabolomic approaches to identify microbe-elicited metabolite production. To rapidly uncover underlying regulatory genes responsible, classical forward genetics using biparental mapping and genome-wide association studies (GWAS) readily reveal metabolite-based quantitative trait loci (mQTL) and ultimately regulatory

genes. Insights from mQTL approaches in maize enabled the identification of unexpected candidate genes involved in the biosynthesis of diverse chemical classes. In vitro biochemical approaches coupled with gene synthesis speeds the confirmation of top candidate genes. To examine entire pathway deletions in vivo, CRISPR/Cas9 mutagenesis enables the creation of knockouts in historically challenging duplicated genes. As an example, using CRISPR/Cas9 we created frame-shift mutations in terpene synthases (Tps) 6/11 to remove the dominant fungal-elicited sesquiterpene acids in maize. Zmtps6/11 mutants display significant susceptibility to attack by both fungi (*Fusarium graminearum*) and bacteria (*Pantoea stewartii*). The development of more comprehensive biosynthetic pathway mutant libraries in maize will enable systematic analyses of metabolite effects on biotic interactions in field environments.

Dissection of metabolic and phenotypic traits in major crops

Jie Luo (jie.luo@mail.hzau.edu.cn)

Huazhong Agriculteral University, Wuhan, China.

Application of a newly developed widely-targeted metabolomics strategy simultaneously detected hundreds of both primary and secondary metabolites in rice and disclosed a number of subspecies-specific metabolites that may reflect, as well as affect, the subspecies differentiation of rice. Distinct and overlapped accumulation was observed and complex genetic regulation of metabolism was revealed in two different tissues by subsequent metabolic QTL (mQTL) mapping and metabolic genome-wide association study (mGWAS). Hundreds of loci with high resolution and large effects were uncovered. Interactive gene/metabolite identification/annotation was facilitated for both functional genomics and metabolomics. Data mining revealed a large number of candidate genes underlying metabolites that are of physiological and agronomical importance, and also can be applied to the bulk identification of tailing enzymes contributing most to metabolic diversity. Similar approaches were also applied to the understanding of the maize kernel metabolome. Furthermore, comparative mGWAS between rice and maize resulted in greatly increased power and resolution in both species. In addition, parallel metabolic and phenotypic GWAS identified new candidate genes responsible for both metabolic and morphologic traits such as grain width and leaf senescence, revealing direct linkage between the metabotype and the phenotype. Our study not only reveals novel biochemical and genetic insights of important aspects of plant and human such as development, stress resistance, and nutrition/health-promoting, but also provides a vast amount of high-quality data for further understanding plant metabolomes and which may help bridge the gap between the genome and phenome. The strategy is a powerful tool for large-scale gene identification, pathway elucidation, and knowledge-based crop genetic improvement.

Metabolomics activity screening

Gary Siuzdak (siuzdak@scripps.edu)

The Scripps Research Institute, La Jolla, CA, USA.

Systems-wide analysis has been designed and implemented into our cloud-based metabolomic platform (XCMSOnline.scripps.edu) to guide large-scale multi-omic experiments. This autonomous approach superimposes metabolomic data directly onto metabolic pathways. These data are then integrated with transcriptomic and proteomic data. To date, the utility of this platform has been demonstrated in thousands of studies. The approach facilitates biomarker discovery, mechanistic data analysis, and metabolomics activity screening (MAS).

Deciphering the role of fungal secondary metabolites within anaerobic microbial communities

Candice Swift (cswift@umail.ucsb.edu)

University of California, Santa Barbara, CA, USA.

Anaerobic fungi thrive in competitive microbial environments such as the digestive tracts of many large herbivores despite being vastly outnumbered by other microorganisms. In addition to secreting powerful biomass-degrading enzymes, these unique non-model organisms also possess a rich array of biosynthetic machinery for producing secondary metabolites, whose functions remain to be determined. We hypothesize that secondary metabolites from gut fungi play important roles in anaerobic communities via microbial defense, communication, or stress tolerance. Here, we have combined complementary approaches in genomics, transcriptomics, mass spectrometry, and NMR to probe the functions of secondary metabolites in the complex anaerobic digestive environment of herbivores. First, we uncovered a plethora of gene clusters encoding biosynthetic enzymes for secondary metabolites from diverse chemical classes by mining the genomes and transcriptomes of three anaerobic fungi from the primitive phylum *Neocallimastogomycota*. Key secondary metabolite clusters include canonical polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). Several of the clusters are expressed under standard laboratory growth conditions, whereas others are silent. RNA sequencing allows us to test environmental conditions that activate the biosynthetic gene clusters that have been identified in the fungal genomes. The environmental conditions we are testing include heat shock, oxidative stress, pH, and nutrient availability. In addition to abiotic factors, we are also co-cultivating the anaerobic fungi with bacteria and archaea to screen interactions that may enhance transcription of the clusters. Finally, we are working with JGI and EMSL to employ analytical techniques like mass spectrometry and NMR that allow us to detect and determine the structures of key secondary metabolites. To date, we have detected ~100 likely secondary metabolites and putatively identified an antioxidant polyketide, baumin, which is also produced by a distantly related fungus from the phylum *Basidiomycota*. Mass spectrometry facilitates high throughput, rapid screening of metabolites within a complex mixture and yields a chemical fingerprint of each molecule that can be compared with reference databases for dereplication with known natural products, while NMR permits detailed structural characterization of isolated compounds. The secondary metabolism of anaerobic fungi represents a completely untapped reservoir of biosynthetic potential, which could be drawn upon for novel therapeutics, new chemical building blocks, and enzymes for bioengineering natural products. Our integrated approach allows us to study secondary metabolism at all levels, from DNA to RNA to metabolites, thus maximizing discovery of novel metabolites and unmasking their native functions.

Metabolomics-guided isolation of significant biological plant, soil, and microbial secondary metabolites

Heino Heyman (heino.heyman@pnnl.gov)

Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA, USA.

One of the grand challenges facing the metabolomics community is the identification of unknown metabolites, with unknown secondary metabolites contributing the most to this challenge. The large chemical heterogeneity of the metabolome has made identification and annotation of biologically significant metabolites one of the most important bottlenecks in untargeted metabolomics analyses. The mass spectrometry-based approaches used for metabolomics and lipidomics analyses are capable of

detecting thousands of molecules in a single run, but comprehensive annotation of the associated metabolites is limited to spectral reference library matching and/or direct comparison to analytical standards. Current tandem mass spectral libraries are small compared to the large number of metabolites found in the biosphere. In cases where metabolites of interest can be matched to reference compounds, MS can give unequivocal identification, but for unambiguous identification of partially or completely unknown molecules, NMR spectroscopy is indispensable. In the diverse applications environment within which the Environmental Molecular Sciences Laboratory operates, with a multitude of different organisms and biological systems under investigation via User Projects, this bottleneck is a common problem and thus leaves a significant part of the interpretation of the metabolomics results ambiguous. By making use of complementary spectral data from both MS and NMR, we are reducing the time for structural elucidation of metabolites by incorporating candidate rejection and substructural conformation. In this talk I will describe the capabilities of a new pipeline, Metabolite Identification and Characterization Pipeline (MICP), currently being developed at EMSL to boost the identification of unknown metabolites and I will present selected applications to fungal and soil studies where we developed a specific directed fractionation and isolation roadmap which was used to purify, isolate, identify, and characterize the fungal and soil unknown metabolites that are of biological importance. The newly identified metabolites will be added to our ever-growing Pacific Northwest National Laboratory metabolite database, which serves as a repository for future validated metabolomics data. The novel metabolites identified by our MICP will be used to improve our understanding of the roles that biotic and abiotic transformations have on plants and soil microorganisms with environmental changes.

Harnessing nature to help farmers sustainably feed the planet

Victoria Knight-Connoni (vknight@indigoag.com)

Indigo Agriculture, Charlestown, MA, USA.

Plants rely on a vast array of natural, beneficial microbes to support their health and productivity. The community of microbes living in and around a plant—its microbiome—works in harmony with the plant to provide life-sustaining benefits throughout the plant's lifecycle. The microbiome helps the plant absorb nutrients in the soil and bolsters its resilience to environmental stresses, in many cases mediated by the secondary metabolites individual microbes produce. Microbes have evolved in conjunction with plants over millions of years, in many cases to optimize their health and maximize their productivity. Development of microbial products to achieve long-term agricultural sustainability is a growing industry. Indigo Agriculture, Inc. (www.indigoag.com) is focused on products that use microbial endophytes, and has developed a large and sophisticated pipeline to discover microbes that enhance plant performance in field trials and farmer fields. Indigo released its first product in 2016, Indigo Cotton™, through a collaboration with Texas A&M led by Dr. Greg Sword. Indigo Cotton™ demonstrated an average yield improvement of 11% in target fields, demonstrating the effectiveness of our discovery and development model. To ensure product safety and efficacy, we have followed different metabolites produced by the product microbes as well as the plants and seeds generated by the introduction of these microbes.

Mining the plant microbiome for novel agricultural pest control solutions

Jill Paulik (jpaulik@agbiome.com)

AgBiome, Research Triangle Park, NC, USA.

At AgBiome, we are focused on delivering innovative solutions for some of the greatest challenges in agriculture and crop protection. We do this by exploring and screening our large and diverse microbial collection via a proprietary GENESIS™ technology platform. Within this platform, ~40,000 individual microbial isolates have been completely sequenced at the whole genome level and are continuously screened for relevant gene sequences and biological activity against major insect and nematode pests, plant diseases, and weeds. Leveraging the knowledge gained by coupling empirical screening via validated screens with genome comparisons facilitates the discovery of the most effective strains for product development. Our first product is being launched this year. It is a unique biological fungicide that is highly effective against a range of soil-borne and foliar fungal diseases.

Strategies to design custom microbes for multiple markets using a foundry model

Johan Kers (jkers@ginkgobioworks.com)

Ginkgo Bioworks, Boston, MA, USA.

There is an emerging demand for sourcing natural products using engineered microbes. While recent advances in synthetic biology and metabolic engineering provide feasible approaches to engineering such organisms, commercial success for developing these “cultured” ingredients presents specific challenges. Unlike biofuels, where efforts can be focused on one particular molecule given the enormous market size, cultured ingredients require developing different organism lines in a rapid and low-cost fashion. This requires a scalable solution for biomanufacturing of organisms, which is provided by our state-of-the-art foundry that continues to innovate and grow. I will describe how organism development at Ginkgo leverages our foundry to accelerate the design/build/test using specific examples. In particular, I will highlight the value of combining computer-aided engineering software tools, low cost gene synthesis, and high resolution-accurate mass LCMS to develop engineered microbes.

Optimizing conditions that induce or repress toxin production using Phenotype MicroArrays

Barry Bochner (bbochner@biolog.com)

Biolog, Hayward, CA, USA.

