71st Annual Meeting and Exhibition 2021

- Unable to attend the meeting

S121 Simplifying biology for a sustainable future: The power of dynamic metabolic control

Z. Ye, DMC Biotechnologies, Durham, NC, USA*

I will present on the dynamic metabolic control technology, its development, optimization, robust and scalable fermentation from µL to m^3. The dynamic metabolic control technology relies on dynamic minimization of the active metabolic network and is implemented in the context of standardized 2-stage bioprocesses. Dynamic metabolic network minimization is accomplished using combinations of CRISPR interference and controlled proteolysis to reduce the activity of multiple enzymes in previously untouchable essential central metabolism. This approach not only results in a design space with greatly reduced complexity, but also in increased metabolic fluxes and production rates as well as in strains which are robust to environmental conditions. Robustness leads to standardized fermentation process and predictable scalability from high-throughput µL-scale screens, all the way to commercial m^3-scale commercial fermenters. We demonstrated the successful applications of the platform for production of several important high volume industrial chemicals at commercially meaningful rates, titers and yields.

S8 From 2 mL to 200 m^3: Strain Development to Product Manufacturing at AB Mauri (R)

J.H. Evans, Ph.D., AB Mauri, St. Louis, MO, USA*

The AB Mauri strain development laboratory uses both classical and advanced ‘omics techniques to generate novel strain lineages for application into bakery, bioethanol, alcoholic beverage, and animal and human nutrition markets. Before laboratory strains become commercial products, a series of scale-up fermentations are performed, starting at 2 mL in 96-well plates in the laboratory and culminating with brews at 200 m^3 scale at commercial manufacturing facilities.

In the laboratory, candidate strains pass through different high-throughput (HT) screens for desired phenotypic and genomic profiles. Fermentation analytes are screened by plate-based HT NIR and genomic profiles by HT capillary electrophoresis. Strains passing through from the screen are then evaluated at 100 mL-scale shake flask and, if passing, at 2L bioreactor scale, with additional analytes evaluated using high-performance liquid chromatography (HPLC). These strain development steps are often iterative before arriving at a final set of candidate strains. Candidate strains exhibiting desired phenotypic performance characteristics are then passed to the product development team.

The product development team uses the received strains to first manufacture active dry yeast (ADY) product at small pilot (1kg) scale using commercial substrates (C and N sources). Small pilot manufacture of ADY determines growth and drying characteristics to inform commercial scale manufacturing. Performance of pilot ADY material is compared to the strain performance and tested in customer applications, also using commercial substrates, to ensure product suitability. These product development steps are often iterative before arriving at a final protocol for product manufacture.

Once passing from small pilot, technology transfer is performed to manufacture 5 to 10 metric tons (mT) product at a commercial facility in 200 m^3 fermenters. ADY is tested for performance as above, and if passing, moves to manufacturing in 72 mT campaigns.

In this presentation, the process of developing new strains and products at AB Mauri for the bioethanol market is presented, with special attention to progressing through the strain development and then product development stages.

S7 Using CFD studies to understand the scale-up of fermentation process with hydrophobic substrates

D. Xie, R. Marx and S. Yoon, University of Massachusetts Lowell, Lowell, MA, USA*

Oils/fats can be used as an important feedstocks to replace sugars for biomanufacturing a series of high-value products. Our group has successfully engineered both E. coli and Y. lipolytica yeast strains for production of high-value products from waste cooking oils. In this presentation, we will introduce the recent progress in using CFD as a tool for bioreactor design, optimization, and scale-up when an oil substrate is contained in the fermentation medium.

S91 Serine recombinase-assisted genome engineering (SAGE) enables high efficiency strain engineering in non-model and undomesticated bacteria

J. Elmore, H. Baldino, R. Francis and R. Egbert, Pacific Northwest National Laboratory, Richland, WA, USA; G. Dexter, J. Huenemann, J. Tweedie, L. Riley and A. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA; D. Coleman-Derr, USDA Agricultural Research Center, Berkeley, CA, USA*

The application of synthetic biology is critical to enable both sustainable enhancements to crop productivity and chemical production from non-traditional feedstocks. However, the genetic tools available to non-model bacteria are frequently insufficient for advanced genetic engineering. Here we present an advanced genome engineering tool – serine recombinase-assisted genome engineering, or SAGE - that performs highly efficient, iterative, and multiplexable genome integration in both non-model strains and undomesticated environmental bacteria. SAGE utilizes transient expression of a serine recombinase to perform unidirectional, site-specific integration of circular DNA into the host chromosome. Integration efficiencies with the eight SAGE recombinases reach up to 10^8 transformants per µg of cargo DNA to generate large strain libraries. Critically, SAGE works without any replicating plasmids in the host organism, and thus can be used in undomesticated hosts without any *a priori* knowledge or additional genetic tools. We utilized SAGE to address a critical challenge of engineering non-model microbes – the identification of genetic elements (*e.g.* transcriptional promoters) for predictable and tunable gene expression. We developed a modified ratiometric barcode sequencing approach to simultaneously assess a library of genome integrated promoters for both magnitude of transcriptional output as well as sensitivity to environmental and genetic context. This approach was applied in 5 diverse bacteria with a 250-promoter library, each with five distinct 5'-UTR barcodes. We identified at least 95 promoters in each organism that passed quality thresholds for context-independent expression with strain library expression ranges between ~2500-fold and ~55,000-fold. Finally, we determined that the magnitude of expression for at least ~1.5% of the tested promoters would have been mis-evaluated by >10-fold if a single barcode were used per promoter. Currently SAGE has been demonstrated in over 10 bacteria comprising diverse phylogenetic groups - proteobacteria (α, β,and γ), actinomyces and firmicutes – and we are actively expand its application to many more.

S73 If scale up were easy, anyone could do it: lessons learned from my industrial biotechnology career

C. Guske, D^2 Biotech Consulting, LLC; CTO-Botany AI; Manuf. Technol. Director - Noblegen, Decatur, IL, USA*

With over 30 years of industrial biotechnology experience in the food, nutraceutical, specialty, and commodity chemicals sectors, including stints at CP Kelco, NutraSweet, and Tate & Lyle, Dr. Guske, an accomplished Biochemical Engineer, Functional/Project Manager, and Consultant, has acquired extensive experience in early process development, piloting, and subsequent translation of those processes to large-scale and commercial production. In his corporate life, Dr. Guske interfaced with/assessed ~100 biotech opportunities/companies and contributed to technology across a broad spectrum of sectors. In over four years as an Independent Consultant, Dr. Guske has assisted some 50 clients, ranging from early-stage startups to multinationals. Tech transfers and scale up are never easy,

but the pain points can be significantly lessened if one knows where “the snakes in the grass” are. Chris will share some of his experience in this panel discussion.

S75 Stefan Schwegmann - abstract

S. Schwegmann, MSU-Bioeconomy Institute*

The Michigan State University Bioeconomy Institute (MSU-BI) offers custom-tailored solutions to scale-up both chemical and biological technologies to commercialization. MSU-BI has operated its fermentation center in Lansing, MI since 1981, and its specialty chemical center in Holland, MI since 2009. In our decades of experience in scale-up we have cultivated a wide range of microbes, range in from anaerobic to highly aerobic, bacteria, yeasts, filamentous fungi, and algae.

In the past 5 years we have developed and scaled over 30 different types of fermentations to produce specialty chemicals, biofuels, proteins, enzymes, and bio-agricultural products. The fermentation center in Lansing houses 14 base units, which control vessels ranging from 1L to 10L working volumes at the bench scale, and a pilot plant with 100L, 150L, and 3000L fermentation vessels alongside appropriately sized feed and hold tanks.

In this panel discussion I will share relevant experiences and the insights we gained while scaling up processes for more than 100 organizations.

S74 Tech transfer in ag-innovation; better biostimulants, biocontrols, and bioprocesses

M. Frodyma, PhD and A. Keley, PhD, New Leaf Symbiotics, St Louis, MO, USA; D. Jimenez, PhD, NewLeaf Symbiotics, St. Louis, MO, USA*

Tech transfer in ag-innovation; better biostimulants, biocontrols, and bioprocesses.

Agricultural biologicals continue to gain market share with double-digit compounded annual growth. As basic microbiome science drives innovation the wealth of novel microbes entice development with exceptional opportunities. However, commercial success requires early attention to process, scale and logistics. In these endeavors Tech Transfer often sits at the inflection point between hope and “halleluiah”. Advances in fermentation and formulation can remove barriers allowing novel microbes, molecules, and consortia to show hope in the laboratory, but significant challenges arise during the delivery of cost-effective solutions. Consumer demand fuels ag-biological investment, but global regulatory harmonization still represents a noteworthy hurdle. Harnessing native microbial attributes and applying contemporary tools to trait selection offers solutions to significant environmental issues, but it all comes down to “does it scale?" From intellectual property to market access the why, who and when of Tech Transfer is often an arbitrator of commercial success.

S32 Presentation title: Leveraging Culture Bioscience's Cloud Bioreactor Lab for High-throughput Formulation Improvement Studies

K. Tyner, Ph.D., M. Galicia and F. Tachea, Culture Biosciences, South San Francisco, CA, USA; A. Roulier and R. Garrett, Joyn Bio, Boston, MA, USA*

Culture Biosciences offers a cloud-based bioreactor platform that customers leverage as either their sole source of bioreactors or to supplement existing internal capacity. Customers across a variety of industries have realized the time-saving benefits of access to Culture’s bioreactor capacity and associated data tools. This includes Joyn Bio, who is developing crop-colonizing nitrogen-fixing microbes in an effort to replace traditional chemical fertilizers, which have been shown to negatively impact the environment. These products will not only reduce the environmental impact of traditional fertilizers, but also have the potential to improve crop performance by providing sustained nitrogen delivery during crop growth. Developing optimal formulation of these microbes to maximize product concentration and viability upon application will be critical to the commercial success of these products. However, development of optimal formulations requires integrated upstream process development and strain improvement, and this work is

often restricted or slowed by limited availability of bioreactors and resources for data collection and analysis. Here we describe how Joyn Bio has leveraged Culture's cloud bioreactors and data visualization tools to improve formulation of their crop-colonizing microbe product through parallel process development and strain screening efforts.

S72 Managing an overseas tech transfer project

S. Iverson, Tepha Inc, Lexington, MA, USA*

Biomanufacturing overseas is an attractive option to a small company with limited capacity, staff, and funding, and to a large company with a need for quick expansion and low cost. Globally, CMO's offer services that could cover the entire product development and manufacturing process. Often the sponsoring company lacks expertise in biomanufacturing that the CMO can provide.
Technical transfer of complicated manufacturing processes is difficult within one's own country and is made inherently difficult at an overseas location where there may be widely differing knowledge, technology, and skill sets. Working together is made more difficult with conflicting time zones as well as language and cultural differences.

It is important that both companies are aligned behind scientific principles and project strategy. A knowledge management system including translation services, frequent site visits, stakeholder alignment, project management, alignment of quality objectives, and a clear definition of success are some of the tools used to manage an overseas tech transfer project.

S123 Epigenome of Sulfate Reducing Bacteria under Copper Stress

S. Rauniyar, P. Thakur, A.K. Tripathi, P. Saxena, R. Singh and R. Sani, Professor, South Dakota School of Mines and Technology, Rapid City, SD, USA*

Epigenetics is a mechanism of gene transcription regulation without changing the DNA sequence and is usually reversible. These changes occur in terms of methylation whose location, abundance, and distribution varies based on environmental stress conditions. Effect of copper stress on the epigenome of a Sulfate reducing bacteria (SRB) is poorly understood. Epigenetic analysis of SRB, *Desulfovibrio alaskensis* G20, under variable copper concentrations (0, 5, 15, and 30µM) were performed to map the differentially methylated (m^5C methylation) genes distributed across CHH, CHG and CPG genomic islands. *Desulfovibrio alaskensis* G20 cell density decreased with an increase in copper ion concentration. The result uncovered 37 genes with de-novo methylation that included methyltransferase genes involved in the transfer of methyl group from S-adenosyl-L-methionine (SAM) to other carbon atoms of differentially methylated genes and the genes involved in sensing extracellular change in environment. Increase in the methylation of transcriptional factor, protein secretion genes, and transmembrane transporter genes suggests their roles under copper stress. The study also revealed increased cytosine methylation in the genes involved in flagellar chemotaxis protein, transcription regulation and chloride transport. M^5C methylation in *Desulfovibrio alaskensis* G20 genome could elucidate the mechanism of gene regulation in SRB in response to metal toxicity.

Monday, August 9

8:00 AM - 11:30 AM Session: 1: Automation, Modeling, and Machine Learning in Metabolic Engineering

Conveners: **Christopher Long**, Ginkgo Bioworks and **Donovan Layton**, Zymergen

Waller Ballroom - Salon C-D, Level 3

8:00 AM S2: Integrated knowledge mining, genome-scale modeling, and machine learning for predicting Yarrowia lipolytica bioproduction

J. Czajka, T. Oyetunde and Y. Tang, Washington University in St. Louis, St. Louis, MO, USA*

Predicting bioproduction titers from microbial hosts has been challenging due to complex interactions between microbial regulatory networks, stress responses, and suboptimal cultivation conditions. This study integrated knowledge mining, feature extraction, genome-scale modeling (GSM), and machine learning (ML) to develop a model for predicting Yarrowia lipolytica chemical titers (i.e., organic acids, terpenoids, etc.). First, Y. lipolytica production data, including cultivation conditions, genetic engineering strategies, and product information, was manually collected from literature (~100 papers) and stored as either numerical (e.g., substrate concentrations) or categorical (e.g., bioreactor modes) variables. For each case recorded, central pathway fluxes were estimated using GSMs and flux balance analysis (FBA) to provide metabolic features. Second, a ML ensemble learner was trained to predict strain production titers. Accurate predictions on the unseen test data were obtained for instances with production titers >1 g/L (R^2 = 0.87). However, the model had reduced predictability for low performance strains (0.01–1 g/L, R^2 = 0.29) potentially due to biosynthesis bottlenecks not captured in the features. Feature ranking indicated that the FBA fluxes, the number of enzyme steps, the substrate inputs, and thermodynamic barriers (i.e., Gibbs free energy of reaction) were the most influential factors. Third, the model was evaluated on other oleaginous yeasts and indicated there were conserved features for some hosts that can be potentially exploited by transfer learning. The platform was also designed to assist computational strain design tools (such as OptKnock) to screen genetic targets for improved microbial production in light of experimental conditions. However, model limitations still exist that should be addressed in future research to obtain more accurate and broader applications for yeast fermentation studies.

8:30 AM BioAutomata: A Self-driving Biofoundry

H. Zhao, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Large-scale data acquisition and analysis are often required in the successful implementation of the design, build, test, and learn (DBTL) cycle in metabolic engineering. However, it has long been hindered by experimental cost, variability, biases, and missed insights from traditional analysis methods. In this presentation, I will describe the application of an integrated robotic system coupled with machine learning algorithms to fully automate the DBTL process for metabolic engineering. As proof of concept, we have demonstrated its capacity by optimizing the lycopene biosynthetic pathway. This fully-automated robotic platform, BioAutomata, evaluates less than 1% of possible variants while outperforming random screening by 77%. A paired predictive model and Bayesian algorithm select experiments which are performed by Illinois Biological Foundry for Advanced Biomanufacturing (iBioFAB). In addition, BioAutomata is being explored for automated protein engineering and metabolic engineering. BioAutomata excels with black-box optimization problems, where experiments are expensive and noisy and the success of the experiment is not dependent on extensive prior knowledge of biological mechanisms.

1. T. Si, R. Chao, Y. Min, Y. Wu, W. Ren, and H. Zhao. “Automated Multiplex Genome-scale Engineering in Yeast.” *Nature Communications*, 8:15187 (2017).
2. R. Chao, S. Miresh, T. Si, and H. Zhao. “Engineering Biological Systems using Automated Biofoundries.” *Metabolic Engineering*, 42, 98-108 (2017).
3. M. HamediRad, R. Chao, S. Weisberg, J. Lian, S. Sinha, and H. Zhao. “Towards a Fully-Automated Algorithm-Driven Platform for Biosystems Design.” *Nature Communications*, 10:5150 (2019).

9:00 AM S3: Genome-wide cutting scores enable sgRNA activity predictions and definition of essential genes in the yeast *Yarrowia lipolytica*

A. Ramesh, D. Baisya, V. Trivedi, S. Lonardi and I. Wheeldon, UC Riverside, Riverside, CA, USA*

Non-conventional organisms are attractive targets for metabolic engineering as they can present a range of desirable traits that help avoid complex and intensive metabolic engineering of less suitable model hosts. As a drawback, their genome and metabolic networks are often less well understood, and they typically lack the range of genetic engineering tools available in conventional hosts. *Yarrowia lipolytica* is one such non-conventional yeast with an abundant native acetyl-CoA pool and a capacity to produce and accumulate lipids to high levels. While there have been significant advances in the metabolic engineering of this yeast for the biosynthesis of oleochemicals and other value-added products, there is also a lack of synthetic biology tools for functional genomic screening and rapid strain development. We have sought to overcome these limitations by developing CRISPR-Cas9/Cas12a systems for gene knockout, integration, regulation, and genome-wide screening. However, prediction of highly active sgRNA which are crucial in effective genome editing and improving confidence in hit calling, remains a challenge. In addition, *Y. lipolytica* lacks a well-defined consensus set of essential genes that would help further our understanding of this organism and ease metabolic engineering efforts. Thus, we constructed two genome-wide libraries, one using SpCas9 and the other using LbCas12a, to target all protein coding sequences. In the absence of DNA repair by non-homologous end joining, screens provided a cutting score (CS) for each guide, while screens in the wild type background provided a fitness score (FS) for each gene. We used the genome-wide CS values to develop a new machine learning based guide-activity prediction algorithm called DeepGuide, and also used these values to provide a guide-activity correction to more accurately determine FS for each gene in the genome. Combined with results from a previously published essential gene set identified using a transposon screen, the outcomes of our CRISPR screens define a consensus set of essential genes for *Y*. *lipolytica*.

9:30 AM Break

10:00 AM S4: Stepping on the Gas to a Circular Economy: Accelerating Development of Carbon-Negative Chemical Production from Gas Fermentation via Automation and Machine Learning

M. Köpke, R. Jensen and S. Simpson, LanzaTech, Skokie, IL, USA*

Climate crisis and rapid population growth are posing some of the most urgent challenges to mankind and have intensified the need for the deployment of carbon recycling technologies. Gas fermentation using carbon-fixing microorganisms offers a solution for transforming waste carbon into sustainable fuels, chemicals and polymers at a scale that can be truly impactful in mitigating the climate crisis. LanzaTech is a pioneer and world leader in gas fermentation, having successfully scaled up the process from the laboratory bench to full commercial scale, with two commercial plants in operation and several additional units in construction.

Compared to other gas-to-liquid processes, gas fermentation offers unique feedstock and product flexibility. The process can handle a diverse range of high volume, low-cost feedstocks. These include industrial emissions (e.g., steel mills, processing plants, refineries) or syngas generated from any biomass resource (e.g., municipal solid waste, agricultural waste, organic industrial waste), as well as CO_2 with green hydrogen. Synthetic biology and metabolic engineering enable conversion of these feedstocks into an array of fuels, chemicals or polymers. Already, direct synthesis of hundreds of molecules has been demonstrated.

In order to develop efficient production strains and accelerate time to market, tools such as automation, modeling and machine learning are critical. LanzaTech has established a first-of-its-kind biofoundry for fully-automated, high-throughput engineering and screening of gas fermenting microbes. Unlike working with traditional hosts, gas fermenting microbes offer a unique set of challenges that needed to be overcome such as the lack of established genetic tools, low transformation and recombination efficiencies, the requirement for anaerobic conditions, and the need for screening in context of toxic and flammable gases. To guide strain engineering, we have augmented the biofoundry with rapid cell-free prototyping workflows and employing a range of advanced models and machine learning approaches,

driven by increasingly large datasets. A couple case studies will highlight how these tools are used and contributing to optimizing strains for high performance.

10:30 AM S5: Artificial intelligence modeling and optimization of cultural bioprocess conditions for prodigiosin production with concomitant feather waste degradation by *Serratia* sp. UCCM 00009

M. Ekpenyong[*]*, A. Asitok, I. Takon and S. Antai, University of Calabar, Calabar, Nigeria*

Concomitant production of two or more microbially-derived value-added biotechnological products on waste substrates is a classical approach towards improved production economics. In the present study, the concomitantly optimized production of keratinase, gelatinase and prodigiosin by the locally-sourced *Serratia* sp. UCCM 00009 using waste poultry feather and waste frying peanut oil as carbon substrates is reported. The red-pigment demonstrated inhibitory activity against clinically-relevant Gram-positive and Gram-negative bacteria but not fungi. It also demonstrated anti-cancer activity against breast and lung cancer, but not T-cell leukemic and normal cell lines. Optimization of bioprocess conditions for concomitant improvement in keratinase activity (Y_1) and gelatinase activity (Y_2) towards improved waste feather degradation (Y_3) and prodigiosin yield (Y_4), involved sequential one-factor-at-a-time (OFAT), regular 2-level factorial design (2-LFD), path of steepest ascent (PSA) and central composite rotatable design (CCRD) matrix. Particle swarm optimization (PSO) of the multi-objective function generated by artificial neural network (ANN) trained with Levenberg-Marquardt algorithm resulted in a composite desirability of 0.955, setting optimum factor levels at: feather substrate (X_1) = 6.4%, waste frying peanut oil (X_2) = 1.5%, peptone (X_3) = 6.8 g/L, temperature (X_4) = 29ºC, pH (X_5) = 8.7 and $ZnCl_2$ (X_6) = 0.055 mg/mL. These conditions yielded an SDS-PAGE purified keratinase with specific activity of 78.73 U/mg; gelatinase with specific activity of 118.64 U/mg, 88.84% waste feather degradation over a 96-h period and an HPLC-purified specific prodigiosin yield of 1.757 g/g. Exploitation of waste feather substrate and waste frying peanut oil for value-added microbial bioactive metabolite production holds promise as a sustainable waste feather management option with considerable production economics.

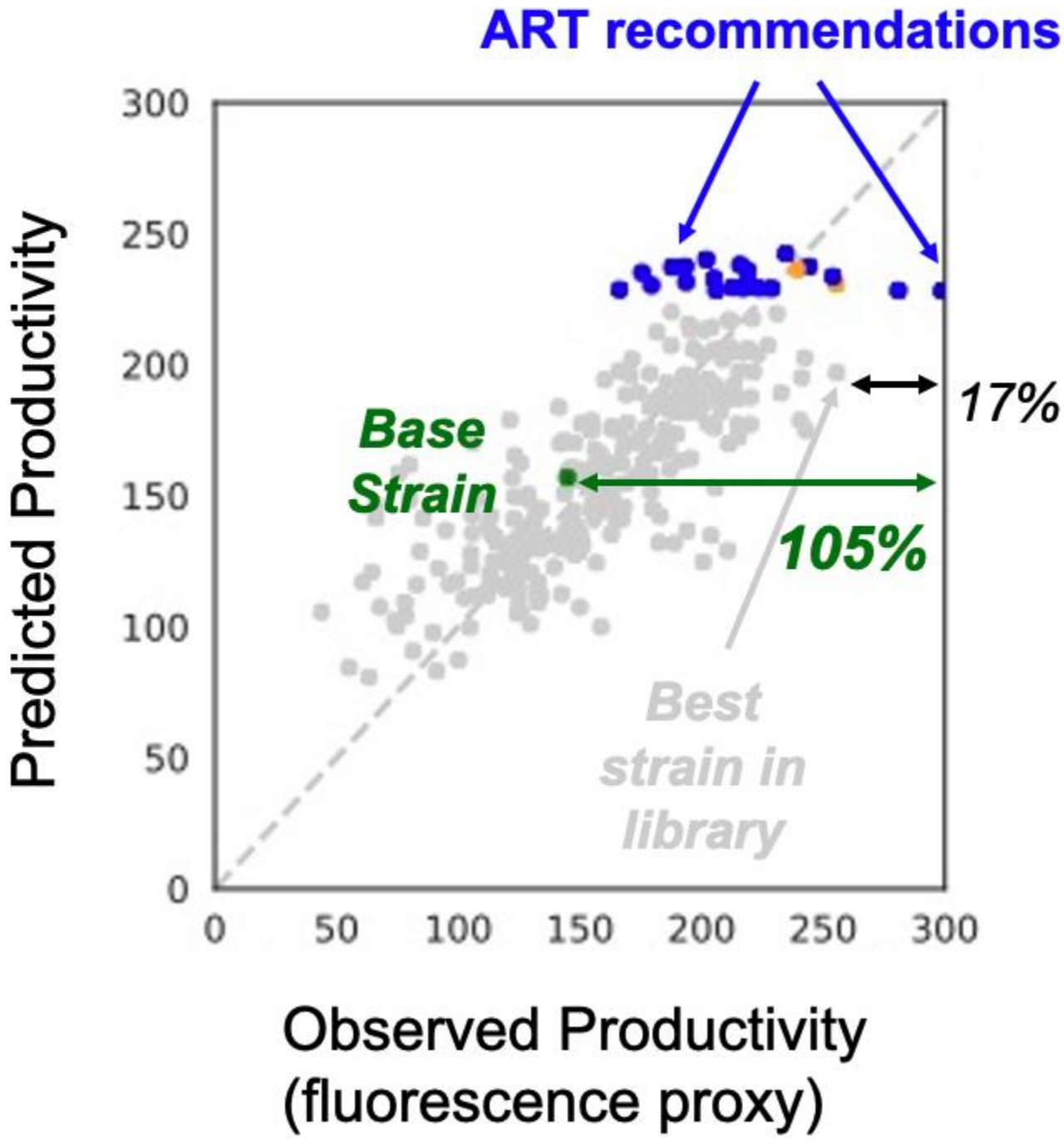
11:00 AM S6: Machine Learning Tools Make Synthetic Biology Predictable (R)

H.G. Martin[*]*, Lawrence Berkeley National Laboratory, Berkely, CA, USA*

Biology has changed radically in the last two decades, transitioning from a descriptive science into a design science. In synthetic biology's effort to design cells to a specification, new tools are now available that promise to disrupt this discipline: from CRISPR-enabled genetic editing, to high-throughput omics phenotyping, and exponentially growing DNA synthesis capabilities.
However, our inability to predict the behavior of bioengineered systems hampers synthetic biology from reaching its full potential.

In this talk, we will present machine learning algorithms and other computational tools that allow us to guide metabolic engineering in an effective fashion. We will discuss the Automated Recommendation Tool (ART): a tool that leverages machine learning and probabilistic modeling techniques to guide synthetic biology in a systematic fashion, without the need for a full mechanistic understanding of the biological system. ART is designed to meet the conditions found in synthetic biology: small data sets, the need to quantify uncertainty and recursive cycles. We will show how to combine it with mechanistic

models to improve tryptophan productivity.



8:00 AM - 11:30 AM Session: 2: Advances in Fermentation Scale-up and Scale-Down

Conveners: **Dr. Dongming Xie**, University of Massachusetts Lowell, Lowell, MA, USA and **Tiffany D Rau**, Rau Consulting LLC

Waller Ballroom - Salon F, Level 3

8:00 AM Break

8:00 AM - 11:30 AM Session: 4: 1st Generation Ethanol: Process Optimization and Recent Advances (Sponsored by POET)

Conveners: **Jennifer Headman**, POET and **Dr. Mohit Bibra**, Zymergen Inc., Emeryville, CA, USA

Waller Ballroom - Salon B, Level 3

8:00 AM S14: Protecting investments: a holistic view to maximize efficiency

J. Forbes, Phibro Ethanol Performance Group, St. Paul, MN, USA*

As investments in technological advancements increase, there becomes a greater incentive to protect these expenditures. This session is designed to focus on product solutions and commercial ethanol plant operations as a holistic system. The discussion will cover relevant decision-making processes in choosing the optimal operational inputs. When looking at fuel ethanol fermentation input costs, grain is always the largest investment. Often the case with biological processing, the primary media substrate is the most costly input. It is highly critical to execute an efficient fermentation to maximize the substrate investment. With the continual development of genetically modified yeast strains, yeast has become a larger investment for the producer compared to the historical norm. There is also a much greater expectation placed on yeast technologies as they are increasingly responsible for more of the fermentation process as compared to a conventional yeast.

The principles of maximizing efficiency are simple: 1) eliminate undesired organisms 2) provide the best environment and support for your primary organism. However, executing on these principles isn't always simple. A producer needs certain products or inputs to do this. But how does one decide which products to select?

This presentation will share economic models on the cost of a compromised fermentation. It will share both lab and commercial data demonstrating the advantages of efficient fermentation. The discussion focuses on fermentation as a holistic system and presents a model where inputs work together to ultimately make the most of each strategic investment. The ultimate goal is to get the best traits from each offering to drive producers toward maximum yield and profitability.

8:30 AM S15: Controlling microbial communities in ethanol bio-refineries requires an understanding of population composition and dynamics

F. Firmino, J. Broadbent and J. Steele, Lallemand Biofuels & Distilled Spirits, Lebanon, NH, USA*

Control of bacterial contaminants in bioethanol fermentations requires a detailed understanding of the organisms present and how they respond to the interventions utilized to control them. The microbial ecology of ethanol biorefineries includes yeast, primarily the strain which is intentionally added, and a fairly diverse population of bacterial contaminants, mainly lactic acid bacteria (LAB). This type of microbial community has occurred in decomposing plant materials since ancient times and hence complex interactions have evolved between these organisms. These interactions include both positive and negative interactions. In ethanol biorefineries, the greatest interest is in interactions which have negative impact on yeast performance, as reduced performance by the yeast can result in significant yield loss. Traditionally, the bacterial contaminants in ethanol biorefineries have been controlled by sanitation and antibiotics. However, there is increasing interest in the development of antibiotic-free solutions.

