2017 Bay Area Plant Microbe Interaction Symposium

Speaker Abstracts

Mapping the pathways of root carbon flow into and through soil microbial food webs
Mary Firestone (University of California, Berkeley), Steve Blazewicz, Javier Ceja-Navarro, Don Herman, Anne Kakouridis, Rachel Neurath, Erin Nuccio, Shengjing Shi, Evan Starr, Jill Banfield, Eoin Brodie, and Jennifer Pett-Ridge

The flow of carbon (C) from roots into soil is controlled by a complex array of interactions. We are addressing how multi-trophic interactions mediate the flow and fate of root C into soil. We use stable isotope probing (SIP) and genome resolved metagenomics to identify and characterize the participants in root-C-based food webs and understand the ecological interactions in the rhizosphere that ultimately control the fate of C entering soil. We track labeled C moving from roots into root-exudate and debris consumers and through the portions of the soil food web supported by these primary consumers. Genome resolved metagenomic analyses of the SIP-isolated DNA then allows us to better understand the functional characteristics of rhizosphere C-transformers and illuminate the carbon basis of these interdomain interactions in soil.

Roots stimulate expression of decomposition transcripts in the soil microbiome
Erin Nuccio (Lawrence Livermore National Laboratory), Ulas Karaoz, Evan Starr, Eoin Brodie, Jizhong Zhou, Susannah Tringe, Rex Malmstrom, Tanja Woyke, Mary Firestone, and Jennifer Pett-Ridge

The soil surrounding roots, the rhizosphere, has long been recognized as a hotspot of belowground carbon cycling. The rhizosphere environment alters the microbial breakdown of plant tissues and root litter, and can accelerate the decomposition of detrital plant biomass, a process commonly termed “priming.” However, the molecular mechanisms underlying rhizosphere C cycling are poorly understood, and the carbohydrate and lignolytic gene transcripts mediating the decomposition of root litter in soil are largely unidentified. We hypothesized that root exudates stimulate the expression of enzymes that are involved in decomposition of macromolecular C compounds. To assess how enzyme-mediated decomposition differs in the rhizosphere relative to the surrounding bulk soil, we analyzed community gene expression (metatranscriptomes) and single cell genomes of rhizosphere and bulk soil associated with wild oat (*Avena fatua*) over time (3, 6, 12, and 22 days). To isolate roots of a defined age in a mature plant, we used microcosms with a transparent experimental sidecar to track roots as they grew. In total, we sequenced 48 soil metatranscriptomes, which contained approximately 40 million high-quality, paired-end mRNA reads per library. We found that transcription of genes involved in decomposition were stimulated in the rhizosphere at all time points, where 96% of the Carbohydrate Active Enzyme (CAZyme) transcripts were significantly elevated in the rhizosphere. These transcripts included cellulose and hemicellulose degradation genes, including beta-glucanases, beta-glucosidases, and xylanases. Additionally, gene transcripts that are potentially involved in decomposing microbial necromass were also elevated in the rhizosphere (e.g., chitinases, lytic murein transglycosylases, peptidoglycan/xylan/chitin deacetylases). The expression of decomposition genes was dynamic and changed as the root grew, and indicates that decomposition is undertaken by a series of different organisms as the root grows. This suggests that rhizosphere priming of plant-derived materials is influenced by the stage of root growth, and that the organisms accomplishing decomposition may be more limited than previously expected based on genomic or metagenomic surveys. This work identifies potential molecular mechanisms that underpin priming in rhizosphere soil.