Phenotype MicroArray (PM) technology allows a biologist to simultaneously culture microbial cells in nearly two thousand microscale culture conditions. It can therefore be used to quickly and efficiently discover culture conditions that induce or suppress production of secondary metabolites including toxins. One approach, taken by Gardiner, Kazan, and Manners to discover inducers of trichothecene toxin by *Fusarium graminearum*, was to use a GFP-toxin gene construct and look for wells with green fluorescence. A second approach, taken by Singh to discover inducers of anti-fungal secondary metabolites, employed scaled-down LCMS analysis of the culture fluid from the microplate wells. Our approach was to develop a general colorimetric cell-based toxicity assay that could be used to screen for conditions that either induce or suppress production of toxins or other toxic secondary metabolites. We first demonstrated the approach by measuring the effect of culture supernatant fluids from *Clostridium*

difficile on CHO cells. We then went on to demonstrate that this approach using CHO cells or other sensitive cells can work for a wide range of toxin-producing bacteria.

Plant growth regulators and plant growth-promoting microbes for crop enhancement

Marci Surpin (Marci.Surpin@valentbiosciences.com)

Valent Biosciences, Walnut Creek, CA, USA.

Valent BioSciences, LLC, develops, manufactures, and markets biorational products globally that help address grower needs in a sustainable manner, safeguard crops postharvest, provide vector control solutions that protect human health, maintain green forests, and deliver measurable value to our customers and stakeholders. Biorational products are typically derived from natural or biological origins and include biological pesticides as well as products for crop stress management, enhanced plant physiology benefits, and root growth management. They are characterized as being highly specific in their activity while delivering distinct economic, health, and/or environmental benefits. This is accomplished via the Biorational Crop Enhancement, Biorational Crop Protection, and Biorational Rhizosphere platforms. This talk will give a broad overview of our discovery pipeline with an emphasis on the roles of secondary metabolites in the Crop Enhancement and Rhizosphere platforms.

Omics approaches to deorphanize fungal natural products

Nancy Keller (npkeller@wisc.edu)

University of Wisconsin, Madison, WI, USA.

Filamentous fungi are renown for the production of a diverse array of secondary metabolites (SMs). These natural products are valued for their bioactive properties stemming from their functions in microbial biology, including protection from abiotic and biotic stress and establishment of a secure niche. This talk will present methods we have taken to address the challenges in activating and connecting chemical product(s) to SM biosynthetic gene clusters, including creation of “fungal artificial chromosome” libraries, creation of inducible heterologous clusters, and transcriptomics/metabolomics assessment of inter-microbial wars.

Membrane trafficking at the host-pathogen interface

Sophien Kamoun (Sophien.Kamoun@sainsbury-laboratory.ac.uk)

The Sainsbury Laboratory, Norwich Research Park, Norwich, UK.

Many plant pathogenic and symbiotic microbes produce specialized structures that invade host cells but remain enveloped by host-derived membranes. The mechanisms underlying the biogenesis and functions of such host–microbe interfaces are poorly understood, but these interfaces are thought to mediate metabolite and macromolecule trafficking enabling inter-organismal communication. The Irish potato famine pathogen *Phytophthora infestans* is an oomycete eukaryotic microbe that infects solanaceous plants. *P. infestans* forms haustoria, which are hyphal extensions that invaginate the host cell membrane. A host-derived membrane, called the extrahaustorial membrane (EHM), separates haustoria from the plant cell and constitutes the haustorial interface. Some *P. infestans* strains infect the model plant *Nicotiana benthamiana* and develop haustoria in infected leaf cells. We have exploited the

N. benthamiana experimental system to perform fast-forward cell biology of the haustorial interface. This revealed dynamic changes in host membrane compartment formation and rerouting in haustoriated plant cells. In particular, we discovered that selective autophagy and other trafficking pathways are diverted to the pathogen interface. Our working model is that *P. infestans*-secreted proteins (effectors) co-opt host membrane trafficking to promote plant colonization.

Comparative phylogenomics identifies fungal secondary metabolites allied with virulence to plant hosts

Gillian Turgeon (bgt1@cornell.edu)

Cornell University, Ithaca, NY, USA.

Filamentous fungi produce chemically diverse secondary metabolites (SMs) that have positive and negative effects on other organisms and are implicated in virulence to hosts and development of structures associated with reproduction. SMs also combat nutritional and environmental stresses in niche situations, making them central to fungal survival and proliferation. SMs are at the very heart of the information networks that play out within a single organism, and between communities of interacting organisms. Sequencing of the genomes of highly aggressive plant pathogens in the genus *Cochliobolus* and in the closely related genus *Setosphaeria* has provided a clearer picture of the plethora of SM gene clusters encoding unknown SMs. Our lab employs a combination of genetic and comparative phylogenomic methods to explore function of SMs to make initial functional predictions (guilt by association). Comparative analyses have revealed that the suites of SM-encoding genes from all *Cochliobolus* species are astoundingly diverse between species but remarkably conserved among isolates of the same species, except for lifestyle-defining examples that generally map to unique genomic regions. This pattern is also found when comparisons are made between the closely related *Cochliobolus* and *Setosphaeria* genera. Functional analysis of several of these strain-unique SMs reveals a strong correlation with a role in virulence for necrotrophs and, surprisingly, also for hemibiotrophs.

Discovery and engineering of novel natural products via synthetic biology

Huimin Zhao (zhao5@illinois.edu)

University of Illinois at Urbana-Champaign, Champaign, IL, USA.

Microorganisms are a major source of new therapeutic agents. My group has been developing new genomics-driven, synthetic biology-enabled strategies to discover and produce novel natural products from sequenced genomes and metagenomes. One strategy is to refactor target cryptic gene clusters in heterologous hosts. As proof of concept, we used this strategy to awaken the silent polyketide spectinabilin pathway from *Streptomyces spectabilis* in *Streptomyces lividans* and activate a cryptic pathway containing a polyketide synthase–non-ribosomal peptide synthetase from *Streptomyces grieseus* in *Streptomyces lividans*, which led to the discovery of two novel tetramic acid natural products that have never been reported in literature. To increase the throughput, we are establishing a fully integrated robotic system to automate all the steps in gene cluster refactoring and product detection. Another strategy is to activate the target cryptic gene clusters in their native hosts by knocking-in strong promoters upstream of the target cryptic gene clusters using a CRISPR/Cas9 system. We successfully activated more than 10 cryptic gene clusters from five different *Streptomyces* and uncovered a number of novel natural products.

Harnessing the chemistry and biology of plant metabolism

Sarah O'Connor (Sarah.Oconnor@jic.ac.uk)

John Innes Centre, Norwich, UK.

Plants, which make thousands of complex natural products, are outstanding chemists. Through the concerted action of enzymes that are assembled into metabolic pathways, nature creates chemical complexity from simple starting materials. In this talk, I will highlight some of the unusual enzymatic transformations that plants use to make complex, bioactive natural products, and will also discuss methods by which these pathways can be harnessed for metabolic engineering. The focus is on the biosynthesis of the monoterpenes called iridoids, and the alkaloids derived from iridoids, known as the monoterpene indole alkaloids. The discovery, functional characterization, and mechanistic study of enzymes involved in the biosynthesis of these important compounds in several medicinal plant species will be discussed.

Metagenomic and synthetic biology approaches for natural product discovery

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Soil microorganisms contain vast reservoirs of bioactive natural products; however, the majority of them are recalcitrant to cultivation in the lab. In this study a large-insert soil metagenomic clone library (~110 kb and 19,200 clones) was constructed from an agricultural soil (Cullars Rotation, Auburn, AL) using a broad host range shuttle BAC vector, pSmartBAC-S. This insert size is capable of harboring many intact secondary metabolite biosynthetic pathways such as Type I PKS pathways that are usually >40 kb. Identification of biosynthetic gene clusters was conducted using multiple methods, including DNA hybridization, PCR, and next-generation sequencing. In the first two methods we targeted a conserved domain of Type I polyketide synthases (PKS) and identified clones by macroarray hybridization or PCR, resulting in 12 and 110 pathway-containing clones, respectively. In addition, we used a strategy in which plates, rows, and columns were separately pooled, and bar-coded DNA sequences from each pool were subjected to Illumina HiSeq sequencing. Contigs were assembled from each pool and screened for secondary metabolite gene clusters using antiSMASH3.0. We identified 593 clones that contained a PKS and/or non-ribosomal peptide synthetase pathway among 1,516 total biosynthetic pathways identified. The cloned pathways are very divergent from known pathways, with the %G+C content varying from 34% to 79% and the nearest BLAST hit of keto-synthase domains ranging from 19% to 95% amino acid identity. Biosynthetic clusters identified via PCR were a subset of the clones identified via next-gen sequencing, which were both numerically more abundant and representative of novel pathways highly divergent from known pathways. 139 identified pathway-containing BAC clones with limited homology to known PKS pathways were transformed into *Streptomyces coelicolor* M1154 and screened for the synthesis of antibacterial compounds by various bioassays against bacterial and fungal human pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Candida albicans*. Clones expressing antimicrobial activity were further characterized by LC/MS analysis. These results indicate that highly novel biosynthetic clusters can be cloned intact from complex metagenomes and heterologously expressed to produce secondary metabolites, thereby expanding our available resources or natural product discovery. Improved methods for capturing, cloning, and overexpressing natural product pathways are being developed to further accelerate the discovery of novel beneficial molecules.

Genome mining for new herbicides

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Due to rapid emergence of weeds that are resistant to commercial herbicides such as glyphosate and glufosinate, the need for new herbicides with a novel mode of action is more urgent than ever. Here we describe recent discoveries in our labs (Tang and Jacobsen). One discovery is a natural product that inhibits dihydroxyacid dehydrogenase (DHAD), a long-sought-after target for herbicide development. The compound was mined from sequenced fungal genomes using a target-based approach. We developed a scalable platform for producing the compound in yeast and showed that it can potently inhibit plant DHAD (Ki ~ 300 nM) and can effectively shut down plant growth in planta. We also found a resistance enzyme that can be used as a transgene for generating transgenic crop plants.

Harnessing synthetic biology for the production of fine and specialty chemicals

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Many microbial genomes encode the machinery to produce diverse bioactive molecules that can be used in healthcare, agriculture, and food. Its natural modularity makes this machinery a particularly attractive target for synthetic biology. The re-engineering of the biosynthetic capacity of microbes requires the development of a wide range of experimental and computational tools. These include orthogonal transcriptional control circuits and bacterial microcompartments, computational tools for the detection and analysis of secondary metabolite biosynthesis gene clusters that enrich our library of parts and building blocks for pathway engineering, and high-resolution mass spectrometry analysis for the debugging of the engineered systems.

In this talk I will explore the possibilities created by the application of the design/build/test/learn cycle of synthetic biology to the engineering of microbial metabolism for the production of high-value chemicals, as implemented in the high-throughput platform of the BBSRC/EPSRC-funded Manchester Synthetic Biology Research Centre, SYNBIOCHEM. I will also discuss the growing toolbox of techniques and approaches, illustrated with concrete application case studies.

Redesigning the genetics of secondary metabolism in *Streptomyces*

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Genetic manipulation of natural product gene clusters in *Streptomyces* is complicated by their large size, complex organization, and a relatively limited toolbox for genetic engineering. We are leveraging recent advances in DNA synthesis and assembly techniques to re-build natural product gene clusters from a collection of characterized genetic elements. We demonstrate the applications of our DNA assembly pipeline toward (1) generating chemical diversity and (2) improving yield using a designed biosynthetic pathway for the neuroprotective natural product serofendic acid.