Additionally, there is growing recognition that LAB can have beneficial impacts on fermentation, including the intentional addition of bacterial ethanologens designed to increase yield through the use of substrates not utilized by the yeast and to decrease losses from contaminating LAB by competitive exclusion. The rationale control of bacteria in bioethanol fermentation requires a detailed understanding of which organisms are present and at what levels.
In ethanol biorefineries, it is common for the most abundant contaminants to be non-detectable using standards methods, such as plating on “non-selective” media. Therefore, culture-independent methods are the methods of choice for the characterization of these microbial communities. We have used these approaches to quantify the relative abundance of LAB before and after treatment with a variety of antimicrobials. Different antimicrobials have different impacts on the microbial ecology of these samples and these differences have a significant impact on level and types of organic acids produced. Additionally, we have used genome sequencing-based methods to detect the abundance of genes encoding antibiotic-resistance. These approaches allow for the rational selection of antimicrobials, including antibiotic-free strategies, for optimal control of LAB in bioethanol refineries.

9:00 AM S16: Improved Yeast Strains for Industrial Ethanol Fermentation

M. Catlett, Novozymes, Davis, CA, USA*

The United States ethanol industry has an annual production capacity of approximately 17 billion gallons (www.eia.gov). To reliably convert ground corn into this massive amount of liquid fuel, ethanol plants require yeast strains that can produce ethanol at high yields and titers and that can also thrive in the stressful and variable conditions encountered during large-scale, industrial fermentations. Novozymes’ Innova yeast products have been developed to meet these needs. This talk will explore the development of the latest strains in the Innova series, where evolution and selection of elite *S. cerevisiae* genetics were combined with strain engineering to produce glucoamylase secreting yeasts with high ethanol yield, reduced glycerol, and market leading toughness.

9:30 AM Break

10:00 AM S17: *Poet’s BPX™ Raw Starch Hydrolysis Fermentation Process Marries Biology with Engineering.*

S. Lewis, POET, Sioux Falls, SD, USA*

Poet operates a raw starch conversion process for the conversion of corn (grain) starch to ethanol. Known as the Poet ***BPX™*** process, the technique avoids the high temperature starch liquefaction process common for starch conversion to fermentable glucose. Milled corn is slurried with process waters and conversion is accomplished using innovative enzymes capable of efficient conversion of granular starch to glucose. The avoidance of high temperatures in ***BPX™*** maintains the activity of the numerous endogenous enzymes naturally present in grain. These enzymes naturally present in the corn feedstock complement the exogenous industrial enzymes added to the process or provided in the in situ fermentation by novel CBP yeast strains. The endogenous enzymes in grain, which include amylases, proteases, and other activities, complement the enzymes provided through Biotechnology derived yeast and enzyme innovations, and enable Poet to operate a high yielding process for both ethanol and corn oil. The endogenous enzymes have also provided Poet with a natural process for the conversion of corn fiber to ethanol since the commercialization of the ***BPX™*** process in 2004. The complementary role of endogenous fiber degrading enzymes in corn grain also supplements the role that industrial cellulases and hemicellulases can provide in the total conversion of carbohydrate and the maximization of yield. In the case of Poet’s ***BPX™*** process, the Biology of the grain enables conversion of fiber in addition to starch, and allows Poet to engineer processes without the same level of CAPEX and OPEX for conversion. Water consumption and energy intensity is also reduced. Engineering advances continue in the processing of feedstocks for the production of fuel alcohol, but are constrained by the Laws of Physics. Advances in Biology that improve bioprocesses are enabled by advances in data management (DNA), much like Moore’s Law has been a guide to the long term advances in the speed and capability of

computers. The prospects for continued engineering advances is dramatically enabled by Poet's strategy of marrying biology with engineering.

10:30 AM S18: An enzymatic solution to reduce foam effects in Brazilian biorefineries (Remote Presentation)

R.F. Alves, Novozymes Latin America, Curitiba, Brazil*

Brazil is the second largest ethanol producer in the world, which accounts for ~30% of global production, primarily from Sugarcane. In this sense, Brazil has been recognized as an attractive place to develop biomass technologies due to the vast availability of sugarcane as feedstock. Moreover, it is important to note that corn ethanol in Brazil is growing, currently corresponding for ~8% of its ethanol production. In most of Brazilian biorefineries, ethanol is produced via the conversion of sugars present in sugarcane juice and molasses into ethanol by the yeast *Saccharomyces cerevisiae* in a non-aseptic fermentation process, which are normally carried out in fed batch mode with cell recycling. In the past, baker´s yeast strains were used to inoculate the vats, however recently companies started purchasing commercially available fuel ethanol strains. Brazilian ethanol fermentation faces several challenges, including bacterial contamination and foam formation. Foam formation is considered one of the main drawbacks of industrial fermentation process, consisting of gas bubble dispersion on liquid, solids and solid/liquid systems influenced by different factors such as medium composition, gas introducing/formation, and strain-specific characteristics of the production organism. The presence of a yeast strain with a foaming phenotype is highly deleterious for the process productivity, since large volume of the vessel is taken up by foam therefore reducing the working volume and increasing the overall costs due to consumption of antifoam agents (AFA). As a more sustainable solution to reduce foam during ethanol production, Novozymes has developed an enzymatic solution called Fermax which consists in a protease that acts in proteins present in the yeast. Fermax has being applied in more than 50 industrial plants in Brazil, corresponding for 15% of total biorefineries, helping to reduce AFA (30-80%) and improving the economic feasible of Sugarcane mills.

8:30 AM - 11:30 AM Scale Up and Scale Down

Conveners: **Tiffany D Rau**, Rau Consulting LLC, West Lafayette, IN, USA and **Dr. Dongming Xie**, University of Massachusetts Lowell, Lowell, MA, USA

Waller Ballroom - Salon E, Level 3

8:30 AM S10: Leveraging Small Scale Models, Definitive Screening Designs and Machine Learning to Progress Innovative Therapies

T.D. Rau, Rau Consulting, LLC, West Lafayette, IN, USA and P. Ramsey, University of New Hampshire, Durham, NH, USA*

Many novel biologic-based therapeutics are progressing through the Chemistry Manufacturing and Control (CMC) pathway towards approval by either the European Medicines Agency (EMA) or the US Food and Drug Administration (FDA) or both. The development of these molecules and the qualification and validation of the manufacturing processes is multi-faceted. There are a number of tools that are used to develop these novel biologic based therapeutics including scale-down models, scale-up models and new ways to analyze data through machine learning initiatives. In this presentation the CMC pathway will be discussed as well as how leveraging small scale models earlier rather than later

partnered with efficient experimental designs and predictive modeling methods from machine learning speeds understanding and has the potential to decrease timelines

9:00 AM S65: Biomanufacturing of high-value products from oils or fats

D. Xie, University of Massachusetts Lowell, Lowell, MA, USA*

The United States produces more than 10 million tons of waste oils and fats each year. Our group at UMass Lowell aims to establish a new biomanufacturing platform that converts plant oils or animal fats into a series of value-added products, such as wax esters as biolubricants, long-chain diacids for high-performance nylons, and omega-3 fatty acids for nutraceutical or pharmaceutical applications. Here we report our recent research results where the metabolically engineered yeast *Yarrowia lipolytica* was used for production of both wax esters and omega-3 EPA from waste cooking oils. To improve the bioreactor performance with the hydrophobic substrates, computational fluid dynamics (CFD) simulation of the bioreactor system was conducted to identify that the extracellular mixing and mass transfer is one of the major limiting factor of the fermentation process due to the insolubility of oil in water. The CFD simulation results helped to optimize the bioreactor design and operating conditions and successfully enhanced oil uptake and bioconversion in fed-batch fermentation experiments. Our study suggests that waste oils or fats can be economical feedstocks for biomanufacturing of many high-value products.

8:30 AM - 11:30 AM Session: 3: Biotechnology for Deep Decarbonization

Conveners: **David Babson**, Advanced Research Projects Agency - Energy U.S. Department of Energy

Waller Ballroom - Salon A, Level 3

8:30 AM S12: Uprooting orthodoxy in wood production: pursuing net-shape growth of tunable plant materials *in vitro*

A. Beckwith and L. Velásquez-García, Massachusetts Institute of Technology, Cambridge, MA, USA; J. Borenstein, Draper, Cambridge, MA, USA*

Each year, global forests lose billions of trees as a result of human activities and natural disasters. This sustained deforestation impacts both environment and economy. Forests are ecologically essential—supporting biodiversity, stabilizing ecosystems, and sequestering carbon. Meanwhile, trees also supply feedstock for building infrastructure, energy generation, production of consumer goods, textile manufacturing, and an increasing range of other economic activities. Biotechnology may hold the key to satisfying the growing demand for wood and wood-based products while staving off further deforestation and environmental disruption. This work explores the use of cell culture to selectively generate plant-based materials without requiring whole-plant cultivation or harvest. The proposed tissue engineering-style approach allows for localized, high-density production, elimination of energy-intensive harvest and hauling, reduced processing, and inherent climate resilience. Targeted and controllable plant material production also opens the door to new possibilities in the growth of specialized plant materials with properties tuned to meet specific application needs.

Employing a Zinnia elegans model system, we provide the first proof-of-concept demonstration of isolated, tissue-like plant material production by way of gel-mediated cell culture. Parameters including hormone concentrations, medium pH, and initial cell density are shown to quantifiably influence cell development and morphology. Differences in cellular-level culture characteristics are then related to changes in final material properties demonstrating the tunability of grown materials at cellular and macroscopic scales. Control over material form is made possible by the casting and bioprinting of cell-laden scaffolds, illustrating the potential of near net-shape plant material production.

This work demonstrates the implementation of plant cell culture in a new application space, proposes and illustrates novel methods for quantification and evaluation of cell development, and characterizes differences in material properties elicited by changes to cellular growth environment. Most importantly, this proof-of-concept illustrates the promise of a new approach to agriculture; with tunable, land-free, net-shape cultivation of plant materials, wood-based products may one day be obtainable without felling a single tree.

9:00 AM S13: CIRCE: Circularizing industries by raising carbon efficiency

M. Ziesack, S. Nangle and P. Silver, Harvard University, Boston, MA, USA*

Our goal is to shift the bioproduction paradigm and to generate highly reduced carbon compounds from simple, inexpensive feedstocks so that the bioeconomy can move beyond niche, high value products and into the high impact commodity space.

A sustainable future relies on minimizing the use of petrochemicals and reducing greenhouse gas emissions. As industrial bioproduction has grown, economies of scale and use of cheaper feedstocks show promising trends towards the delivery of commodities, such as fuels. Some of the least expensive and most sustainable feedstocks are gases (H_2, O_2, CO_2). Compared to commonly used carbohydrate-based feedstocks, gaseous feedstocks have the potential to be more cost-effective, less land-intensive, have fewer restrictions to delivery in large volumes and have smaller carbon footprints. They represent a transformative shift in our approach to bioproduction.

We use synthetic biology tools to genetically engineer the metabolically flexible *Cupriavidus necator* and develop efficient mixotrophic and lithoautotrophic production modes with state-of-the-art fermentation technology. We are developing a carbon-neutral precision fermentation platform to produce various products including sugars, bioplastics, fertilizers and lipids with applications in fuel and other high impact commodity markets.

This approach allows for co-location at CO_2 and organic carbon sources (e.g., corn ethanol plants) to enhance carbon efficiency and product value of these established bioproduction processes. We will translate research in the lab into real world opportunities and processes for carbon-efficient bioproduction.

9:30 AM Break

10:00 AM S11: Decarbonization Opportunities and Impacts in the Agricultural Sector (R)

C. Scown, PhD, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

The agricultural sector is crucial to achieving deep decarbonization in the United States. Improved land management holds the promise of increasing soil organic carbon sequestration and reduced nitrous oxide and methane emissions. Improved soil quality can in turn improve crop yields and the water holding capacity of soil, thus perpetuating a virtuous cycle for at least a few decades. This presentation will cover a wide range of results, including the impacts of biochar and compost application on agricultural lands, the feasibility of expanding high-yielding bioenergy crop production as a strategy for achieving deep decarbonization, and the practical considerations for carrying out more ambitious strategies such as widespread use of crops engineered for increased carbon sequestration or nitrogen use efficiency, or altering soil microbial communities.

1:00 PM - 4:30 PM Session: 5: Synthetic and Systems Biology for Natural Products Research

Conveners: **Yousong Ding**, University of Florida, Gain, FL, USA and **Joshua Blodgett**

Waller Ballroom - Salon A, Level 3

1:00 PM S22: New regulatory insights from old molecules: using polycyclic tetramate macrolactam comparative metabologenomics to reveal crypticity mechanisms

J. Blodgett[*]*, Washington University in St. Louis., St. Louis., MO, USA*

The genomes of filamentous actinomycetes are rich with biosynthetic gene clusters (BCGs) predicted to encode for drug-like metabolites. However, many BGCs are thought to be silent, where host strains fail to produce genomically-predicted compounds. Understanding and manipulating silent BGCs for biotechnology is a priority goal for the field. In this talk I will review several insights into silent metabolism that our research group has gained by studying various *Streptomyces* bacteria that produce polycyclic tetramate macrolactam (PTM) family antibiotics. PTMs have been known to natural products chemistry since the 1970s. Many congeners are now documented from phylogenetically diverse bacteria, and PTMs are increasingly targeted for synthetic biology due to their BGC simplicity and interesting array of bioactivities. Here we posit that common PTM BGCs offer an exceptional model system for deep regulatory understanding via comparative metabologenomics, and show that promoter heterogeneity and BGC crosstalk within individual strains can strongly tune PTM production outcomes.

1:30 PM S1: Rapid Discovery of Novel Natural Products

H. Zhao[*]*, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Natural products have been a prolific source of bioactive compounds such as antibacterial and anticancer drugs and fungicides. However, despite the pressing need for new bioactive compounds, the rate of natural product discovery is diminishing. Fortunately, the genomics revolution has made it clear that natural product producing microorganisms have the genetic capacity to produce a far greater number of natural products than have been isolated to date. In this presentation, I will briefly introduce a number of strategies my lab developed in the past 10 years for discovering novel natural products with a particular focus on a direct cloning strategy named “Cas12a Assisted Precise Targeted Cloning using *in vivo* Cre-*lox* Recombination” (CAPTURE). This CAPTURE method was used to clone 47 uncharacterized natural product biosynthetic gene clusters (BGCs) ranging from 10 to 113 kb from both Actinomycetes and Bacilli with ~100% efficiency. Heterologous expression of these cloned BGCs led to the discovery of 15 novel natural products including six unprecedented cyclic head-to-tail heterodimers with a unique 5/6/6/6/5 pentacyclic carbon skeleton, designated as bipentaromycins A–F. Four of the bipentaromycins showed strong antimicrobial activity to both gram positive and negative bacteria such as methicillin-resistant *Staphylococcus aureus,* vancomycin resistant *Enterococcus faecium,* and bioweapon *Bacillus anthracis*.

2:00 PM S20: Mining bioactive natural products that mediate interactions in the human skin microbiome

J. Claesen[*]*, Department of Cardiovascular and Metabolic Sciences Cleveland Clinic Lerner College of Medicine, CWRU, Cleveland, OH, USA*

Human skin is a setting for active competition between Actinobacteria and Firmicutes. Our lab is interested in characterizing the bioactive small molecules involved in these microbe-microbe interactions, as well as the molecular mechanisms by which select members of our skin microbiota are linked to disease. We use a combination of activity-driven and genomics-guided approaches and identified compounds with antibacterial, biofilm inducing and immunomodulatory functions [1, 2].
While *Corynebacterium spp.* are dominant members of our skin microbiota, little is known about how they are sensed by our immune system. Our genetic analysis identified corynomycolic acids as a driver that causes increased numbers and activation of γδ T cells in mouse skin [1]. Under normal conditions, corynomycolic acids did not elicit an inflammatory response. However when *Corynebacterium* was applied to mice on a high fat diet, they presented with psoriasis-like skin inflammation, linking the immune interaction of the skin microbiota to host metabolic state [1].

We identified a biosynthetic gene cluster (BGC) encoding a thiopeptide antibiotic in the genome of select *Cutibacterium acnes* isolates [2]. Analysis of longitudinal metagenomic data from multiple skin sites revealed that this BGC is widely distributed across skin sites. We characterized the BGC product in *C. acnes* liquid culture and heterologous expression allowed for structural elucidation of the thiopeptide antibiotic, dubbed cutimycin. Nanomolar amounts of cutimycin inhibited many *Staphylococcus aureus* and *epidermidis* strains, but not other common members of the skin microbiome. We detected *in vivo* cutimycin production in the pooled hair follicle content of healthy individuals and observed a positive correlation between the presence of the cutimycin BGC and reduced *Staphylococcus* colonization [2].

Identification of molecular interactors produced by human commensals contributes to a better understanding of the complex interplay that takes place in our microbiome. This will facilitate the development of therapeutic strategies aimed at immune modulation or microbial community alteration and pathogen elimination.

1. Ridaura, *et al*. (2018) J Exp Med, 215:785-799.
2. Claesen, *et al*. (2020) Sci Transl Med, 12:eaay5445.

2:30 PM Break

3:00 PM S21: Predicted cyclic peptide natural products for antibiotic discovery

M. Hostetler, C. Smith, S. Nelson, Z. Budimir, R. Modi, I. Woolsey, A. Frerk, B. Baker, J. Gantt and E. Parkinson, Purdue University, West Lafayette, IN, USA*

Identification of novel bioactive molecules is critical to the development of new therapeutics. Natural products (NPs) have historically been a bountiful source of bioactive molecules, with NPs and their derivatives making up 64% of FDA-approved small molecule drugs. Unfortunately, discovery of novel bioactive NPs is very challenging due to a myriad of issues including cryptic biosynthetic gene clusters, low titers, arduous purification schemes, and difficult to culture organisms. Herein, we describe a novel method for the identification of bioactive molecules inspired by NPs. This method significantly expedites the discovery of bioactive molecules from bacterial sources by combining bionformatics predictions of non-ribosomal peptide synthetase (NRPS) biosynthetic gene clusters with chemical synthesis of the predicted NPs (pNPs). We utilize a recently discovered peptide cyclase, the penicillin binding protein (PBP)-like cyclase, as the bioinformatics lynchpin for the discovery and development of a library of cyclic peptide pNPs. Mining of publicly accessible genomes in the NCBI database revealed that 396 of the top 500 BGCs containing PBP-like cyclases co-occurred with NRPS genes. Further analysis of the NRPS containing biosynthetic gene clusters resulted in the identification of 131 unique and novel cyclic peptide pNPs. Solid phase peptide synthesis and in-solution cyclization allowed easy access to 52 diverse pNPs. Antibacterial testing of these molecules revealed 14 pNPs with antibiotic activity, including several with activity against multidrug resistant Gram-negative bacteria. Overall, we demonstrate the power of combining bioinformatics predictions with chemical synthesis to greatly accelerate the discovery of bioactive molecules.

3:30 PM S23: Manufacturing malonyl-CoA derived bioproducts, from malonates to cannabinoids

A. Conley, Lygos Inc., Berkeley, CA, USA*

Lygos is a leading innovator in developing and commercializing technologies for the manufacturing of bio-advantaged chemicals and polymers, replacing environmentally harmful and expensive petrochemical processes with cost-competitive and sustainable biological processes. Lygos uses acid-tolerant yeasts to fermentatively produce organic acids that serve as platform chemicals for conversion into other products. For example, malonic acid and its dimethyl and diethyl esters are high-performance, versatile chemicals used in the production of a variety of novel industrial and consumer products including biopolymers,

industrial coatings, flavours, fragrances and pharmaceuticals. Given the tremendous complexity of biological systems and to advance the performance of the malonate strains, Lygos has recently incorporated time-series multi-omics data analysis and machine learning to provide a predictive model of pathway dynamics and to accelerate microbe optimization. As an extension of this work, Lygos is now leveraging its modular yeast platform and its expertise and capabilities in malonyl-CoA metabolism to enable economical production of numerous highly pure and consistent cannabinoids for consumer and pharmaceutical applications. In this presentation, some of the key technical challenges related to the production of these malonyl-CoA derived products will be discussed.

4:00 PM S24: CRAGE enables rapid activation of biosynthetic gene clusters in undomesticated bacteria (R)

Y. Yoshikuni, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

It is generally believed that exchange of secondary metabolite biosynthetic gene clusters (BGCs) among closely related bacteria is an important driver of BGC evolution and diversification. Applying this idea may help researchers efficiently connect many BGCs to their products and characterize the products' roles in various environments. However, existing genetic tools support only a small fraction of these efforts. Here, we present the development of chassis-independent recombinase-assisted genome engineering (CRAGE), which enables single-step integration of large, complex BGC constructs directly into the chromosomes of diverse bacteria with high accuracy and efficiency. To demonstrate the efficacy of CRAGE, we expressed three known and six previously identified but experimentally elusive non-ribosomal peptide synthetase (NRPS) and NRPS-polyketide synthase (PKS) hybrid BGCs from *Photorhabdus luminescens* in 25 diverse γ-Proteobacteria species. Successful activation of six BGCs identified 22 products for which diversity and yield were greater when the BGCs were expressed in strains closely related to the native strain than when they were expressed in either native or more distantly related strains. Activation of these BGCs demonstrates the feasibility of exploiting their underlying catalytic activity and plasticity, and provides evidence that systematic approaches based on CRAGE will be useful for discovering and identifying previously uncharacterized metabolites.

1:00 PM - 4:30 PM Session: 6: Metabolic Engineering for Fuels and Chemicals I (commodity chemicals)

Conveners: **Rajib Saha**, UNL

Waller Ballroom - Salon C-D, Level 3

1:00 PM S25: Systems and synthetic biology advancements to improve *Synechocystis* sp. PCC 6803 strain engineering in the industrially-relevant condition of diurnal light-dark cycles

C. Peebles, Colorado State University, Fort Collins, CO, USA*

Cyanobacteria are an interesting chassis for industrial chemical production due to their ability to utilize sunlight and carbon dioxide as substrates. However, much of the strain engineering has been done under low- and continuous- light laboratory conditions as opposed to the realistic day/night cycle of outdoor sunlight availability. Our lab previously demonstrated that engineered free fatty acid production is decreased in daily light-dark cycles as opposed to continuous light relative to wild-type. This observation motivated system and synthetic biology developments to improve strain engineering efforts specifically in realistic day/night cycles. Toward this goal, we have improved systems biology understanding and developed synthetic biology tools for use in day/night cycles. Specifically, we discovered and characterized four native *Synechocystis* sp. PCC 6803 promoters which enable light-activated gene expression in daily light-dark cycles. We engineered a photobioreactor system which enables diurnal

sinusoidal light cycles with peak-light intensities reaching over 1,500 µmol photons m^{-2} s^{-1}. We developed and implemented a multi-platform 'omics study investigating the dynamic behavior of *Synechocystis* sp. PCC 6803 in sinusoidal day/night cycles. We observed widespread oscillations of metabolism in these conditions. Microbial growth displayed distinct lag, biomass accumulation, and cell division phases of growth. During the lag phase, amino acids (AA) and nucleic acids (NA) accumulated to high levels per cell followed by decreased levels during the biomass accumulation phase, presumably due to protein and DNA synthesis. We have also engineered and optimized bisabolene production in *Synechocystis* sp. PCC 6803 reaching 7.8 mg/L titers after 5 days and have investigated strategies to improve reliable protein expression in cyanobacteria. Together, these advances contribute to the advancement of *Synechocystis* sp. PCC 6803 as an industrially-relevant chassis for chemical production.

1:30 PM S26: Engineering organisms for oleochemical production

Q. Yan and B. Pfleger, University of Wisconsin, Madison, WI, USA*

Microbial production of oleochemicals from renewable feedstocks remains an attractive route to produce high-energy density, liquid transportation fuels and high-value chemical products. Metabolic engineering strategies have been applied to demonstrate production of a wide range of oleochemicals, including free fatty acids, fatty alcohols, esters, olefins, alkanes, ketones, and polyesters in both bacteria and yeast. The majority of these demonstrations synthesized products containing long-chain fatty acids. These successes motivated additional effort to produce analogous molecules comprised of medium-chain fatty acids, molecules that are less common in natural oils and therefore of higher commercial value. Substantial progress has been made towards producing a subset of these chemicals, but significant work remains for most. The other primary challenge to producing oleochemicals in microbes is improving the performance, in terms of yield, rate, and titer, of biocatalysts such that economic large-scale processes are feasible. Common metabolic engineering strategies include blocking pathways that compete with synthesis of oleochemical building blocks and/or consume products, pulling flux through pathways by removing regulatory signals, pushing flux into biosynthesis by overexpressing rate-limiting enzymes, and engineering cells to tolerate the presence of oleochemical products.
In this talk, I will present our efforts on engineering gatekeeper enzymes to improve chain-length selectivity. In the first half, I will describe designed and tested metabolic pathways in *Escherichia coli* to specifically produce 2-heptanone, 2-nonanone and 2-undecanone. We achieved substantial production of each ketone by introducing chain-length specific acyl-ACP thioesterases, blocking the β-oxidation cycle at an advantageous reaction, and introducing active β-ketoacyl-CoA thioesterases. In second part of the talk, I will highlight the development of a novel acyl-ACP:CoA transacylase strategy for producing medium-chain oleochemicals in bacteria. Through bioprospecting, mutagenesis, and metabolic engineering, we developed strains of *Escherichia coli* capable of producing over 1 g/L of medium-chain free fatty acids, fatty alcohols, and methyl ketones using the transacylase strategy. I will conclude with commentary on the remaining challenges in the field where further research investment could prove fruitful.

2:00 PM S27: Development of emerging model microorganisms: *Megasphaera elsdenii* for biomass and organic acid upgrading to fuels and chemicals

N. Wood, Z. Obenhoff and J. Westpheling, University of Georgia, Athens, GA, USA; L. Riley and A. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

The development of industrial processes that rely on biological systems often requires the use of non-model microbes that have the natural ability to do things impossible to engineer. The native ability of *M. elsdenii* to condense acetyl-CoA to efficiently generate C4 to C8 compounds makes it a compelling platform for the production of next-generation, drop-in fuels and chemicals from lactate and plant carbohydrates. Our objective is to develop *M. elsdenii* as a platform for the conversion of lignocellulosic biomass and organic acids to long chain alcohols and other valuable chemicals. *M. elsdenii* produces organic acids, (including butyric (C4), valeric (C5), hexanoic (C6), and in some cases octanoic (C8) acid)

when grown on lactate or glucose, likely via a chain elongation pathway using acetyl-CoA. Fuel properties improve with carbon chain length, making hexanol an appealing target as a next-generation gasoline blend stock. Previous efforts to make such products in *E. coli* have been moderately successful, but production of C6 and larger products remains low, suggesting that extending the chain elongation pathway beyond a single cycle remains a significant challenge in model organisms. We developed a method for DNA transformation of *M. elsdenii* via methylome analysis and heterologous expression of DNA methyltransferases in *E. coli* to protect DNA from restriction on transformation into *M. elsdenii.* Heterologous expression of an *adhE2* gene from *Clostridium acetobuticulum* resulted in the producton of 5.3 mM butanol in *M. elsdenii* ATCC 25940 grown on lactic acid, representing 7.4% of the detected fermentation products. We developed a gene deletion system based on a counter-selectable genetic marker, and deletion of a propionyl-CoA transferase in the *M. elsdenii* chromosome resulted in increased production of long chain fatty acids. *This is the first demonstration of metabolic engineering in Megasphaera and proof of concept that this approach may lead to the accomplishment of our longer-term goals.*

2:30 PM Break

3:00 PM S28: Second level onboarding of Yarrowia lipolytica for the production of sustainable chemicals

M. Blenner, University of Delaware, Newark, DE, USA*

Strain engineering is necessary to produce biochemical products at industrially relevant titers and rates. Host onboarding is the process of establishing tools and genomic knowledge to allow basic genetic manipulations. Yarrowia lipolytica has advanced beyond this point but still significantly lags behind several bacterial hosts and the model yeast S. cerevisiae. This talk describes our work towards second level onboarding, in the form of advanced genetic engineering tools for Yarrowia lipolytica and their application to better understanding and engineering of cellular metabolism. We will describe novel enzyme localization tags for spatial control of biocatalysis, new efficient pathway integration tools, finely tuned promoter libraries, and genome scale screens for improving cellular phenotypes.

3:30 PM S29: Metabolic engineering of *Lactococcus lactis* to produce biorenewable chemicals from lignocellulosic biomass

S. Rothstein, S. Sen and T. Mansell, Iowa State University, Ames, IA, USA*

Lignocellulosic biomass is an abundant source of carbon, especially in agricultural areas like Iowa. Fast pyrolysis is one method that can be used to create fermentable sugars from cellulosic sources, but product of this process, bio-oil, is rich in (1) levoglucosan, an anhydrosugar form of glucose, and (2) phenolic and other inhibitory compounds. Here we report the engineering of *Lactococcus lactis* to utilize purified levoglucosan for the production of enantiomerically pure L-lactic acid and, by expressing a heterologous three-gene pathway, 1,2-propanediol. We also report media conditions that allow the production of these chemicals from diluted bio-oil and strategies to engineer tolerance to bio-oil and other "dirty" substrates. In addition, we have developed an in trans reporter system to rapidly integrate transcription factors, enabling tunable heterologous protein expression. Finally, we report the development of CRISPR-assisted genome-scale engineering and library creation tools in this important industrial organism.