Timing of microbial mutualist arrival has a greater effect on seedling growth than interspecific competition

Kabir Peay (Stanford University)

While plant-microbe interactions play a critical role in structuring communities, the temporal dimensions of these interactions are generally ignored or considered implicitly in their study. Experimental studies of plant-microbe interactions are generally carried out in greenhouses to adequately control the microbial community, but most greenhouse experiments examine only the effects of microbial presence or absence. For naturally recruiting plants, though, symbiont availability more likely varies as a function of time due to ongoing dispersal of the microbial partners. While presence-absence studies capture the extremes of partner arrival time, they may not reflect the most common interaction scenarios. I conducted a growth chamber experiment to test the relative importance of timing and biotic context on mutualistic interactions using an ectomycorrhizal fungus, its pine host, and the pines' major competitor. Over 9 months I varied the timing of ectomycorrhizal inoculation (Inoculation Time), presence of a competitor (Competition), and measured their effects on pine seedling growth. The experiment was conducted in live field soil from two sites (Soil Origin) differing in their proximity to adult pines. Seedlings were harvested at three time points (Harvest Time) to capture key phases in the temporal dynamics of plant-microbial interactions. I found that ectomycorrhizal colonization varied substantially across treatments as evidenced by a significant three-way interaction between Soil Origin x Harvest Time x Inoculation Time ($F_{1,6} = 10.79$, $P < 0.001$). Colonization was uniformly high for seedlings growing in soils collected near pines, but in soils collected away from established pines seedling colonization was determined by the timing of ectomycorrhizal spore inoculation. The treatments also generated substantial variation in pine seedling biomass as evidenced by a significant three-way interaction for Soil Origin x Harvest Time x Inoculation Time ($F_{1,6} = 25.84$, $P < 0.001$). The overall pattern of biomass effects largely mirrored those for ectomycorrhizal colonization. Notably, for seedlings growing in soils collected away from established pines, biomass decreased as delay in ectomycorrhizal inoculation increased. Competition treatments had significant negative effects on pine seedling growth. However, the competition effect was much smaller than the effect of delayed mutualist arrival. These results have two important implications. First, the importance of spore arrival time suggests that plants may experience mutualist limitation more frequently than previously expected. Second, the magnitude of seedling responses to mycorrhizal fungi and competing plants show that mutualism is likely of equal or greater importance compared with interspecific competition during community assembly.

Genetic determinants of bacterial adaptation to plants

Asaf Levy (DOE Joint Genome Institute), Isai S. Gonzalez, Sur H. Paredes, Dale Pelletier, Sharon L. Doty, Susannah G. Tringe, Tanja Woyke, and Jeff Dangl

Plants intimately associate with an array of diverse bacteria. Plant-associated (PA) bacteria have evolved a gene set enabling adaptation to the plant environment. However, the identity and functions of these genes are poorly characterized. Here, we sequenced 484 genomes of bacterial isolates from the roots of *Brassicaceae*, poplar, and maize. We then performed a large-scale comparative genomics analysis encompassing 3837 bacterial genomes to identify PA and root-associated genes and operons. PA bacterial genomes are larger, and encode more carbohydrate metabolism functions and fewer mobile elements than related non-plant associated genomes. Novel PA proteins include members of a predicted pathogen-specific type VI effector family and a phage-like secretion system. We also identified 113 PA protein domains that are apparent mimics of plant domains, many of which are also shared with PA fungi and oomycetes. This work significantly expands the genome-based understanding of plant-microbe interactions and could open new avenues of efficient and sustainable agriculture through microbiome engineering.

Comparative genomics of lichen mycobionts

Alan Kuo (DOE Joint Genome Institute), Dave Culley, Olaf Mueller, Paul Dyer, Jon Magnuson, François Lutzoni, and Igor Grigoriev

Lichens are mutualistic symbioses usually between an alga (the photobiont) and a fungus (the mycobiont). The photobiont contributes fixed carbon to the partnership, while the mycobiont provides a physical structure that encloses the photobiont, roots the partners to a substrate, and acquires nutrients often of air-borne origin. Lichens are an ancient (possibly 600 Mya) and highly successful adaptation, covering 8% of terrestrial ecosystems, and comprising as much as 20% of fungal species diversity. The lichen lifestyle has evolved multiple times and occurs in at least 5 fungal classes. As part of an ongoing effort to represent the full phylogenetic and ecological diversity of fungi in the JGI's MycoCosm and the 1000 Fungal Genomes Project, we have partnered with members of the lichen research community to sequence, assemble, and annotate several lichen mycobionts and photobionts. Initial comparison of 5 publicly available mycobiont genomes from 3 distinct lichen-forming clades with their nearest non-lichenizing siblings reveal varied combinations of secondary metabolite synthase, transporter, and protease gains and losses. The diversity of gains and losses suggest that the independent evolutionary transitions to the lichen lifestyle may have involved different gene-level adaptations. We expect to expand on these findings by examining additional functional characters, and by sequencing more genomes, as well as importing more genomes from our partners and from the public domain.