Discovery of novel signaling secondary metabolites from *Clostridium*

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Medicinally active natural products have functional group arrays and scaffold architectures that offer advanced platforms for the development of successful new drugs. The study of biosynthetic routes to natural products will facilitate the production of target molecules including commercial drugs and key starting materials for chemical derivatization and semisynthesis. Our lab is interested in studying the biosynthesis of various pharmaceutically important natural products, including but not limited to antimycin-type depsipeptides, the manumycin family, and pyrroloindole alkaloids. We are also developing general platforms of in situ natural products labeling for various applications. Small-molecule secondary metabolites (SSMs) are often employed by microbes to access information about both their intracellular physiological status and their extracellular environment, and they are often critical in controlling complex processes including morphological differentiation, multicellularity, biofilm formation, secondary metabolite production, and virulence. In order to block the bacterial communication leading to pathogenicity, it is important to unveil the identity and function of the hidden SSMs in pathogenic bacteria. We are interested in unveiling hidden SSMs from mycobacteria, followed by mode of action studies. The characterization of enzymatic machinery for the biosynthesis of SSMs and their functional network will promote the development of new anti-bacterial treatments. Additionally, we are also interested in uncovering the identity and function of hidden signaling SSMs in *Clostridia*, with the goal of improving ABE fermentation performance. Bacterial volatiles represent a source for new biofuel compounds in addition to the traditional bioethanol and plant-oil-derived biodiesel. The relevant volatile compounds that have been identified include various short- to medium-chain alkanes, alkenes, alcohols, and isoprenoids, which have great potential to replace or supplement petroleum-derived chemicals and fuels. However, little is known about the enzymatic logic for the biosynthesis of many of the volatile organic compounds (VOCs) produced by various bacterial cultures. In order to develop more sustainable and economically feasible biochemicals and biofuels, it is important to fully characterize the enzymatic mechanisms of biosynthesis and secretion of these hydrocarbons as well as to engineer their heterologous production with increased yield and efficiency in preferred hosts.

Interkingdom signaling: a *Populus* case study

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Experimental evidence pointing to inter-kingdom signaling between *Populus* and its endophytic community will be presented specifically related to *Laccaria* colonization, small secreted protein signaling, and quorum sensing. These data initially emerged from the JGI-based, sequenced, assembled and annotated *Populus* genome, where over 35 archaeal, bacterial, and fungal genomes were detected and assembled. Since then over 500 bacterial endophytic and 35 fungal associates have been sequenced, assembled, and annotated from *Populus*. As a result of these efforts, *Populus* lectin receptor-like kinase (RLK) has been identified that control *Laccaria* colonization. The *Populus* transgenes have been transformed into *Arabidopsis*, which resulted in the formation of a Hartig-net, the first report of a mycorrhizal association in *Arabidopsis*. The PtRLK gene induces metabolic changes in *Arabidopsis* that mimic fungal challenge but in the presence of *Laccaria* these responses subside. From the *Populus*

genome assembly over 200 small secreted proteins have been identified and characterized. Several of these are actively taken up by fungal associates and are then subsequently localized to the nucleus by *Laccaria*. The presence of the *Populus*-secreted proteins in the *Laccaria* nuclei causes changes in *Laccaria* hyphal branching. From the *Populus*-based endophytic bacterial collection, several genera, i.e., *Rhizobium, Rahnella, Albidiferax* and *Pseudomonas*, were found to contain quorum-sensing genes that respond to *Populus* leaf macerate. The plant signal is actively transported and is most likely a dipeptide, resembling a D-Ala-D-Leu dimer. It appears that *Populus* has metabolic signals that attract favorable bacteria via co-option of their quorum sensing machinery. Strategies for leveraging this information indicate we may be able to intentionally and specifically manage the *Populus* microbiome in an environmentally relevant manner. Inter-kingdom signaling between a plant host and its microbiome, through exchange of metabolites and proteins, appears to be pervasive.

Poster Presentations

Streptomyces' dynamic role in the root of *Arabidopsis thaliana*

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While consistent presence of *Streptomyces* within roots of a number of plant species suggests specific selection, mechanisms that facilitate internal root colonization are uncharacterized. Here we seek to tease apart plant from microbe-derived mechanisms, and more specifically reveal *Streptomyces'* ability to positively and negatively influence co-occurring microbes. We hypothesize that select soil *Streptomyces* are influential members of the *Arabidopsis thaliana* root community, capable of harnessing their metabolic potential to join and sculpt the root microbiome. To address this, we explore the potential of select *Streptomyces* isolates as drivers of root community establishment, employing antimicrobial metabolics to selfishly target potential microbial competitors. We utilized the model organism *Arabidopsis thaliana* to investigate isolate-specific influences on plant phenotype. Plant-*Streptomyces* experiments reveal isolate-dependent phenotypes, with altered root structure and varying colonization potential. Microbe-microbe experiments have shown *Streptomyces*' ability to inhibit growth of select soil microbes, including several Bacilli isolates. Preliminary experiments exploring colonization of seedlings inoculated with *Streptomyces* and a natural mixed community differ in biomass, plant growth, and root structures. Sequencing of the 16S gene will further elucidate community compatibility and structural dynamics, providing evidence of shifts in microbial relative abundances. Obtaining full genome sequences of these *Streptomyces* isolates allows targeted investigation of potential chemical mechanisms driving interactions and continue to inform experiment design and interpretation of findings. Initial experiments investigating the role of bacterially-derived melanins, phenazine, and indole products, their influence on plant root colonization, and ability to affect microbial colonizers provide evidence of differential production, potential microbial growth influence, and consistent plant root interaction phenotypes. Two of four *Streptomyces* isolates uniquely capable of producing melanin consistently out-compete other microbes for colonization within the root. We are currently conducting targeted experiments to understand the potential role of this metabolite in colonization. Additionally, preliminary investigation of the indole derivative auxin suggests auxin-independent root phenotypes, indicating a potential role for isolate-specific non-indole metabolites in root growth promotion. Together, the preliminary and anticipated findings suggest microbe and host relationship dynamics responsible for microbial community modification. These findings further our current understanding of *Streptomyces*' activities in the root of *Arabidopsis thaliana*.

Seeds of antagonism: An ABC transporter and its adjacent transcription factor in *Fusarium verticillioides* are required for pyrrocidine B tolerance

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The prevalent seed-borne maize endophyte, *Fusarium verticillioides*, is the causal agent of severe kernel rot disease. Its production of fumonisin mycotoxin is a worldwide food safety concern, as contaminated

corn is associated with human and animal toxicosis. Another maize endophyte, *Sarocladium zeae*, cohabits the ecological niche of maize seeds with *F. verticillioides*. Within maize kernels, *F. verticillioides* is primarily confined to the pedicel, while *S. zeae* is observed in embryos. In vitro competition assays have indicated *S. zeae* can inhibit the growth of *F. verticillioides*. A lactam-containing antibiotic produced by *S. zeae*, named pyrrocidine B, is associated with the antagonism. LC-MS analysis of liquid cultures indicated that *F. verticillioides* can defend itself by degrading pyrrocidine B, when the concentration doesn't significantly impede its growth. To explore the antagonistic mechanism on the side of *F. verticillioides*, RNA-seq experiments were conducted by challenging the *F. verticillioides* liquid culture with pyrrocidine B at subinhibitory concentrations to induce transcriptomic changes. Ten genes with dramatic induction in pyrrocidine B treatment were selected as targets to generate gene deletion mutants. Phenotypic analyses revealed that deletion of an ABC transporter gene (FVEG_11089) or its adjacent transcription factor (FVEG_11090) elevated sensitivity of *F. verticillioides* to pyrrocidine B. Quantitative PCR showed 13,530-fold induction of ABC transporter gene in response to pyrrocidine B exposure, and the induction was dependent on the adjacent transcription factor. Hence, we theorize that FVEG_11089 functions in pyrrocidine B resistance by extruding the antibiotic out of the cell, and FVEG_11090 positively regulates induction of FVEG_11089 to a high level in response to pyrrocidine B exposure. The exploration of the antifungal resistance mechanism addresses the competitive relationships of the two maize seed endophytes colonizing the same ecological niche.

Exploring the soybean microbiome using complementary spatial metabolomics techniques

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In an effort to attain more sustainable agricultural practices, there is a great interest in understanding metabolic processes within plant systems known to acquire nitrogen through biological nitrogen fixation. The symbiotic association between nitrogen-fixing soil bacteria (*Rhizobiaceae*) and plants of the family *Leguminosae* are one such system of interest. This symbiosis generates specialized organs, called root nodules, where rhizobia reduce N_2 into bioavailable products accessible to the host plant, and in exchange the plant provides a carbon source to the bacteria to ensure (among other things) sufficient energy for nitrogen fixation. However, little is known about the array of metabolites involved, and their spatial distribution, which influence the rhizobia-legume association. Since these biological processes are inherently three-dimensional phenomena, where localized metabolism can exist within different anatomical compartments of the root nodule, we describe the use of both laser ablation electrospray ionization (LAESI) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) methods to spatially observe the array of metabolites that influence the rhizobia-legume association of *Bradyrhizobium japonicum* and soybean (Glycine max Williams 82). We were able to identify and map a number of molecules involved in biological nitrogen fixation, such as heme B, riboflavin, and adenine. We could also visualize the distributions of secondary metabolites (e.g., flavonoids, saponins, and glucosides) that modulated this symbiosis. Tandem MS, pre-mass analysis ion mobility separation, and high mass resolution and mass accuracy measurements of the isotopic envelope permitted us to move beyond providing putative identifications based on accurate measured mass alone, and provided higher confidence in the identity and localization of metabolites. We further applied this information to

elucidate the active metabolic pathways within the soybean root nodule, which afforded a better view of actual metabolism than obtained from proteomics and transcriptomics alone.

Enzymatic transformation of the siderophore pyochelin through imaging mass spectrometry of bacteria-fungi interaction

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Many species of plant-associated bacteria secrete natural products that inhibit the development or growth of plant pathogens. In turn, pathogens may develop resistance to antagonistic molecules. However, little is known about enzymatic transformations of secreted metabolites that occur during interactions between bacteria and fungi. *Burkholderia cenocepacia* strain 869T2, an endophytic bacterium, showed a promising antagonistic effect against *Phellinus noxius*, a pathogen that causes brown root rot disease. To understand the functional bacterial-fungal interaction comprehensively, it is a good strategy to monitor metabolite secretion and gene expression profiles of both interacting organisms simultaneously. Pyochelin is one siderophore of *Pseudomonas aeruginosa* and *Burkhoderia* sp. that is able to induce plant ISR (induction of systemic resistance) and has been identified as an antifungal antibiotic. Through IMS and RNA-seq, we found that instead of inhibiting the gene expression of pyochelin in strain 869T2, *P. noxius* could modify pyochelin (m/z 325) to m/z 383, while *P. noxius* faced to *Burkholderia cenocepacia* strain 869T2. We then isolated pyochelin and pyrrolnitrin, an antifungal compound from 869T2, and found that the inhibited zone caused by pyrrolnitrin decreased over time. However, the combination of pyochelin and pyrrolnitrin has shown a longer inhibitory effect against *P. noxius*. This result might explain why *P. noxius* tried to modify the structure of pyochelin. We also found 869T2 could transform m/z 393 to m/z 409 and inhibit some metabolites secreted from *P. noxius* in response. Thorough understanding of this bacterial/fungal interaction would further the development of biological control strategies, and may lead to the discovery of a method to control brown root rot disease.