4:00 PM S30: Developing a synthetic biology toolbox for a lignin-consuming bacterium and its application to mevalonate biomanufacturing

K. Tyo, PhD, Northwestern University, Evanston, IL, USA*

Utilization of lignin, an abundant renewable resource, for synthesis of fuels and chemicals is limited by its heterogenous composition and complex structure. Biological valorization of lignin has the potential to more readily deal with this heterogeneity than chemical approaches. To this end, several microbes have been investigated for the biomanufacturing using lignin and other aromatic substrates. In this talk, we will present work using the soil microbe *Acinetobacter baylyi* ADP1 which has unique advantages for lignin valorization. Aside from possessing the β-ketoadipate pathway to consume lignin-related aromatics, ADP1 has the unique abilities of being naturally competent (take up DNA under normal growth conditions) and highly recombinogenic with its genome. These properties make it highly amenable to engineering. To enable sophisticated engineering strategies in ADP1, we first sought to optimize several standard engineering work flows to create a synthetic biology toolbox for future efforts. We optimized the transformation protocol to allow both easy plasmid ligation and integration into the genome. We generated a library of 15 promoters and 5 ribosome binding sites that enabled more than 100 fold range of expression, and we mapped 30 genomic locations for reliable integration. Most significantly, we developed a Cas9-based single-step marker-less and scar-less genomic integration method. This Cas9 method makes many serial genomic modifications possible without the need for multiple genetic markers. We used this synthetic biology toolbox to evaluate the synthesis of mevalonate, a polymer precursor, from aromatic substrates in ADP1. While initial mevalonate titers were less than 10 mg/L, we found that the process was highly amenable to fed batch pushing titers to greater than 100 mg/L. After knocking out a competing wax ester pathway, mevalonate titers were further boosted to greater than 800 mg/L in fed batch. This work establishes a foundation for future efforts to engineer improved production of mevalonate and derivatives from lignin-derived aromatics using ADP1.

1:00 PM - 4:30 PM Session: 7: Fermentation and impact on Formulation and shelf life stability

Conveners: **Farzaneh Rezaei**, Pivot Bio and **Mark Mikola**

Waller Ballroom - Salon F, Level 3

1:00 PM S31: Development of a single-use fermentation technology for extending shelf life of living microbial products.

J. Fife, CTO, 3BAR, 3Bar Biologics, Columbus, OH, USA*

Microbial technologies that exploit plant-microbe interactions for increasing nutrient use efficiency and crop productivity present a new paradigm in sustainable crop production. Translating microbial technologies that work well in a research setting into a commercial product remains challenging. Often cited reasons for not moving forward with commercialization is the lack of effective formulations for shelf life, particularly for gram-negative, non-spore forming microbes. Typically, 99%+ of viable microbes die or become compromised during processing, shipping, and storage in the supply chain. Current microbial products are fermented in large stainless-steel vessels, then often concentrated in order to increase populations above label limits to account for the loss of viable microbes in the supply chain. Additionally, refrigeration is often required for distribution and storage. As the production scale gets larger, variations in fermentation conditions (temperature, dissolved oxygen, nutrients, shear stresses) create stressful conditions for the microbes leading to challenges in maintaining microbe stability and risk of contamination during scale up in the large vessel during production. To overcome these challenges, 3Bar Biologics developed a disposable, single-use fermentation system involving fermentation in the final packaging; thus, eliminating scale-up issues and providing increased flexibility and lower risk of contamination during biomanufacturing. The patented technology consists of a proprietary cap that holds the stabilized microbe dry formulation and a second chamber containing a liquid nutrient medium and gas. After aseptic transfer (“activation”) of the inoculum from the cap into the liquid medium, the microbes grow exponentially and within 24-48 hours the product is ready for use. Consistent fermentation of the microbes in the single-use system shows growth of populations ranging from 1E8 CFU/mL to 1E9

CFU/mL, depending on the microbe and nutrient medium used. Product shelf life of greater than one-year has been demonstrated for a commercialized gram-negative microbe in an agricultural supply chain.

1:30 PM S33: When Fermentation KPI becomes Kreate Product Innovation (R)

N. Kreamer, Ph.D., PivotBio, Berkeley, CA, USA*

The majority of time in biotechnology, the ultimate goal in fermentation is to produce an enzyme or chemical product. While production of whole cell, biomass as final product is gaining more attention, still it is less common and mainly focused on spore forming gram positive bacteria or fungi. A greater challenge is posed when the product is a live gram-negative microbe that requires robust shelf-life sufficient to support commercial business operations. Unlike gram positive spore formers, gram negatives are very sensitive and can rapidly die in package, representing a unique challenge to ferment and formulate. Another challenge is lack of biomarkers or indicators for whole cells that could predict a cell's ability to withstand harsh conditions necessary to generate shelf stable products. We leveraged the standard approach of optimizing for product yield in fermenter and translated that to a key performance indicator (KPI) of high cell titer both at the end of fermentation and in package. This approach resulted in the need to redefine KPI to Kreate Product Innovation, that indicates a technology and knowledge gap in defining fermentation KPIs which support formulation stability when dealing with whole cell gram negative as a product.

2:00 PM Break

1:00 PM - 4:30 PM Session: 8: Genomic and molecular biology tools across prokaryotic and eukaryotic systems

Conveners: **Fiona Crocker**, US Army Engineer Research and Development Center, Vicksburg, MS, USA and **Kang Wu**, Chemical Engineering, University of New Hampshire, NH, USA

Waller Ballroom - Salon E, Level 3

6:00 PM S34: Microbial trait data predicts differential soil carbon processes in forested wetlands and tidal marshes

E. Weingarten, ORISE Fellow - US Army Engineer Research and Development Center, Vicksburg, MS, USA; C. Jung, N. Hurst, F. Crocker, J. Berkowitz and K. Indest, US Army Engineer Research and Development Center, Vicksburg, MS, USA*

Landscape perturbations occur over a wide range of spatiotemporal scales due to natural and anthropogenic events. These can occur suddenly (e.g. storm surge) or gradually (e.g. sea level rise). Collectively, these processes can amplify terrain degradation, impacting site assessments and emergency operations in the short-term and ecosystem services in the long-term. The objective of this effort is to develop innovative processes for measuring, analyzing, and predicting microbial traits (biomass, respiration, diversity, and community composition and function) across environmental gradients or soil taxonomies. These data will be integrated with additional data layers pertaining to the geochemistry of soils and sediment, plant cover, and other physicochemical characteristics to provide meaningful visualizations and data products to assist decision makers and emergency managers in coastal regions in assessing terrain modification over different time periods.
The microbiomes of four wetland vegetation types on the U.S. Gulf coast, which exist along a salinity gradient, were examined by high-throughput sequencing and found to differ in composition ($p<0.001$).

Microbial richness ($p<0.001$) and diversity ($p<0.001$) differed between vegetation types; higher in intermediate salinity *Typha spp.* and tidal shrub wetlands. Highest salinity *Juncus spp.* marsh had higher β-glucosidase activity ($p\leq0.015$) than any other vegetation type. Microbial biomass was influenced by vegetation ($p=0.035$) and was highest in forested wetlands. However, microbial growth was slowest in forested sediment ($p<0.001$). Potential methanogenesis was lower in forest than in marsh ($p<0.001$), while aerobic respiration was higher in forests than in marsh ($p<0.001$). This coincided with lower carbon fixation in forest sediment.

Forested wetlands were lower in diversity, microbial growth, and carbon degradation activity than emergent intermediate-to-brackish salinity marsh. This correlated with higher belowground biomass. Potential methanogenesis from forests was lower than from marsh, but aerobic respiration was higher. These findings demonstrate a fundamental difference in soil microbial processes between forested swamps and tidal marshes. Incorporation of rich microbial trait data will be critical to developing better soil carbon models, particularly in vulnerable coastal wetlands.

1:30 PM S35: Why is my lake the same color as my lawn? An ecophysiological examination of cyanobacterial harmful algal blooms in western Lake Erie

T. Davis, Bowling Green State University, Bowling Green, OH, USA*

Understanding the ecological and climatic drivers of CHAB development and toxin concentration are of growing importance, especially for freshwater systems that supply drinking water such as western Lake Erie. While it is well known that cultural eutrophication is a primary driver of CHABs, there is still debate over the roles of nitrogen (N) and phosphorus(P) in stimulating the growth and the production of toxins. While it has been shown that estimates of bloom size can be made using spring P-loading values from the Maumee River to Lake Erie, to date no such model exists for estimating bloom toxin concentration. I will present the results from long-term ship-based and in situ monitoring, microcosm experiments and advanced genetic techniques. Initial metagenomic and metatranscriptomic results have shed light on why a non-N-fixing cyanobacterium (*Planktothrix*)can dominate in a chronically N limited system and reveal mechanisms that *Microcystis* can use to outcompete other phytoplankton during times of P limitation. Furthermore, omics approaches have elucidated the ecophysiological drivers that initiated the 2014 Toledo water crisis and have highlighted the importance of the phycosphere to bloom-forming organisms like *Microcystis*. Finally, shifts in regional climatology, specifically warming and more extreme hydrologic events, leading to increased nutrient loading is predicted to exacerbate the western Lake Erie CHABs. However, one factor that is often overlooked is the potential shift in the initiation and duration of the bloom that may accompany earlier increases in spring water temperatures and warmer waters later into the fall, respectively. Our findings suggest that if the biogeochemical conditions for bloom formation in western Lake Erie persist, rising temperatures will lead to an earlier onset and longer growing season for the *Microcystis*-dominated blooms. In conclusion, CHABs in western Lake Erie are complex and recent studies highlight the need to develop dual nutrient control strategies to effectively mitigate them. If action is not taken soon, climate change will likely impact bloom formation, toxin concentration and duration which will make mitigation efforts more difficult.

2:00 PM S36: 'Omics analyses of the hydraulically-fractured shale isolate *Halanaerobium* highlights membrane modifications that underpin adaptation under deep subsurface biogeochemical drivers (R)

F. Colosimo and P. Mouser, PhD, University of New Hampshire, Durham, NH, USA; S.O. Purvine, H. Olson, A. Wong, E. Eder, R. Chu and D. Hoyt, Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, USA; S. Callister and J. Kyle, Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA*

The Gram-positive *Halanaerobium* spp. is a dominant bacterial genus across geographically distinct fractured shale formations, which are increasingly used for natural resource extraction. These bacteria

encounter harsh physicochemical conditions in the deep terrestrial biosphere, including high temperatures, brine-level salinities, anoxia, and elevated pressures. Microbial membranes act as the first line of defense against these environmental stressors, and maintaining membrane functionality during changing environmental conditions requires careful regulation of intact lipid composition and membrane-embedded proteins. To investigate membrane response to varying growth rates and temperatures, we cultivated *Halanaerobium congolense* WG10 in continuous culture (chemostats) for the first time. 'Omics analysis of metabolites, proteins and lipids for steady state cells revealed that *Halanaerobium* alters its cell membrane to maintain membrane fluidity while maximizing growth rate. *Halanaerobium* modulates the ratio of phospholipid headgroups in response to changes in temperature, and increases cardiolipin and phosphatidylethanolamine polar lipid abundance with increasing carbon availability. We also observed higher abundance of neutrally charged simple glycerol lipids at lower temperature and growth rates, while glycerophospholipids with larger polar heads and zwitterionic lipids prevail at warmer temperatures and faster growth rates. Proteomics analysis identified a total of 2,227 out of 2,800 predicted protein-coding genes. Among these, 356 proteins were found to be significantly higher in abundance in one or more treatments, including known stress regulators involved in cellular envelope homeostasis such as cold shock proteins (CspA), a typA, bipA GTP binding protein involved in stress response, and a nucleotide-binding universal stress protein (UspA). We also identified lipid-A synthesis proteins at lower temperature or high growth rate, a lipopolysaccharide endotoxin uncommonly found in Gram-positive bacteria. Collectively, our 'omics continuous culture approach sheds new light on the metabolism and membrane features of the halotolerant bacterium *Halanaerobium* under biogeochemical drivers relevant to engineered shale, with implications on membrane charge, permeability, and metabolism.

2:30 PM Break

1:00 PM - 4:30 PM Session: SO: Student Oral Session

Conveners: **Katherine Chou**, NREL and **Stephanie Gleason**

Waller Ballroom - Salon B, Level 3

1:00 PM S124: Gene expression profiling of sulfate reducing bacteria under varying copper concentrations

A.K. Tripathi, P. Saxena, P. Thakur, S. Rauniyar, R. Singh, V. Gopalakrishnan and R. Sani, Professor, South Dakota School of Mines and Technology, Rapid City, SD, USA*

Microbial induced corrosion (MIC) accounts for 50% of the annual corrosion costs in USA with sulfate reducing bacteria (SRB) contributing to ~$5 billion dollar of the costs. Copper (Cu) has been the material of choice for piping in industries because of its antimicrobial and corrosion resistant properties. However, certain species of SRB induce MIC. Our research focuses on a model SRB (i.e., *Desulfovibrio alaskensis* G20, DAG20) which corrodes Cu. The molecular response mechanisms of DAG20 to Cu exposure is not well understood. Here, we combine physiological, and transcriptomic analysis to provide, for the first time, a comprehensive view on the pathways activated in DAG20 in response to toxic levels of Cu under planktonic conditions. Transcriptomics analyses show that 47.4% genes were downregulated and 52.6% were upregulated in 5µM Cu (vs Control), while 49.7% genes were upregulated and 50.3% were downregulated in 15µM Cu (vs Control). Detailed analysis of the differently expressed genes suggests involvement of novel putative molecular mechanisms (heavy-metal translocation and metal-ion binding) in DAG20 response to Cu. Radical SAM-domain containing protein which is responsible for metal ion binding was upregulated , while the flagellar basal body protein which helps in motility, was downregulated in the presence of Cu. Moreover, results indicated that important transcription factor families associated with stress responses were differentially expressed in Cu. Our analysis has allowed

for the identification of possible molecular mechanisms adopted by DAG20 to cope with Cu toxicity. These findings are expected to accelerate the development of MIC mitigation strategies involving SRB.

6:00 PM S125: Quantifying sulfate reduction rates in biofilm on shale fracture walls of a microfluidic reactor

L. Zhou and C.J. Werth, The University of Texas at Austin, Austin, TX, USA; G.A. Fried, R.A. Sanford, M. Sivaguru, A.S. Bhattacharjee and B.W. Fouke, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Microbial sulfate reduction in hydraulically fractured shale is problematic as sulfate reducing bacteria (SRB) growth promotes bioclogging in preferential flow paths and decreases hydrocarbon production, while hydrogen sulfide production creates a pipeline corrosion and human health hazard. Few studies have quantified biomass growth and activity in shale fractures due to the complexity of studying processes at this scale, resulting in a knowledge gap regarding the ability of microbes to colonize shale fracture surfaces and clog these oil and gas flow conduits. To address this gap, a natural shale sample is shaped to create two inlets that feed into a single 250 µm-wide fracture, all contained within a microfluidic platform. This shale-based microfluidic flowcell reactor is inoculated with a field culture dominated by SRB, followed by incubation and increase in nutrient flux. Microscopic images and effluent samples are analyzed to assess biofilm growth and biocide inhibition, with a numerical model developed to quantify sulfate reduction rates. The results indicate that biomass grows as biofilms on shale surfaces with little mass transfer limitations, and that accurate quantification of sulfate reduction rates depends on quantifying this biomass.

6:00 PM S126: Tinkering in the Third Domain: Using Extremophilic Archaea as a Production Platform and Enzyme Source for Synthesis of Renewable Bioproducts

S. Carr and N. Buan, University of Nebraska Department of Biochemistry, Molecular Trait Evolution, LLC, Lincoln, NE, USA; P. Blum, University of Nebraska School of Biological Sciences, Molecular Trait Evolution, LLCUniversity of Nebraska - Lincoln, Lincoln, NE, USA; S. Kopf, Grinnell College, Grinnell, IA, USA*

Archaea have adapted over millions of years to thrive in nearly every environment on Earth. Amongst the most resilient of these are extremophiles, microbes which thrive in environments that push the boundaries of life. Extremophiles can be a rich resource of unique enzymes and have promising potential as bioproduction platforms. Here I discuss two cases in which conventional biotechnologies were improved by exploring the distinctive physiologies of extremophilic archaea. Methanogens are obligately anaerobic archaea noteworthy for producing methane from C1 compounds and acetate. The energetic limitations of these low-energy substrates require methanogens to utilize a highly efficient central metabolism which greatly favors respiratory byproducts over biomass. This metabolic strategy creates high substrate:product conversion ratios which is industrially relevant for the production of biomethane, but may also allow for the production of value-added commodities. Like all archaea, methanogens synthesize isoprenoid membranes utilizing the high flux mevalonate pathway, making them ideal to produce terpenoids. We found that the engineered methanogens directed up to 4% of total carbon towards isoprene production with increased biomass. To aid in biomass conversion to value added products, we developed an enzyme cocktail from thermoacidophilic archaea, EXTREMASE, which liberation of sugar from cellulosic feedstocks at extreme temperature and pH conditions. High rates of monosaccharide (glucose and other sugars) were liberated from a broad range of energy crop plants including corn, sorghum, wheat and most recently nut shells (almond). By optimizing production of thermophilic enzymes in mesophilic organisms, we allow for extremely heat tolerant cellulases to be produced without specialty culturing equipment. In this presentation I discuss how organisms are chosen for their unique physiology and how extremophilic archaea could be used to improve existing biotechnology processes.

2:00 PM Break

6:00 PM S127: Discovery of the non-proteinogenic amino acid homoarginine provides a key to unlock cryptic natural product biosynthetic pathway

I. Mohanty, S.G. Moore, D.A. Gaul, N. Garg and V. Agarwal, Georgia Institute of Technology, Atlanta, GA, USA*

L-Homoarginine (hArg) is a non-proteinogenic amino acid present in the human metabolome. Together with the proteinogenic amino acid, L-arginine, hArg is a substrate for nitric oxide synthases leading to the production of the vasodilator nitric oxide. Due to its role in nitric oxide production, the abundance of hArg in blood plasma is negatively correlated with cardiovascular and renal dysfunction. While the function of hArg and its relevance as a disease biomarker in mammalian physiology is well validated, the presence and role of hArg in other biological systems has received lesser attention. We recently reported the detection of hArg in the benthic marine invertebrate animals - marine sponges. Sponges and their symbiotic bacteria produce small organic molecules called natural products. These natural products are produced to perform specific biological functions which make them suitable lead candidates for pharmaceuticals. Two such classes of biologically active natural products were detected in the sponges within our study - pyrrole-imidazole alkaloids and brominated tyrosine alkaloids. They are a large and chemically complex class of sponge natural products with many congeners. Despite their varied diversity, insights into their biosynthesis are scant. Querying the sponge metabolomes for plausible intermediates in their biosynthetic pathways, hArg was discovered in high abundance. We rationalized hArg to be a biosynthetic precursor for pyrrole-imidazole and two brominated tyrosine alkaloids, serving as a critical branch point to connect primary metabolite lysine with the production of these natural products. Further, hArg is a substrate for the production of nitric oxide, a metabolite with effects on marine larval settlement and development. Our study provides the framework for further investigation of the biosynthesis and role of hArg in marine sponge holobionts. We have used less than 1 g of biomass for each sponge specimen, highlighting the applicability of contemporary -omic technologies in transcending the limitation of biomass availability.

2:40 PM S3: Genome-wide cutting scores enable sgRNA activity predictions and definition of essential genes in the yeast *Yarrowia lipolytica*

A. Ramesh, D. Baisya, V. Trivedi, S. Lonardi and I. Wheeldon, UC Riverside, Riverside, CA, USA*

Non-conventional organisms are attractive targets for metabolic engineering as they can present a range of desirable traits that help avoid complex and intensive metabolic engineering of less suitable model hosts. As a drawback, their genome and metabolic networks are often less well understood, and they typically lack the range of genetic engineering tools available in conventional hosts. *Yarrowia lipolytica* is one such non-conventional yeast with an abundant native acetyl-CoA pool and a capacity to produce and accumulate lipids to high levels. While there have been significant advances in the metabolic engineering of this yeast for the biosynthesis of oleochemicals and other value-added products, there is also a lack of synthetic biology tools for functional genomic screening and rapid strain development. We have sought to overcome these limitations by developing CRISPR-Cas9/Cas12a systems for gene knockout, integration, regulation, and genome-wide screening. However, prediction of highly active sgRNA which are crucial in effective genome editing and improving confidence in hit calling, remains a challenge. In addition, *Y. lipolytica* lacks a well-defined consensus set of essential genes that would help further our understanding of this organism and ease metabolic engineering efforts. Thus, we constructed two genome-wide libraries, one using SpCas9 and the other using LbCas12a, to target all protein coding sequences. In the absence of DNA repair by non-homologous end joining, screens provided a cutting score (CS) for each guide, while screens in the wild type background provided a fitness score (FS) for each gene. We used the genome-wide CS values to develop a new machine learning based guide-activity prediction algorithm called DeepGuide, and also used these values to provide a guide-activity correction to more accurately

determine FS for each gene in the genome. Combined with results from a previously published essential gene set identified using a transposon screen, the outcomes of our CRISPR screens define a consensus set of essential genes for *Y. lipolytica*.

3:00 PM S129: Development of a point-of-use treatment strategy for *Legionella pneumophila* and amoebae hosts using microwave radiation and silver ions or nanoparticles

S. Marien, C. Ayres, M.J. Kirisits, N. Saleh, D. Lawler and E. Cambronne, The University of Texas at Austin, Austin, TX, USA*

The opportunistic human pathogen *Legionella pneumophila* poses a unique health hazard because it can persist and proliferate in premise plumbing systems despite upstream drinking water treatment. This virulent bacterial pathogen can cause a severe and deadly form of pneumonia, called Legionnaires' disease, when contaminated aerosols are inhaled. Within the water sector, this public health risk is exacerbated by the proliferation of *L. pneumophila* in premise plumbing within free-living amoebae. These protozoan hosts provide significant resistance to residual disinfection and serve as a vector for *Legionella* of enhanced infectivity. Both *L. pneumophila* and associated amoebae hosts are however, vulnerable to thermal treatment, an established emergency protocol for *Legionella* control in hotels and hospitals. We aim to implement thermal treatment at the point-of-use using microwave (MW) radiation as the energy source, utilized in concert with silver. We hypothesize that MW irradiation used in concert with silver could lead to enhanced inactivation through facilitated ion uptake and/or localized heating on the cellular surface. In this study, planktonic *L. pneumophila* cells are exposed to ionic silver (Ag^+) or silver nanoparticles (AgNPs) in the presence or absence of MW radiation. While neither silver treatment alone is effective for inactivating *L. pneumophila* over a short exposure period (18 min), a combined treatment of silver with MW radiation successfully produces 3-4 log inactivation of *L. pneumophila* within 6 min of irradiation. Additional inactivation is observed when *L. pneumophila* is pre-exposed to either treatment (i.e., MW radiation or silver) prior to exposure to the other. We observed a rapid association of ionic silver and AgNPs with *L. pneumophila* cells during the pre-exposure period; silver at the cell surface could provide localized heating upon MW radiation (due to the high thermal conductance of silver) and also could act as a local source for silver transport into the cell. Data presented support the development of a *L. pneumophila* inactivation device that harnesses MW radiation and can potentially mitigate this public health risk.

4:30 PM - 5:30 PM Korean Society for Microbiology and Biotechnology (KMB) Lecture

Conveners: **Kun-Soo Kim**, Korean Society for Microbiology and Biotechnology, Seoul, Korea, Democratic People's Republic of (North)

Waller Ballroom - Salon C-D, Level 3

4:30 PM A Diketopiperazine-Mediated Signaling Pathway in Pathogenic Vibrio species: A Novel Target for Anti-pathogenic Bacterial Agents

K.S. Kim, Korean Society for Microbiology and Biotechnology, Seoul, Korea, Democratic People's Republic of (North)*

Cyclic dipeptides, also called 'diketopiperazines,' are known to be synthesized by numerous microorganisms and have various biological activities. Pathogenic *Vibrio* species produce cyclic-phenylalanine-proline [cyclo(phe-pro), cFP], and the roles of this small compound in pathogenicity have been recently emerging. Here, we have elucidated a signal transduction pathway associated with cFP and the underlying regulatory mechanism. cFP binds to the inner membrane protein ToxR and triggers

signaling via LeuO, the cytoplasmic master regulator. The signal is transduced to histone-like proteins HU-ab and subsequently to the alternative sigma factor RpoS. Each of these components gives cues to its own target genes associated with the virulence of the pathogen. We also found that iron strongly represses expression of almost all components associated with quorum sensing pathways in species of *Vibrio*—not only the cFP-mediated pathway but also the classical AI-2 mediated pathway—and that the main cognate modulator is Fur. These results suggest that well-orchestrated balancing of gene expression in response to cell density and iron is important to obtain optimized physiological conditions for infection.
Furthermore, our findings indicate that the cFP-signaling pathway is closely associated with the virulence of *Vibrio* spp., and that this could be a good target for development of novel anti-virulence agents, which could be free of antibiotic resistance issues. We constructed a *lux* reporter-based screening system and then screened for chemicals interrupting cFP-mediated signaling, thereby specifically inhibiting the virulence of pathogenic *Vibrio* species. Since screening of various chemical analogs of cFP was futile in this screening strategy, we next screened more than 6,500 chemicals in the chemical library collection provided by the Korean Chemical Bank. After three rounds of screening, we narrowed down to four hits that showed inhibition activities. Among them, the two compounds SGC-0010 and SGC-0020, which did not show any harmful effect on human cells, were selected as final hits. We currently are synthesizing various analogs of these two compounds to obtain candidates with improved activities.

6:00 PM - 8:00 PM Session: PS: Poster Session

Moontower Hall, Level 2

S124 Gene expression profiling of sulfate reducing bacteria under varying copper concentrations

A.K. Tripathi, P. Saxena, P. Thakur, S. Rauniyar, R. Singh, V. Gopalakrishnan and R. Sani, Professor, South Dakota School of Mines and Technology, Rapid City, SD, USA*

Microbial induced corrosion (MIC) accounts for 50% of the annual corrosion costs in USA with sulfate reducing bacteria (SRB) contributing to ~$5 billion dollar of the costs. Copper (Cu) has been the material of choice for piping in industries because of its antimicrobial and corrosion resistant properties. However, certain species of SRB induce MIC. Our research focuses on a model SRB (i.e., *Desulfovibrio alaskensis* G20, DAG20) which corrodes Cu. The molecular response mechanisms of DAG20 to Cu exposure is not well understood. Here, we combine physiological, and transcriptomic analysis to provide, for the first time, a comprehensive view on the pathways activated in DAG20 in response to toxic levels of Cu under planktonic conditions. Transcriptomics analyses show that 47.4% genes were downregulated and 52.6% were upregulated in 5µM Cu (vs Control), while 49.7% genes were upregulated and 50.3% were downregulated in 15µM Cu (vs Control). Detailed analysis of the differently expressed genes suggests involvement of novel putative molecular mechanisms (heavy-metal translocation and metal-ion binding) in DAG20 response to Cu. Radical SAM-domain containing protein which is responsible for metal ion binding was upregulated , while the flagellar basal body protein which helps in motility, was downregulated in the presence of Cu. Moreover, results indicated that important transcription factor families associated with stress responses were differentially expressed in Cu. Our analysis has allowed for the identification of possible molecular mechanisms adopted by DAG20 to cope with Cu toxicity. These findings are expected to accelerate the development of MIC mitigation strategies involving SRB.

P1 Digital Genome Engineering: Unlocking the Unlimited Potential of Biology

T. Miller, ; E. Abbate, A. Brooks, N. Krishnamurthy, K. Krouse, B. Leland, J. Rosains and T. Shepherd, Inscripta, Boulder, CO, USA*

CRISPR-based genome engineering has the potential to accelerate discovery. Unfortunately, current approaches suffer from limitations in scalability, diversity of edit types, and accessibility. Onyx™, a benchtop genome engineering platform, overcomes these limitations by providing the capability to generate up to 10,000 precisely edited and trackable strain variants in *E. coli* or up to 6,000 such variants in *S. cerevisiae*, in a single run. This enables novel approaches for the engineering of gene, pathway, and genome-wide targets by simplifying the complex editing workflow, from the design of various edit types to the creation and genotyping of the engineered edited cell library. The Onyx™ platform provides the software, reagents, bench top instrument, and analysis to streamline the editing workflow. Here, we present 3 applications including high-throughput gene target discovery, metabolic engineering, and accelerated protein optimization. These applications resulted in the identification of new gene targets for improved glycerol utilization, creating an E. coli strain with >10,000-fold increase in the production of lysine, and creating GFP mutants with increased intensity and spectral characteristics. The Onyx™ platform will usher in the next era of genomics, enabling the researcher to transition from primarily observational studies to truly engineering biology. This will have far-reaching benefits for biology, bio-industrial science, agriculture, healthcare, and alternative energy.