Discovery, transfer, and characterization of novel phytase genes for utilization of recalcitrant phosphate

Christine Shulse (DOE Joint Genome Institute), Mansi Chovatia, Carolyn Agosto, G Wang, Y Lei, M Hamilton, Sam Deutsch, Yasuo Yoshikuni, Adam Deutschbauer, and Matt J Blow

High yield agricultural plant growth is currently dependent on costly and environmentally damaging phosphate fertilizers. One approach to alleviating this dependency is to develop bacterial strains that convert existing phosphorus sources in the soil to soluble forms available for plant uptake. Among potential sources, phytic acid is an abundant organic phosphorus-containing compound accounting for up to 40% of soil phosphate in some agricultural soils. We therefore aimed to engineer plant-associated bacteria with the ability to hydrolyze phytic acid, and release plant-available phosphate. We first searched all available microbial genomes and environmental metagenomes in the Integrated Microbial Genomes database and selected 92 sequences that represent the diversity of phytase genes. Using JGI DNA synthesis program capabilities, we refactored these sequences for optimal expression in Proteobacteria, synthesized the genes and engineered them into the genomes of four known plant-associated bacteria. We next determined the ability of these engineered strains to solubilize phytic acid in liquid culture assays. While host strains have no or low native phytase activity, a total of 29 engineered strains were capable of high levels of phytic acid hydrolysis, with at least one representative for each of the host strains used. Finally, we tested 10 strains in plant assays and identified 4 strains from two hosts that confer a significant growth advantage on the model plant Arabidopsis thaliana Col-0 when phytate is the sole phosphate source. These data provides proof of principle that DNA synthesis approaches can be used to generate plant associated strains with novel capabilities benefitting plant growth, and are a first step in the development of alternative approaches to sustainable phosphorus use in agriculture.

At the Interface: Arabinogalactan Peptides and GIPC Sphingolipids are Required for Symbiosis in Medicago truncatula

William Moore (Joint Bioenergy Institute), Candace Chan, Toshiki Ishikawa, Emily Rennie, Jennifer Mortimer, Oge Nnadi, Paul Hussey, Nathan Hillson, and Henrik Scheller

In nature plants interact with beneficial root associated bacteria and fungi that aid in nutrient uptake and promote plant growth and resilience. Here we use *Medicago truncatula* as a model plant to investigate the role of the plant cell surface in beneficial plant-microbe interactions with arbuscular mycorrhizal fungi (AMF) and nitrogen-fixing Sinorhizobium. The plant cell surface plays a key role in symbiosis by forming a specialized plant-microbial interface composed of plant-derived membranes, cell wall polysaccharides, and protein complexes, through which nutrients and information are bi-directionally exchanged. It has previously been reported in the literature through the use of glycan-directed monoclonal antibodies that arabinogalactan proteins (AGPs) aggregate at the symbiotic interface in a variety of plant-microbe mutualisms, however, the identity of the genes encoding these glycoproteins has remained unknown. Here we report the discovery of several AGP encoding genes in *M. truncatula* that are specifically expressed during symbiosis with either AMF or Sinorhizobium meliloti. Functional studies using RNAi-mediated knockdown of specific AGPs result in drastic symbiosis defective phenotypes. In parallel we have identified a glycosyltransferase enzyme that is also required for AM symbiosis and root nodulation. Biochemical evidence from Arabidopsis indicates that this GT is a HexN(Ac) transferase involved in glycosyl inositol phosphorylceramide sphingolipid biosynthesis. RNAi mediated knockdown of this GT specifically impairs the development of symbiotic plant-derived membranes that establish the plant-microbial interface. Our data indicate that Arabinogalactan peptides and GIPC sphingolipids are necessary for establishing and maintaining symbiosis in M. truncatula.