Metabolic engineering a probiotic yeast to increase astaxanthin production

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In this study, an astaxanthin-biosynthesis *Kluyveromyces marxianus* strain Sm23 was first constructed, which could produce 31 μg/g DCW astaxanthin. Then, genome integration of the key astaxanthin-biosynthesis genes Hpchyb and bkt was done to increase gene copy number and astaxanthin yield. Four improved strains were obtained and the yield of astaxanthin and the total yield of carotenoids in a strain increased with the copy numbers of Hpchyb and bkt. To improve the yield further, the gene Hpchyb from *Haematococcus pluvialis* was modified by site-directed mutagenesis to increase the enzyme efficiency and/or to prevent the heterologous protein degradation by ubiquitination. Using repeated-integration approach of bkt and the mutated Hpchyb into Sm23, the S3-2 strain was obtained and shown to produce the 3S, 3'S-astaxanthin at 9972 μg/g DCW in a 5 L fermentor.

Efflux transporters contribute to virulence and host compatibility of *Pseudomonas syringae* B728a

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Plant pathogens such as *Pseudomonas syringae* encounter diverse plant-produced metabolites during colonization of both the apoplast and surfaces of plants. Successful infection or epiphytic colonization requires tolerance to such chemicals by either detoxification or active efflux from the cell. *Pseudomonas syringae* pv. syringae B728a harbors approximately 120 multidrug resistance transporters, representing several protein families. The majority of these genes are uncharacterized. Since different plant species produce chemically diverse antimicrobial compounds (phytoalexins), one major objective is to address the extent to which different efflux pumps are required in different host plants and the extent of redundancy in this trait. MexB (a homolog of AcrB in *E. coli*) is a multidrug resistance efflux transporter in the Resistance-Nodulation-Division protein family. It has previously been shown to contribute to tolerance to many toxicants in vitro. In the bean apoplast, a MexB mutant grows to a lower total population than wild type, but this difference is only visible after at least four days. This is approximately the time required for bean to produce phytoalexins in a compatible interaction, and therefore MexB is likely a major pump responsible for survival. *P. syringae* B728a was originally isolated from common bean (*Phaseolus vulgaris*), and is also pathogenic on *Nicotiana benthamiana*. Interestingly, it appears to be able to grow in the apoplast of many additional plant hosts, including other members of the *Fabaceae* as well as hosts in the *Solanaceae* and *Asteraceae*. While the mutant strain lacking MexB is less virulent than wild type in bean leaves, it grows as well as the wild type strain in several other plant species, suggesting that these additional host plants do not produce antimicrobial compounds that are removed by the MexB transporter. A bar-coded TnSeq approach is being used to determine the contribution of each of the transporters singly, and in combination with MexB, to the fitness of *P. syringae* in a variety of host plants. Infected leaf tissue is being interrogated by LC-MS/MS to determine the extent to which plant species differ in their complement of toxic metabolites that must be overcome by potential pathogens, and thus their need for efflux pumps.

Volatile terpene secondary metabolism in switchgrass roots in the biotic/abiotic environment

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As a prairie grass, switchgrass (*Panicum virgatum*) produces an extensive fibrous root system, which is exposed to a dynamic biotic and abiotic belowground environment. Switchgrass roots are rich in volatile terpenoid secondary metabolites, particularly the monoterpenoids (-)-borneol and its derivative (-)-camphor. We investigate the multiple functions of these compounds in beneficial or antagonistic interactions with soil-borne organisms. While it is known that volatile terpenoids affect colonization by epiphytic bacteria in aboveground tissues, the role of these compounds in interaction with microbes in the rhizosphere and endosphere of roots is largely unknown. We hypothesize that root-produced terpene volatiles function as host-specific chemo-selective factors that affect root microbial community composition as growth inhibitors or C sources. To assess the specific use of terpenoids as C sources, culture-based methods are applied to identify terpenoid-metabolizing microbes in the switchgrass

endosphere and rhizosphere. To further determine the effect of volatile terpenoids on the root microbial community in vivo, we are characterizing the terpene synthase (TPS) gene family of switchgrass. Within the TPS-b subfamily, we have identified PvTPS04 as a leaf- and root-expressed terpene synthase that forms (-)-borneol as a major product. RNAi-based approaches are in progress to reduce the production of borneol and camphor and determine modifications of the root microbiome associated with changes in the root chemical environment. In an attempt to assess the effect of abiotic (drought) stress on terpenoid dynamics in switchgrass roots, we found an unexpected accumulation of sesquiterpenoids and diterpenoids indicating changes in the root chemical environment specific to drought stress episodes. The role of these chemical modifications in root-microbial associations and stress protection needs to be further addressed.

A protein-protein interaction network centered on c-di-GMP signaling in the plant growth promoting rhizobacteria *P. fluorescens*

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Plant growth promoting (PGP) rhizobacteria achieve their beneficial effects through direct and indirect interactions with plant roots. While PGP activities are extensively described at a physiological level, the underlying mechanisms by which bacteria colonize plant roots, sense nutrient composition, and make compounds available to the plant remain poorly characterized. To address this knowledge gap, we deciphered a key protein-protein interaction network involving a family of proteins known to regulate bacterial sensing and signaling during colonization of plant roots by *Pseudomonas fluorescens*.

Pseudomonads are abundant soil bacteria with characterized PGP effects, including the production of auxins (plant hormones), siderophores (iron uptake) and secondary metabolites facilitating phosphate solubilization. Although successful root colonization by fluorescent Pseudomonads is a critical initial step in PGP, other mechanisms are also involved. In many bacterial species, the chemical messenger c-di GMP has emerged as a key player in the control of many cellular processes involved in developmental transition and cell fate, production of exopolysaccharides and other metabolites, and control of virulence. In bacteria, c-di-GMP homeostasis involves the balance activities of diguanylate cyclases (DGCs) and phosphodiesterases (PDEs). These enzymes are characterized by the presence of catalytic domains displaying signature motifs (GGDEF/ EAL/HP-GYP) often linked with additional domains for signaling inputs and outputs. Upon binding c-di-GMP, these proteins exert a regulatory action at transcriptional, post-transcriptional or post-transductional levels.

This study combines identification of protein complexes involved in c-di-GMP regulatory pathways with a systematic CRISPRi-based functional analysis. A high-quality binary protein-interaction map centered on c-di-GMP signaling in *P. fluorescens* SBW25 was built from very high confidence interactions. Targeted on 10 c-di-GMP binding proteins involved in bacteria-root associations, our protein-protein interaction network is composed of 94 proteins connected by 130 interactions clustered in connected functional modules, revealing connections between the c-di-GMP signaling and various cellular processes. It exhibited a remarkable structural organization that revealed functional associations connecting c-di-GMP binding proteins to particular metabolic pathways and cellular machineries relevant with plant-root interactions, such as cell signaling, transcriptional regulations, cell adhesion, and transport of various nutrients. This network is of high biological significance and provided a wealth

of hypotheses for further deciphering of the relationships between c-di-GMP signaling and plant (Aspen)-root colonization by *P. fluorescens*.

Nanoscale clustering of enzymes in a fungal sesquiterpene biosynthetic pathway

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The mevalonate pathway leads to the synthesis of farnesyl diphosphate, which serves as a precursor for both primary and secondary terpenoid metabolites. Since these different products draw upon the same starting materials, how do cells apportion the supply of shared molecular precursors to primary and secondary metabolic pathways? Cellular compartmentalization may serve to sequester pathways and to channel metabolites for particular purposes. Trichothecenes are conditionally expressed sesquiterpene mycotoxins produced by the fungus *Fusarium graminearum*. Upon induction of trichothecene synthesis, enzymes of the trichothecene pathway as well as upstream mevalonate pathway enzymes co-localize to highly remodeled endomembrane structures called organized smooth endoplasmic reticulum (OSER). Based on super-resolution fluorescence structured illumination microscopy (SIM) and fluorescence resonance energy transfer (FRET), it can be inferred that these enzymes form a multi-protein complex. Nanoscale localization of proteins demonstrates that integral ER membrane proteins from both pathways are brought together along with cytoplasmic enzymes captured within the three-dimensional cellular architecture of OSER. To identify additional proteins associated with these structures, fluorescence-activated cell sorting (FACS) has been used to enrich for fluorescently labeled OSER for proteomic analysis. Proteomics revealed additional proteins involved in the trichothecene biosynthetic pathway as well as a number of conserved ER proteins. Efforts currently are underway to destabilize the trichothecene biosynthetic complex and to mislocalize component proteins to test how clustering of enzymes and ancillary proteins influence trichothecene synthesis and other terpenoid pathways within the cell.

Drought stress results in a compartment-specific restructuring of rice-root-associated microbiomes

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Plant roots support complex microbial communities that can influence plant growth, nutrition, and health. While extensive characterizations of the composition and spatial compartmentalization of these communities have been performed in different plant species, there is relatively little known about the impact of abiotic stresses on the root microbiota. Here, we have used rice as a model to explore the responses of root microbiomes to drought stress. Using four distinct genotypes, grown in soils from three different fields, we tracked the drought-induced changes in microbial composition in the rhizosphere (the soil immediately surrounding the root), the endosphere (the root interior), and unplanted soils. Drought significantly altered the overall bacterial and fungal composition of all three communities, with the endosphere and rhizosphere compartments showing the greatest divergence

from well-watered controls. The overall response of the bacterial microbiota to drought stress was taxonomically consistent across soils and cultivars, and was primarily driven by an enrichment of multiple *Actinobacteria* and *Chloroflexi*, as well as a depletion of several *Acidobacteria* and *Deltaproteobacteria*. While there was some overlap in the changes observed in the rhizosphere and endosphere communities, several drought-responsive taxa were compartment-specific, a pattern likely arising from preexisting compositional differences, as well as plant-mediated processes affecting individual compartments. These results reveal that drought stress, in addition to its well characterized effects on plant physiology, also results in restructuring of root microbial communities, and suggest the possibility that constituents of the altered plant microbiota might contribute to plant survival under extreme environmental conditions.

The rhizosphere of the beachgrass *Ammophila breviligulata* as a model for plant-microbiome interactions

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The American beachgrass *Ammophila breviligulata* is a salt-loving plant considered to be an important sand dune architect of barrier islands and other coastal environments in the mid-Atlantic and Northeast. A variety of interesting adaptations allow it to colonize and trap sand in order to promote the formation of dunes and offer protection to these coastal ecosystems. My research group is interested in understanding the role that the root-associated microbiomes play in healthy beachgrass growth. We have characterized the microbiomes associated with the soils and roots of beachgrass samples collected along the south shore of Long Island under a variety of beachgrass growth and health conditions. We are learning that the microbial communities associated with beachgrass are remarkably diverse and extremely well structured across soil microenvironments. More importantly, we have also identified a variety of patterns in microbial community composition that are associated with the health of the plant. In addition, we have also been able to isolate and characterize plant-growth-promoting microbes that could play a key role in healthy beachgrass root growth. Genome sequencing of key isolates has allowed us to identify pathways that may play a role in their molecular signaling exchanges with beachgrass through the production of indole acetic acid, siderophores, terpenes, and other secondary metabolites. Our goal is to use the beachgrass *Ammophila breviligulata* as a model system to study plant-microbe interactions, laying the groundwork for the utilization of specific isolates and microbiome configurations to promote the health of beachgrass and other plants facing similar environmental challenges.