S129 Development of a point-of-use treatment strategy for *Legionella pneumophila* and amoebae hosts using microwave radiation and silver ions or nanoparticles

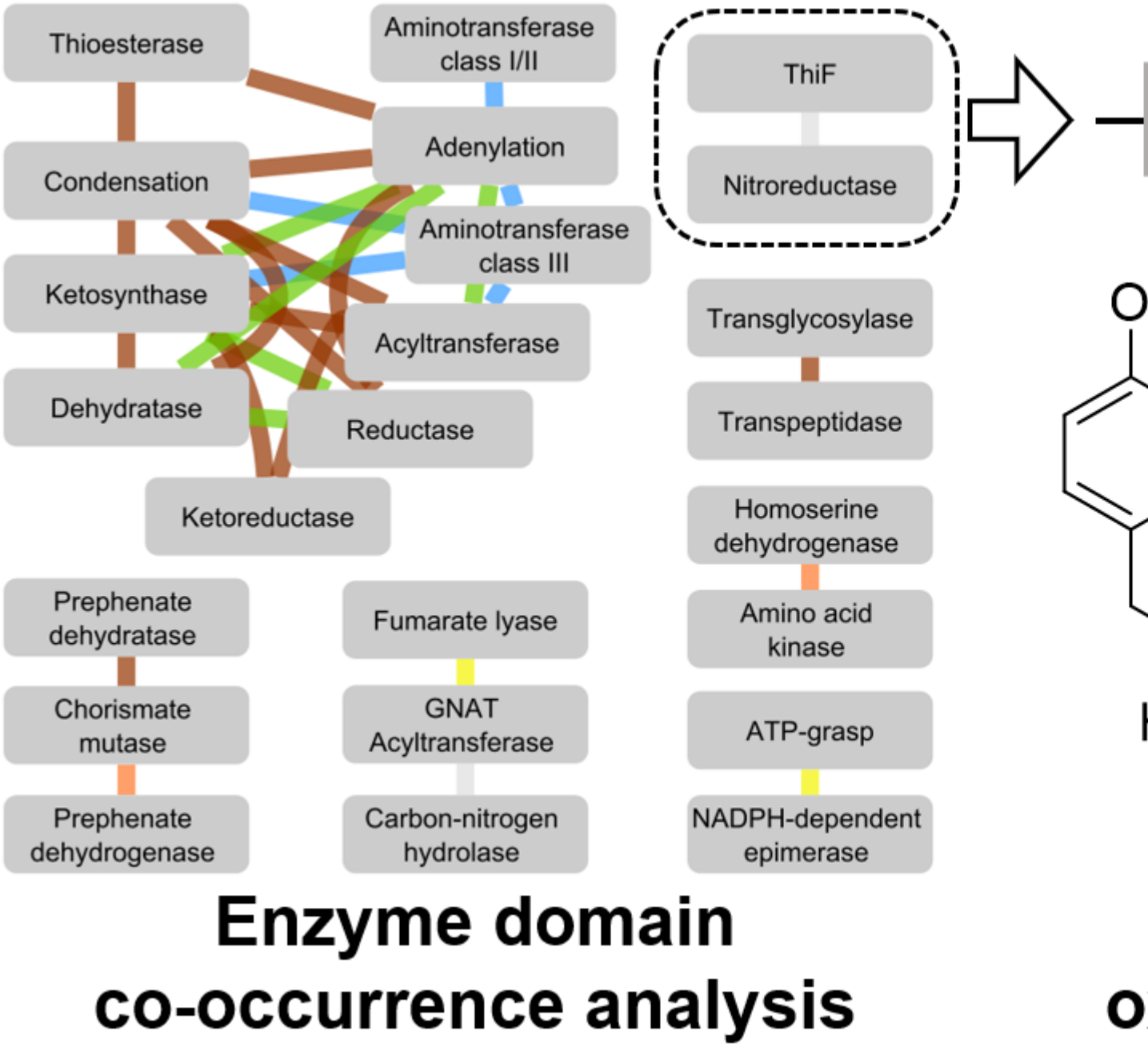
S. Marien[*], *C. Ayres*, *M.J. Kirisits*, *N. Saleh*, *D. Lawler and E. Cambronne*, *The University of Texas at Austin*, *Austin*, *TX*, *USA*

The opportunistic human pathogen *Legionella pneumophila* poses a unique health hazard because it can persist and proliferate in premise plumbing systems despite upstream drinking water treatment. This virulent bacterial pathogen can cause a severe and deadly form of pneumonia, called Legionnaires' disease, when contaminated aerosols are inhaled. Within the water sector, this public health risk is exacerbated by the proliferation of *L. pneumophila* in premise plumbing within free-living amoebae. These protozoan hosts provide significant resistance to residual disinfection and serve as a vector for *Legionella* of enhanced infectivity. Both *L. pneumophila* and associated amoebae hosts are however, vulnerable to thermal treatment, an established emergency protocol for *Legionella* control in hotels and hospitals. We aim to implement thermal treatment at the point-of-use using microwave (MW) radiation as the energy source, utilized in concert with silver. We hypothesize that MW irradiation used in concert with silver could lead to enhanced inactivation through facilitated ion uptake and/or localized heating on the cellular surface. In this study, planktonic *L. pneumophila* cells are exposed to ionic silver (Ag^+) or silver nanoparticles (AgNPs) in the presence or absence of MW radiation. While neither silver treatment alone is effective for inactivating *L. pneumophila* over a short exposure period (18 min), a combined treatment of silver with MW radiation successfully produces 3-4 log inactivation of *L. pneumophila* within 6 min of irradiation. Additional inactivation is observed when *L. pneumophila* is pre-exposed to either treatment (i.e., MW radiation or silver) prior to exposure to the other. We observed a rapid association of ionic silver and AgNPs with *L. pneumophila* cells during the pre-exposure period; silver at the cell surface could provide localized heating upon MW radiation (due to the high thermal conductance of silver) and also could act as a local source for silver transport into the cell. Data presented support the development of a *L. pneumophila* inactivation device that harnesses MW radiation and can potentially mitigate this public health risk.

P3 Co-Occurrence of Enzyme Domains guides the discovery of an oxazolone synthetase

T. de Rond, Ph.D.[*] *and B. Moore, Ph.D., University of California, San Diego / Scripps Institution of Oceanography, La Jolla, CA, USA*

Billions of years of evolution has provided immense genetic – and hence, biochemical – diversity. Some of this genetic diversity is starting to be explored through genome mining of biosynthetic gene clusters containing homologs of known core biosynthetic genes of established classes of natural products. We developed a contrasting genome mining approach for the discovery of biochemical transformations through the analysis of co-occurring enzyme domains (CO-ED) in a single protein. Guided by CO-ED, we targeted an unannotated predicted ThiF-nitroreductase di-domain enzyme found in more than 50 proteobacteria, leading to the discovery of a series of natural products containing the biologically rare oxazolone heterocycle and the characterization of their biosynthesis. Notably, we identified the di-domain enzyme as an oxazolone synthetase. Oxazolones (also known as Erlenmeyer azlactones) are versatile synthetic intermediates and, as such, we are exploring the capacity of this enzyme and its homologs to catalyze the transformation of diverse N-acyl amino acids into oxazolones.



P8 Growth of *Saccharomyces cerevisiae* 09-448 under industrially relevant stressors

B. Jones, A. Gutierrez-Cano, J. Macario, S. Martin and M. Edwards, St. Edward's University, Austin, TX, USA; K. Boundy-Mills, University of California at Davis, Davis, CA, USA*

Finding more sources of biomass for ethanol production increases the efficiency and reliability of biofuel production; one such source is known as pectin-rich biomass. Pectin-rich biomass is normally treated as a waste product, but it can be fermented by microbes to produce ethanol for biofuels. Phaff Yeast Culture Collection strain *Saccharomyces cerevisiae UCDFST 09-448,* hereafter called 09-448*,* is able to break down pectin into oligosaccharides and free fermentable sugars. 09-448 does not require the commercial enzymes normally needed to degrade pectin, making the process more cost effective. In this study, 09-448 is compared to an industrial active dry yeast strain (ADY) to determine how 09-448 is able to grow under industrial conditions and understand its current limitations. Conditions tested include pH, ethanol concentrations, osmotic stress, and its ability to use different sugars (glucose, fructose, sucrose, arabinose, xylose and galacturonic acid) as a carbon source. To characterize the growth, a plate reader was used to generate growth curves under varying conditions. OD600 was recorded at 15-minute intervals after inoculation and used to calculate generation time and Vmax. ADY grew better than 09-448 in industrial conditions, with shorter generation times than 09-448 in tryptic soy broth containing 0-10% (v/v) ethanol and sugar solutions (glucose, fructose, and sucrose). ADY also reached a higher cell density at 12 hours than 09-448 in the ethanol and sugar media. This suggests that ADY is better at growing in industrial conditions than 09-448. Based on these results, 09-448 is not currently a viable industrial ethanologen. However, we have begun to optimize 09-448 for survival under increased ethanol stress through adaptive evolution. 09-448 was first grown in media containing 2.5% (v/v) ethanol, once growth improves under these conditions, ethanol concentrations will be incrementally increased in the growth medium. The aim is to isolate derivatives of UCDFST 09-448 capable of growing at up to 18% (v/v) ethanol, thus improving prospect of using 09-448 as an industrial strain.

P9 Enhancing production of propionic acid with NuCel® 632 LQ yeast extract in optimized culture medium for *Acidipropionibacterium acidipropionici* fermentations

S. Nelson, Ph.D., Procelys, Milwaukee, WI, USA; J. Aldridge, Ph.D., Procelys, Cedar Rapids, IA, USA; R. Rangel, Ph.D. and A. Sourabié, Ph.D., Procelys, Maisons-Alfort, WI, France*

Propionic acid is an organic acid that is capable of preventing the growth of some fungal and bacterial species. As a result of this property, it is used as a preservative in the feed and food industries. Production of propionic acid has increased in recent years with demand for clean label products by consumers. Fermentation has long been used to produce propionic acid, but its acceptance has been hampered by poor yields. Here we describe a study which sought to increase the yield of propionic acid as produced in *Acidipropionibacterium acidipropionici* This study specifically looked at the contribution of yeast based nutrients (YBN) in culture medium and its effects on cell growth and propionic acid yield. Our findings reveal that by optimizing media with NuCel® 632 LQ, the fermentative yield of propionic acid can be increased by 36% while experiencing a 62% reduction in lag time.

P11 Conversion of High-Solids Hydrothermally Pretreated Bioenergy Sorghum to Lipids and Ethanol Using Yeast Cultures

M.H. Chen, Ph.D., Y.S. Jin, J. Shin and V. Singh, University of Illinois Urbana Champaign, Urbana, IL, USA; B. Dien, Ph.D., S. Thompson, P. Slininger, Ph.D. and N. Qureshi, Ph.D., NCAUR-ARS-USDA, Peoria, IL, USA*

Glucose and xylose are the major sugars present in cellulosic hydrolysates. The cellulosic sugars can be used for the production of platform chemicals. In this study, productions of lipid and ethanol by yeasts were compared for concentrated bioenergy sorghum syrup. Bioenergy sorghum was hydrothermally

pretreated at 50% w/w solids in a continuous industrial reactor and sequentially mechanically refined using a burr mill to improve biomass accessibility for hydrolysis. Fed-batch enzymatic hydrolysis was conducted with 50% w/v solids loading and cellulase cocktail (50 FPU/g biomass) to achieve 230 g/L sugar concentration. Various strains of *Rhodosporidium toruloides* were evaluated for converting sugars into lipids, and strain Y-6987 had the highest lipid titer (9.2 g/L). The lipid titer was improved to 19.0 g/L by implementing a two-stage culture scheme, where the first stage was optimized for yeast growth and the second for lipid production. For ethanol production, the engineered Saccharomyces cerevisiae SR8ΔADH6 was utilized to coferment glucose and xylose. Ethanol fermentation was optimized for media nutrients (YP, YNB/urea, and urea), cellulosic sugar concentration, and sulfite conditioning to maximize the ethanol concentration from sorghum syrups. Fermentation of 70% v/v concentrated hydrolysate conditioned with sulfite produces 50.1 g/L ethanol from 141 g/L of sugars.

P12 Detectability of Rogue SynBio in Complex Matrices

C. Jung, M. Carr and K. Indest, US Army CoE - ERDC, Vicksburg, MS, USA; L. Carrigee, US Army CoE - ERDC - ORISE, Vicksburg, MS, USA*

Synthetic biology (SynBio) aims to rationally engineer or modify traits of organisms into a singular functional organism through advanced genetic engineering techniques. As researchers continue to explore this emerging technology it is important to accurately assess the effect of SynBio materials on the environment yet the fate and transport of SynBio is largely unknown. To assess the detectability and persistence of SynBio products environmental fate studies were carried out using microcosms representing different environmental matrices (soil, water, wastewater). A synthetic genetic construct (SC) of the cadaverine gene was prepared as either naked DNA or in a plasmid chassis system for two separate host organisms as a model rogue SynBio element. Persistence and proliferation of the SC in the matrices over time were determined by PCR and chemical analysis of the functional production of cadaverine via HPLC. The SC persisted throughout microcosms containing either naked DNA or plasmid within a bacterial host, suggesting that SC released into the environment may persist and leave diagnostic signatures. Detectability decreased when ratios of water:soil increased while wastewater liquor demonstrated a proliferative environment for SC persistence. Currently, very little is known about the potential for rogue SynBio to functionally integrate into the natural microbial communities of complex environmental matrices. The US Army and its adversaries are actively invested in SynBio and as such it is important to gain foundational understanding of potential rogue SynBio constructs or products in the environment, which would in turn be useful in developing tools to track adversaries on the bioterrorism front.

P13 Presence of multi-drug resistant pathogens and antibiotic resistance genes in the recreational fishes of southeast Louisiana

R. Boopathy and E. Naquin, Student, Nicholls State, Thibodaux, LA, USA*

The spread of antibiotic resistance is a growing global concern in recent years. Improper usage and disposal of antibiotics by consumers, hospitals, and industries has furthered the emergence of antibiotic resistance in the waterways of Southeast Louisiana, namely Bayou Lafourche and Bayou Terrebonne, a main source of drinking water impacting over 70,000 individuals that live along its banks. Additionally, there are existing reports of exposure to antibiotic-resistant bacteria through contact with the wetlands, as well as the presence of antibiotic resistance genes. In Louisiana, one out of every seventy jobs are related to the seafood industry, and this region exports roughly 1 billion pounds of seafood each year at a value of 2.4 billion USD. In this study, water samples were collected as well as samples from various species of freshwater fish and shellfish—common seafood caught in the area—and the occurrence of antibiotic-resistant bacteria was monitored. The results of this study showed the presence of multi-drug resistant bacteria exhibiting resistance to all antibiotics tested. Furthermore, the tet(S) and tet(41) genes, genes for tetracycline drug resistance, qnrB56, a gene for fluroquinolone resistance, aac(6')-lc, a gene for aminoglycoside resistance, and the oqxB gene, a gene for olaquindox resistance, were identified. Furthermore, liquid chromatography analyses of three water samples showed the presence of antibiotics in a considerable amount in both waterways tested.

P15 Metagenomics insights into the distribution of Firmicutes across Black hill's region of South Dakota, USA

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Firmicutes constitute a phylum of spore forming Gram-positive bacteria, which as such are ubiquitous in nature. Many of its members are an important part of flora associated with biomass degradation and carbohydrate metabolisms. The context is necessary when ligninolytic bacteria and enzymes are desired. In this work, with the help of 16S high throughput metagenomics, we have aimed to better understand microbial diversity, biogeographical distribution, and functions of Firmicutes across two different sample types located in the Black hill's region of South Dakota, USA: South Dakota Landfill Compost (SDLC, 60°C), and Sanford Underground Research Facility sediments (SURF, 45°C), and present a case for a temperature-based ecotype diversification. In both the samples, there was a dominance of Firmicutes, followed by Proteobacteria. The abundant classes of bacteria in the SDLC compost site, within the phylum Firmicutes, were Bacilli (83.20%), and Clostridia (2.90%). In comparison the sample from the SURF mine was dominated by the Clostridia 40 (45.80%) and then Bacilli (20.10%). Subsequently, culture isolations of endospore forming Firmicutes members from these samples and identification based upon 16S rRNA and gyrB gene sequence analysis was performed. Depending on the similarity indexes, we present a case that gyrB sequence is more useful than 16S rRNA sequence analysis for inferring intra- and some intergeneric relationships between the isolates.
Keywords: Black Hills, Distribution, Firmicutes, *Geobacillus*, Metagenomics.

P16 Biogenic synthesis and characterization of nano-inclusions by gram negative bacteria

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The use of biological systems for the synthesis of biogenic derived metals is being exploited by the field of nanotechnology as the demand for critical natural resources increases. Certain microbes have an intrinsic ability to reduce metallic ions and, in some cases, accumulate these metals as inclusion bodies usually as a detoxification response or for energy generation. These processes may also offer a cost-effective green solution to metals reclamation to benefit industry and the environment. In this study, several gram-negative bacteria were evaluated for the ability to produce metallic inclusion bodies when grown in media containing 1-2 nM of iron, nickel, cobalt, selenite and selenate. All strains achieved growth over 48 hrs in the presence of selenite, selenate and iron, but with little to no growth observed in the presence of nickel or cobalt. Supernatant, hexane derived fractions, and fixed cells were subjected to TEM and EDS analysis to determine if metal inclusions were present intracellularly and/or in the media. *Pseudomonas stutzeri*, an organism known to reduce and uptake selenium ions, was chosen for TEM analysis to ensure growth parameters and metal exposure were sufficient for inclusion formation. Analysis of *P. stutzeri* showed reduction of selenate to selenium in the supernatant and hexane fractions and detection of internal spheroidal inclusions in fixed whole cells measuring ~200-300 nm. *Shewanella oneidensis* was able to reduce iron ions and exhibited a collection of clover-shaped clusters of iron nanospheres (each nanosphere measuring ~5-10 nm and each cluster measuring ~10-15 nm) associated with the plasma membrane, however no internal inclusions were confirmed. The biogenic synthesis of nano-inclusions has the potential to become a green source of metal nanoparticles thereby advancing the fields of applied microbiology and nanotechnology.

P17 Influence of Environmental Parameters on Macromolecule Degrading *Bacillus* species

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Microbes which can degrade macromolecules such as starch, protein, lipids and lignocellulose biomass to their subunits produce enzymes that can be deployed in various industrial processes including cleaning. Depending on application, microbial cleaning products used in domestic, commercial, industrial, and recreational settings may contain enzymes such as amylase, cellulase, lipase, protease, and xylanase; and related enzymes that act in synergy. Whole microbial cells that produce biocatalysts can also be employed in cleaning as in muck removal from lakes and ponds. We had isolated amylolytic, cellulolytic, proteolytic, lipolytic and xylanolytic microorganisms for production of enzymes that can be used in cleaning products with biological activity. The isolates were identified by 16s ribosomal RNA gene sequence analysis. Two *Bacillus* species M13 and T21 with potential for macromolecule degradation in water were selected. *Bacillus* species M13 and T21 produced several macromolecule depolymerizing enzymes. The influence of temperature, pH, osmotic pressure and UV light on growth of the isolates in water were examined. Both T21 and M13 displayed promising growth in moderate environmental conditions, suggesting potential application for macromolecule degradation in water. Nonetheless, further studies are required to test the efficacy of *Bacillus* T21 and M13 for muck removal in pond water under field conditions.

P18 Utilization of human feces and urine with concomitant production of biogas in confined environments.

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Efficient and environmentally safe utilization of human waste in a confined environment possesses a certain challenge. It is particularly important for space flights and establishing of settlements on the Moon and Mars. The goal of this project was to select a stable microbial community capable of an effective digest of human wastes concomitant with production of biogas. Dog feces (in lieu of human feces) were incubated anaerobically with human artificial urine in batch reactors for up to four months. Production of molecular hydrogen, methane and carbon dioxide were monitored by gas chromatography and production of fermentation products were monitored by HPLC. The composition of the established microbial communities was determined by 16S rRNA gene analyses. It was established that butyric fermentation is the key fermentation process during incubation. Hydrogen production initially tends to increase but then its concentration substantially drops until it is consumed and methane was not produced during most of experiments. Addition of a *Clostridium butyricum* culture to the fermentation does not improve production of molecular hydrogen and/or methane. Analyses of the microbial communities in incubation vessels indicate that a substantial change in the microbial community composition takes place over the time of incubation and added clostridia successfully survived in the fecal-urine environment used for the project.

P19 A screening approach for identifying bacterial isolates with efficacy for controlling cyanobacteria

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Harmful algal blooms (HABs), predominantly cyanobacteria (cyanoHABs), are a growing global concern negatively impacting ecological systems in freshwater lakes and rivers. During a bloom event, odors are released, dead zones can form in the water column, and dangerous toxins are released into the ecosystem that are detrimental to aesthetics and harmful to environmental and public health. As a result, HABs impact local and regional economies by limiting fisheries resources, restricting recreational and commercial waterways, and raising costs of treatment for drinking water. To control HABs, researchers are developing short- and long-term mitigation strategies by exploiting bacterial products for targeted chemical control reagents to reduce the impact severity of HABs. In this study we screened the commercially available, biological relevant compounds trytpoline, tryptamine, isatin, and ginsenoside

using *Microcystis aeruginosa* UTEX 2385 via combination of semisolid plate assays and liquid assays. Tryptoline inhibited *Microcystis* growth most effectively followed by tryptamine, isatin and ginsenoside. In addition to established compounds, 6 isolates were further screened for algicidal properties using liquid assays. Preliminary screening demonstrated that sterile filtrate of BLCC-B42 and BLCC-B43 had activity against the colony-forming cyanobacterium *Microcystis aeruginosa* BLCC-F188 and diatom *Fistulifera alcalina* BLCC-F88. Collectively, bacterial isolates that result in at least a 50% reduction in algal biomass after 72 hours will be used to generate data to investigate safety factors based on effective application dosages to achieve target efficacy relative to effect levels on non-target species. Future work will be focused on characterizing and scaling-up algaecidal compounds to further evaluate the effects on non-target species (i.e., zooplankton, invertebrates, fish).

P20 Selection of soil bacteria for augmentation of bioelectricity production in microbial fuel cell

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Microbial fuel cells deploy microbial metabolism of nutrients in anaerobic environments to produce electricity by releasing electrons from nutrients. In a microbial fuel cell (MFC) the electrons are captured by the anode and travel to the cathode where electrons reduce oxygen to water. The ability of microbes to produce electricity by this mechanism has gained increasing attention. Evolution of modern technology and unique equipment enabled validation of the theory of microbial production of electricity. A notable example is the microbial fuel cell (MFC) in which electrochemically active microorganisms transfer electrons for bioelectricity production. In this study we have deployed the MudWatt MFC to screen soils for presence of electrochemically active microbes that produce bioelectricity. Lignocellulose biomass is an abundant natural resource that can be hydrolyzed to sugars for bioelectricity production. Facultative anaerobic bacteria isolated from the soil samples were first tested for production of cellulase and xylanase in a complex lignocellulose biomass medium. Higher cellulolytic and xylanolytic activities were present in microbes isolated from soils producing bioelectricity in the MFC. Selected cellulolytic and xylanolytic bacteria from soil are being evaluated for augmentation of bioelectricity production in microbial fuel cell.

S34 Microbial trait data predicts differential soil carbon processes in forested wetlands and tidal marshes

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Landscape perturbations occur over a wide range of spatiotemporal scales due to natural and anthropogenic events. These can occur suddenly (e.g. storm surge) or gradually (e.g. sea level rise). Collectively, these processes can amplify terrain degradation, impacting site assessments and emergency operations in the short-term and ecosystem services in the long-term. The objective of this effort is to develop innovative processes for measuring, analyzing, and predicting microbial traits (biomass, respiration, diversity, and community composition and function) across environmental gradients or soil taxonomies. These data will be integrated with additional data layers pertaining to the geochemistry of soils and sediment, plant cover, and other physicochemical characteristics to provide meaningful visualizations and data products to assist decision makers and emergency managers in coastal regions in assessing terrain modification over different time periods.
The microbiomes of four wetland vegetation types on the U.S. Gulf coast, which exist along a salinity gradient, were examined by high-throughput sequencing and found to differ in composition ($p<0.001$). Microbial richness ($p<0.001$) and diversity ($p<0.001$) differed between vegetation types; higher in

intermediate salinity *Typha spp.* and tidal shrub wetlands. Highest salinity *Juncus spp.* marsh had higher β-glucosidase activity ($p \leq 0.015$) than any other vegetation type. Microbial biomass was influenced by vegetation ($p=0.035$) and was highest in forested wetlands. However, microbial growth was slowest in forested sediment ($p<0.001$). Potential methanogenesis was lower in forest than in marsh ($p<0.001$), while aerobic respiration was higher in forests than in marsh ($p<0.001$). This coincided with lower carbon fixation in forest sediment.

Forested wetlands were lower in diversity, microbial growth, and carbon degradation activity than emergent intermediate-to-brackish salinity marsh. This correlated with higher belowground biomass. Potential methanogenesis from forests was lower than from marsh, but aerobic respiration was higher. These findings demonstrate a fundamental difference in soil microbial processes between forested swamps and tidal marshes. Incorporation of rich microbial trait data will be critical to developing better soil carbon models, particularly in vulnerable coastal wetlands.

P22 Phylogenetic diversity and distribution of bacteria catalyzing aristolochic acid detoxification

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Plants used in traditional medicine can at times produce harmful toxins that prevent the utilization of the beneficial compounds. *Aristolochia* is a genus of herbaceous twining vine found in tropical and sub-tropical regions worldwide. They are a rich source of antioxidants and are widely used in traditional medicine. Unfortunately, Aristolochic acids (AA), toxic nitro phenanthrene carboxylic acids, are produced by many species. AA are activated by key enzymes in the liver and kidney to create reactive aristolactam nitrenium ions that bind covalently to purine bases in DNA. Aristolactam-DNA (AL-DNA) adducts are irreversible and their accumulation leads to acute kidney injury, end stage renal failure and urothelial carcinomas collectively referred to as aristolochic acid nephropathy (AAN). The presence of AL-adducts as a biomarker has confirmed that AAN has spread throughout China, Taiwan, Japan, South Korea, and the Balkans. Although a great deal is known about mammalian toxicology and metabolism of AA, its biodegradation has not been reported. We used selective enrichment to isolate bacteria able to biodegrade AA from 3 species of *Aristolochia*. Isolates including *Pseudomonas, Bradyrhizobium, Pedobacter, and Variovorax* species can grow on aristolochic acid as the sole source of carbon, nitrogen, and energy. Accumulation of nitrite indicates that the initial attack on the molecule involves an oxygenase catalyzed elimination of the nitro group. Transient accumulation of a denitrated early metabolite provides additional evidence for the oxidative pathway rather than the alternative reductive mechanism. The phylogenetic diversity of the isolates suggests that they are opportunistic rather than involved in a close symbiotic relationship with the plants. Discovery of bacteria able to degrade AA suggest the potential for elimination of the AA and thus the toxicity during submerged fermentation of plant tissue catalyzed by the bacterial isolates. The molecular details of the catabolic pathway and the potential for application in detoxification are under investigation.

S125 Quantifying sulfate reduction rates in biofilm on shale fracture walls of a microfluidic reactor

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Microbial sulfate reduction in hydraulically fractured shale is problematic as sulfate reducing bacteria (SRB) growth promotes bioclogging in preferential flow paths and decreases hydrocarbon production, while hydrogen sulfide production creates a pipeline corrosion and human health hazard. Few studies have quantified biomass growth and activity in shale fractures due to the complexity of studying processes at this scale, resulting in a knowledge gap regarding the ability of microbes to colonize shale fracture surfaces and clog these oil and gas flow conduits. To address this gap, a natural shale sample is shaped to create two inlets that feed into a single 250 μm-wide fracture, all contained within a microfluidic platform. This shale-based microfluidic flowcell reactor is inoculated with a field culture dominated by

SRB, followed by incubation and increase in nutrient flux. Microscopic images and effluent samples are analyzed to assess biofilm growth and biocide inhibition, with a numerical model developed to quantify sulfate reduction rates. The results indicate that biomass grows as biofilms on shale surfaces with little mass transfer limitations, and that accurate quantification of sulfate reduction rates depends on quantifying this biomass.

P27 Bioconversion of plastic wastes into value-added products using thermal oxo-degradation

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Over 8 billion tons of plastics have been produced, and the available strategies for its disposal have proven insufficient due to economic and environmental sustainability limitations. As a result, most of all plastic ever made has ended or will end in landfills and our ecosystems. Plastics are not biodegradable because the breakdown of the polymers into molecules that microorganisms can utilize is estimated to take thousands of years. However, combining thermal oxo-degradation (TOD) with biocatalytic conversion can drastically reduce the rate-limiting step of plastic degradation and open an avenue for plastic waste upgrading. TOD rapidly deconstructs polymer backbones while adding oxygen functionalities to the fragmented products. The products of this process with plastic feedstocks are mixtures of fatty substrates - including fatty acids and fatty alcohols – and hydrocarbons. In this study, a screening process was carried out to select a suitable microorganism for the bioconversion of thermally oxo-degraded high-density polyethylene (HDPE), the world's most common plastic. The TOD products were mostly hydrophobic and not emulsified before testing carbon utilization, resulting in a solid floating at the surface of the culture. Remarkably, the selected organism overcame the mass transfer limitations in the liquid culture and grew with TOD of HDPE products as its sole carbon source. The growth profile was even comparable to glucose controls. This microorganism could be leveraged as a microbial cell factory, or its biomass could be turned into single cell protein, resulting in waste upcycling. These findings serve as proof-of-concept and showcase the potential of our novel approach to solving the world's plastic pollution problem.

P28 Multiplexed Serine recombinase Assisted Genome Engineering (mSAGE) as tool kit for high throughput genetic engineering in *Pseudomonas putida* KT2440

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Pseudomonas putida KT2440 has emerged as a promising host for the pursuit of biological conversion of lignocellulosic biomass. With diverse catabolic capabilities and resistance to many bioprocess stressors, *P. putida* KT2440 can serve as a platform strain on which to expand industrial capabilities for bioproduction of fuels and chemicals. While advances in synthetic biology have significantly improved that rate at which novel DNA elements can be produced, many non-model hosts are still limited by the rate of stable genomic integration of these elements. In this work we have expanded the capabilities of the Serine-recombinase Assisted Genome Engineering (SAGE) toolkit to create the multiplexed SAGE (mSAGE) suite of engineering tools. We have established a base *P. putida* KT2440 strain that contains engineered landing pads enabling the genomic integration of up to 9 unique DNA elements via orthogonal serine recombinases. These serine recombinases function at high efficiencies exceeding traditional homologous recombination and are highly site specific. Beyond the use of individual recombinases, we have demonstrated that 3 plasmids can be simultaneously integrated at high efficiency. Via co-transformation of 6 total plasmids, 3 that integrate and 3 that transiently express recombinases, we have achieved simultaneous integration of three plasmids at an efficiency of $1.2x10^6$ cfu/ug of DNA and 100% accuracy of site-specific integration. To further enhance these tools, we have also established a temperature sensitive plasmid-based system that can excise non-essential plasmid elements and antibiotic markers with a 100% effective rate of removal. Combined, this toolkit enables rapid, high-

throughput genomic integration of up to 9 unique DNA elements and combinatorial pathway assembly in *P. putida*. The mSAGE toolkit will accelerate the engineering of *P. putida* and other non-model organisms for a wide range of applications.