Individual-based ecology of the phyllosphere

Johan Leveau (University of California, Davis)

The term phyllosphere refers to leaf surfaces as a habitat for microorganisms. My lab is interested in the assembly, size, composition, and function of leaf-associated microbiota, not only as they pertain to food security and food safety, but also in the context of using the phyllosphere as a model habitat for understanding microbial ecology at the scale that matters most to microbes. We take an individual-based approach to leaf colonization and use experimental tools (GFP-based bioreporters, biomimetic leaf surfaces) that allow us to interrogate microbes for their individual experience of the leaf surface and get a qualitative and quantitative appreciation for the factors that drive and constrain the ability of microbes to attach, detach, disperse, multiply, sense, and communicate along the leaf surface.

Insect herbivory re-shapes the distribution and abundance of bacteria in the phyllosphere
Parris Humphrey (Harvard University) and Noah K. Whiteman

Native plants face a high risk of attack by insect herbivores. Herbivory can induce several plant defenses that alter plant phenotypes relevant to microbial fitness, potentially impacting the probability of infection and the local abundance of plant-associated microbes, including phytopathogens. For natural populations, we have a poor understanding of (1) what drives variation in insect herbivory, and (2) how such herbivory impacts the distribution of abundance of plant-associated microbes. We conducted manipulative field experiments in each of two years in sub-alpine habitats using a native plant–insect system to explore how insect herbivory, and the plant defenses they induce, alters the distribution and abundance of phyllosphere bacteria. We randomized replicate patches of our focal plant species to treatment with various plant defense hormones and subsequently quantified variation in herbivory by an abundant native herbivore. In parallel, we Illumina sequenced bacterial 16S from >500 randomly sampled surface-sterilized leaves from across our plant treatment plots and performed parallel culture-based validation of bacterial counts in tissue sub-samples of ~100 of the same leaves. Using peptic nucleic acid "PCR clamps", we dramatically increased our sequence coverage of bacterial 16S over plant organelle 16S. Our analysis exploits the remaining organelle 16S reads as an internal index of overall bacterial abundance: organelle 16S read counts were negatively correlated with bacterial colony-forming units in our paired culture-based analysis. Thus, we used information from organelle 16S read counts in our analysis to document variation in relative and absolute abundances of bacterial taxa. Across both study years, insect herbivory dramatically increased both the prevalence and abundance of over half of the ~151 bacterial taxa we delineated by minimum entropy decomposition. This finding validated prior observational work showing that herbivory is positively correlated with the presence and abundance of *Pseudomonas syringae* and *P. fluorescens*, among other bacteria. In general, leaves harbored low diversity microbial assemblages dominated by groups whose abundance varied dramatically with insect herbivory; several taxa also changed under plant defense hormone regimes independently of insect herbivory, although effect sizes were smaller. Our plant defense hormone treatments significantly impacted the prevalence and intensity of insect herbivory itself, indicating that phenotypic variation in plant defenses can influence subsequent herbivory rates and, thus, the local abundance of plant-associated microbes—including phytopathogenic *P. syringae*. Our study helps us understand the causes of variation in local abundances of microbes among their hosts, which is an important step for moving from coarse-grained community-level descriptions to mechanistic models of microbial population-level dynamics.