Hydroxycinnamic acid amides are exported by a MATE transporter in *Arabidopsis*

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Arabidopsis thaliana is able to prevent colonization by *Phytophthora infestans*, the causal agent of late blight disease of potato. This nonhost resistance depends on multilayered defense responses. To address the role of plant surface-localized secondary metabolites for pathogen defense, untargeted

metabolite profiling was performed. In addition to indolic compounds, the hydroxycinnamic acid amide coumaroylagmatine was among the secreted compounds. Microarray analyses revealed *P. infestans*-activated and highly co-expressed genes, encoding an agmatine coumaroyl transferase (ACT) and a MATE transporter. In leaves of *P. infestans*-inoculated ACT knockout mutants, no coumaroylagmatine was detectable, suggesting that biosynthesis was impaired. In MATE mutants, coumaroylagmatine accumulated intracellularly, but not extracellularly, indicating that the MATE transporter is required for the export of coumaroylagmatine.

In *Solanum tuberosum*, coumaroylagmatine accumulates in response to *P. infestans* infection in leaves, but not extracellularly, suggesting that potato is not able to secrete coumaroylagmatine efficiently. Expression of both AtACT and AtMATE in transgenic potato plants leads to high levels of extracellular agmatine and putrescine conjugates. This suggests that the MATE transporter is specific for a role of AtMATE for the export of a specific subset of hydroxycinnamic acid amides.

Mapping the colonization of a synthetic microbial community inoculum from sugarcane microbiome in maize and soybean plants

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Plants live in association with a highly complex community of bacteria and fungi groups. Recent advances in microbial studies have shown that host genotypes influence the diversity, structure, and composition of plant microbiomes. However, the genetic and molecular mechanisms involved in plant-microbe communication that are responsible for establishing the microbial community in plant tissues remain unknown. Unraveling the traits involved in microbe-host interaction during microbiome assemblage is an imperative step towards building biotechnological tools to transfer microbes and their beneficial functions to economically relevant plants. In this work, we sought to explore the genetic and molecular mechanisms involved in cross-species microbial compatibility by studying the colonization of two important crop plants from distinct physiological groups, maize and soybean. Our main strategy involves transferring a bacterial community to a new C_4 plant and to a C_3 plant to evaluate if the bacterial members of this community are capable of colonizing both plants with contrasting physiological profiles. First, we designed a synthetic consortium of microorganisms composed of the most abundant bacterial groups from sugarcane, a C_4 bioenergy crop. Secondly, we established a platform for inoculation assays using maize, another C_4 crop, as a plant model. Maize seeds are germinated and planted in sterile conditions and are inoculated with the synthetic community. With this platform, we can evaluate the effect of the presence of the bacterial community in plant growth and also test different nutrient solution concentrations or stress conditions. The presence of the synthetic community improves plant growth even under low nutrient availability. We also tested whether this community would be able to colonize the plant tissues. By using culture-independent techniques to profile inoculated and non-inoculated plants, we show that only a part of the synthetic community robustly colonizes the maize plants. This data indicates that even among phylogenetically close plants there might exist traits underplaying host colonization by specific microbial groups. Additionally, plants inoculated with the synthetic microbial community had their total fresh and dry biomass on average up to three times increased when compared with uninoculated plants. We sought to investigate if the synthetic community is also able to colonize soybean plants with a similar pattern and a beneficial plant

response. Dual RNA-seq of plant and microbial community will reveal genes and pathways involved in the establishment of the microbial community and its beneficial functions.

Deciphering the role of fungal secondary metabolites within anaerobic microbial communities

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Anaerobic fungi thrive in competitive microbial environments such as the digestive tracts of many large herbivores despite being vastly outnumbered by other microorganisms. In addition to secreting powerful biomass-degrading enzymes, these unique non-model organisms also possess a rich array of biosynthetic machinery for producing secondary metabolites, whose functions remain to be determined. We hypothesize that secondary metabolites from gut fungi play important roles in anaerobic communities via microbial defense, communication, or stress tolerance. Here, we have combined complementary approaches in genomics, transcriptomics, mass spectrometry, and NMR to probe the functions of secondary metabolites in the complex anaerobic digestive environment of herbivores. First, we uncovered a plethora of gene clusters encoding biosynthetic enzymes for secondary metabolites from diverse chemical classes by mining the genomes and transcriptomes of three anaerobic fungi from the primitive phylum *Neocallimastogomycota*. Key secondary metabolite clusters include canonical polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). Several of the clusters are expressed under standard laboratory growth conditions, whereas others are silent. RNA sequencing allows us to test environmental conditions that activate the biosynthetic gene clusters that have been identified in the fungal genomes. The environmental conditions we are testing include heat shock, oxidative stress, pH, and nutrient availability. In addition to abiotic factors, we are also co-cultivating the anaerobic fungi with bacteria and archaea to screen interactions that may enhance transcription of the clusters. Finally, we are working with JGI and EMSL to employ analytical techniques like mass spectrometry and NMR that allow us to detect and determine the structures of key secondary metabolites. To date, we have detected ~100 likely secondary metabolites and putatively identified an antioxidant polyketide, baumin, which is also produced by a distantly related fungus from the phylum *Basidiomycota*. Mass spectrometry facilitates high throughput, rapid screening of metabolites within a complex mixture and yields a chemical fingerprint of each molecule that can be compared with reference databases for dereplication with known natural products, while NMR permits detailed structural characterization of isolated compounds. The secondary metabolism of anaerobic fungi represents a completely untapped reservoir of biosynthetic potential, which could be drawn upon for novel therapeutics, new chemical building blocks, and enzymes for bioengineering natural products. Our integrated approach allows us to study secondary metabolism at all levels, from DNA to RNA to metabolites, thus maximizing discovery of novel metabolites and unmasking their native functions.

Root-associated microbiome of a diterpene-deficient maize mutant

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Plants deploy specialized metabolites to communicate with other organisms and cope with environmental challenges. This includes interactions with microbial communities, in which plants exchange sugars for available nutrients as well as protection against environmental stressors. However, the molecular mechanisms by which a plant recruits its particular microbial community and the role of specialized metabolites in this communication are poorly understood. Here, we report that maize root diterpenes, a group of specialized metabolites with versatile functions in stress resilience, influence rhizosphere bacterial communities. In addition to the previously described kauralexin metabolites with key roles in maize pathogen and environmental stress resistance, we recently elucidated a novel group of maize-specialized diterpenes, termed dolabralexins, which also show activity against biotic and abiotic stress. Distinct from the gibberellin biosynthesis pathway, both kauralexins and dolabralexins are synthesized via the copalyl diphosphate synthase ZmAn2 before branching into separate pathways. The an2 (anther ear 2) maize mutant is deficient in forming both kauralexin and dolabralexin metabolites, and exhibits enhanced stress susceptibility. Using 16S rRNA sequencing, we determined the bacterial community compositions of the an2 mutant compared to its wild type sibling. Under well-watered conditions, distinct bacterial communities and diversities were observed between mutant and wild type plants, whereas the microbiome compositions became indistinguishable under drought conditions. These findings suggest that diterpenes play an important role in shaping the rhizosphere microbiome, while alternate mechanisms may be dominant under drought stress.

Genome sequence of an abundance-driven microbiome synthetic community with beneficial effects on plant development

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The sugarcane-associated microbial community has long been explored for its capability of supporting plant development under diverse conditions. These surveys have mostly focused on specific bacterial groups such nitrogen-fixing bacteria by approaches based on isolation on defined culture media and inoculation of a single bacteria. However, the sole use of methods based on cultivation is strongly biased and may not reflect the real composition of the bacterial community in the plant. Furthermore, the inoculation of a single bacteria does not represent the highly complex dynamic of a plant microbiome. As a result, we lack fundamental information regarding the microbial assemblage and its functional role in association with the sugarcane plant. The goal of this work is to explore the genetic traits in the untapped microbial community that are responsible for plant development. Our group has adopted a strategy that concomitantly use culture-dependent and culture-independent techniques to target dominant microbial groups from the sugarcane microbiome. By using culture-independent techniques, we found a core microbiome composed of less than 20% of the total microbial richness and that sums up to over 90% of the total microbial relative abundance in roots, stalks, and leaves of sugarcane. The core microbiome is mostly formed of bacterial and fungal groups whose functions in association with

plants have never explored. Unlike traditional approaches that investigate the beneficial effects of the microbiota by selecting microbial candidates solely based on taxonomical identity, we designed a synthetic bacterial community by choosing naturally dominant groups in the sugarcane microbiome, mostly poorly explored in terms of association with the sugarcane plant. Bacterial candidates were derived from a collection of microorganisms from over 5000 isolated communities from roots and stalks of sugarcane. Our results shows that the synthetic community robustly colonized plants, stimulated the root development, and tripled plant biomass. The community profile shows that each microbe in the synthetic community displays a pattern of colonization that does not correlate with phylogenetic relationship. We have sequenced the genome of the synthetic community members to evaluate potential genes and pathways conserved among the robust colonizers.

Community-based culture collection as a strategy for targeting beneficial plant-associated bacteria from the sugarcane microbiome

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Recent advances in microbiome studies have linked microbial diversity to biological functions associated with plant development and biotechnological processes. The soil-plant ecosystem harbors a vast microbial diversity that varies according to the soil type, plant species, organs, and genotypes, and most bacterial groups are difficult to cultivate. Exploring the microbial community members requires methods for isolation, annotation, and cross-referencing with community assemblage data to target organisms of interest such as beneficial plant-associated (BPA) microorganisms. However, microbial isolation traditionally requires several rounds of picking and streaking to obtain pure colonies. These methods are time-consuming and costly, and may result in losses of relevant biological information. As an alternative method for microbiome assessment, we recently introduced the concept of community-based culture collections (CBC). This approach was used for isolating, identifying, and investigating novel BPA bacteria from the sugarcane microbiome. Colonies from primary platings of the sugarcane root and stalk microbiota were stored regardless of whether they were formed by single or multiple microorganisms. A multiplex amplicon sequencing strategy of full-length microbial 16S rRNA genes was developed using the PacBio platform. The cross-referencing of the CBC (culture-dependent approach) with the sugarcane microbiome profile (culture-independent approach) revealed that the CBC recovered 399 unique bacteria representing 15.9% of the rhizosphere core microbiome and up to 65.3% of the endophytic core microbiomes of the sugarcane stalks. A synthetic community comprised of highly abundant bacteria from the root and stalk sugarcane core microbiomes was inoculated in maize and stimulated root growth and increased plant biomass by 3.4-fold. The natural microbiota profile was dramatically changed in inoculated plants both in diversity and abundance. The bacteria of synthetic inoculum displaced the natural microbiota and robustly colonized maize plants, summing up to 53.9% of the total microbial abundance in roots. The CBC method can be used to recover larger fractions of microbiota from any environment preserving their putative interactions, as it overcomes significant limitations of current microbiome research related to microbiota culture collection. Furthermore, the concomitant use of culture-dependent and culture-independent techniques allowed targeting highly abundant and beneficial bacterial groups from the sugarcane microbiome that have yet been poorly explored.