P29 Fine-tuning gene expression in bioproduction pathways in diverse bacterial hosts

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Climate change and energy security concerns are driving the need for biobased products to be brought to market. However, only a handful of host microorganisms have been sufficiently “domesticated” to efficiently make bio-products. To realize biomanufacturing’s full potential, we must expand the scope of domesticated hosts, particularly those more suited to industrial conditions and with the metabolic versatility to broaden the types of feedstocks used and chemicals produced. One underdeveloped area of synthetic biology is precise gene regulation. Traditionally, biosynthetic pathways have been modified in a binary fashion, whereby genes are either turned on or off. However, the ability to moderate gene expression, between 0-100%, would greatly expand our ability to control metabolic flux, and ultimately product yields. To fill this void, we created a random, semi-degenerate suite of small, non-coding RNAs, called riboregulators, that modulate gene expression at varying, discrete levels. Our riboregulators, or cis-repressors (CRs), limit RNA translation by occluding the ribosomal binding site in a synthetic hairpin. By varying the thermodynamic stability of this hairpin, gene expression can be tuned to distinct levels. To validate the activity of our riboregulators across diverse systems, we coupled CRs with a reporter protein (*sfGFP*) and established our CR-sfGFP plasmid suite in three industrially-relevant hosts: *Escherichia coli*, *Pseudomonas putida,* and *Corynebacterium glutamicum.* Comparing cellular fluorescence within and across species, we found that our CRs repressed gene expression from 20-100% in *E. coli,* 35-100% in *P. putida*, and 50-100% in *C. glutamicum.* Additionally, we found that specific CRs consistently inhibit *sfGFP* expression to similar levels regardless of organism. To demonstrate the utility of our CRs in producing value-added chemicals, we integrated four CRs in *P. putida* to modulate the expression of phosphoenolpyruvate synthase enzyme (*ppsA*), which is used to produce muconate. We found that moderate-expression of ppsA increased the muconate titer, compared to full-expression. Overall, these findings highlight the vast utility that this synthetic engineering tool can have on an array of bacteria in the biomanufacturing sector.

P30 Tuning gene expression by phosphate in the methanogenic archaeon *Methanococcus maripaludis*

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Methanococcus maripaludis is a rapidly growing, hydrogenotrophic, and genetically tractable methanogen with unique capabilities to convert formate and CO_2 to CH_4. The existence of genome-scale metabolic models and an established, robust system for both large-scale and continuous cultivation make it amenable for industrial applications. Additionally, its ability to synthesize unusual coenzymes and capability of performing reactions with extremely low redox potentials make it an attractive industrial workhorse. However, the lack of molecular tools for differential gene expression have hindered its application as a microbial cell factory to produce biocatalysts and biochemicals. In this study, a library of differentially regulated promoters was designed and characterized based on the *pst* promoter, which responds to inorganic phosphate concentration in the growth medium. Gene expression increases by 4 to 6-fold when the medium phosphate drops to limiting concentrations as growth progresses into stationary phase. Hence, this regulated system decouples growth from heterologous gene expression without the need of adding an external inducer. Regulation dynamics were similar when either formate or H_2/CO_2 was used as the growth substrate. The minimal *pst* promoter was identified and contains a conserved AT-rich region, factor B recognition element and TATA box for phosphate-dependent regulation. Rational changes to the factor B recognition element and start codon had no significant impact on expression. However, changes to the transcription start site and 5' untranslated region resulted in differential protein

production with regulation remaining intact. Compared to a previous expression system based upon a histone promoter, this regulated expression system resulted in significant improvements in expression of a key methanogenic enzyme complex, methyl-coenzyme M reductase, and the potentially toxic arginine methyltransferase MmpX. This work further expands the genetic toolbox for methanogenic archaea, making them a more efficient cell factory workhorse.
KEYWORDS: heterologous gene expression, gene regulation, 5' untranslated region, *Methanococcus maripaludis*

P31 Design of temperature-responsive controllers for regulating gene-expression

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Transcription factors (TFs) are frequently used in synthetic biology for regulating gene expression to promote the production of valuable medicines and chemicals, act as sentinels in the environment, and much more. The ideal TF would have tunable inputs and outputs with clear design rules allowing engineers to rapidly prototype desired behaviors as needed. Currently used TFs, however, are products of evolution making them difficult to tune rationally. In this work, I design and test a modular and programmable TF, by fusing elastin like polypeptides (ELPs) as a tunable sensor module and a swappable TF to control output. ELPs aggregate reversibly in response to changes in environmental conditions such as temperature or pH at thresholds encoded in the ELP structure. ELP aggregation removes the fused TF partner from solution modulating gene expression. Using these ELP-TFs, I demonstrate that ELP aggregation can reduce protein expression by up to 83% in E. coli. To improve the performance of the ELP-TFs, I am building feedback control circuits to maintain the expression levels of ELP-TFs irrespective of temperature changes. Successful completion of this work will generate ELP-TFs that allow easy, economical, and contamination-free temperature-dependent control; fast and reversible regulation; and rapid tuning of TF levels. This will be a new paradigm for the regulation of cellular processes, with the potential to introduce autonomous feedback for optimal bioproduction.

P32 Engineering and evolution of muconic acid production from glucose and xylose in *Pseudomonas putida* KT2440

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Muconic acid is a bioprivileged molecule that can be converted into direct replacement chemicals or performance-advantaged bioproducts. To date, metabolic engineering efforts in *Pseudomonas putida* KT2440 have enabled muconate production from glucose, and separately, *P. putida* strains have been developed that can co-consume glucose and xylose, which are often the primary carbohydrates in lignocellulosic hydrolysates. Here, we combine metabolic engineering and adaptive laboratory evolution (ALE) to produce muconate efficiently from glucose and xylose. The D-xylose isomerase pathway was incorporated to a strain previously engineered to produce muconate from glucose, followed by ALE to increase utilization rates on xylose. Muconate production in the best-performing isolate achieved a 26% yield (mol/mol), a titer of 12.3 g/L, and productivity of 0.10 g/L/h in bioreactors on mixed sugars. Two missense point mutations in xylose transporter XylE and a point mutation in the promoter region of a putative transporter, together with a 227.8 kB duplicated region, were identified by resequencing of the best-performing isolate from ALE, all of which were shown to be important to muconate production on xylose. To obtain a strain with a clean genetic background, the three point mutations were engineered into the unevolved strain, followed by overexpression of the *aroB* gene from the duplicated region. The resulting rationally engineered strain, *P. putida* LC224, outperformed its evolved counterpart in both yield (50%), titer (26.8 g/L), and productivity (0.28 g/L/h) on glucose and xylose in bioreactor cultivations. These strain engineering modifications may also improve production of other shikimate pathway related products from mixed sugars in *P. putida*.

S126 Tinkering in the Third Domain: Using Extremophilic Archaea as a Production Platform and Enzyme Source for Synthesis of Renewable Bioproducts

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Archaea have adapted over millions of years to thrive in nearly every environment on Earth. Amongst the most resilient of these are extremophiles, microbes which thrive in environments that push the boundaries of life. Extremophiles can be a rich resource of unique enzymes and have promising potential as bioproduction platforms. Here I discuss two cases in which conventional biotechnologies were improved by exploring the distinctive physiologies of extremophilic archaea. Methanogens are obligately anaerobic archaea noteworthy for producing methane from C1 compounds and acetate. The energetic limitations of these low-energy substrates require methanogens to utilize a highly efficient central metabolism which greatly favors respiratory byproducts over biomass. This metabolic strategy creates high substrate:product conversion ratios which is industrially relevant for the production of biomethane, but may also allow for the production of value-added commodities. Like all archaea, methanogens synthesize isoprenoid membranes utilizing the high flux mevalonate pathway, making them ideal to produce terpenoids. We found that the engineered methanogens directed up to 4% of total carbon towards isoprene production with increased biomass. To aid in biomass conversion to value added products, we developed an enzyme cocktail from thermoacidophilic archaea, EXTREMASE, which liberation of sugar from cellulosic feedstocks at extreme temperature and pH conditions. High rates of monosaccharide (glucose and other sugars) were liberated from a broad range of energy crop plants including corn, sorghum, wheat and most recently nut shells (almond). By optimizing production of thermophilic enzymes in mesophilic organisms, we allow for extremely heat tolerant cellulases to be produced without specialty culturing equipment. In this presentation I discuss how organisms are chosen for their unique physiology and how extremophilic archaea could be used to improve existing biotechnology processes.

P34 Targeting global regulatory responses using precise and programmable *cis*-riboregulators

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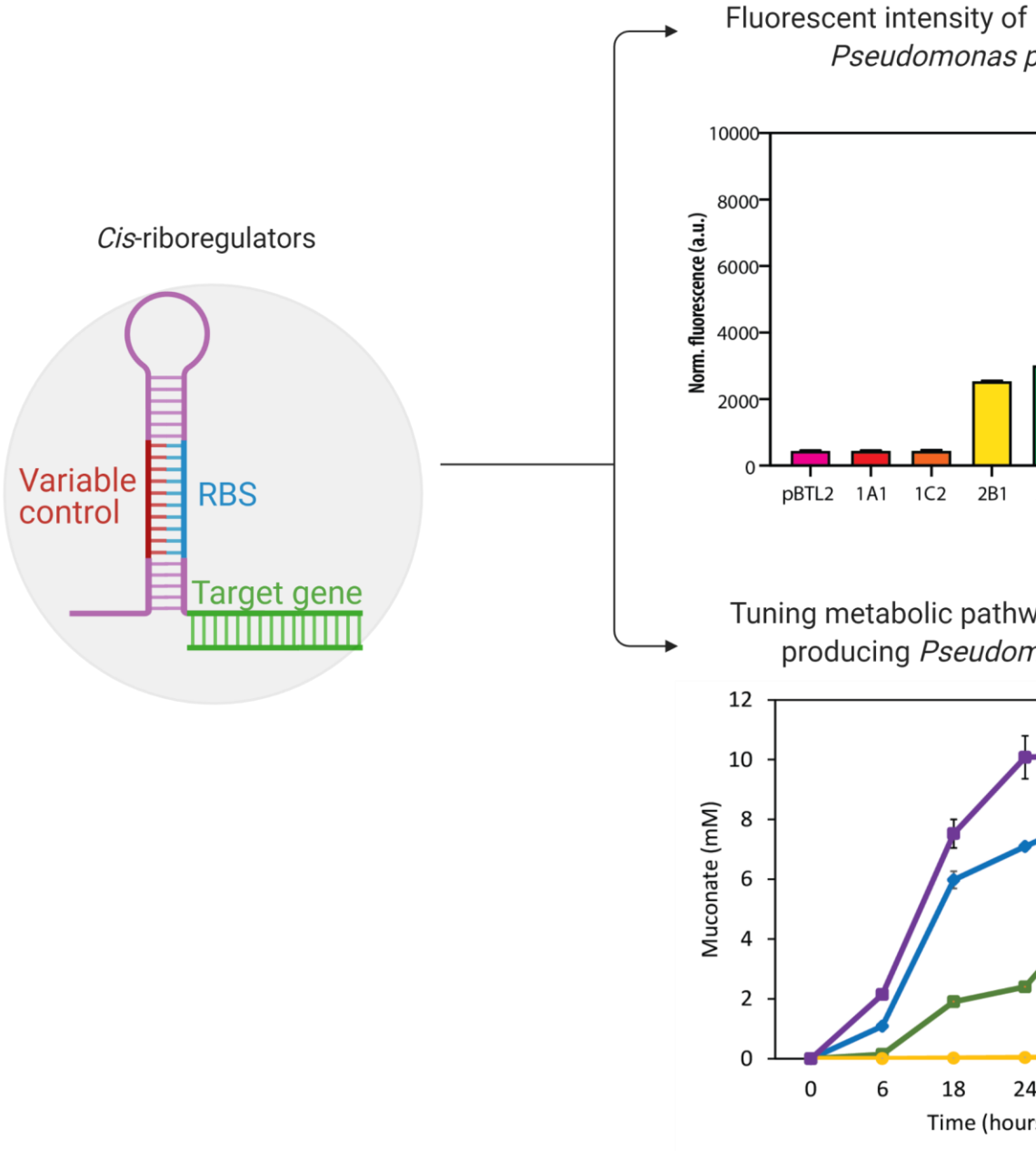
One of the hurdles in host engineering is balancing the energy trade-off between growth and protein production. Energy-intensive pathways can be a drain to achieving optimal production yields and productivity of new metabolic pathways. Additionally, metabolism slows down when microbes encounter a stress or enter stationary growth phase. For example, *Pseudomonas putida* has been synthetically engineered to convert lignocellulosic mass into muconic acid, a high-value acid precursor to bioplastics. As *P. putida* has been increasingly engineered to produce more muconate (up to 200% improvement in productivity), the overall growth of the organism has been effected.

Notably, research in manipulating the microbial host's reaction to various global responses such as stationary growth phase, quorum sensing and stress response has been limited. A key challenge to manipulating microbes in this way is that many global response regulator pathways are often interconnected and essential for the cell's survival; therefore, a fine-tuned approach is required. Here, we report a class of prokaryotic *cis*-regulators that provide ultra-specific RNA detection capabilities. By changing the variable control nucleotides of the stem-loop structure of the riboregulator, a range of programmable regulators were created that provide over a 100-fold differences in gene expression in response to target RNAs.

By exploiting this programmable design, we implemented these *cis*-riboregulators to target a range of global response regulators, including a carbon storage regulator CsrA/RsmA, the "magic" spot of bacterial growth economy by RelA, as well as other muconate "ON" pathway targets. By leveraging these targets in muconate-producing *P. putida*, we were able to uncouple muconate productivity from growth. Not only

were we able to manipulate the gene expression of essential genes to near-digital control, but we were able to evaluate a combination of *cis*-repressed genes. Furthermore, we could target genes that were unsuccessful for gene combination knockouts. Ultimately, the fine-tuning the expression of multiple targets in *P. putida* resulted in up to 3x improvement in muconate productivity, an industrial precursor with a global demand of >$40 B.



P35 Investigating the Role of the Transcriptional Regulator Ure2 in the Metabolism of *Saccharomyces cerevisiae*

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The production of lipids and valuable oleochemicals in yeast is affected by changes in nitrogen availability. Lipid accumulation occurs under nitrogen limiting conditions, the response to which is mediated by the nitrogen catabolite repression (NCR) pathway. Ure2 is a key transcriptional regulator of this pathway. To investigate the role of this regulator, gene expression and intracellular metabolite concentrations were measured in a *ΔURE2* strain of *Saccharomyces cerevisiae* through RNA sequencing and gas chromatography/mass spectrometry (GC/MS). Transcriptomic changes consistent with both nitrogen starvation and autophagy, including the upregulation of allantoin, urea, and amino acid uptake and utilization pathways and selective autophagic machinery were observed in the *ΔURE2* strain. This mutant strain also accumulated less trehalose and glycogen, and it produced more lipid and ethanol. *URE2* is therefore a potential target for engineering yeast strains capable of lipid accumulation on nitrogen-rich substrates.

P36 "Multi-omics analysis to investigate carbon and lipid metabolism in two oleaginous yeasts"

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Oleaginous yeasts are promising hosts for the production of biofuels and bioproducts such as biodiesel, organic acids, polyols, and fatty alcohols from renewable lignocellulosic biomass. Oleaginous yeasts such as *Rhodosporidium toruloides* and *Lipomyces starkeyi* can naturally utilize a variety of carbon sources, including lignocellulosic hydrolysates and lignin-derived aromatics These yeasts accumulate triglycerides during growth under nitrogen, phosphorous, or sulfur limitation Despite its ability to produce a wide range of value-added products, little is known about the physiology of these organisms.
In our study, we evaluated the growth of *L. starkeyi* and *R. toruloides* on different carbon sources and performed transcriptome and metabolome analysis to understand the underlying mechanisms of sugar metabolism. We have performed transcriptome and metabolome analysis of *R. toruloides* during growth on glucose, xylose, acetate, and lipids, and *L. starkeyi* during growth on glucose, xylose and cellobiose. We mapped these changes onto the governing metabolic pathways to better understand how these yeasts reprograms its metabolism to enable growth on tested substrates. These results revealed that different metabolite pathways are activated under different carbon sources. The results provide a better understanding of the mechanism of substrate utilization and the identification of the key genes governing the lipogenesis process. We also identified and functionally characterized a few putative sugar transporters from these yeasts in *Saccharomyces cerevisiae* for co-consumption of glucose and xylose. The integration of the metabolite data of central carbon metabolism with gene regulation offers us a better understanding of the metabolic response of both organisms on different substrates. These results could provide the future directions for metabolic engineering of oleaginous yeasts to produce biofuels and bioproducts.

P37 Leveraging epigenetics to improve CAZyme expression in anaerobic fungi

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Anaerobic fungi (Neocallimastigomycota) are native to the digestive tracts of ruminant and hindgut fermenting animals where they are central to the digestion of crude lignocellulosic materials. Anaerobic fungi secrete the largest known diversity of lignocellulolytic carbohydrate active enzymes (CAZymes) in the fungal kingdom (>300 CAZymes), which unaided can degrade up to 60% of the ingested plant material. To this end, our lab is building a suite of genetic and epigenetic tools to enable engineering in these organisms. Efforts include cataloging the most abundant histone post translational modifications, which has never been studied in this phylum. To better document the global extent of epigenetic regulation in gut fungi we are leveraging multiple epigenetic inhibitors (e.g. SAHA, 5’ Azacytidine) to disrupt histone post translational modification state. These treatments enable control of global H3K4 and H3K27 trimethylation and are correlated with up to a 100% increase in xylanase activity. RNA sequencing and differential gene expression for these transcriptomes provides insight into the extent of epigenetic

regulation of anaerobic fungi and enables elucidation of engineering strategies that will be employed in future efforts. Our studies show for the first time that early diverging anaerobic fungi use epigenetic mechanisms for gene expression and establish their role in lignocellulose hydrolysis. Our work provides insight into the mechanisms that control anaerobic fungal plant-degradation abilities and develops facile tools to enhance activity for efficient plant degradation.

P38 Expanding biological routes for ester biosynthesis by engineering promiscuity of thermostable chloramphenicol acetyltransferase

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Robust and efficient enzymes are essential modules for metabolic engineering and synthetic biology strategies across biological systems to engineer whole-cell biocatalysts. By condensing an acyl-CoA and an alcohol, alcohol acyltransferases (AATs) can serve as an interchangeable metabolic module for microbial biosynthesis of a diverse class of ester molecules with broad applications as flavors, fragrances, solvents, and drop-in biofuels. However, the current lack of robust and efficient AATs significantly limits their compatibility with heterologous precursor pathways and microbial hosts. To develop a robust and efficient microbial ester biosynthesis platform compatible with a wide range of microbial hosts and their metabolism, we engineered promiscuity of a robust chloramphenicol acetyltransferase (CAT) to function like an AAT. Through bioprospecting and rational protein design, we developed a highly thermostable CAT with a broad substrate range, capable of synthesizing at least 168 potential esters from whole cell biocatalysts. As a proof-of-concept, we engineered mesophilic *Escherichia coli* for isoamyl acetate production from glucose. We further demonstrated consolidated bioprocessing of ester production by engineering a thermophilic bacterium *Clostridium thermocellum* expressing the engineered CAT, producing about 1 g/L of isobutyl esters from cellulose at elevated temperatures (e.g., 55^{o}C). The high thermostability and promiscuity of the engineered CAT make it a versatile platform for microbial biosynthesis of designer esters from various renewable resources including lignocellulosic biomass.

P39 Development of Emerging Model Microorganisms: *Megasphaera elsdenii* for biomass and organic acid upgrading to fuels and chemicals

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The native ability to efficiently generate C4 to C8 compounds makes *M. elsdenii* a compelling platform for the production of fuels and chemicals from lactate and plant carbohydrates. Our objective is to develop *M. elsdenii* as a platform for the conversion of lignocellulosic biomass and organic acids to long chain alcohols and other valuable chemicals. *M. elsdenii* produces organic acids, (including butyric (C4), valeric (C5), hexanoic (C6), and in some cases octanoic (C8) acid) when grown on lactate and glucose, likely via a chain elongation pathway using acetyl-CoA. As the carbon chain length increases, fuel properties improve, making hexanol a target as a next-generation gasoline blend stock. Previous efforts to make such products in *E. coli* have been moderately successful, but production of C6 and larger products remains low, suggesting that extending the chain elongation pathway beyond a single cycle remains a significant challenge in model organisms. The use of non-model microbes such as *M. elsdenii* to efficiently push carbon through its native chain elongation pathway is a promising approach. We are engineering *M. elsdenii* to efficiently produce next-generation, drop-in lignocellulosic fuels such as hexanol at high yield and titer as a general bioengineering platform. We developed a method for DNA transformation of two strains of *M. elsdenii* via methylome analysis and heterologous expression of DNA methyltransferases in *E. coli* to protect DNA from restriction on transformation into *M. elsdenii.* Heterologous expression of an *adhE2* gene from *Clostridium acetobutylicum* resulted in the production of 5.3 mM butanol in *M. elsdenii* ATCC 25940 grown on lactic acid, representing 7.4% of the detected fermentation products. We developed a gene deletion system based on a counter-selectable genetic marker, and deletion of a propionyl-CoA transferase in the *M. elsdenii* chromosome resulted in increased production of long chain fatty acids. *This is the first demonstration of metabolic engineering in*

Megasphaera and proof of concept that this approach may lead to the accomplishment of our longer-term goals.

P40 *Geobacillus*: a New Platform for High-throughput *in vivo* Discovery and Optimization of Thermophilic Enzymes for Pathway Engineering

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A key challenge in engineering thermophilic micro-organisms is the lack of well characterized proteins that function at relevant temperatures. The vast majority of characterized enzymes, pathways, and genetic tools originate from mesophilic organisms and do not function at high temperatures limiting the genetic tools and enzymes needed for metabolic engineering of thermophilic bacteria. For example, there are no known thermophilic versions of key metabolic enzymes for engineering new pathways for alcohol production in thermophiles. Modeling approaches to increase the thermotolerance of mesophilic proteins have met with limited success. We are developing an *in vivo* platform for screening and *selecting*thermophilic protein variants in *Geobacillus thermoglucosidasius*, taking advantage of its unique ability to grow both aerobically and anaerobically over a temperature range of 37 – 70°C. In addition to generating thermostable variants of key metabolic enzymes, our long-term goals are to improve existing genetic tools to make thermophilic organisms easier to use, and demonstrating their utility by using a combination of rational enzyme design, high throughput construction of mutants, and adaptive evolution to convert mesophilic metabolic enzymes and mesophilic genetic tools into versions that function at thermophilic temperatures. This new platform will open new approaches to creating the thermophilic enzymes needed for current and future microbial engineering efforts. Here we report our progress in engineering strains for the SELECTION of enzymes that function at high temperature. We have constructed deletions of genes that result in strains that REQUIRE isobutanol production for viability during anaerobic growth, allowing the selection of thermostable versions of key pathway enzymes.

P41 Dynamic Control of the Beta-ketoadipate pathway, In-situ Efflux Pump Engineering, and High-throughput Functional Genomics in Pseudomonas putida KT2440 with CRISPR-Cas9 Tools

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Pseudomonas putida KT2440 is a promising chassis for the renewable conversion of lignin into commodity chemicals. While this organism has a wealth of genetic tools available, high-throughput (HTP) experiments that enable forward engineering have not yet been published in this organism. As current microbial engineering efforts rely on the Design, Build, Test cycle to generate desirable production phenotypes, it is critical to deploy HTP capabilities in this organism to domesticate this host. Towards this goal, this work seeks to leverage CRISPR-Cas9 technologies to demonstrate HTP capabilities on various biotechnologically relevant targets.

First, CRISPR-interference was optimized by screening inducible promoters expressing dCas9 on various targets. The arabinose and lactose inducible promoter systems expressing dCas9 preformed best and the dynamic range of of these toolsets were determined across rich and minimal media conditions for four guide designs. Single cell time lapse microscopy coupled with bulk culture experiments charaterize the dynamic response of knockdown and reveal population heterogeneity when targeting fluorescent, essential, and metabolic genes. The CRISPRi system was then used to accumulate 4.75 times more beta-ketoadipate, a nylon precursor, when feeding p-coumarate by inducing the knockdown of *pcaIJ*. This initial study demonstrating the usefulness of CRISPRi has laid the foundation for the study of a gRNA library, constructed through a collaboration with JGI, targeting every coding region in the KT2440 genome. Preliminary data are shown.

Second, the CRISPR-Cas9 genome editing toolset first developed by Sun. et al 2018 has been optimized for library scale mutagenesis. The minimum homology length requirements for efficiently introducing both deletion and single nucleotide polymorphism (SNP) mutations was determined and the transformation protocol was optimized to increase the number of edited cells 100-fold while keeping the editing efficiency at 100%. This updated design was used to introduce libraries of SNPs into the TolC-like multidrug efflux pump, ttgABC, that confers antibiotic and solvent tolerance to *P. putida.* Preliminary selection experiments with piperacillin reveal enhanced growth.

P42 Comparative omics analysis of *Saccharomyces cerevisiae* strains with different isobutanol pathways

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Isobutanol has emerged as a promising next-generation biofuel due to its higher energy density and lower vapor pressure compared to ethanol. The industrial bioethanol producer, *Saccharomyces cerevisiae*, has been identified as a suitable host for isobutanol production due to its high alcohol tolerance and genetic tractability. In this experiment, we aimed to understand how two different metabolic engineering strategies, compartmentalization and cofactor balancing, could affect the performance and physiology of an isobutanol producing *S. cerevisiae* strain. The first strategy entails localizing the five enzymatic steps involved in isobutanol biosynthesis into a single compartment: either the mitochondria or the cytosol. The second strategy involves cofactor balancing the pathway with glycolysis by using an NADH-dependent ketoacid decarboxylase (KARI); glycolysis produces two NADH per molecule of glucose, while isobutanol fermentation naturally consumes one NADPH via KARI and one NADH via ADH. In our work, we equipped yeast with isobutanol cassettes which had either a mitochondrial or cytosolic compartmentalized isobutanol pathway and used either a cofactor imbalanced (NADPH-dependent) or cofactor balanced (NADH-dependent) KARI enzyme; a multiomics analysis was then performed on the engineered strains to elucidate the functional differences between them.
We found that pathway compartmentalization had a greater effect on isobutanol production than cofactor balancing. The strain harboring the mitochondrial localized isobutanol pathway outperformed the cytosolic version by 3.7-fold, while the strain harboring the cofactor imbalanced pathway outperformed the cofactor balanced pathway by 1.5-fold. Furthermore, the transcriptome and proteome of the strains harboring the cytosolic localized pathways were significantly altered; some of the most dramatically altered genes/proteins were involved in sulfur-related pathways (sulfate assimilation and cysteine/homoserine/siroheme biosynthesis). Since one of the enzymes in the isobutanol pathway, dihydroxy-acid dehydratase, requires a 2Fe-2S cluster, we hypothesize the altered sulfur metabolism is related to this cluster requirement. Our current objective is to use the knowledge gained from this multiomics study to inform the design of the next isobutanol producing strain.

P43 Stereospecifically controlling the formation of complex chemical scaffolds remains very challenging. Here, we study the biocatalytic mechanism of how enzymes control the 3*R* or 3*S*-spirooxindole formation in natural products.

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Although stereochemistry plays a crucial role in the efficacy and safety of chiral drugs, stereospecifically controlling the formation of complex chemical scaffolds remains very challenging. Prenylated indole alkaloids featuring characteristic spirooxindole rings possess a 3*R* or 3*S* carbon stereocenter, which determines the bioactivities of these compounds. Despite the stereoselective advantages of spirooxindole biosynthesis compared with those of asymmetric organic synthesis, the biocatalytic mechanism of how to control the 3*R* or 3*S*-spirooxindole formation has been elusive. Here, we report the biochemical characterization of a new oxygenase/semipinacolase CtdE that specifies the 3*S*-spirooxindole construction in the biosynthesis of 21*R*-citrinadin A. High-resolution X-ray crystal structures of CtdE with the substrate and flavin adenine dinucleotide cofactor, together with site-directed mutagenesis and computational studies reveal the catalytic mechanisms for the possible β-face epoxidation followed by a regioselective collapse of the epoxide intermediate, which triggers semipinacol rearrangement to form the

3*S*-spirooxindole. Dynamic repositioning of the flavin adenine dinucleotide upon substrate-binding is also observed in the co-crystal structures. Comparing with PhqK, which specifically catalyzes the formation of the 3*R*-spirooxindole, we revealed an evolutionary branch of CtdE in specific 3*S* spirocyclization to enrich the structural diversity and complexity of prenylated indole alkaloids. Our study provides deeper insights into the stereoselective catalytic machinery, which is important for the biocatalysis to synthesize spirooxindole pharmaceuticals.

P44 New Tools For Targeted Cloning And Over Expression Of Biosynthetic Gene Clusters

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110 biosynthetic gene clusters (BGCs) ranging from 12 to 150 kb from 95 diverse bacterial and fungal strains were successfully captured and cloned using CRISPR-Cas9 to precisely excise the pathway of interest. To improve the success of heterologous expression, we developed a new *Streptomyces* BGC expression vector (pDualP) which uniquely includes two inducible promoter elements, one flanking each side of the cloning site. As a proof of concept, we cloned the ACT and RED BGCs from *S. coelicolor* in both orientations of the pDualP vector, integrated them into *S. lividans* ΔredΔact, and observed inducible production of the blue ACT cluster product and the red RED cluster product but not from the native promoters. Second, we observed a substantial enhancement of the antimicrobial activity of heterologously-expressed, soil-derived metagenomic BGCs through induction with pDualP. Additional clusters cloned and introduced to *Streptomyces* and *Bacillus* heterologous hosts include nystatin, erythromycin, vancomycin, difficidin, bacillusin A, and dozens of novel clusters. Finally, we de-orphaned the stravidin BGC from *Streptomyces* sp. NRRL S-98 in two months using the same approach. These results indicate that virtually any sequenced BGC can be cloned intact from complex genomes, and that direct cloning to a dual-inducible expression vector can greatly accelerate downstream small molecule characterization.