Finding plant beneficial microbes with commercial value using a novel computational biology platform
Victor Kunin (DuPont Pioneer), Matthew Ashby, Stewart Scherer, and Nasstasia Patin

Plants display myriad interactions with microbes, many of which are beneficial to plant health and yield performance. The challenge remains how to identify the most beneficial microbes in a background of thousands to tens of thousands of distinct microbial species. The task is further complicated by the organization of these communities into functional consortia which precludes their characterization through traditional screens of individual microbes. 16S rRNA gene profiling of rhizosphere microbial communities can produce large data sets that reflect the vibrant species diversity/richness and species interactions present in these environments. A description of the bioinformatics platform we developed that enables teasing out microbes and microbial consortia with commercial potential for enhancing productivity and sustainability of large acre row crops will be presented.

Laboratory ecosystem fabrication for examination of plant-microbe interactions

Trent Northen (DOE Joint Genome Institute), Jian Gao, Joelle Schlapfer, Dominique Loque, Adam Deutschbauer, Romy Chakraborty, Yasuo Yoshikuni, John Vogel

In recent years, our understanding of human, animal and plant microbiomes in a range of diverse environments has grown dramatically. However, one of the major challenges for generalized understanding of microbiomes in these complex ecosystems is extreme variation in microbiomes and environmental conditions. We are developing methods for ecosystem fabrication (EcoFAB) with the aim of constructing model soil ecosystems to allow for reproducible laboratory plant microbiomes that can be experimentally manipulated and disseminated between labs. Our approaches are based on recent workshop findings from a diverse cross-section of scientists (www.eco-fab.org). Broad scientific community acceptance of a few of these model ecosystems would no doubt exponentially increase our understanding of microbial communities as a whole by focusing diverse expertise and capabilities on the same systems. Each EcoFAB system is contained within a sterile plant-sized container with independent lighting. 3D printing is used to create root chambers attached to microscope slides, enabling the use of hydroponics, soil, or sand as substrate, as well as high-resolution rhizosphere imaging. The integrated fluidics system facilitates selective sampling and introduction of microbes, metabolites, etc. We have shown that these systems are suitable for growth of diverse plants, including *Brachypodium distachyon*, Arabidopsis thaliana, and bioenergy crop switchgrass for >1mo. Chemiluminescent imaging was used to examine localization and movement of labeled Pseudomonas fluorescens within the rhizosphere. Metabolomic analysis of EcoFAB culture was used for determination of the effects of microbiome composition on exudate plant exudate composition, and mass spectrometry imaging-based localization of root metabolites. Efforts are underway using individual bacterial mutants to investigate the biochemical ecology of specific exuded metabolites—a reductionistic approach typically not possible in natural ecosystems that enables determination of causal mechanisms of plant-microbe interactions. Future efforts are focused on extending EcoFABs to better reflect environmental processes e.g. to study nutrient cycling and plant growth promotion through low-input agriculture.

Poster Abstracts

Comparative Genomics and Transcriptomics of Russulaceae

B Looney, MJ Piatek, D Weighill, P Jones, P Meidl, K Barry, **Alan Kuo (DOE Joint Genome Institute)**, I Grigoriev, F Martin, D Jacobson, J Labbe

Russulaceae is a diverse fungal family mostly made up of the genera Russula, Lactarius, Lactifluus, and Multifurca, and composes one of the most widespread and species rich ECM lineages. In a recent collaborative effort, the Joint Genome Institute has sequenced genomes and transcriptomes of representative groups across Russulaceae, including a saprotrophic outgroup. Here is an overview of the first insight into the dense genome sampling within the family to explore and capture specific genomin features.

Genome-wide Identification of Bacterial Plant Colonization Genes

Benjamin J. Cole (DOE Joint Genome Institute), M. E. Feltcher, Robert J. Waters, Kelly M. Wetmore, Tatiana S. Mucyn, Elizabeth M. Ryan, Gaoyan Wang, Sabah Ul-Hasan, Meredith McDonald, Yasuo Yoshikuni, Rex R. Malmstrom, Adam M. Deutschbauer, Jeffery L. Dangl and Axel Visel