Metabolomics-guided isolation of significant biological plant, soil, and microbial secondary metabolites

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One of the grand challenges facing the metabolomics community is the identification of unknown metabolites, with unknown secondary metabolites contributing the most to this challenge. The large chemical heterogeneity of the metabolome has made identification and annotation of biologically significant metabolites one of the most important bottlenecks in untargeted metabolomics analyses. The mass spectrometry-based approaches used for metabolomics and lipidomics analyses are capable of detecting thousands of molecules in a single run, but comprehensive annotation of the associated metabolites is limited to spectral reference library matching and/or direct comparison to analytical standards. Current tandem mass spectral libraries are small compared to the large number of metabolites found in the biosphere. In cases where metabolites of interest can be matched to reference compounds, MS can give unequivocal identification, but for unambiguous identification of partially or completely unknown molecules, NMR spectroscopy is indispensable. In the diverse applications environment within which the Environmental Molecular Sciences Laboratory operates, with a multitude of different organisms and biological systems under investigation via User Projects, this bottleneck is a common problem and thus leaves a significant part of the interpretation of the metabolomics results ambiguous. By making use of complementary spectral data from both MS and NMR, we are reducing the time for structural elucidation of metabolites by incorporating candidate rejection and substructural conformation. In this talk I will describe the capabilities of a new pipeline, Metabolite Identification and Characterization Pipeline (MICP), currently being developed at EMSL to boost the identification of unknown metabolites and I will present selected applications to fungal and soil studies where we developed a specific directed fractionation and isolation roadmap which was used to purify, isolate, identify, and characterize the fungal and soil unknown metabolites that are of biological importance. The newly identified metabolites will be added to our ever-growing Pacific Northwest National Laboratory metabolite database, which serves as a repository for future validated metabolomics data. The novel metabolites identified by our MICP will be used to improve our understanding of the roles that biotic and abiotic transformations have on plants and soil microorganisms with environmental changes.

Evolution and diversity of a biosynthetic gene cluster for production of a vinylglyine

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The biological herbicide and antibiotic 4-formylaminooxyvinylglycine (FVG) was originally identified in strains of *Pseudomonas fluorescens* isolated from the rhizosphere of wheat and other grasses. Biosynthetic enzymes, regulatory factors, and transporters essential for FVG production and accumulation are encoded by the gvg biosynthetic gene cluster. In order to understand more about the diversity of FVG production, we investigated the evolution of the gvg cluster. We sequenced the genomes of multiple FVG-producers and combined these data with mining of sequence databases. We

identified >30 isolates with gvg clusters, including *P. fluorescens*, *P. syringae*, and several non-pseudomonads. By observing the pattern of distribution of the gvg cluster, examining its genetic context in different strains, and comparing gene and species trees, we concluded that the gvg cluster has been inserted multiple times in different lineages through horizontal gene transfer. The fate of gvg clusters after insertion reveals examples of gene loss and gene decay but little rearrangement. The frequency in which the gvg cluster appears in unrelated strains suggests a useful function for FVG for diverse bacteria living in a variety of environmental habitats.

Changes in root exudation and microbiome recruitment by maize in response to phosphate limitation

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Beneficial microbial communities are important for supporting plant growth and productivity, particularly under non-ideal nutrient conditions. Modulation of root exudate composition is one way in which plants can influence their rhizosphere microbial communities and potentially recruit beneficial microbes in response to different growth conditions. Domestication and breeding for high input agricultural production may have affected the ability of maize to adapt to nutrient-limited conditions. Here we present an investigation into the response of maize plants to phosphate limitation in terms of exuded metabolites, rhizosphere microbiome recruitment, and plant growth. We first performed an experiment with a single modern maize hybrid grown with and without available phosphate (provided as 85 mg/L KH2PO4). Phosphate limitation resulted in significant differences in exuded metabolites, with approximately 10% of detected metabolites showing differential exudation between the two growth conditions. Phosphate limitation also resulted in a shift in biomass allocation from shoot to root production, with roots comprising 36% and 58% of total biomass for growth with and without phosphate, respectively. We then performed an additional experiment with an evolutionary panel of four maize accessions grown under three phosphate conditions (low phosphate [5 mg/L KH2PO4], high phosphate [85 mg/L KH2PO4], and insoluble phosphate [1.5 g FePO4.2H2O per 1.5 L pot]). The panel included one teosinte (wild relative), one ancient landrace, one inbred parent of modern maize hybrids, and one modern maize hybrid. Plants were grown in sterilized sand and inoculated with a microbial community derived from low phosphorus (13 ppm P_i) soil from unfertilized wheat plots at Russell Ranch in Davis, California. Growth with low phosphate again resulted in more root biomass compared with high phosphate and insoluble phosphate (51% vs. 43% and 44% of total biomass, respectively). The total phosphorus content of plant leaves indicated that for all four accessions, plants grown with insoluble phosphate were able to uptake more phosphate than the low phosphate condition, but less phosphate than the high phosphate condition. Overall, root exudates from plants grown with low phosphate had lower levels of total organic carbon (12 ppm TOC/g root dry weight) compared with plants grown with high phosphate or insoluble phosphate (16 and 15 ppm TOC/g root dry weight, respectively). This trend was apparent in the teosinte, landrace, and inbred accessions, but was different for the modern hybrid, for which the insoluble-phosphate growth condition produced the lowest average TOC exudation. Initial plate assays for microbial phosphate solubilization indicate differences in microbiome recruitment between different accessions, with the teosinte being the most effective at consistently recruiting phosphate solubilizing microorganisms. Further analysis of root exudate metabolites and microbial community composition from this experiment are ongoing.

Microbial variation among high and low methane emitting rice cultivars

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Rice cultivation accounts for more than 15% of the global anthropogenic emissions of methane, a potent greenhouse gas, due to methanogenic archaea in the soil that are supported by rice root exudates. The rate of methane emission in rice has been found to be dependent on both genotype and location. In this study, a high methane emitting cultivar (Sabine), and low emitting cultivar (CLXL745) were grown in a field site in Arkansas, and the microbiome of the soil directly surrounding the root (rhizosphere) and the interior of the root (endosphere) were sampled throughout the growing season. Surprisingly, the relative abundance of methanogenic archaea was higher in the late season measurements, after the period of peak methane emissions. There was a statistically significant increase in the relative abundance of methanogens associated with the high emission cultivar Sabine relative to the low emission cultivar CLXL745 at this stage. Future experiments will examine the role of physiology, structure, and root exudates in driving the cultivar differences in methane emission.

Use of LC-MS/MS in the characterization of protein bioactives from *Bacillus* spores

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Bayer Biologics is working to deliver integrated solutions for farmers, including biological products, for management of plant pests and diseases as well as enhancing crop vigor and yield potential. Research at Bayer Biologics focuses on screening a large microbial strain collection for new bioactives. My research focuses on the characterization of *Bacillus* spore proteins that are of interest as bioactives. Proteins are first isolated from spores and LC-MS/MS methods for identification and quantitation are used in protein characterization. Successful recovery of spore proteins is achieved using a trichloroacetic acid extraction protocol. Proteins are then trypsin-digested and analyzed on an AB Sciex TripleTOF 4600 mass spectrometer for protein identification or an AB Sciex QTrap 4000 mass spectrometer for protein quantitation. These analyses allow for the precise and specific tracking of unmodified spore proteins and are essential in the characterization of novel protein bioactives.

Strigolactone impacts on soybean rhizosphere microbial community assembly

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Recent research increasingly suggests an intimate and mutualistic interaction between plants and their associated microbiomes. The rhizosphere, the narrow region between plant and soil, is the most dynamic and active interface, with intensive communication between a plant and its microbiome. In this region, a general enrichment of the microbial community is triggered by plant exudates, which is followed by host-specific differentiation of microbiota that thrive on the rhizoplane and in the

endosphere. A growing body of studies suggests root exudates, especially secondary metabolites, are important compounds mediating plant and microbe communication. Strigolactones are a novel class of carotenoid-derived phytohormones that control many aspects of root and shoot development. In terms of rhizosphere communication, strigolactones have been demonstrated to be involved in germination of parasitic plant seeds, induction of hyphal branching of arbuscular mycorrhizal fungi, and promotion of nodulation. We postulate strigolactone could simultaneously influence the rhizosphere microbial community recruitment directly by acting as a signal molecule after being exuded into soil or indirectly through regulation of root morphological architecture. To explore the impact of strigolactones on soybean rhizosphere microbiome recruitment, three highly expressed genes (D14, MAXI, and MAXII) that participate in strigolactone biosynthesis and downstream perception were identified. Both overexpression and RNAi silence constructs of Glycine max Williams 82 will be generated using a transgenic hairy root system, and confirmed based on a green fluorescent protein (GFP) reporter. After screening, transgenic lines and empty vector control lines will be grown in the greenhouse. At flowering stage, rhizosphere soil will be harvested and used for DNA extraction. 16S ribosome RNA amplicon sequencing targeted at the V3_V4 region will be used to characterize the rhizosphere microbial community. The results of this experiment will elucidate whether increased or decreased strigolactone production impacts rhizosphere microbiome structure and provide initial evidence on which bacteria are most actively responding to this particular root exudate.

Identification of unknown secondary metabolites by hybrid NMR/MS approach: application to studying the flowering time in *Arabidopsis thaliana*

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Despite recent progress in metabolomics, a large number of secondary metabolites in soil, microbial, and plant systems are still unknown, i.e., they are not identified or available in any metabolomics database. These unknown secondary metabolites must be structurally characterized and identified in an efficient and accurate manner. With the increased resolution of mass spectrometers (MS), the determination of accurate masses of individual known and unknown secondary metabolites is becoming increasingly routine. This information allows one to deduce the molecular formula of metabolites that underlies each peak in the complex mixture mass spectrum. However, knowledge of molecular formulas does not easily translate to the identification of individual secondary metabolites because of the large degeneracy of the structural space belonging to a given molecular formula. With increasing mass, this degeneracy increases exponentially. This makes identifying unknown secondary metabolites very challenging by MS alone. On the other hand, NMR spectra can differentiate significantly between isomers. Therefore, integration of NMR information with MS opens up new opportunities to address the structure elucidation challenge. Recently, we proposed a hybrid NMR/MS metabolite identification strategy. This strategy first identifies the chemical formulas of the mixture components from accurate masses by MS and then generates all feasible structures that are consistent with these chemical formulas. Next, NMR spectra of each member of the feasible structures are predicted and compared with the experimental NMR spectra of the same mixture to identify the molecular structures that best match the information obtained from both the MS and NMR techniques. We demonstrate the approach on the identification of unknown secondary metabolites in *Arabidopsis thaliana* in the context of determining the metabolic underpinnings of shifts in flowering time in response to atmospheric CO_2 rise,

which will have major implications on a global scale, since CO_2 is expected to disrupt carbon cycling within ecosystems. The hybrid NMR/MS approach has been routinely used at Environmental Molecular Sciences Laboratory (EMSL) for identification of unknown soil, microbial, and plant secondary metabolites. EMSL's capabilities are available to researchers worldwide through a peer-reviewed proposal process, typically at no cost. For more information, visit https://www.emsl.pnl.gov/emslweb/.