P45 An *E. coli*-based Biosynthetic Platform Expands the Structural Diversity of Natural Benzoxazoles

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Benzoxazoles are frequently found in prescribed pharmaceuticals and medicinally active natural products. To facilitate benzoxazole-based drug development, an eco-friendly and rapid platform for benzoxazole production is required. In this study, we have completed the biosynthesis of benzoxazoles in *E. coli* by co-expressing the minimal set of enzymes required for their biosynthesis. Moreover, by coupling this *E. coli*-based platform with precursor-directed biosynthesis, we have shown that the benzoxazole biosynthetic system is highly promiscuous in incorporating fluorine, chlorine, nitrile, picolinic, and alkyne functionalities into the scaffold. Our *E. coli*-based system thus holds the promise of understanding of substrate selection and incorporation governed by benzoxazole biosynthetic machinery while also paving the way for future generation of novel benzoxazole analogues through protein engineering and combinatorial biosynthesis.

P46 Mechanistic studies into PtmT3 and PtmT1, two bacterial diterpene synthases from the *ptm* BGC

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The diterpenoid family of natural products, represented by the chemotherapeutic paclitaxel, are a diverse family with over 16,000 members; however, currently there are less than 200 characterized bacterial diterpenoids. This family of natural products demonstrates a remarkable amount of structural diversity in part due to the diterpene synthases (DTSs) that are responsible for setting the backbone of these natural

products; moreover, they are commonly able to produce multiple scaffolds. The platensimycin-platencin-platensilin BGC (*ptm* BGC) is an example of a bacterial diterpenoid pathway that encodes for the biosynthesis of three highly functionalized bioactive natural products as a result of three DTSs, two of which work on the shared intermediated created by the previously studied *ent*-CPP synthase, PtmT2. DTSs are enzymes that control the carbocation-mediated cyclization of the C_{20} precursor geranylgeranyl diphosphate (GGPP) and its derivatives. Previous in vivo studies support PtmT3 and PtmT1 as DTSs that are responsible for converting *ent*-CPP to the *ent*-beyerene, *ent*-kauranol, and *ent*-atiserene scaffolds observed in the final natural products of the *ptm* BGC. Although both of these enzymes are both capable of producing *ent*-atiserene, PtmT3 is able to produce all three scaffolds and shares high structural and sequence homology to canonical type I DTSs, while PtmT1 is a dedicated *ent*-atiserene synthase that instead belongs to the newly emerging UbiA family of DTSs. PtmT3 thus provides an excellent opportunity to learn how this family of enzymes can control the formation of these three *ent*-CPP derived scaffolds. Comparisons can subsequently be made to PtmT1 to probe the differences and similarities between canonical and non-canonical type I DTSs are able to produce the same scaffold.

P47 Deciphering how *cis*-acyltransferase assembly line ketosynthases gatekeep for processed polyketide intermediates

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With the redefinition of polyketide synthase (PKS) modules, a new appreciation of their most downstream domain, the ketosynthase (KS), is emerging. In addition to performing its well-established role of generating a carbon-carbon bond between an acyl-CoA building block and a growing polyketide, it may gatekeep against incompletely processed intermediates. Here, we investigate 737 KSs from 100 primarily actinomycete, *cis*-acyltransferase assembly lines. A comparison of 32 active site residues from KSs separated into 16 families (based on the chemistries at the a- and b-positions of their polyketide substrates) revealed unique sequence fingerprints. Surprisingly, distinct fingerprints were also observed from KSs separated into groups based on the chemistries at the a-, b-, and g-positions. To better understand the roles of gatekeeping residues, representative KSs were modeled bound to their natural polyketide substrates. A key glutamine was investigated through mutagenesis within a model triketide synthase. Insights from these studies advance our understanding of the logic employed by PKSs as well as our ability to engineer them.

P48 Engineering Tata Box to Create a Promoter Library to Balance Heterologous Pathways in *Y. Lipolytica*

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Metabolic engineering requires precise control of gene expression to improve flux through target molecules. Oleaginous yeast *Yarrowia lipolytica* has been emerging as an industrial workhorse due to its high flux lipids biosynthetic pathways; however, fewer tools to control gene expression are available compared to model yeast *S. cerevisiae*. To date, most approaches to control gene expression use endogenous promoters or hybrid promoters. Both types of promoters have difficult to anticipate responses to different substrates and genetic contexts. Here, we develop a tunable expression system in *Y. lipolytica* by mutating the TATA Box region in different promoters. The 8bp TATA Box region was randomized through degenerate bases and promoter libraries created to express *hr*GFP. We used FACS to isolate TATA Box mutants resulting in different expression levels under the context of TEF and UAS1B8-TEF promoter. To demonstrate the utility of finely tuned expression systems, we used the expression system to balance a multienzyme heterologous pathway for deoxyviolacein synthesis. This work generates a simple system for heterologous pathway balancing in *Y. lipolytica*

P49 Total biosynthesis of triacsin featuring an *N*-hydroxytriazene pharmacophore

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Triacsins are an intriguing class of specialized metabolites possessing a conserved *N*-hydroxytriazene moiety not found in any other known natural products. Triacsins are notable as potent acyl-CoA synthetase inhibitors in lipid metabolism, yet their biosynthesis has remained elusive. The *N*-hydroxytriazene moiety, containing three consecutive nitrogen atoms, is rare in nature and has only been identified in the triacsin family of natural products. Through extensive mutagenesis and biochemical studies, we here report all enzymes required to construct and install the *N*-hydroxytriazene pharmacophore of triacsins. Two distinct ATP-dependent enzymes were revealed to catalyze the two consecutive N-N bond formation reactions, including a glycine-utilizing hydrazine-forming enzyme, Tri28, and a nitrite-utilizing *N*-nitrosating enzyme, Tri17. This study paves the way for future mechanistic interrogation and biocatalytic application of enzymes for N-N bond formation.

P50 Preparative production of an enantiomeric pair by engineered polyketide synthases

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Modular polyketide synthases (PKSs) synthesize stereochemically complex, bioactive compounds. Reprogramming them would enable the production of designer polyketides in an efficient and environmentally friendly manner. Here we report engineered synthases that furnish 2-stereocenter triketide lactones of opposite handedness with >99% enantiomeric excess (*ee*). The synthases were constructed with the updated module boundary from the genes encoding the pikromycin PKS. After both synthases demonstrated activity *in vitro* and *in vivo*, production was optimized in shake flasks. The activity of one synthase increased 20-fold when it was split into 2 polypeptides through the insertion of docking motifs. Both synthases were at least twice as active compared with synthases designed with the traditional module boundary. In optimized conditions the synthases generated 0.22 g of one enantiomer and 0.39 g of the other per liter of *E. coli* K207-3 culture after 6 days. Both triketide products were readily purified and characterized, including by chiral chromatography and crystallography.

P51 Biosynthesis and heterologous production of mycosporine-like amino acid palythines

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Mycosporine-like amino acids (MAAs) are a family of over 30 water-soluble UV filters isolated mainly from marine organisms, and may also ameliorate UV-induced oxidative stresses, which provides a novel two-pronged strategy for sun protection and presents a new scaffold of next-generation anti-UV agents. However, their broad application is limited by the lack of effective synthesis routes. Recent biochemical and genetic studies have elucidated the biosynthesis of mono- and di-substituted MAAs and supported their biosynthetic production. In this regard, advanced biosynthetic understanding of diverse MAA analogs with different UV protection properties (e.g., palythine and palythene) is significant to further develop this family of natural products as sunscreen ingredients. Herein, we combined different bioinformatic approaches to assess the distribution of the MAA biosynthesis in microbial genomes and identified 92 putative MAA gene clusters mainly from cyanobacterial genomes. Further genome neighborhood network analysis revealed a nonheme iron(II)- and 2-oxoglutarate-dependent oxygenase (MysH) as a potential, new MAA biosynthetic enzyme. We then selected a putative *mysH*-containing gene cluster from *Nostoc linckia* NIES-25 and created multiple refactored clusters to characterize gene functions. High resolution (HR)-LC-MS analysis revealed the production of three disubstituted MAA analogs porphyra-334, shinorine and mycosporine-glycine-alanine in *E. coli* cells expressing refactored *mysH*-free gene clusters. When coexpressed, MysH converted these disubstituted MAAs into corresponding palythines. The UV absorption maxima of palythines are 320 nm, lower than that of disubstituted MAAs at 334 nm. The yield of the most abundant product palythine-Thr reached 2.7 ± 0.3 mg/L. Furthermore, our biochemical characterization for the first time revealed the substrate preference of recombinant MysD, a d-Ala-d-Ala ligase-like enzyme for the formation of disubstituted MAAs. MysD utilized six of 20 natural amino acids to synthesize four known disubstituted MAAs and two novel analogs, highlighting its biocatalytic potential.

Collectively, this work fills a critical gap in the biosynthesis of advanced MAAs, showcases genome-based discovery of new biosynthetic enzymes, and opens new opportunities to developing next-generation sunscreens for broad UV protection.

P52 Thioacid biosynthesis and incorporation: the late stages of the thioplatensimycin-thioplatencin-thioplatensilin pathway

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Natural products have always been at the forefront of new drug discovery, and with the advent of the post-genomic era, they will remain so well into the future. During this process, thousands of natural products have been characterized, but of these, less than ten have been characterized to contain the thioacid functional group. Three thioacid-containing diterpenoid fatty acid synthase inhibitors, thioplatensimycin (tPTM), thioplatencin (tPTN), and thioplatensilin (tPTL), originate from the *ptm* biosynthetic gene cluster (BGC) in *Streptomyces platensis*. Recently, our lab has demonstrated that the thioacid functional group is introduced by a pair of enzymes, encoded by a two-gene cassette, that catalyze the transfer of sulfur from a sulfur carrier protein to a CoA-linked natural product. In tPTM-tPTN-tPTL biosynthesis, the resultant 3-amino-2,4-dihydroxybenzoic thioacid is then coupled to the varied diterpenoid ketolide-CoAs by a third enzyme, PtmC, a novel member of the widespread arylamine *N*-acetyltransferase family. Despite the apparent rarity of characterized thioacid-containing natural products, a survey of bacterial genomes indicates more than 300 distinct putative BGCs containing this thioacid gene cassette. Therefore, the questions of how and why the unstable thioacid moiety is synthesized and incorporated into natural products becomes more general and less niche.

P53 Evaluation of Sulfhydralase Domains and Persulfide Intermediates in the Biosynthesis of Leinamycin and Guangnanmycin

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Polyketides are the most structurally diverse family of natural products, and they traditionally consist of carbon, hydrogen and oxygen atoms. Sulfur atoms are not typically found within the scaffolds of polyketide natural products. The leinamycin (LNM) family of natural products, which includes guangnanmycin (GNM), contains multiple sulfur atoms embedded within a hybrid peptide-polyketide scaffold. The sulfur atoms are key to the biological activity of the natural products, and their incorporation by a polyketide synthase (PKS) represents an unprecedented type of chemistry for PKSs. We have investigated the origin of the sulfur atoms in the biosynthesis of LNM and GNM and characterized a sulfhydralase (SH) domain within the *lnm* and *gnm* PKS assembly lines. Using a combination of bioinformatics, genetics, microbial fermentation, organic synthesis, and enzymology, we have shown that the SH domains are responsible for the formation of persulfides within their respective PKS assembly lines. This discovery has provided key insights into the sulfur incorporation mechanism for the LNM family of natural products and led us to propose a new model for their biosynthesis.

S41 Predicted metabolites from multiple microbiome sequencing data modulate Autism Spectrum Disorder phenotype in mice

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Introduction:
Gut microbiome dysbiosis is associated with central nervous system disorders such as autism spectrum disorder (ASD). The impact of intestinal microbial metabolism in ASD can be estimated from multiple cohorts then tested in vivo.

Methods:

Ten 16S rRNA amplicon, three shotgun metagenomics and one metatranscriptomics sequencing data from stools of 588 ASD and 518 age- and/or sex-matched neurotypical pediatric subjects were analyzed. Differentially abundant microbial metabolites associated with ASD were predicted, followed by meta-analysis to identify concordance across datasets. Selected metabolites were tested in CNTNAP2 mice for modulation of brain RNA-seq pathways and behavior associated with ASD.

Results:

We identified two bile acids, Glycodeoxycholate (GDC) and Ulsodeoxycholate (UDC), and other metabolites as concordantly decreased in ASD across datasets. Brain RNA-seq of the mice fed the metabolites exhibited enrichment of neuronal system and neurotransmitter receptors and postsynaptic signal transmission pathway gene expressions. Increased habituation exploration and decreased anxiogenic behavior were observed with GDC and UDC fed CNTNAP2 mice, respectively.

Conclusion:

By applying prediction algorithms and meta-analysis to a large set of microbiome sequencing data, we were able to identify metabolites that modulate behaviors in mice. This approach accelerates the drug discovery process in ASD and can be translated to other disease areas of interest.

S127 Discovery of the non-proteinogenic amino acid homoarginine provides a key to unlock cryptic natural product biosynthetic pathway

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L-Homoarginine (hArg) is a non-proteinogenic amino acid present in the human metabolome. Together with the proteinogenic amino acid, L-arginine, hArg is a substrate for nitric oxide synthases leading to the production of the vasodilator nitric oxide. Due to its role in nitric oxide production, the abundance of hArg in blood plasma is negatively correlated with cardiovascular and renal dysfunction. While the function of hArg and its relevance as a disease biomarker in mammalian physiology is well validated, the presence and role of hArg in other biological systems has received lesser attention. We recently reported the detection of hArg in the benthic marine invertebrate animals - marine sponges. Sponges and their symbiotic bacteria produce small organic molecules called natural products. These natural products are produced to perform specific biological functions which make them suitable lead candidates for pharmaceuticals. Two such classes of biologically active natural products were detected in the sponges within our study - pyrrole-imidazole alkaloids and brominated tyrosine alkaloids. They are a large and chemically complex class of sponge natural products with many congeners. Despite their varied diversity, insights into their biosynthesis are scant. Querying the sponge metabolomes for plausible intermediates in their biosynthetic pathways, hArg was discovered in high abundance. We rationalized hArg to be a biosynthetic precursor for pyrrole-imidazole and two brominated tyrosine alkaloids, serving as a critical branch point to connect primary metabolite lysine with the production of these natural products. Further, hArg is a substrate for the production of nitric oxide, a metabolite with effects on marine larval settlement and development. Our study provides the framework for further investigation of the biosynthesis and role of hArg in marine sponge holobionts. We have used less than 1 g of biomass for each sponge specimen, highlighting the applicability of contemporary -omic technologies in transcending the limitation of biomass availability.

P56 Biosynthesis of valinophos uncovers a new route for phosphonic acid natural products

Y. Zhang, L. Chen, J. Wilson, T. Pham, C. Kayrouz and K.S. Ju, The Ohio State University, Columbus, OH, USA*

Valinophos, produced by *Streptomyces durhamensis*, is an unusual phosphonopeptide natural product composed of (*R*)-2,3-dihydroxypropylphosphonic acid (DHPPA) and N-acetyl L-valine connected via an ester bond. The structural novelty and potential antimicrobial activity of DHPPA prompted us to investigate its biosynthetic origins. Bioinformatic analysis of the *S. durhamensis* genome revealed a

seven gene neighborhood surrounding phosphoenolpyruvate mutase that we hypothesized as the biosynthetic gene cluster for valinophos. Cloning and heterologous expression of these genes in *S. lividans* resulted in production of both DHPPA and valinophos, supporting their role in biosynthesis. The function of each gene was subsequently determined through deletion analysis, the identification of accumulated intermediates, and biochemical reconstitution of each proposed step. Overall, a combination of five distinct isomerization, reduction, and phosphorylation reactions is required to transform phosphoenolpyruvate into DHPPA, to which L-valine is ligated. Notably, the DHPPA pathway represents a previously unknown biosynthetic avenue towards phosphonic acid natural products. Genomic surveys revealed the genes for DHPPA to be broadly encoded within the genomes of diverse microbes including *Streptomyces*, *Nocardia*, *Pantoea* and *Pseudomonas*, suggesting many other phosphonic acid natural products may derive from this new biosynthetic route.

P57 Combinitorial engineering of pikromycin polyketide synthase modules

J. Lutgens, R. Desai and A. Keatinge-Clay, The University of Texas at Austin, Austin, TX, USA*

Modular polyketide synthases (PKSs) can be rationally engineered to expand upon the medicinal chemical space that we can access. Therefore, decades of research have aimed to understand and exploit the principles by which PKS modules organize and operate. The ability to perform such novel organic synthesis efficiently, however, has largely eluded investigators. We thus used combinatoric engineering of new PKSs as a solution. Using the recently updated PKS module boundaries and a modular cloning platform, we re-ordered modules of the PKS that produces pikromycin to engineer novel PKSs. As proof-of-principle, we first created a four-module tetraketide synthase whose product was detected by liquid chromatography-mass spectrometry. Such proof enabled the use of combinatoric engineering to explore the specificity of module-module compatibility within the pikromycin PKS. Keeping the first and seventh modules of this synthase in place, we engineered all five triketide synthases and all 25 tetraketide synthases that were accessible by reordering modules in between. Our results elucidate the semi-specific nature of PKS modules, helping explicate the rules that govern interactions between modules of the pikromycin PKS and understand solutions to existing limitations of PKS engineering.

P58 Combinatorial engineering of polyketide synthases using updated module boundaries

K. Ray, The University of Texas at Austin, Austin, TX, USA*

Type I Polyketide Synthases (PKSs) are modular enzymatic assembly lines responsible for producing a variety of secondary metabolites in bacteria that have proved useful in medicine and agriculture. Their assembly line nature is due to their composition of several modules: groups of enzymatic domains that work to extend and modify the growing polyketide intermediate. Evolutionary co-migration has revealed that the functioning module unit has the ketosynthase (KS) domain at the downstream end of the module, rather than the traditional position at the upstream end. This has put the KS domain into a powerful position to gatekeep against incorrect intermediates. This redefinition of the module boundary has allowed us to rapidly engineer the modules of the pikromycin synthase using BioBrick style assemblies. To gain more insight to PKS engineering, we are working on a library of tetraketide and pentaketide products that take advantage of the updated boundary of the modules of the pikromycin synthase. In particular, tetraketides with the structure of 1YZ7 and pentaketides with 1XYZ7, where X, Y, and Z represent modules 2-6 of the pikromycin synthase will be constructed. Polyketide products will be characterized by LC-MS and purified for NMR and/or small molecule X-Ray Diffraction (XRD). Combinatorial assemblies of modules can be assessed using the high-throughput method of Mass-Spec Imaging (MSI). Synthases that are non-functional provide a platform to investigate KS gatekeeping and residues thought to interact with and exclude the non-natural substrate will be mutated to attempt to engineer substrate specificity. This work is testing the limits of combinatorial polyketide biosynthesis and PKS engineering.

P59 Engineering polyketide synthases to produce triketides and tetraketides

R. Bista[*] and A. Keatinge-Clay, The University of Texas at Austin, Austin, TX, USA

Type I Polyketide Synthases (PKSs) are enzymatic assembly lines composed of several modules. These modules work together to produce secondary metabolites which have potential in medicine. We utilize the updated module boundary (downstream ketosynthase as the end of a module instead of the upstream ketosynthase as in traditional boundary) of these megadalton structures to engineer several triketides and tetraketides synthases. We plan to understand the full potential of these modular assemblies in producing even larger products. We designed different strategies to engineer the triketides and tetraketides synthases and test them for the production of expected products.
In first strategy, tetraketide synthases Pik 1X67 were comprised of 3 polypeptides (docking domains were added where necessary). The DNA encoding these polypeptides were inserted on 3 expression plasmids. The three plasmids of each synthase were transformed into E. coli K207-3 cells. The production of tetraketides was tested in vivo until 6 days.

In second strategy, we utilized a combinatorial approach for the engineering of several triketide and tetraketide synthases. DNA encoding the modules was used to genetically combine the modules in a BioBrick fashion to design the synthesis of triketide Pik 147 and tetraketides Pik 1X47 (pyrones). The production was analyzed in vivo until 6 days in a shaker at 19 degrees. The triketide and tetraketides were characterized by LC-MS and HPLC. To optimize the synthases that had no activity, we are currently analyzing our gatekeeping residues in the downstream ketosynthase domains.

Tuesday, August 10

8:00 AM - 11:00 AM Session: 10: Plastics: synthesis, deconstruction, and conversion

Conveners: **Allison Werner**, National Renewable Energy Laboratory, USA, Golden, CO, USA and **Adam Guss**, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Waller Ballroom - Salon F, Level 3

8:00 AM S43: Characterization and engineering of the two-enzyme system from *Ideonella sakaiensis* for poly(ethylene terephthalate) depolymerization

E. Erickson[*], B. Knott, J. Gado and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; T. Shakespeare, R. Graham, A. Pickford and J. McGeehan, University of Portsmouth, Portsmouth, United Kingdom

The continued accumulation of single-use plastic waste products presents a serious and growing risk to natural environments around the globe. The possibility of harnessing enzymatic hydrolysis of waste PET to develop a chemical recycling or upcycling process holds promise for mitigating accumulation of this abundant polyester. A number of PET-hydrolyzing enzymes have been identified and characterized to date, including the PETase enzyme from *Ideonella sakaiesis* 201-F6*.* PETase, together with a second native enzyme, MHETase form a two-enzyme system for the conversion of PET to its constituent monomers, terephthalic acid (TPA) and ethylene glycol (EG). This two-enzyme system affords the bacterium an ability to use PET as a major carbon and energy source. Using the two *Ideonella* enzymes as model biocatalysts for biological PET conversion, we have characterized the catalytic, structural, and thermal properties of the enzymes individually. When both enzymes are used in combination, we observe a highly synergistic relationship for PET conversion. Using the mechanistic insights from these studies to guide enzyme engineering, we have also identified enzyme variants with improved catalytic performance. Despite their utility for PET hydrolysis, the wild-type *Ideonella* PETase and MHETase enzymes are not optimized for industrial application. Nevertheless, study of these enzymes provides an opportunity to elucidate fundamental structural and mechanistic characteristics that permit biological depolymerization of an otherwise recalcitrant synthetic polyester material.

8:30 AM S44: Advancing sustainable and renewable solutions that address plastic pollution with biodegradable counterparts

T. Cooper, Danimer Scientific, Bainbridge, GA, USA*

Danimer Scientific is changing the biodegradability market. After our recent IPO, we are aggressively working with our partners to deliver sustainable solutions for plastics. The challenge of ending plastic pollution is a difficult one. But Danimer Scientific is taking positive steps toward meeting it with PHA. Sustainable, renewable, bio-based and completely biodegradable, PHA holds enormous promise for replacing plastics made from petrochemicals. Danimer Scientific biopolymer resins can be adapted to a wide range of products. Because of this, we can create customized mixtures of biopolymers, tailored to our customers' needs. Our PHA Resin has been certified as biodegradable and compostable in all mediums, including soil, compost, freshwater and saltwater environments. It is also FDA approved as safe for use in food-contact applications.

9:00 AM Break

9:30 AM S45: Near complete depolymerization of polyesters with nano-dispersed enzymes

C. DelRe, PhD and T. Xu, PhD, University of California, Berkeley, Berkeley, CA, USA*

Successfully interfacing enzymes with polymers affords new plastics with programmable depolymerization at the material's end-of-life. However, achieving depolymerization down to the polymer's small molecule building blocks, and maintaining enzymatic latency during material usage, requires controlled solid-state biocatalysis. Embedding enzyme microparticles is known to speed up polyester degradation, but compromises host properties during usage and unintentionally accelerates the formation of microplastics with partial polymer degradation due to random chain scission. Here we show that by nanoscopically embedding enzymes with deep active sites, semi-crystalline polyesters can be degraded on-demand primarily via chain end-mediated processive depolymerization akin to polyadenylation-induced messenger RNA decay. The chain end-mediated mechanism enables programmable enzymatic latency and material integrity during usage and provides control over degradation temperature by modulating the plastic's crystalline lamellae thickness. Furthermore, polyesters formulated with certain additives resist processive degradation due to hierarchical changes in crystalline morphology; however, synergistic enzyme mechanisms can be exploited for near complete depolymerization. Our work provides molecular guidance towards enzyme–polymer pairing and the selection of enzyme-protecting additives to modulate substrate selectivity and optimize biocatalytic pathways.

10:00 AM S46: Biological upcycling of deconstructed poly(ethylene terephthalate) to β-ketoadipic acid

A. Werner, R. Clare, I. Pardo, K. Ramirez, S. Haugen, F. Bratti, C. Johnson, N. Rorrer, D. Salvachua and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; T. Mand, Q. Peabody, J. Huenemann, G. Dexter, J. Elmore and A. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

As the most abundantly consumed synthetic polymer, poly(ethylene terephthalate) (PET) is a major source of plastic waste. Towards open-loop upcycling of PET, here we combine chemocatalytic PET depolymerization with subsequent biological transformation of the monomers to higher-value products. Specifically, we metabolically engineer *Pseudomonas putida* KT2440 to (i) utilize ethylene glycol more efficiently, (ii) catabolize terephthalate (TPA), (iii) catabolize bis(2-hydroxyethyl) terephthalate (BHET), and (iv) convert BHET to a performance-advantaged bioproduct, β-ketoadipic acid (βKA). The resulting strain produced 15.1 g/L βKA from BHET at 76% molar yield in bioreactors. We further demonstrate conversion of chemocatalytically depolymerized PET to βKA. Overall, this work highlights the potential of tandem catalytic deconstruction and biological conversion as a means to upcycle waste PET.

8:00 AM - 11:00 AM Session: 9: New Modes of Natural Product Bioactivity

Conveners: **Dr. Ashu Tripathi**, University of Michigan Life Sciences Institute Rogel Cancer Center, MI, USA and **Dr. Jan Claesen**, Department of Cardiovascular and Metabolic Sciences Cleveland Clinic Lerner College of Medicine, CWRU, Cleveland, OH, USA

Waller Ballroom - Salon A, Level 3

8:00 AM S40: *Trachymyrmex septentrionalis* ants promote fungus garden hygiene using *Trichoderma*-derived metabolite cues

S.P. Puckett, K.E. Kyle, R.M. Samples, J.L. Klassen and M.J. Balunas, University of Connecticut, Storrs, CT, USA; A.M. Caraballo-Rodríguez and P.C. Dorrestein, University of California San Diego, La Jolla, CA, USA*

Trachymyrmex septentrionalis ants are fungus-growing ants primarily located along the eastern seaboard of the United States. The ants are part of a multipartite symbiosis wherein ants bring leaves and/or other organic material to be digested by the fungus garden, and the garden, in turn, differentially grows gongylidia sacs the ants use as their main food source. Since this garden is crucial for ant survival, the ants have adopted various techniques to keep their gardens healthy, such as maintaining garden hygiene by physically removing pieces of compromised fungus garden known as weeding. However, how ants distinguish healthy from unhealthy garden is not known. The goal of this project was to determine if chemical cues induce weeding and if so, what metabolites contribute to this behavior. Using a combination of genomics, metabolomics, and infection analyses, *Trichoderma* fungi were determined to be present and potentially pathogenic in both field and laboratory experiments. Peptaibols, a class of common *Trichoderma* metabolites, were found to be highly abundant in bioactive fractions and purified peptaibols were confirmed to induce ant weeding behavior. Future experiments will explore if cues are sensed by the ants directly or if weeding behavior is the result of cues from infected fungus gardens and thus comprises an extended defense response.

8:30 AM S39: Targeting microRNA by natural products as therapeutic strategy against human diseases

Y. Zhuang, O. Mohamed, F. Yu and A. Garner, University of Michigan, Ann Arbor, MI, USA; A. Tripathi, University of Michigan Life Sciences Institute Rogel Cancer Center, MI, USA*

RNA-targeted drug discovery is an exciting, yet challenging area of medicinal chemistry. A major bottleneck toward promoting this field has been the discovery of new chemical space for targeting RNAs and methods that will enable such discovery efforts. Over the past few years, in collaboration with Prof. Amanda Garner at the University of Michigan, we have developed platform assay technology capable of identifying RNA-binding, selective inhibitors of disease-relevant human miRNAs. Using this approach, we have revealed natural products as a promising area of chemical space for the discovery of such molecules. The impact of natural products in RNA targeting is significant, as many of the clinically approved ribosome-targeted antibiotics are natural products, and these compounds are the only RNA-targeted drugs that have received FDA approval. Moreover, natural products have been invaluable for developing front-line drugs against cancer, infectious diseases, and parasites, and as tools for furthering our understanding of cellular biology. In our lab, we have developed a unique “E-PurE” meta-data based approach to rapidly identify and characterize chemical entities from complex microbial extracts. By combining the power of our screening technology and “E-PurE” platform with the rich chemical diversity represented by natural products, we will discover and develop novel, first-in-class miRNA-targeted therapeutics for the treatment of cancer. Targets of interest include miR-21, -155, -10b and -17~92. Herein we describe our first efforts in natural product inhibitor discovery leading to the identification of a depsipeptide class of natural products as RNA-binding inhibitors of Dicer-mediated miRNA processing.

6:00 PM S41: Predicted metabolites from multiple microbiome sequencing data modulate Autism Spectrum Disorder phenotype in mice

T. DeSantis, M.S.[*]*, SECOND GENOME, INC., Brisbane, CA, USA*

Introduction:

Gut microbiome dysbiosis is associated with central nervous system disorders such as autism spectrum disorder (ASD). The impact of intestinal microbial metabolism in ASD can be estimated from multiple cohorts then tested in vivo.

Methods:

Ten 16S rRNA amplicon, three shotgun metagenomics and one metatranscriptomics sequencing data from stools of 588 ASD and 518 age- and/or sex-matched neurotypical pediatric subjects were analyzed. Differentially abundant microbial metabolites associated with ASD were predicted, followed by meta-analysis to identify concordance across datasets. Selected metabolites were tested in CNTNAP2 mice for modulation of brain RNA-seq pathways and behavior associated with ASD.

Results:

We identified two bile acids, Glycodeoxycholate (GDC) and Ulsodeoxycholate (UDC), and other metabolites as concordantly decreased in ASD across datasets. Brain RNA-seq of the mice fed the metabolites exhibited enrichment of neuronal system and neurotransmitter receptors and postsynaptic signal transmission pathway gene expressions. Increased habituation exploration and decreased anxiogenic behavior were observed with GDC and UDC fed CNTNAP2 mice, respectively.