Diverse soil-resident bacteria can contribute to plant growth and health, but the molecular mechanisms enabling them to effectively colonize their plant hosts remain poorly understood. We used randomly barcoded transposon mutagenesis sequencing (RB-TnSeq) in Pseudomonas simiae, a model root-colonizing bacterium, to establish a genome-wide map of bacterial genes required for colonization of the Arabidopsis thaliana root system. We identified 115 genes (2% of all P. simiae genes) whose function is required for maximal competitive colonization of the root system. Among the genes we identified were some with obvious colonization-related roles in motility and carbon metabolism, as well as forty-four other genes that had no or vague functional predictions. Independent validation assays of individual genes confirmed colonization functions for 20 of 22 (91%) cases tested. To further characterize genes identified by our screen, we compared the functional contributions of P. simiae genes to growth in 90 distinct in vitro conditions by RB-TnSeq, highlighting specific metabolic functions associated with root colonization genes. Our analysis of bacterial genes by sequence-driven saturation mutagenesis revealed a genome-wide map of the genetic determinants of plant root colonization and offers a starting point for targeted improvement of the colonization capabilities of plant-beneficial microbes.

Surveying the genetic and genomic diversity of lentil nodulating Rhizobia

Y. Gai (University of California, Davis), B.K. Riely, M. H. Rashid, M. Wink, D. R. Cook

Lentil (Lens culinaris ssp culinaris) is a pulse legume crop that is an important source of protein for millions of people worldwide. Lentil's high protein content is a bi-product of its ability to form a symbiotic relationship with several species of nitrogen fixing bacteria from the genus Rhizobium. Rhizobium isolates vary in the efficiency with which they fix nitrogen and consequently bacterial genes and genomes can impact both the vigor and yield potential of the host. The identification of efficient nitrogen fixers that are adapted to different agro-ecological environments may help boost lentil production in a sustainable manner. We are establishing a collection of lentil nodulating bacteria and are implementing WGS to determine the level of genetic and genomic diversity present within our collection. These data will allow us to rationally select diverse strains for greenhouse and field experiments interrogating nitrogen fixation efficiency and strain competitiveness under different field conditions. To date, we have sequenced approximately 150 genomes from strains originating in seven different countries. Our phylogenetic analyses demonstrate significant diversity among the core genomes that appears to be largely a function of the geographic origin of the strains. In contrast, the symbiotic genes are monophyletic relative to other symbiovars, consistent with the horizontal transfer of the symbiosis genes conferring host specificity among different rhizobium species. Our current progress in characterizing the strains and their genomes will be reported.

Human pathogens interaction with lettuce

J. Montano, **Cristian Jacob (University of California, Davis),** S Porwollik, M McClelland, M Melotto

Pathogens that cause foodborne illness pose a challenge to food safety and security, as crops that are vectors for these human diseases may appear healthy, resulting in their integration to marketplaces throughout the world. Although some human pathogens that are introduced to the phyllosphere elicit an immune response within the plant, *Salmonella enterica* serovar Typhimurium strain SL1344 has been shown to disrupt plant innate immune signaling (including stomatal immunity) and survive for long periods of time inside the leaf. We have determined that, like SL1344, *S. enterica* serovar Typhimurium strain 14028S (14028S) also disrupts stomatal immunity after 4 hours post-inoculation (hpi). A forward genetic screen of 14028S multi-gene deletion (MGD) mutants identified *Salmonella* Pathogenicity Island (SPI)-1 and SPI2 as essential genomic elements for stomatal aperture modulation. Evaluation of apoplastic populations of MGD mutants lacking SPI1 and SPI2 determined that only SPI2 is essential for durable apoplastic persistence. Furthermore, investigation of single-gene deletion (SGD) mutants lacking Type Three Secretion System (T3SS) structural genes found on SPI1 and SPI2 were able to persist at higher titers in the apoplast, suggesting that these structures have a negative impact on 14028S survival in the apoplast. Additionally, the interaction between human pathogens (14028S and *Escherichia coli* O157:H7) and Lettuce (*Lactuca sativa* L.) cultivars and *Lactuca serriola* L. genotypes are being studied in this project. Bacterial leaf attachment and internalization assays were conducted along with stomatal density and aperture characterization. Preliminary data indicates the existence of differential interactions between lettuce genotypes and these human pathogens.