Examining the secondary metabolite activity in the lichen community

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Lichens are some of the longest-living organisms known, despite their slow growth, and they very rarely appear to die of disease. The lichenized fungus establishes the main lichen thallus in association with an alga or cyanobacterium. This scaffold becomes a niche for a variety of other filamentous ascomycetes and basidiomycetes, yeasts, bacteria, and, occasionally, insects. Lichens are known to produce a plethora of unique secondary compounds. Their longevity and robustness, despite a close association with diverse microbes, provides an interesting study system to view the role of secondary metabolites in managing a microbial community. We isolated extracts from 72 lichen species and tested for their effects on sporulation, hyphal growth, and secondary metabolite production in fungal cultures. The structure of these compounds is under investigation, as is the identification of the microbial source. Interestingly, the most common activity by far among the lichen extracts had the effect of arresting secondary metabolite production. This finding suggests that lichens attenuate negative interactions with the incumbent fungi through their ability to regulate secondary metabolism.

Changes in the root metabolome of citrus plants infected with *Candidatus Liberibacter asiaticus*

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Huanglongbing (HLB) is a severe, incurable disease affecting citrus plants that is believed to be caused by the bacterium *Candidatus Liberibacter asiaticus* (CLas). Citrus roots may be preferentially colonized by CLas prior to other tissues; however, little is known about the impact of CLas infection on plant metabolism in the root system. One-year-old greenhouse-grown Lisbon Lemon and Washington Navel orange citrus trees were graft-inoculated with citrus budwood infected with CLas. Roots were obtained from healthy (n = 17) and infected (n = 17) trees 46 weeks post-inoculation and analyzed via 1H NMR spectroscopy to identify and quantify water-soluble root metabolites. Mann-Whitney U testing and partial least squares discriminant analysis (PLS-DA) were used to determine significant differences in metabolite concentrations and to identify distinct metabolite patterns. Overall, several metabolites were significantly different in roots obtained from plants infected with CLas compared to healthy control plants and it was possible to determine infection status through differentiated metabolomics profiles. This study demonstrates that a discreet metabolome arises in the roots of citrus infected with CLas.

Assessing microbial community contribution to plant abiotic stress tolerance: a case study in serpentine soils

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Metal-contaminated and drought-prone soils have characteristics similar to those found in serpentine soil. Because of these similarities and adapted plant communities, serpentine is an excellent model system for studying plant and microbial adaptation to these stressors. Serpentine soils contain high concentrations of heavy metals, such as nickel, cadmium, copper, and lead, but low concentrations of calcium relative to magnesium and nitrogen. As a further challenge to plants, serpentine soils also have low water holding capacity, which means that many plants must adapt to drought stress as well. Using a model system of serpentine soils, we propose to define a core microbiome and metagenome associated with California native plant congeners within the genus *Linanthus* grown on serpentine and non-serpentine soil. We aim to determine the functional attributes of the plant core microbiome associated with each soil type and to understand how microbiome assembly is influenced by abiotic factors associated with serpentine soil. Under greenhouse and field conditions, using a reciprocal transplant design, I will transplant sterilely germinated *Linanthus* seedlings from serpentine and non-serpentine soil into each soil type. I will collect microbial DNA from the plant rhizosphere and endosphere at 1, 5, and 21 days post transplant (dpt). To determine biodiversity, all samples will have the 16S rRNA gene region and marker genes associated with stress tolerance sequenced. Additionally, whole shotgun sequencing will be conducted on the 21 dpt field sample to assess the taxonomic composition and diversity and determine the functional role of microbial communities in serpentine systems. There are a variety of outcomes to this experiment that will help both academic and industry researchers highlight microbes or genes for future research. Additionally, results will help researchers understand community assembly dynamics, which will aid in microbiome management for agricultural production and phytoremediation. Microbiome management is one way to help farmers optimize resource use to ensure the continued use of arable land.

Metabolomics to understand and detect *C. Liberibacter asiaticus* infection

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Metabolomics is an analytical method that comprehensively measures metabolites to provide a snapshot of the metabolic state of an organism. Metabolite composition changes in response to stress, including infection and disease. Metabolomic analysis of citrus can therefore be used as an indirect method to determine if a pathogen is present, since it measures the host response; this is in contrast to direct methods that require detecting the pathogen itself. Huanglongbing (HLB) is a devastating citrus disease caused by the bacterial pathogen C*andidatus Liberibacter asiaticus* (CLas). Early detection of CLas in trees is critical to control the spread of HLB. However, since CLas is not evenly distributed throughout the tissue of trees and can be present at very low levels, direct methods often fail to detect the pathogen. We have previously shown that metabolomics can detect metabolite changes in fruit from citrus infected with CLas regardless of the presence of visible symptoms. Here, we report on metabolomic analysis of citrus roots and leaves upon CLas exposure and citrus leaves exposed to the insect vector of CLas, the Asian citrus psyllid (ACP). Metabolite differences were observed between roots

from healthy and infected greenhouse trees. Infected field trees were also distinguished from healthy trees based on their metabolite pattern. Since ACPs are the main mode of CLas transmission in the field, the effects of ACP feeding in the absence of CLas were also studied. The changes due to CLas-free ACP feeding were different from changes measured during CLas infection. Together, these results suggest a role for metabolomics for improving detection of CLas.

Chemistry of the plant exudation and substrate utilization preferences of soil microorganisms underlying rhizosphere microbiome assembly in annual and perennial grasses

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Plant-soil-microbial interactions regulate the availability of nutrients and carbon (C) transformation in soil. Plants exude a diverse range of compounds into the soil surrounding their roots; these exudates are thought to attract and support microorganisms that may improve plant nutrient acquisition, drought tolerance, and resistance to pathogens. Additionally, plant-microbial interactions could define the future fate of root C, and specifically whether it is respired to the atmosphere or stabilized in soil. Here we used mass-spectrometry-based metabolomics to identify key metabolites that we suggest can be important players in bidirectional plant-microbe interactions in soil. These include exudation patterns of two annual and perennial grasses, metabolite exchange between plant and rhizosphere microorganisms, and potential chemical mechanisms of rhizosphere community assembly.

Taking a multi-scale approach including field, greenhouse, and highly controlled lab experiments, our goal is to determine the processes that underlie plant-soil-microbial relationships. We identified exudation patterns of the annual grass *Avena barbata* and linked changes in *Avena* exudate composition to substrate utilization preferences of bacterial isolates from the *Avena* rhizosphere. Similarly, we analyzed successional changes in exudation profiles of the perennial grass switchgrass. Currently we are studying a large collection of switchgrass isolates and the exudation profile of switchgrass to define metabolic mechanisms underlying switchgrass rhizosphere assembly, including possible beneficial effects of rhizosphere bacteria on plant nutrition and drought tolerance.

In our study, we propose that long-term relationships between plants and rhizosphere organisms are mediated by dynamic root exudation and microbial substrate selectivity.

Broad-host-range expression reveals native and host regulatory elements influencing heterologous antibiotic production in Gram-negative bacteria

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Heterologous expression has become a powerful tool for studying microbial biosynthetic gene clusters (BGCs). Although Gram-positive heterologous hosts such as *Streptomyces* have been developed and optimized to support diverse secondary metabolic reactions, there has been comparatively less work on Gram-negative hosts, some of which grow faster and are easier to work with. Here, we extend the transformation-associated recombination cloning and heterologous expression platform for microbial BGCs to include Gram-negative hosts. Using a broad-host-range expression platform, we test the implicit assumption that biosynthetic pathways are more successfully expressed in more closely related heterologous hosts. Cloning and expression of the violacein BGC from *Pseudoalteromonas luteoviolacea* 2ta16 revealed robust production in two proteobacterial hosts, *Pseudomonas putida* KT2440 and *Agrobacterium tumefaciens* LBA4404, but very little production of the antibiotic in various laboratory strains of *Escherichia coli*, despite their closer phylogenetic relationship. We identified a non-clustered LuxR-type quorum sensing receptor from *P. luteoviolacea* 2ta16, PviR, that increases pathway transcription and violacein production in *E. coli* by ~60-fold independently of acyl-homoserine lactone autoinducers. Although *E. coli* harbors the most similar homolog of PviR identified from all hosts tested, overexpression of various *E. coli* transcription factors did not result in a statistically significant increase in violacein production, while overexpression of two *A. tumefaciens* PviR homologs significantly increased production. Thus, this work not only introduces a new genetic platform for heterologous expression of microbial BGCs, but it also challenges the assumption that host phylogeny is an accurate predictor of host compatibility. We argue for the use of a diverse set of heterologous hosts, which may also provide insights into biosynthetic mechanism and biological function.

Tomato Rhiz'OMICS

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The rhizosphere microbial community affects the host physiology, and vice versa. However, the intricate processes, i.e., environmental and host molecular factors combined, that shape the microbiome of the rhizosphere are still greatly unknown. Here, two approaches were used in order to correlate the two major components of the tomato rhizosphere: the tomato root and the soil bacterial diversity. First, we assessed the genetic factors that affect the rhizosphere bacterial composition in a set of 76 introgression lines (ILs) of *Solanum lycopersicum* carrying only a single chromosome segment from the wild species *Solanum pennellii* (LA0716). For that tomato population, a core microbiome was defined based on 16S rRNA amplicon sequencing, consisting of 154 abundant OTUs that changed quantitatively across most ILs. Testing of these OTUs' abundances for cosegregation, 4 host quantitative trait loci (QTL) show significant linkage with relative abundances of specific bacterial OTU. These QTL affect bacterial OTU by

increasing its abundance, either controlling an individual OTU or various OTUs spanning a diverse taxonomic range. In addition, in order to understand how the root exudation influences the tomato rhizosphere microbiota, tomato roots were challenged with soil microbial communities established using a dilution-to-extinction approach. The metabolic patterns, analyzed by LC-MS and GC-MS, of tomato roots and exudates are tailored by soil microbial diversity and composition. The metabolic changes of host plants in response to soil microbial diversity might also affect the rhizosphere microbial ecology.

Functional genomics-guided discovery of cryptic metabolites involved in pathogenic plant-microbe interactions

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Plant pathogenic fungi produce an arsenal of hydrolytic enzymes, proteinaceous effectors, and secondary metabolites to facilitate their infection of hosts. Compared to our understanding of proteinaceous effectors, our understanding of the roles of secondary metabolites in pathogenic plant-microbe interactions is still poor. Our understanding is partially hindered by conditional expression of biosynthetic gene clusters (BGCs) and lack of efficient tools for translating BGCs to metabolites. Guided by established biosynthetic logics, we used a combination of functional genomics, synthetic biology, and chemical ecology tools to uncover these cryptic metabolites and their functions. In particular, the BGCs were prioritized based on gene expression data of pathogens during infection of plant hosts and heterologous hosts (*A. nidulans* or *S. cerevisiae*) were used for reconstruction of selected BGCs. Some examples from our group's recent studies are presented here, including the discovery of mellein, elsinochrome, and cytochalasin (in progress) pathways in the wheat pathogen *Parastagonospora nodorum*, and their possible roles in plant-microbe interactions.