Conclusion:

By applying prediction algorithms and meta-analysis to a large set of microbiome sequencing data, we were able to identify metabolites that modulate behaviors in mice. This approach accelerates the drug discovery process in ASD and can be translated to other disease areas of interest.

9:30 AM Break

10:00 AM S42: Reimagining Druggability using Chemoproteomic Platforms (Remote)

D. Nomura[*]*, University of California, Berkeley, Berkeley, CA, USA*

The Nomura Research Group is focused on reimagining druggability using chemoproteomic platforms to develop transformative medicines. One of the greatest challenges that we face in discovering new disease therapies is that most proteins are considered “undruggable,” in that most proteins do not possess known binding pockets or “ligandable hotspots” that small-molecules can bind to modulate protein function. Our research group addresses this challenge by advancing and applying chemoproteomic platforms to discover and pharmacologically target unique and novel ligandable hotspots for disease therapy. We currently have three major research directions. Our first major focus is on developing and applying chemoproteomics-enabled covalent ligand discovery approaches to rapidly discover small-molecule therapeutic leads that target unique and novel ligandable hotspots for undruggable protein targets and pathways. Our second research area focuses on using chemoproteomic platforms to expand the scope of targeted protein degradation technologies. Our third research area focuses on using chemoproteomics-enabled covalent ligand discovery platforms to develop new induced proximity-based therapeutic modalities. Collectively, our lab is focused on developing next-generation transformative medicines through pioneering innovative chemical technologies to overcome challenges in drug discovery.

8:00 AM - 11:30 AM Session: 11: Unconventional Fermentations and feedstocks - (e.g. solid phase, continuous, etc)

Conveners: **Katy Kao**, San Jose University and **Ching Leang**, Lanzatech

Waller Ballroom - Salon C-D, Level 3

8:00 AM S47: Formyl-CoA elongation (FORCE) pathways for orthogonal C1 bioconversion and synthetic methylotrophy

A. Chou, S.H. Lee, F. Zhu, J. Clomburg and R. Gonzalez, University of South Florida, Tampa, FL, USA*

One-carbon (C1) compounds represent low-cost and abundant feedstocks for the chemical industry [1]. Due to the often dilute and/or disperse nature of these feedstocks, biochemical processes have the potential to be effective technologies for C1 utilization by enabling lower capital expenditure and distributed manufacturing in ways that current chemical technologies are limited [1]. While C1 molecules can be utilized by microorganisms for growth, the biological production of industrial chemicals from C1 feedstocks as well as the efficient growth of industrial organisms on C1 substrates remain as open challenges.

All metabolic engineering efforts reported to date for C1 bioconversion have exploited the canonical architecture of metabolism, which involves pathways for C1 assimilation, central carbon metabolism, and product synthesis. In this work, we develop an alternative approach based on synthetic pathways for C1 bioconversion that are orthogonal to the host metabolic network, thus minimizing crosstalk with native metabolism and enabling more efficient biocatalysts. The engineered pathways are based on formyl-CoA elongation (FORCE) reactions catalyzed by the enzyme 2-hydroxyacyl-CoA lyase [2] and generate multi-carbon metabolites directly from C1 elongation units in the form of formyl-CoA. FORCE pathways include aldose elongation, α-reduction, and aldehyde elongation and can be used directly for product synthesis or for the generation of substrates for biocatalyst growth or maintenance. We design and assess the feasibility of pathway variants for orthogonal C1 bioconversion and synthetic methylotrophy using thermodynamic and stoichiometric analyses. We further evaluate their ability to support product synthesis in resting and growing cultures of the non-methylotrophic bacterium *Escherichia coli*. Finally, we demonstrate that the pathways can support methylotrophy in a two-strain *E. coli* co-culture system. FORCE pathways are envisioned to serve as a facile way to establish C1 bioconversion and synthetic methylotrophy for biotechnological applications with significant advantages over other natural or synthetic platforms.

***1**-Clomburg, J. M., Crumbley, A. M. & Gonzalez, R.. Science 355, aag0804 (2017).*

***2**-Chou, A., Clomburg, J. M., Qian, S. & Gonzalez, R. Nat. Chem. Biol. 15, 900–906 (2019).*

8:30 AM S48: High throughput gene-to-trait discovery in Pseudomonas putida KT2440 to improve growth on lignin

C. Eckert, CU Boulder, Boulder, CO, USA*

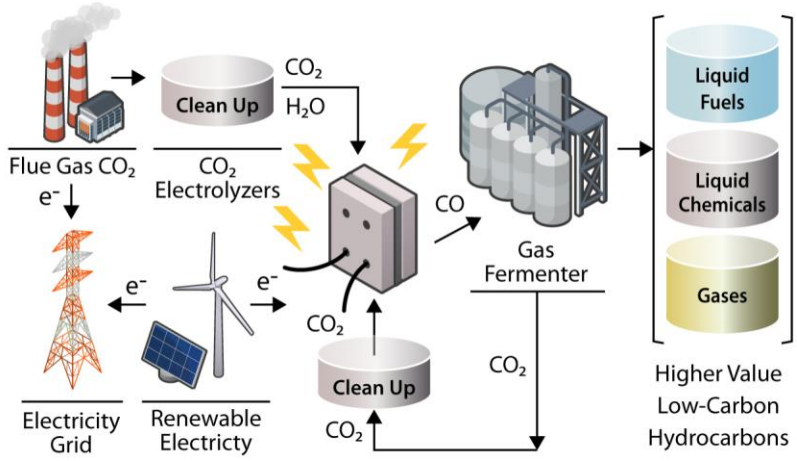
Pseudomonas putida KT2440 is emerging as a top microbial chassis for the conversion of lignin into a variety of valuable compounds. Over the last few years, the number of genetic tools available has grown substantially, although their implementation for high-throughput (HTP) studies has been limited. We are utilizing developed CRISPR-Cas9 technologies to demonstrate HTP capabilities to identify genotype-phenotype relationships to improve utilization and tolerance of lignin feedstocks and to engineer improved production of target chemicals. We have optimized CRISPR interference (CRISPRi) and determined the dynamic range across a variety of conditions. In addition, we performed single cell time lapse microscopy as well as bulk culture experiments to better characterize the dynamic response of knockdown, revealing population heterogeneity when targeting fluorescent, essential, and metabolic genes. We also utilized

CRISPRi to increase production of beta-ketoadipate using p-coumarate as a sole feedstock. To better understand guide design for genome-scale experiments, we have additionally constructed a guide RNA library, in collaboration with JGI, targeting every coding region in the KT2440 genome. We are also developing a CRISPR-based method for library scale protein mutagenesis that has been utilized to introduce libraries of SNPs into the TolC-like multidrug efflux pump, ttgABC, to identify mutations that lead to increased antibiotic and solvent tolerance as an initial proof-of-concept.

9:00 AM S49: Rewiring Today's Biorefineries to Improve Carbon Efficiency

M. Resch, E. White, L. Magnusson, L. Ford and M. Guarnieri, NREL, Golden, CO, USA; Z. Lui and R. Masel, Dioxide Materials, Boca Raton, FL, USA; S. Simpson, LanzaTech, Skokie, IL, USA*

To reduce atmospheric CO_2 emissions, we are investigating an integrated bioelectrocatalytic approach to produce fuels and chemicals from industrial flue gas streams. In partnership with Dioxide Materials and LanzaTech, we have integrated CO_2 electrolysis and gas fermentation. This presentation will highlight our research to understand the effects of variable flue gas compositions and efforts to improve the energy and carbon efficiency of the integrated bioprocess. We discuss our observations on the effects of sulfur compounds on the Membrane Electrode Assembly (MEA) and CO selectivity on an Ag cathode, as well as Adaptive Laboratory Evolution (ALE) of chemically mutated *C. autoethanogenum* under different ratios of CO:H_2 to define process limitations and achieve enhanced performance. This bolt-on-capable technology has the potential to increase the carbon efficiency, sustainability, and profitability of 1st and 2nd generation ethanol biorefineries by converting more of the feedstock carbon into fuels and chemicals.



9:30 AM S50: Small microorganisms - big impact: Industrial-scale Biomethanation with Archaea (R)

D. Hafenbradl, Electrochaea GmbH, Planegg, Germany*

Electrochaea's biomethanation technology produces methane from CO_2 and H_2 using a patented archaea strain. The CO_2 can come from a purified stream or a feedstock that already contains CH_4, such as biogas. When H_2 is produced by electrolysis, the process is called power-to-gas because electrical energy is converted into chemical energy. The second step in this power-to-methane process transfers the chemical energy into CH_4 molecules. When renewable power is used, the resulting methane is a renewable replacement for natural gas. Thus, intermittent sources of renewable energy can be stored for later use. In addition, the renewable methane can be used to green the gas grid and serve to recycle CO_2. Operational characteristics of the biomethanation process have been demonstrated at two industrial scale pilot plants in Denmark and Switzerland. They both typically operated with a 15-25 Nm^3 output of product gas when pure CO_2 was used as an input gas. The approximate 3000 L reactor represents more than a 7,000-fold increase in scale from the original laboratory biomethanation experiments. Of the CO_2 fixed by the archaeal biocatalyst, as little as 1.5% is used for biocatalyst regeneration and maintenance.

Greater than 97% of the CO_2 or H_2 introduced into the reactor is recovered as CH_4. The product gas quality exiting the methanation reactor reaches greater than 97% methane content without any separation procedures. The content of the product gas is not altered by changing flow rate of the feed gases, as long as the ratio of the two substrates, H_2 and CO_2, is maintained at 4:1. The gas quality and quantity is also maintained during repeated start and stop sequences. The demonstrated flexibility indicates that the system is suitable for load following or intermittent operation that would be required for operation using PV or wind power. Electrochaea is currently completing design work for a commercial, scalable biomethanation plant, with an additional 10-50-fold increase in throughput, supported by the European Union Horizon 2020 Programme (grant 101010276).

8:00 AM - 11:30 AM Session: 13: Biodegradation and Bioremediation: Emerging Contaminants and Novel Technologies

Conveners: **Kevin Finneran**, Clemson University

Waller Ballroom - Salon E, Level 3

8:00 AM S55: Bioremediation 3.0 - The new frontier in environmental biotechnology

K. Sorenson, PhD, PE and D. Saran, Allonnia, LLC, Boston, MA, USA*

The use of bioremediation to treat wastewater dates back to the Romans, circa 600 BC. Arguably its first intentional use was over 100 years ago with the invention of activated sludge. We refer to this use of naturally occurring microorganisms to treat wastewater as Bioremediation 1.0. In the late 1960s, bioremediation was first used commercially to treat an oil spill and Bioremediation 2.0 was launched, the use of microorganisms to treat contaminated sites. Over the last 50+ years we have seen genetic engineering lead to the first U.S. patent for an engineered organism (*Pseudomonas putida*), discovery of and bioaugmentation with the novel dehalogenator, *Dehalococcoides spp.*, and the widespread use of in situ biodegradation for diverse organic and inorganic contaminants. Nevertheless, some contaminants remain recalcitrant to biodegradation (e.g., polychlorinated biphenyls and dioxins), and emerging contaminants, especially per- and polyfluorinated alkyl substances, or PFAS, pose a new set of challenges.

While nature will likely find a way to degrade these compounds eventually, that could take decades or more. Recent advances in synthetic biology and automated genetic engineering have created an opportunity to accelerate nature's ability to process these contaminants. The intersection of an established practice of bioremediation with these recent advances opens the door for Bioremediation 3.0 – the rapid development of transformative biology building on microorganisms and degradation pathways found in nature to solve the most difficult problems in contamination and waste.

Results from three different proof-of-concept studies will be presented to illustrate the possibilities of this new era of bioremediation. One application looks at methods being used to develop a biological solution to detoxify produced water from oil and gas operations. The second application explores the development of biological strains that facilitate ore beneficiation to remove impurities and decrease the carbon footprint of ore processing. The third application explores the identification of enzymes capable of degrading PFAS constituents. While much work remains to be done, the preliminary results suggest an exciting future for environmental biotechnology.

8:30 AM S56: Membrane PLFA remodelling in *Halanaerobium congolense* WG10 and mixed enrichment cultures grown across subsurface environmental and laboratory engineered gradients

C. Ugwuodo, F. Colosimo and P. Mouser, PhD, University of New Hampshire, Durham, NH, USA*

The subsurface is being engineered at unprecedented scales for numerous purposes including energy recovery, CO_2 storage, and environmental mitigation. The hydraulic fracturing of black shale formations for natural gas and oil recovery is a subsurface engineering technique increasingly being used to meet rising global energy demands. Halotolerant anaerobic microbial communities play an important role in altering system biogeochemical dynamics, with implications for effective hydrocarbon recovery and shale well longevity. However, we lack a mechanistic understanding of biomass-fluid-mineral interactions across changing subsurface conditions. The microbial membrane protects the cell from external stressors and mediates critical cellular functions such as transport, metabolism, aggregation into biofilms and attachment to surrounding matrices. In this study, we applied mass spectrometry to investigate the membrane phospholipid fatty acids (PLFAs) of pure *Halanaerobium congolense* WG10 and mixed enrichment cultures from hydraulically fractured wells in West Virginia, grown for the first time in continuous culture (using a 1-L Sartorius Biostat® Q-plus system) under four growth rates (hydraulic retention times (HRTs) of 72 h, 48 h, 24 h and 19.2 h), two temperatures (40°C and 25°C), and three salinities (7%, 13% and 20% NaCl). Initial findings indicate that these subsurface microbes substantially remodel their membrane PLFA composition and chemistries across the controlled environmental and engineered gradients. This involves changes in saturation/unsaturation, acyl chain length modification and the incorporation of stress-induced fatty acids such as hydroxy- and cyclopropane fatty acids. These alterations influence the structural configuration and dynamic properties of the membrane including packing, thickness, phase behavior, permeability, diffusivity, and bending rigidity, all of which are relevant to membrane-associated cellular processes such as respiration and attachment. This study provides new insight into our understanding of subsurface microbial membrane mechanics and PLFA-driven biophysical changes under varying engineered shale conditions, both of which are important for elucidating biomass-matrix interactions essential for maintaining effective energy capture.

9:00 AM S57: Bacterial remediation of microcystin-HAB toxins

C. Jung, M. Carr, K. Indest and N. Harms, US Army CoE - ERDC, Vicksburg, MS, USA*

Harmful cyanobacterial/algal blooms (HABs) are a worldwide problem. The immediate and direct effects of bloom events occur when the cyanobacteria biomass clog waterways and fish gills and physically impair aquatic wildlife movement or oxygen exchange. As cyanobacteria die and decompose, nutrients are released, allowing bacteria to flourish and drive available oxygen to critically low levels. Furthermore, toxin producing cyanobacteria have been documented in almost all states and are a high priority concern for inland waterways. The most commonly occurring cyanobacterial HABs are from the genera *Microcystis*, *Anabaena*, and *Planktothrix* (*Oscillatoria*), with microcystins (MCs) being the primary toxins reported in freshwater. MCs are cyclic peptides and known hepatotoxins that can cause liver damage, heart failure, and death. Biological degradation of MCs by bacteria may be an effective form of toxin remediation that has not yet been fully utilized. Naturally-occurring populations of bacteria can degrade MC toxins most typically through the *mlrABCD* gene cluster. The MlrA enzyme linearizes the cyclic structure of MC, greatly reducing its toxicity and the molecule is then further degraded sequentially by MlrB, MlrC, and MlrD. Optimization of the production of MlrA and MlrB in a laboratory organism (*E. coli*) has yielded promising results. In this study codon optimized and synthetically produced *mlrA*, *mlrB*, and bicistronic *mlrAB* were cloned into *E. coli* which produced highly effective enzymes against MC. Each enzyme functioned predictably and either transformed MC to the linear MC (MlrA and MlrAB) or from the linear MC to the tetrapeptide (MlrB and MlrAB). Active cultures and cell-free filtrates were shown to degrade high concentrations (10 ppm) MC within 24 hours. The goal of this research is to produce the enzyme(s) in sufficient quantity that it could be delivered to HAB toxin-impacted sites to reduce toxicity to cleanup crews and wildlife. If successful, this would be a high-impact tool for the fight against HABs.

9:30 AM Break

10:00 AM S58: Unique RDX-degrading bacteria isolated by in situ cultivation from RDX soil enrichments

K. Clark, M. Michalsen, M. Fuller and F. Crocker, US Army Engineer Research and Development Center, Vicksburg, MS, USA*

Metagenomics studies of explosive-degrading microcosms have identified taxa with the potential to degrade hexahydro-1,3,5,-trinitro-1,3,5-triazine (RDX). These taxa (known only by their 16S rRNA sequences) have yet to be cultured and represent an unexplored resource that could reveal novel and highly-efficient strains or enzymes for explosives biotransformation in soils. A novel in situ soil diffusion array was used to cultivate bacteria in RDX-degrading soil enrichments. The metagenomics sequencing of two RDX-enriched soils indicated that *Sphingomonadales*, *Pseudomonadales*, *Xanthomonadales*, *Burkholderiales*, and *Sphingobacteriales* increased in abundance in a surface soil, while *Actinomycetales* and *Rhizobiales* significantly increased in abundance in subsurface sediments. In addition, an increase in *Solibacterales* in the surface soil and *Caulobacterales* in the subsurface soil represented the first inference of a role in RDX biodegradation for these taxa. Approximately 600 bacterial isolates were cultivated from the soil diffusion array and screened for an ability to degrade RDX. About 17 % of the isolates used RDX as a nitrogen source for growth. The majority of the RDX-degrading isolates were identified as *Actinomycetales* genera. *Gordonia* and *Microbacterium* isolates, similar to previously known RDX-degrading bacteria, were cultivated. In addition, *Aeromicrobium, Arthrobacter, Nocardioides,* and *Paenarthrobacter*, were identified as RDX-degrading bacteria for the first time. The RDX-degrading gene, *xplA*, wasn't identified in isolates from these four genera, which further indicated the uniqueness of these strains. Thus, the *in situ* soil cultivation approach was successful at isolating novel species of RDX-degraders.

10:30 AM S59: Biological treatment of nitrogen rich wastewater using circular economy concept

R. Boopathy, Nicholls State, Thibodaux, LA, USA*

The circular economy is an economic system aimed at eliminating waste and the resources are used continuously. In other words, nothing is wasted everything in the production process is reused, recycled or repurposed. In this paper I describe circular economy in the shrimp production process. The wastewater from shrimp aquaculture industry contains high concentration of nitrogen and carbon. The new technology for shrimp farming is called recirculating raceway system. This is a zero-water exchange system capable of producing high-density shrimp yields. However, this system produces wastewater characterized by high levels of ammonia, nitrite, and nitrate due to 40% protein diet. The high concentration of nitrate and nitrite (>25 ppm) are toxic to shrimp causing high mortality of shrimp. So treatment of this wastewater is imperative in order to make shrimp farming viable. One simple method of treating high nitrogen wastewater is the use of a sequencing batch reactor (SBR). A SBR is a variation of the activated sludge process, which accomplishes many treatment events in a single reactor. Removal of ammonia and nitrate involved nitrification and denitrification reactions by operating the SBR aerobically and anaerobically in sequence. Initial SBR operation successfully removed ammonia, but nitrate concentrations were too high because of carbon limitation in the shrimp production wastewater. Specifically, the initial chemical oxygen demand concentration of 1996 mg/L was reduced to 4 mg/L within eight days of reactor operation. Ammonia in the wastewater was nitrified within three days. The denitrification of nitrate was achieved by the anaerobic process and more than 99% removal of nitrogen was observed. The treated water is recycled into the production system and sludge is repurposed for coastal restoration activities.

8:30 AM - 11:30 AM Session: 12: Successful Strategies for Lab to Commercialization for Biofuels and Bioproducts

Conveners: **Nigel Mouncey**, Joint Genome Institute and **Aditi David**, Zymergen, Inc.

Waller Ballroom - Salon B, Level 3

8:30 AM S51: Pollution to Products – Recycling Carbon at Commercial Scale

M. Köpke and S. Simpson, LanzaTech, Skokie, IL, USA*

Waste carbon (ending up as greenhouse gas in the atmosphere or polluting the environment) is generated in many manufacturing processes (e.g. steel production) or can come from gasification of agricultural, forest and unsorted, unrecyclable municipal wastes. LanzaTech has pioneered a gas fermentation process enabling us to convert this waste carbon into fuels and everyday products, turning our carbon problem into an economic opportunity keeping the skies and oceans clean and blue for all. This presentation will address the various waste gas resources available and touch upon the gas fermentation pathway as a technology enabler for low-cost manufacturing of fuels, chemical-building blocks, materials and food from sustainable resources.

During the gas fermentation process, carbon oxides are fixed by autotrophic microorganisms. The Wood-Ljungdahl pathway of anaerobic acetogens is believed to be the oldest biochemical pathway on Earth and considered as the most efficient carbon oxide fixation pathway. In nature, acetogens only make a very limited range of products and not even 10 years ago were considered genetically inaccessible. Advancements in Synthetic Biology now enable efficient reprogramming of acetogens. LanzaTech has established advanced genetic parts, tools, algorithms and models including a unique biofoundry that enables automated strain engineering of anaerobic organisms and strain screening in context of flammable and toxic CO and H_2 gases. Through this platform, LanzaTech has demonstrated direct conversion of waste gases to over 100 different molecules of different chemistries. The first wave of products has been optimized for commercial rollout. LanzaTech has successfully scaled up the gas fermentation process from the laboratory bench to full commercial scale and is operating two commercial scale plants with additional units under construction.

9:00 AM From the Lab Bench to Market – Crafting a Commercialization Strategy

S. Lancaster, South Dakota Innovation Partners*

In order to commercialize technology, the so called “right” approach depends on the technology and what stage of development. Good science and technology are a start, but it will only be a piece of the puzzle. This presentation will discuss real world experience focused on funding an early-stage idea and how to de-risk that idea focused on five main areas (1) Technology (2) Team (3) Business Model (4) Finance and (5) Intellectual Property.

9:30 AM Break

10:00 AM S53: The BioManufactory at Berkeley Lab: Catalyzing Innovation in Sustainable Biomanufacturing

N. Mouncey, Joint Genome Institute*

Significant barriers stand in the way of more effectively harnessing biological systems to produce fuels, chemicals, therapeutics, food, and feed. To overcome these challenges, Lawrence Berkeley National Laboratory (Berkeley Lab) has developed a set of comprehensive and integrated capabilities that can accelerate the discovery and development of bio-products while diminishing risk for industry. The BioManufactory is designed to rapidly transition from biological hypotheses to pre-commercial applications. This platform is a combination of four different DOE-funded programs at the Berkeley Lab: (i) the Joint Genome Institute (JGI), (ii) the Joint BioEnergy Institute (JBEI), (iii) the Agile BioFoundry (ABF), and (iv) the Advanced Biofuels and Bioproducts Process Development Unit (ABPDU). By combining sequencing, multi-omics technologies, predictive design of metabolic pathways and host development, predictive scale-up, and large-scale fermentation and downstream processing capabilities,

with high-performance computing, bioinformatics, and modeling infrastructure, the BioManufactory can be the one-stop-shop for innovators and entrepreneurs to transform their ideas to prototype products and for mature companies to further optimize process performance and economics. Techno-economic and life-cycle analyses ensure that processes are aligned with the sustainability goals of our collaborators and society. The BioManufactory at Berkeley Lab is well-positioned to catalyze sustainable biomanufacturing powered by synthetic biology at industrially relevant scales of operations. In this presentation, we will review several case studies that demonstrate our successes to date.

10:30 AM S54: Production Cost and Carbon Footprint of Biomass-Derived Dimethylcyclooctane as a High Performance Jet Fuel Blendstock (R)

N. Baral, PhD, A. Mukhopadhyay, VP, Biofuels and Bioproducts, JBEI, T.S. Lee, PhD and C. Scown, PhD, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; M. Yang, PhD, Lawrence Berkeley Nation, Berkeley, CA, USA; B. Harvey, PhD, United States Navy, Naval Air Warfare Center Weapons Division (NAWCWD), China Lake, CA, USA; B.A. Simmons, Joint BioEnergy Institute, Emeryville, CA, USA*

Near-term decarbonization of aviation requires energy-dense, renewable liquid fuels. Biomass- derived 1,4-dimethylcyclooctane (DMCO), a cyclic alkane with a volumetric net heat of combustion up to 9.2% higher than Jet-A, has the potential to serve as a low-carbon, high- performance jet fuel blendstock that may enable paraffinic bio-jet fuels to operate without aromatic compounds. DMCO can be produced from bio-derived isoprenol (3-methyl-3-buten-1- ol) through a multi-step upgrading process. This study presents detailed process configurations for DMCO production to estimate the minimum selling price and life-cycle greenhouse gas (GHG) footprint considering three different hydrogenation catalysts and two bioconversion pathways. The platinum-based catalyst offers the lowest production cost and GHG footprint of $9.0/L-Jet-Aeq and 61.4 gCO2e/MJ, given the current state of technology. However, when the conversion process is optimized, hydrogenation with a Raney nickel catalyst is preferable, resulting in a $1.5/L-Jet-Aeq cost and 18.3 gCO2e/MJ GHG footprint if biomass sorghum is the feedstock. This price point requires dramatic improvements, including 28 metric-ton/ha sorghum yield and 95-98% of the theoretical maximum conversion of biomass-to-sugars, sugars-to-isoprenol, isoprenol-to-isoprene, and isoprene-to-DMCO. Because increased gravimetric energy density of jet fuels translates to reduced aircraft weight, DMCO also has the potential to improve aircraft efficiency, particularly on long-haul flights.

1:00 PM - 3:45 PM Session: 17: DEI Session: Tools to fight biases in the workplace and scientific community- Zoom Info Meeting ID: 965 3363 6202 Passcode: SIMBDEI

Conveners: **Laura Jarboe**, Iowa State University, Ames, IA, USA and **Noel Fong**, Nucelis

Waller Ballroom - Salon E, Level 3

1:00 PM S76: (Remote) Tools to Fight Biases in the Workplace and Scientific Community

A.G. Parangan-Smith, San Francisco State University, CA, USA*

The world of Diversity, Equity, and Inclusion is constantly evolving to find new solutions and tools effective at reducing unconscious biases.
Studies have increasingly shown the importance of these efforts in the sciences, where expanding the inclusivity and diversity of workplaces has had direct effects on boosting the diversity of thought and accelerating innovation. In this session, Audrey Parangan-Smith, PhD [Co-Director of SF BUILD (Building

Infrastructure Leading to Diversity) at San Francisco University] and Janie Pinterits, PhD [Inclusion, Diversity, Equity, & Accountability (IDEA) Program Manager at Lawrence Berkeley National Lab] will discuss the tools they have found useful in their programs and lead interactive discussions on implementation and use of these tools in the workplace and scientific community.

1:30 PM S77: (Remote) 50 Ways to Fight Bias: Hybrid Demonstration and Discussion

*J. Pinterits**

This experiential presentation will examine use of Lean In's 50 Ways to Fight Bias card deck with hybrid teams. We will review this accessible, research-grounded tool, its resources, and discuss its pros and cons across different settings.

2:00 PM Break

1:00 PM - 4:30 PM Session: 14: Unusual Enzymology in Natural Product Biosynthesis

Conveners: **Wenjun Zhang**, UC Berkley, CA, USA and **Hui Zhou**, Ginkgobio Works

Waller Ballroom - Salon A, Level 3

1:00 PM S60: Enhancing activity of a commercial microbe for agriculture by increasing the productivity of its native biosynthetic gene clusters

A. Roulier, G. Barbier, L. Carriedo and B. Stanton, Joyn Bio, Boston, MA, USA; B. Traag, Zymergen, Emeryville, CA, USA; Y. Cao, Ginkgo Bioworks, Boston, MA, USA; M. Goettge, DoubleRainbow Biosciences, Cambridge, MA, USA; R. Horst, Amazon, Seattle, WA, USA; W.O. Ng, Formely joyn Bio, Boston, MA, USA*

Joyn Bio is striving to fulfill the large unmet need for new sustainable products in agriculture. Biologics are playing an ever-increasing role in aiding farmers to grow crops more sustainably. An overwhelming majority of these biologics have been wild-type microbes isolated for their natural ability to provide protection from pests, promote plant growth, or fix nitrogen. While there have been many successful products launched from these wild type strains, their ability to compete with existing chemistries on application rate and cost in the field is limited. Joyn Bio is addressing the efficacy concerns of wild-type microbes by using Synthetic Biology. In this discussion, we will describe how Joyn is using Synthetic Biology, Bioinformatics, and Enzymology to increase the activity of a commercial strain.

1:30 PM S61: Leveraging microbial strain collection to discover new enzymology in natural product biosynthesis

B. Shen, Scripps Research, Jupiter, FL, USA*

The unique structural features of natural products have traditionally inspired the discovery of novel enzymology in their biosynthesis. Genomics approaches to natural product biosynthesis have provided unprecedented opportunities to discover new enzymology by directly mining their biosynthetic gene clusters (BGCs). By comparing and contrasting BGCs to eliminate specific biosynthetic steps in natural product biosynthesis, candidate genes encoding novel enzymology could be identified and prioritized for functional characterization. The Scripps Research Institute hosts one of world’s largest actinobacteria collection, totaling >125,000 strains. One of the current efforts has been focused on sequencing the strain collection to establish the Natural Products Genomics Resource Center for the broad scientific community

to enable natural product discovery, characterization, development, and associated applications. Selected examples from our current studies will be presented to highlight how to leverage the strain collection, and the natural product BGCs encoded thereof, to discover new enzymology.

2:00 PM S62: Biosynthesis of Triacsins with an *N*-hydroxytriazene Pharmacophore

W. Zhang, University of California Berkeley, Berkeley, CA, USA*

Triacsins are a family of natural products possessing a conserved *N*-hydroxytriazene moiety not found in any other known secondary metabolites. Though many studies have examined the biological activity of triacsins in lipid metabolism, their biosynthesis has remained unknown. Here I will present our recent work on elucidating chemical logic and enzymatic machinery for triacsin biosynthesis. Through genome mining, extensive mutagenesis, incorporation experiments, and biochemical studies, we have revealed all enzymes required to construct and install the *N*-hydroxytriazene pharmacophore of triacsins. Two distinct ATP-dependent enzymes were revealed to catalyze the two consecutive N-N bond formation reactions, including a glycine-utilizing hydrazine-forming enzyme, Tri28, and a nitrite-utilizing *N*-nitrosating enzyme, Tri17.