Potential for endophytic nitrogen fixation along a soil fertility gradient
Dianne Quiroz (University of California, Merced), Lara Kueppers

Old-growth temperate and boreal coniferous forests often occur in nutrient poor conditions suboptimal for plant growth. In these systems, the origin of accumulated nitrogen is unclear because N-fixing plants occur at low abundance, yet there is more nitrogen (N) in soil and vegetation than can be explained by known sources. The disparity between accumulated nitrogen and low abundance of N-fixing plants suggests an unknown diversity of N-fixing bacteria in these ecosystems. Recent findings indicate that a novel nitrogen uptake pathway involving endophytic bacteria may account for some of the missing nitrogen. To probe the ubiquity of this pathway and potential host and soil constraints, we examined the potential for foliar nitrogen fixation in bishop pine (Pinus muricata) and lodgepole pine (Pinus contorta ssp. bolanderi and ssp. contorta) along a natural soil fertility gradient at the “ecological staircase” in Mendocino, California. Our analysis suggests lodgepole and bishop pine have active nitrogenase enzymes, capable of fixing N2, strengthening the case for atmospheric nitrogen uptake through this pathway. Rates of ethylene production ranged from 0.3 – 0.7 nmol hr-1 g-1 and there appears to be no strong effect of soil fertility or of species ($p = 0.635$, $p = 0.995$). To better understand the ecological significance of this association future work will focus on scaling up and calibrating rates of fixation to determine system level contributions of this fixation route.

Bioremediation potential of the endogenous microbiome informs landscape architecture around the Gowanus Canal, Brooklyn’s hippest toxic waste dump
Elizabeth Henaff (Weill Cornell School of Medical Sciences), Ian Quate, Matthew Seibert, Christopher Mason

The Gowanus Canal in Brooklyn is scheduled to undergo dredging and sub-aquatic capping as part of the US Environmental Protection Agency Superfund Cleanup plan beginning in 2017. Historically a productive estuary, the waterfront was claimed by industrial and cultural needs that dramatically altered the habitat and ecological flow. Beginning when Gowanus Creek was dredged into Gowanus Canal in 1869, and perpetuated until 2013 when the record of decision was reached designating the Gowanus Canal Superfund Site, the canal has collected 150 years of industrial byproducts. To this day raw sewage overflows into the system following each rain event, adding to the unique slurry of hydrocarbons and heavy metals mixed with estuarine silt deposits. Driven by a citizen scientists’ curiosity regarding the extant environment which will be effectively destroyed by cleanup efforts, we have sampled the Canal’s sediment for short-read metagenomic analysis over four seasons of the last year. Functional analysis of these data have revealed that these populations encode bioremediation functions related to the historical use of the Canal, including degradation of hydrocarbons and industrial solvents. We discuss ways to exploit this genetic solution to the challenge of contamination and to inform the design of the built environment for rehabilitation of the Canal.

Syringafactin production by Pseudomonas syringae, is contact-dependent and affects the local environment via its hygroscopicity

Monica Hernandez (University of California, Berkeley), Steven Lindow

Pseudomonas syringae produces the biosurfactant syringafactin which has hygroscopic properties. The contribution of syringafactin to the water availability around cells was assessed using a proU promoter, that is induced under low water conditions. When the proU promoter was fused with gfp, it showed that even under humid conditions syringafactin-deficient cells have less water available for use, with a gfp fluorescence of 2707.02, compared to cells that produce syringafactin, having a gfp fluorescence of 2367.81. The contribution of syringafactin to cell viability caused by using propidium iodide staining reveals that syringafactin-deficient cells have an overall higher proportion of cell death of 25.34% compared to syringafactin-producing cells which had an average cell death of 10.81%. Furthermore, the water absorption properties of syringafactin are quite hygroscopic especially when the relative humidity is in the range of 93% to 100%. It has also been shown that syringafactin production is a contact-dependent trait by examining the induction of the syfA gene, which encodes for syringafactin production. The syfA gene is induced at a basal level in liquid culture, but when cells are placed on a surface, syfA induction increases by at least two-fold. SyfA induction is induced on a variety of surfaces including glass, plastic, paper, parafilm, leaves, agar, and membrane filters. The syfA gene has also been shown to be induced in liquid culture containing 5% Dextran.