Chemical diversity generation using RiPP pathways

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RiPPs (ribosomally synthesized and post-translationally modified peptides) are a ubiquitous family of natural products derived from ribosomally synthesized peptides. As such, RiPP biosynthetic pathways have been used in the synthesis of large libraries of derivatives. Here, I will describe how cyanobactin RiPP pathways can be exploited to generate designed derivatives through rational engineering. These derivatives can be synthesized in vitro or in cells for synthetic biology applications.

Impact of HLB on the metallome and metabolome of Citrus 1H

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NMR-based metabolomics has recently been used to study the citrus host response to infection by *Candidatus Liberibacter asiaticus* (CLas), the bacterium associated with the destructive citrus disease Huanglongbing (HLB; syn. citrus greening disease). Metabolomics is a promising method for the early detection of CLas in plants. Metal ions, including zinc, magnesium, potassium, and iron, are rich in citrus plant tissues and some of these can bind to certain metabolites, broadening their signals and making quantification difficult via 1H NMR. Here we longitudinally sampled and analyzed leaves from navel orange trees graft-inoculated with CLas with inductively coupled plasma-mass spectroscopy (ICP-MS). We observed changes in Mg^{2+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, and Zn^{2+} concentration with infection over time, with the greatest difference between control and $CLas^{+}$ plants occurring with severe HLB symptoms. Additionally, to characterize the impact of varying concentrations of metal ions on the metabolite NMR spectrum, these same leaves were analyzed by ICP-MS and 1H NMR. High concentrations of Mg^{2+}, Ca^{2+}, and Fe^{2+} broadened some metabolites, making identification and quantification more difficult. We therefore investigated if the addition of ethylenediaminetetraacetic acid (EDTA), a common chelating agent, could improve the quality of the NMR spectrum and help quantify the metal concentration in citrus leaf samples. Our observations show that the addition of EDTA removes the impact these ions have on identifying and quantifying metabolite NMR resonances, and allows the concentrations of Mg^{2+}, Ca^{2+}, and Fe^{2+} to be measured without the need for using ICP-MS.

Investigating a genome-to-phenotype pipeline for model grasses

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Although the effects of elevated CO_2 on plant growth, physiology, and metabolism has been investigated thoroughly, the underlying integrated organismal, cellular, and molecular mechanisms of these changes are less understood. Also, two major plant photosynthesis types, C_3 and C_4 plants, are often each affected in different ways by all global change parameters. In this study, accessions of *Brachypodium distachyon* Bd21 (C_3 model grass) and *Setaria viridis* A10.1 (C_4 model grass) were grown under current and elevated CO_2 levels in growth chambers. Detailed growth-stage-based phenotypic analysis revealed different above and below-ground morphological and physiological responses of C_3 and C_4 grasses to the enhanced CO_2 levels condition. Based on our preliminary results and screening values of total biomass, water use efficiency (WUE), root to shoot ratio, root system architecture (RSA) parameters, and net assimilation rates, we postulated a three-phase physiological mechanism (RootPlus, BiomassPlus, and YieldPlus phases) for grass growth under the elevated CO_2 condition. To characterize additional physiological changes, we used a novel non-invasive, image-based dynamic environmental photosynthesis imager (DEPI) chamber capable of revealing new transient or environment-specific phenotypes. The generated images and revealed photosynthesis-specific parameters including

photosystem II (PSII) quantum efficiency (ΦII), light-driven linear electron flow (LEF), dissipative non-photochemical quenching (NPQ) of absorbed light energy, and its components (qE and qI responses) will be presented. Moreover, these comprehensive sets of morphological and process-based observations are currently in use to develop, test, and calibrate biophysical whole plant models and in particular to simulate leaf-level photosynthesis at various developmental stages of C_3 and C_4 using the model BioCro. Also, the whole plant phenotypic observations will be complemented with the stomatal density, stomatal area, epidermal cell area, and omics data to further link the observed phenotypic traits at the organismal level to tissue and molecular levels.

Establishing a genome-to-phenotype pipeline for model grasses

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The integrated Plant-Atmosphere-Soil Systems (iPASS) Initiative is a Pacific Northwest National Laboratory (PNNL) Laboratory-Directed Research and Development (LDRD) project aimed at deciphering fundamental principles that govern the plant ecosystem, from plant genotype through multiple scales to ecosystem traits and responses. A key to obtaining mechanistic understanding of plant responses to environmental perturbations is to link phenotypic traits at organismal and ecosystem scales to molecular-scale phenotypes under a variety of conditions. Plants and associated microbiota emit a diverse array of volatile organic compounds (VOCs) into the atmosphere. VOCs are a vital element of a plant's phenotype and are a central character in the plant ecosystem due to their role as ecological signals and their influence on atmospheric chemistry. VOCs are very diverse and consist of various organic classes such as isoprenes, terpenes, fatty acid derivatives, alcohols, alkanes, alkenes, esters, and acids. VOCs actively participate in plant growth and protection against biotic and abiotic stresses, and, therefore, the intensity and composition of VOC emissions are strongly dependent on environmental conditions. Many studies have shown production of VOCs is strongly regulated by genetics, making VOC emissions highly species specific. Also several studies have shown variability among VOC emissions with genotypes of cultivated and wild plants. In this study, accessions of *Brachypodium distachyon* Bd21 (C_3 model grass) and *Setaria viridis* A10.1 (C_4 model grass) were grown under current and elevated CO_2 levels in growth chambers. Detailed growth-stage-based phenotypic analysis revealed different above- and below-ground morphological and physiological responses in C_3 and C_4 grasses to enhanced CO_2 levels condition. Based on our preliminary results and by screening values of total biomass, water use efficiency (WUE), root:ratio allometry, root system architecture (RSA) parameters, and net carbon assimilation rates, we postulated a three-phase physiological mechanism (RootPlus, BiomassPlus, and YieldPlus phases) for grass growth under the elevated CO_2 condition. To characterize additional physiological changes, we used a novel non-invasive, image-based dynamic environmental photosynthesis imager (DEPI) chamber capable of revealing new transient or environment-specific phenotypes. Moreover, these comprehensive sets of morphological and process-based observations are currently in use to develop, test, and calibrate biophysical whole-plant models and in particular to simulate leaf-level photosynthesis at various developmental stages of C_3 and C_4 grasses using the BioCro model. Also, the whole-plant phenotypic observations will be complemented with data for stomatal density, stomatal area, epidermal cell area, and omics to further link the observed phenotypic traits at

the organismal level to tissue and molecular levels. The preliminary data of identified VOC metabolites emitted by *Brachypodium* plants measured by dynamic vegetation enclosure will be also presented.

Characterization of the microviridin biosynthetic gene cluster from *Chryseobacterium*

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The rapidity and ease of genome sequencing has enabled bioinformatics-guided natural product discovery and characterization. The Plant-Microbe Interfaces project at Oak Ridge National Laboratory aims to identify natural products that influence interactions in the *Populus* Many microbial genomes encode the machinery to produce diverse bioactive molecules that can be used in healthcare, agriculture and food. Its natural modularity makes this machinery a particularly attractive target for synthetic biology. The re-engineering of the biosynthetic capacity of microbes requires the development of a wide range of experimental and computational tools. These range from orthogonal transcriptional control circuits and bacterial microcompartments, to computational tools for the detection and analysis of secondary metabolite biosynthesis gene clusters that enrich our library of parts and building blocks for pathway engineering, and high-resolution mass spectrometry analysis for the debugging of the engineered systems.
In this talk I will explore the possibilities created by the application of the design/build/test/learn cycle of synthetic biology to the engineering of microbial metabolism for the production of high-value chemicals, as implemented in the high-throughput platform of the BBSRC/EPSRC-funded Manchester Synthetic Biology Research Centre, SYNBIOCHEM. I will also discuss the growing toolbox of techniques and approaches, illustrated with concrete application case studies. microbiome, and to determine how microbiome structure affects the health of the plant host. In collaboration with the Joint Genome Institute, 254 bacterial isolates from the rhizosphere of *Populus trichocarpa* and *Populus deltoides* have been sequenced; this has enabled a bioinformatics-driven characterization of the biosynthetic potential of the *Populus* microbiome. The biosynthetic gene cluster responsible for microviridin biosynthesis was found within nearly 75% of the sequenced *Chryseobacterium* strains, but is absent in all other sequenced organisms. Here, we characterize the secondary metabolite clusters within the 18 *Chryseobacterium* isolates from the *Populus* rhizosphere and draw comparisons to known microviridins. Initial MS-based screening demonstrated the production of the predicted cyclic depsipeptides in vitro. Antagonism was observed in spot-on-lawn assays, which may in part be the result of microviridin production.

Survey of the biosynthetic potential of the *Populus* microbiome

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Thousands of bacterial species have been isolated from plant root microbiomes, making the study of microbe-microbe and microbe-plant interactions a challenge. *Bacilli* and *Streptomyces* are ubiquitous in soil and make up a large percentage of the microbial community in the rhizosphere. These bacteria have been studied for use as biocontrol agents due to their genetic potential to produce complex natural products with antibiotic activity; however, the role of these compounds in microbe-microbe and plant-microbe interactions remains largely unknown. Rhizosphere isolates from *Populus* were analyzed for biosynthetic gene clusters and the production of bioinformatically predicted natural products, and

Streptomyces strains were selected for further analysis based on an abundance of predicted clusters. Screening of bacterial extracts showed a number of bioinformatically predicted compounds were produced under laboratory conditions, including one lasso peptide, siamycin I. Using chemical imaging, production of the lasso peptides was observed in *Plantae*. Significant plant growth effects were observed when plants were treated with the bacterial isolates, but constructed bacterial communities on plant roots were not dramatically altered with the addition of these strains, suggesting a nuanced and multifaceted role for these natural products in the rhizosphere.

Engineering the biocatalytic selectivity of iridoid production in *Saccharomyces cerevisiae*

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Monoterpene indole alkaloids (MIAs) represent a structurally diverse, medicinally essential class of plant-derived secondary metabolites that have recently been produced in *Saccharomyces cerevisiae*. However, the irreversible reduction of α,β-unsaturated carbonyl pathway intermediates results in a non-recoverable loss of carbon, which has a strong negative impact on metabolic flux. In this study, we sought to characterize and engineer the determinants of biocatalytic selectivity that control flux towards the iridoid scaffold from which MIAs are derived. In vitro reconstitution of previously uncharacterized shunt pathways enabled the identification of two distinct routes to a reduced shunt product including endogenous “ene”-reduction and non-productive reduction by iridoid synthase. To this end, deletion of five genes involved in α,β-unsaturated carbonyl metabolism resulted in a five-fold increase in biocatalytic selectivity of the desired nepetalactol over reduced shunt product. We anticipate that our engineering strategies will play an important role in the development of *S. cerevisiae* for sustainable production of iridoids and MIAs.

Genome mining of fungal natural products

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Natural products are major sources for drug discovery; however, because of increasing drug resistance to existing molecules and a dwindling pipeline of new drug leads, our need to uncover novel natural products is becoming ever more critical. Here we proposed and applied a new strategy, target-guided mining, to discover new natural products with desired bioactivities. Glyceraldehyde-3-phosphate dehydrogenase, known as a target for anti-anaerobic bacteria and antimalarial and anti-cancer agents, was used as an input to carry out target-guided genome mining, and we successfully discovered and verified a known inhibitor of this target as an output. This example shows the feasibility of target-guided genome mining of bioactive natural products.