Structural changes in JAZ4 lead to functional insight of jasmonic acid signaling during plant defense and development

Paula Oblessuc (University of California, Davis), Logan DeMott, Maeli Melotto

Jasmonic acid (JA) signaling is an important process involved in the balance between growth and development of plants and defense against pathogens. Imperative to the regulation of jasmonate signaling are the Jasmonate ZIM-domain (JAZ) transcriptional regulators involved in active repression of JA signaling through direct interaction with various transcription factors. JA signaling is upregulated by JAZ protein degradation via the 26S proteasome upon recognition of JA-isoleucine, or its structural mimic coronatine (COR), by the F-box protein CORONATINE INSENSITIVE1 (COI1). Previously, we found that a jaz4 null mutant of Arabidopsis is more susceptible to Pseudomonas syringae pv. tomato (Pst) strain DC3000 and display developmental defects such as shorter roots and delayed flowering, suggesting that JAZ4 plays an important role in both plant development and defense. Here, we show that transgenic Arabidopsis plants overexpressing the structural variant JAZ4ΔJas have a dominant-negative JA-insensitive phenotype, displaying more resistance to Pst DC3000 compared with wild-type plants. In addition, JAZ4ΔJas protein lacks proteasome-dependent degradation upon COR treatment. Interestingly, we identified a naturally occurring isoform of the JAZ4 transcript, JAZ4.2 that encodes for a protein with truncated Jas domain similar to JAZ4ΔJas. Structure-function analyze of these proteins should advance our understanding of the role JAZ4 variants play in plant growth and defense.

Phylogenetic analysis and phenotypic characterization of a living collection of Turkish chickpea associated rhizobacteria

Rabia Mufti (University of California, Davis), Brendan Riely, Ping Song, Lei Feng, Alex Greenspan, Asghari Bano, Douglas R. Cook

The rhizosphere is a complex ecosystem containing diverse microbiota capable of associating with the plant. Some of these microbes exhibit plant growth promoting properties and are potential tools for improving the sustainability of agriculture. Chickpea (Cicer arietinum) is an important grain legume that was domesticated from its wild progenitor, Cicer reticulatum, around 11,000 years ago in Southeastern Turkey. Here we describe the isolation of microbes that have co-evolved with wild chickpea, reasoning that some of these may exhibit activities optimized by natural selection to benefit cultivated chickpea. We developed a living collection of approximately 700 Cicer associated rhizobacteria by growing wild C. reticulatum accessions in their native Turkish soils and isolating bacteria from within and on the surfaces of their roots and nodules. We used 16S sequencing as an initial means to characterize the collection and to select a diverse subset of 182 strains were selected for Illumina whole genome sequencing and in vitro phenotyping. Phylogenetic analysis revealed that 19 genera are represented in the collection spanning all four phyla of the Bacteria. We subsequently tested these strains for activities known to be associated with plant growth promoting properties of rhizobacateria, including phosphate solubilization, 1-aminocyclopropane carboxylic acid (ACC) deamination, indole acetic acid (IAA) catabolism, and antifungal activity against the chickpea soil borne pathogen Fusarium oxysporum f.sp. ciceris. Phenotyping nominates a subset of 14 strains with strong phenotypes, all of which are effective phosphate solubilizers, 3 of which have strong antifungal activity, and 1 each that grow actively on ACC or IAA as the sole nitrogen source. Future work will test these activities in planta and assess the impact of these bacteria on plant growth during biotic and abiotic stress.