2:30 PM Break

3:00 PM S63: New PLP-dependent enzymes from biosynthetic pathways as potential biocatalysts

Y. Tang and M. Chen, University of California - Los Angelas, Los Angeles, CA, USA

Enzymes from natural product biosynthetic pathways catalyze the biosynthesis of complex molecules. While the core enzymes prepare the carbon backbones of natural products, enzymes involved in precursor biosynthesis and tailoring steps are particularly attractive as potential biocatalysts. These enzyme include group transferases, oxidative enzymes and pericyclases, etc. Many of the observed regio-, stereo-, and peri-selective reactions are synthetically challenging or without synthetic parallels. In this presentation, I will present enzymes discovery efforts in my laboratory in recent years, with an emphasis on pyridoxal phosphate (PLP) enzymes in fungal biosynthetic pathways. We recently identified PLP-dependent enzymes involved in enantioselective synthesis of alkyl-substituted pipecolic acids, which are frequently used building blocks in drug synthesis. The new group of PLP-dependent enzymes catalyze gamma-substitution, carbon-carbon bond forming reactions. The substrate promiscuity of the enzymes make them excellent candidates as biocatalysts and starting points for protein engineering. I will present the source of discovery through genome mining, characterization and application of the these enzymes.

3:30 PM S64: Highly unusual and promiscuous enzymes in secondary metabolism in *Streptomyces pactum*

T. Mahmud, Oregon State University, Corvallis, OR, USA*

The soil bacterium *Streptomyces pactum* ATCC 27456 is known to produce a number of specialized metabolites. Some of them are structurally intriguing and highly bioactive with distinct mechanisms of action. The most well-studied metabolite from this strain is pactamycin, a broad-spectrum aminocyclitol-derived antitumor antibiotic that has antibacterial, antifungal, anti-plasmodial, and anti-tumor activities. This antibiotic is synthesized by three major pathways: the shikimate, the amino sugar, and the acetate pathways. Pactamycin biosynthesis involves a number of unusual and promiscuous enzymes, including those that can catalyze the glycosylation and the post-glycosylation modification reactions of acyl carrier protein (ACP)-bound intermediates. The tailoring processes in pactamycin biosynthesis involve several highly promiscuous enzymes. The strain also produces NFAT-133, conglobatins, and piericidins. NFAT-

133 is an aromatic polyketide compound with immunosuppressive, antidiabetic, and anti-trypanosomal activities. It is derived from modular type-I polyketide synthases (PKSs) whose genes in *S. pactum* are highly disorganized and inconsistent with the co-linearity of modular PKS gene clusters seen in many other secondary metabolites. Conglobatins are macrodiolide antitumor compounds that bind to heat shock protein 90 (Hsp90) that result in the inhibition of cell proliferation and the induction of apoptosis. Conglobatins are synthesized by modular type-I PKSs, involving a cyclase/thioesterase (TE) domain that acts iteratively, couples two monomers head-to-tail, rebinds the dimer product, and then cyclizes it. This TE domain is also unusual and highly promiscuous, leading to the production of many hybrid products.

1:00 PM - 4:30 PM Session: 15: Metabolic Engineering for Alternative Feedstocks

Conveners: **Thomas Eng, PhD**, Lawrence Berkeley National Lab, Berkeley, CA, USA and **Paul Opgenorth, PhD**, Invizyne Inc, Monrovia, CA, USA

Waller Ballroom - Salon C-D, Level 3

1:00 PM S66: Elucidating aromatic utilization mechanisms in engineered Rhodococcus opacus strains for lignin valorization

W. Anthony, G. Roell, B. Wang, J. Ning and G. Dantas, Washington University in St. Louis School of Medicine, St Louis, MO, USA; R. Carr, J. Diao, A. Ponukumati, Y. Hu, K. Davis, D. DeLorenzo, Y. Tang, M. Foston, F. Zhang and T.S. Moon, Washington University in St. Louis, St Louis, MO, USA; H.G. Martin, Lawrence Berkeley National Laboratory, Berkely, CA, USA*

Our project goal is to combine adaptive evolution, gene deletion analysis, and multi–omics approaches to identify aromatic tolerance and utilization mechanisms in the promising biofuel production strain *Rhodococcus opacus* PD630 (*R. opacus*). Our approach provides insights into the catabolic potential of *R. opacus* as a chassis for the conversion of lignocellulose, specifically catalytically depolymerized lignin (i.e., substituted phenolics), into valuable products.

R. opacus is naturally tolerant to aromatic compounds found in lignin-derived mixtures. We have demonstrated the potential of *R. opacus* for increased survivability in high concentrations of aromatics through adaptive evolution. Through genomic and functional characterization of wild-type (WT) and adapted (mutant) strains, pathways for aromatic degradation and funneling into central metabolism have been elucidated. Expression profiles have only been generated for select carbon sources, however, limiting our understanding of aromatic utilization and tolerance.

To increase our knowledge of aromatic utilization and tolerance, we grew WT *R. opacus* PD630 and mutant strains in minimal media supplemented with model lignin breakdown products at a total aromatic concentration permissive to WT growth. Additionally, we grew the mutant strains at higher concentrations of the relevant aromatics to examine the transcriptional changes which supported the increased-tolerance phenotype. Additionally, ^{13}C metabolic flux analysis and targeted metabolomics were completed for WT/mutants growth on aromatics to rigorously measure and compare how aromatic substrates were consumed.

We have been performing multi–omics analyses and gene deletion experiments to determine mechanisms of aromatic tolerance and utilization. Specifically, we have utilized transcriptomics, machine learning-based transcript-to-flux prediction models, and recently developed synthetic biology tools to elucidate the intriguing mechanisms of aromatic utilization. This study will deepen our understanding of aromatic tolerance and utilization mechanisms in diverse *R. opacus* mutants by expanding the list of aromatic compound mixtures. In addition, this work will enable us to provide a genome-scale model of *R. opacus* to facilitate the development of the promising biofuel production organism.

1:30 PM S67: Toward carbon negative chemicals by synthetic biochemistry upgrading of ethanol

H. Liu, Ph.D., University of California, Los Angeles, Los Angeles, CA, USA*

It is now possible to efficiently fix flue gas CO/CO_2 into ethanol using acetogens, thereby making carbon negative ethanol. While the ethanol could be burned as a fuel, returning the CO_2 to the atmosphere, it might also be possible to use the fixed carbon for more diverse chemicals, thereby keeping it fixed. Moreover, converting carbon negative ethanol into more valuable chemicals could increase ethanol markets. We are developing a synthetic biochemistry approach to upgrading ethanol that can be ultimately be powered by electrochemically via formate oxidation. I will describe progress on a simple synthetic biochemistry approach for converting carbon negative ethanol into the synthetic building block chemical 1,3 butanediol and more sophisticated cell-free systems capable of converting ethanol into isoprenoids, opening up to an even wider range of value-added commodity chemicals.

2:00 PM S68: Biological upgrading of thermochemical wastewater towards performance advantaged bioproducts

W. Henson, A. Meyers, N. Rorrer, B. Black, A. DeCapite, L. Jayakody, W. Michener, C. Johnson and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; C. Hoyt, National Renewable Energy Laboratory, USA, Golden, CO, USA*

Waste streams are an alternative feedstock to biomass-derived carbohydrates that can improve atom efficiency and process economics for biological production of fuels, chemicals, and materials. Catalytic fast pyrolysis (CFP), which is used to convert biomass to gasoline and diesel blendstocks, generates a carbon rich aqueous waste stream that could be upgraded to valuable co-products, but its complex composition and toxicity is a barrier for valorization. Using detailed compositional analysis of CFP wastewater, we apply a biological upgrading approach to engineer Pseudomonas putida to consume major components of CFP wastewater, including acids, alcohols, ketones, and aromatic compounds. Because of the high toxicity of these streams, we implement native toxicity tolerance machinery to improve tolerance towards major stream components. As aromatic compounds comprise a large fraction of the total carbon in the waste stream, we target muconic acid and similarly structured molecules as a target product from these streams for polymer applications. Our approach to match a microbe's catabolic potential to stream composition to produce a performance advantaged bioproduct can be applied to valorize other waste streams in addition to CFP wastewater.

2:30 PM Break

3:00 PM S69: A light driven synthetic CO_2 fixation cycle

T. Miller, T. Schwander, C. Diehl, M. Sc., R. McLean, M. Sc., T. Chotel, M. Sc., N. Socorro Cortina, P. Claus and T.J. Erb, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany; T. Beneyton, M. Girault and J.C. Baret, University of Bordeaux, CNRS, Centre de Recherche Paul Pascal, Pessac, France*

Engineering carboxylases has become a cornerstone in synthetic biology as part of the effort to achieve a carbon-neutral economy that will utilize atmospheric CO_2 as a sustainable carbon source. To advance this technology, we developed a microfluidic platform for prototyping multi-enzyme reaction cascades, which we demonstrated by integrating a 17-enzyme novel CO_2-fixation pathway. Here we show that the crotonyl–coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle as a synthetic network for carbon dioxide conversion is amenable to this approach. By using natural and synthetic parts we created a structural and functional mimic of a chloroplast that continuously converts CO_2 into the organic feed-stock compound glycolate using light as an energy source. In this work, a photosynthetic energy module was developed and optimized based on thylakoid membranes of spinach chloroplasts. The thylakoid energy module was then used to power different enzymatic reactions and complex enzyme cascades. Microfluidic-based encapsulation of the photosynthetic energy module together with the CETCH cycle enzymes generated cell-sized droplets that in essence performed a novel type of photosynthesis, which could be analyzed for catalytic efficiency in multiplex and provided a readout in real-time. The activity of the micro-droplets can be programmed and controlled by adjusting their internal compositions (e.g. thylakoid membranes, enzyme, substrate, and co-substrate concentrations) as well as

using light as an external trigger. Therefore, this approach enables continuous, multi-step synthesis of compounds that can be combined with additional modules *in vitro* for the production of diverse molecules in a plug-and-play manner. Furthermore, the optimization of complex synthetic pathways *in vitro* can allow for a streamlined integration into *in vivo* hosts. These pioneering efforts pave the way to realize a diverse CO_2-fixation biochemistry that can find application in biocatalysis, green biotechnology, and artificial photosynthesis.

3:30 PM S70: Engineering *Pseudomonas putida* for efficient conversion of lignin derived sugars and aromatics into bioproducts and biofuels

D. Banerjee, PhD, T. Eng, PhD and A. Mukhopadhyay, VP, Biofuels and Bioproducts, JBEI, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Strain engineering for efficient bioconversion goes beyond pathway engineering. There are two major challenges for scaling up of any microbial process, first to maintain desirable final product titer rate and yield across scales and second is production variability due to inherent differences in the conditions used to cultivate a microbe in a shake flask versus a bioreactor. We address these challenges using two powerful approaches, high throughput fitness profiling and genome scale metabolic modeling to engineer *Pseudomonas putida* KT2440 for production of a heterologous non-ribosomal peptide, indigoidine. Using these approaches, we engineered *P. putida* strains that demonstrated desirable production phenotypes across scales ranging from shake flasks to 250 mL ambr® and 2 L bioreactors. These strains can convert galactose to indigoidine at around 26 g/L titers. We also demonstrate an 8-fold improvement in production of indigoidine when fed *para*-coumarate in an ambr® 250 platform. Our computationally driven product substrate pairing pipeline and experimentally driven high throughput fitness profiling-based method complement and better inform each other at systems level. These two approaches are powerful strategies for advanced strain engineering to maintain desirable final product titers, rates, and yields across scales.

4:00 PM S71: Engineering the tryptophan synthase β-subunit for the synthesis of noncanonical amino acids

E. Watkins-Dulaney, Aralez Bio, San Leandro, CA, USA*

Noncanonical amino acids (ncAAs) are valuable "last-step" precursors to natural products and therapeutics. Despite their bioactive potential, applications are limited by their availability: chemical synthesis often requires multi-step routes that suffer from poor yields and low overall stereoselectivity. One solution is to harness natural enzymes such as amino acid synthases, which can catalyze the formation of complex molecules directly from simple precursors with excellent regio- and stereoselectivity. To this end, Aralez Bio has engineered an *in vitro* process utilizing the β-subunit of the PLP-dependent enzyme tryptophan synthase (TrpB) to couple a diverse array of indole analogs with serine, generating over 100 tryptophan analogs. We have further expanded the TrpB substrate scope using an in vivo continuous evolution method, called OrthoRep, by selecting only on the native TrpB activity. The resulting sequence-diverse TrpB variants span a range of substrate profiles useful in industrial biocatalysis and suggest that the depth and scale of evolution that OrthoRep affords will be generally valuable in enzyme engineering and the discovery of new biomolecular functions.

1:00 PM - 4:30 PM Session: 18: Biocatalysis of Biomaterials

Conveners: **Jennifer Headman**, POET

Waller Ballroom - Salon B, Level 3

1:00 PM S78: Toward a metabolic engineering platform for conversion of natural gas liquids to higher value products: Engineering *Escherichia coli* to synthesize alkylsuccinates.

P. Cirino[*]*, University of Houston, Houston, TX, USA*

Short-chain alkanes are abundant carbon sources for production of fuels and chemicals, but their efficient utilization is met with many technical and economic hurdles relating to their low energy density, high cost of transportation and storage, and most notably the catalytic challenges associated with selective and controlled functionalization of these hydrocarbons. While biological routes to alkane functionalization are promising, those requiring oxygen still suffer from significant energy and carbon inefficiencies. In contrast, a variety of anaerobic alkane-degrading bacteria (largely nitrate and sulfate reducers) activate sub-terminal C-H bonds via fumarate addition. Through metabolic engineering, the “alkylsuccinate” products of this reaction can be converted into biofuels or other value-added chemicals, with co-regeneration of fumarate in a cyclic pathway.

Alkylsuccinate synthases are multi-subunit enzymes that require activation by a partner “activase”, which utilizes an iron-sulfur cluster to reductively cleave AdoMet, generating a 5’-dAdo· radical that in turn generates a catalytic glycyl radical on the synthase. Functional, heterologous expression of these finicky enzyme systems has proven difficult. With *E. coli* as the host microorganism, chosen for its ease of genetic manipulation, we have established anaerobic growth and gene expression conditions that support continued biosynthesis of alkylsuccinates using the *mas* gene system from *Azoarcus* sp. str. HxN1. I will describe key experimental considerations and results that led to production of compounds such as 1-methyl-pentylsuccinate (from hexane) and 1-methyl-ethylsuccinate (from propane). This includes host strain engineering, gene expression optimization, subunit analyses, identification of appropriate culture conditions, and method development for product analysis. Steps toward the directed evolution of alkylsuccinate synthases for improved activity and altered substrate specificity will also be described.

1:30 PM S79: Benefits of using yeast-based nutrients for production of biomaterials via fermentation.

S. Nelson, Ph.D.[*]*, Procelys, Milwaukee, WI, USA, J. Aldridge, Ph.D., Procelys, Cedar Rapids, IA, USA and A. Sourabié, Ph.D., Procelys, Maisons-Alfort, WI, France*

Biomaterials produced by fermentation encompass a wide range of products used for many applications, from microbial metabolites for crop protection, to precursors to blockbuster drugs. This presentation will highlight how yeast-based nutrients (YBN) play a critical role in feeding microbial fermentations. Whether the target microbe is yeast, bacteria, fungi, or algae, YBN provide nutrients in the form of free amino acids, protein peptides of variable lengths, minerals, vitamins, and other trace elements required for growth in organisms. Case studies will be presented, the first of which demonstrates the use of YBN to increase the yield of propionic acid as produced in *Acidipropionibacterium acidipropionici* fermentations. This study highlights the contribution of YBN in culture medium and its effects on cell growth and propionic acid yield. The second study demonstrates the influence of YBNs as a media supplement for recombinant protein production in *Pichia pastoris.* The results of this trial show an increase in both growth rate and protein production by adding YBN to a chemically-defined medium.

2:00 PM S80: Leveraging strain engineering for developing a commercially viable fermentative process for vegetable oil alternatives

B. Uranukul[*]*, C16 Biosciences, New York, NY, USA*

Palm oil and palm kernel oil are among the world’s most versatile raw ingredients for a wide range of industries, including food, personal care, energy, pharmaceutical, and industrial materials. Due to slash-and-burn practices at palm plantations, their production causes a variety of environmental issues. Unfortunately, no conventional agricultural alternative has been able to compete with the twin advantages of palm-based oils: high productivity per unit land and flexible chemical properties enabling broad

applications across consumer product classes. One strategy to overcome these issues associated with palm oil production is the development of microbial oils with equivalent properties. C16 Biosciences has developed a scalable process to brew a sustainable alternative to palm oil from microbes. To improve on key cost drivers toward achieving an economically viable, scalable process, we focus our R&D efforts on three technology areas – biochemistry and pathway engineering, fermentation development, and product recovery. This talk will highlight how strain engineering can be leveraged to improve cost drivers in these technology areas and draw on specific examples of how we employ an iterative Design-Build-Test-Learn (DBTL) framework for engineering problem-solving.

2:30 PM Break

3:00 PM S81: Metabolic and Bioprocess Engineering of *Lipomyces starkeyi* for Lipid Based Commercial Products

D. Mokry, PhD, A. Gluth, BS, R. Taylor, BS, A. Zitzow, B.S., T. Kelleher, PhD and T. Jeffries, Ph.D., Xylome Corporation, Madison, WI, USA*

The oleaginous yeast *Lipomyces starkeyi* is of biotechnological importance due to its exceptional capacity to accumulate lipid and utilize diverse feedstocks. Once limited by the lack of a tractable genetic system, recent advances by academic entities and Xylome have enabled strain development of this yeast through metabolic engineering strategies. The lipid produced in this organism is of particular interest, exhibiting a profile remarkably similar to palm oil, which is currently being supplied at unsustainable rates. In order to increase lipid titers to economically feasible yields, we systematically evaluated overexpression or deletion of roughly 40 genes related to lipid metabolism, and implemented a two-stage bioprocessing approach that includes a rapid growth phase followed by a lipogenic phase. The resulting strain of *Lipomyces starkeyi* produced over 100 grams per liter of lipid when cultured under these conditions. The lipid profile also changed, resulting in increased palmitic and decreased oleic acid ratios relative to the wild-type strain. During the lipogenic phase, a large intracellular lipid body is generated from which oil can be directly extracted and used in downstream processes, such as a palm oil substitute. Alternatively, the lipid bodies themselves have commercial value in the cosmetic and pharmaceutical industries due to their unique chemistry and composition. During the course of these studies, the feedstock choice was also investigated, and included byproducts derived from industrial ethanol production. Both the wild-type and engineered strains were able to consume all of the organics present in the feedstocks examined, including the sugars released by enzymatic saccharification of fiber derived from corn stover and stillage. Although *Lipomyces starkeyi* lacks a native cellulase consortium, it is possible to rationally engineer such a system into this organism to broaden the number of feedstocks available for use.

3:30 PM S82: Key learnings from multiple successful commercial scaleups

L. Suominen, Genomatica Inc., San Diego, CA, USA*

How do you take your lab-scale work and make it successful at commercial scale? Genomatica will share key learnings from its successes in scaling three intermediate or specialty chemicals. The first, our process to make biobased 1,4-butanediol (BDO) was further validated recently by a commitment to build a second commercial plant, bringing total capacity to about 100,000 tons per year; the second, for 1,3-butylene glycol (our Brontide® BG) has produced over a thousand tons; and we've publicly shared news of our production of the world's first ton of nylon-6 precursor – along with plans for our demonstration scaleup. More is coming, including in long-chain chemicals. These biobased chemicals are gaining market traction as drop-in replacements that can impact existing products and value chains, quickly, with up to 93% savings in greenhouse gases.

Key ideas we'll cover include a 'whole process' approach, ensuring that fermentation and downstream issues are intimately interwoven and co-optimized with microorganism engineering, from the start of a program; and scaling down for more effective scaling up.

Wednesday, August 11

8:00 AM - 11:30 AM Session: 19: Peptidic Natural Products

Conveners: **Jason Crawford**, Yale University, West Haven, CT, USA and **Yi Tang**, University of California - Los Angelas, Los Angeles, CA, USA

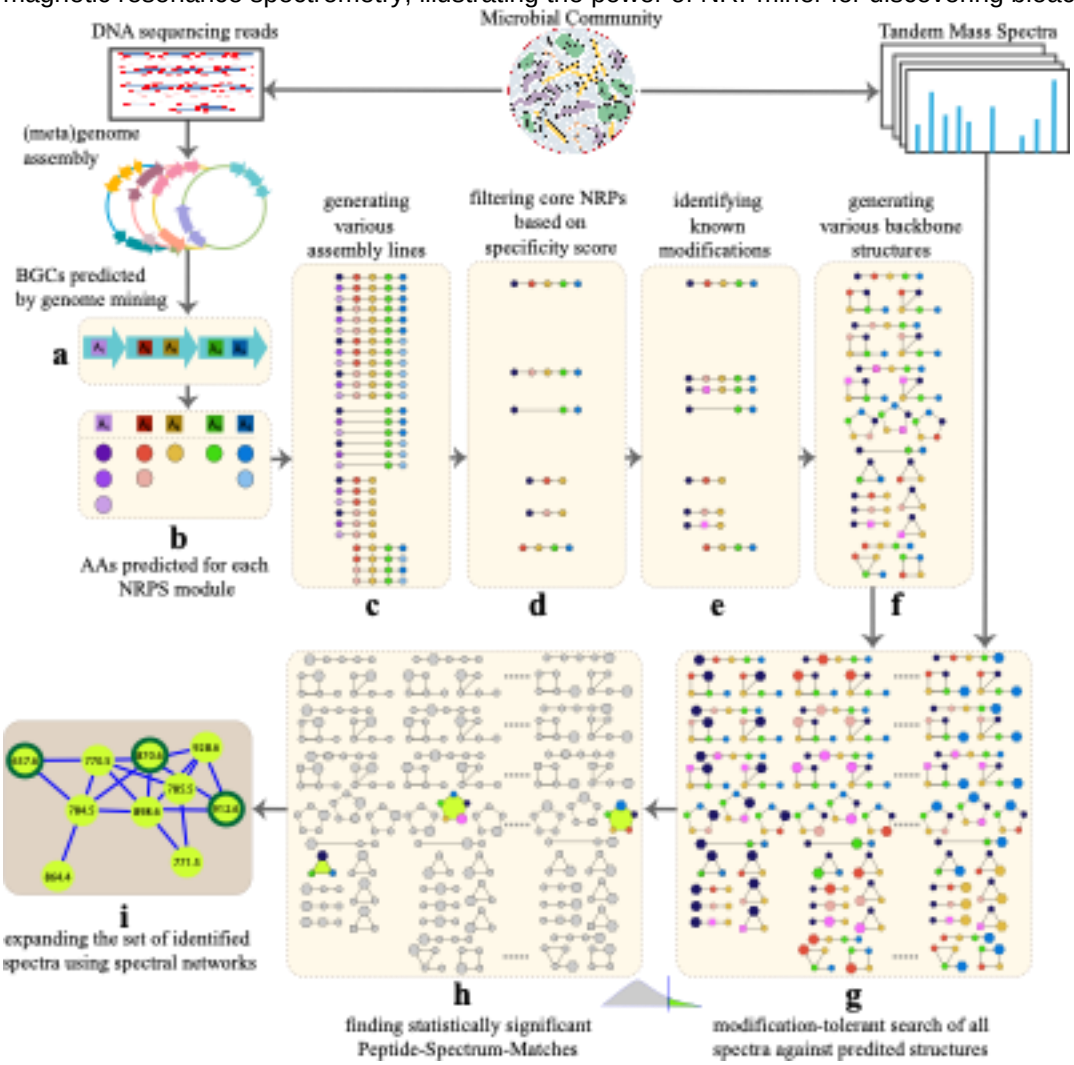
Waller Ballroom - Salon A, Level 3

8:00 AM S83: Integrating genomics and metabolomics for scalable non-ribosomal peptide discovery

H. Mohimani and B. Behsaz, Carnegie Mellon University, Pittsburgh, PA, USA*

Non-Ribosomal Peptides (NRPs) represent a biomedically important class of natural products that include a multitude of antibiotics and other clinically used drugs. NRPs are not directly encoded in the genome but are instead produced by metabolic pathways encoded by *biosynthetic gene clusters* (BGCs). Since the existing genome mining tools predict many putative NRPs synthesized by a given BGC, it remains unclear which of these putative NRPs are correct and how to identify post-assembly modifications of amino acids in these NRPs in a blind mode, without knowing which modifications exist in the sample. To address this challenge, we developed NRPminer, a modification-tolerant tool for NRP discovery from large (meta)genomic and mass spectrometry datasets. We show that NRPminer is able to identify many NRPs from different environments, including four previously unreported NRP families from soil-associated microbes and NRPs from human microbiota. Furthermore, in this work we demonstrate the anti-parasitic activities and the structure of two of these NRP families using direct bioactivity screening and nuclear

magnetic resonance spectrometry, illustrating the power of NRPminer for discovering bioactive NRPs.



8:30 AM S84: Development of a unified cross-kingdom expression platform and application in decoding a new class of nucleotide analogs from the human microbiome.

J. Patel, J. Oh, F. Isaacs and J. Crawford, Yale University, West Haven, CT, USA*

Recent advances in microbial synthetic biology have found applications in specialized metabolite discovery, bioproduction of valuable materials, living therapeutics, and bioremediation. To this end, an unresolved challenge is in disseminating and expressing genetic cargo across highly diverse microbial chassis. Here, we develop a hybrid platform for the cross-kingdom mobilization, integration, and expression of synthetic genetic elements (SGEs) into versatile microbial hosts, including yeast, Gram-negative and Gram-positive bacteria. This versatile host-range is achieved through a series of technological developments. Using this strategy to achieve multi-host heterologous expression, we demonstrate utility in deorphanizing biosynthetic gene clusters (BGCs) from the human microbiome. In particular, we successfully activate an orphan BGC from the human commensal *Lactobacillus iners* and uncover an unprecedented route for the biosynthesis of previously undescribed acylated- and deacylated-nucleotide analogs featuring an orthoester moiety. The deacylated-nucleotide inhibits protein translation, suggesting a hydrolytic prodrug activation mechanism. Remarkably, genetic studies support a biosynthetic pathway that invokes a new class of Amadori synthases, as well as an unexpected role for

“abortive” aminoacyl-tRNA synthetases in orthoester formation. The adaptable platform developed for the domestication of diverse microbial chassis overcomes bottlenecks in the expression and decoding of multigene systems and establishes a general paradigm for mobilizing functionally-active genetic elements broadly into both eukaryotic and bacterial microbial communities.

9:00 AM S85: Amide-backbone α-*N*-methylation in RiPP biosynthesis

M. Freeman, University of Minnesota, St. Paul, MN, USA*

Amide-bond α-*N*-methylations impart favorable characteristics to otherwise labile peptides that include increased structural rigidity, proteolytic resistance, and biological membrane permeability. Beginning in the 1970s, characterizations of enniatin and cyclosporine biosynthetic enzymes helped solidify the assumption that amide backbone α-*N*-methylations were exclusive to non-ribosomal peptides. However, in the past several years we and others have described the nematotoxic omphalotins as the first ribosomally encoded borosin peptide natural products to harbor amide backbone α-*N*-methylations. Here we report our progress in identifying putative borosin RiPP gene clusters incorporating α-*N*-methylations. Through these discoveries we highlight the peculiar and highly variable protein architectures of borosin methyltransferases and RiPP precursor peptides. Our results help shed light on the strategies taken to alter the properties and composition of protein and peptide backbones in nature.

9:30 AM Break

10:00 AM S86: Engineering polyketide assembly lines using the updated module boundary

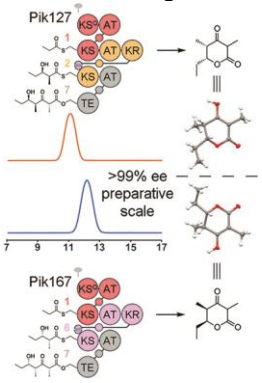
A. Keatinge-Clay, T. Miyazawa, J. Zhang, M. Hirsch, K. Ray, J. Lutgens, R. Bista, B. Fitzgerald and R. Desai, The University of Texas at Austin, Austin, TX, USA*

The set of domains that most closely work together and evolutionarily co-migrate in modular polyketide synthases was recently found to be different than the traditional definition of a module. The updated module positions ketosynthases at the downstream rather than the upstream end where they can function as gatekeepers to select intermediates properly processed by upstream enzymes. Our lab has employed the updated module boundary to engineer triketide, tetraketide, and pentaketide synthases. The triketide synthases, Pik127 and Pik167, generated from modules of the pikromycin synthase, are active within an engineered strain of E. coli that converts supplied propionate to methylmalonyl-CoA building blocks. Optimization of the synthases and the culture conditions has enabled yields of 0.2-0.4 g/L from shake flasks. Products are readily isolated and characterized, including by crystallography. The use of docking domains to split up large polypeptides has proved especially beneficial.

We devised a strategy to more rapidly construct engineered synthases, both in directed and combinatorial fashions. DNA encoding an array of modules that embeds DNA encoding an array of docking domains was used to genetically combine modules in BioBrick fashion. Within a month, 5 triketide and 25 tetraketide synthases were constructed from modules of the pikromycin synthase and tested. Half of the synthases produce the anticipated product. To optimize synthases with weak to no activity, residues within ketosynthase domains suspected of gatekeeping are being mutated.

To better understand gatekeeping, a bioinformatics/structural study was performed on 710 ketosynthases from well-characterized modular polyketide synthases. Sets of residues that help bind substituents on the alpha- and beta-carbons were also identified. Sets of residues that bind small primer units or diketides were identified. Our investigation also revealed that sets of residues can recognize features beyond the beta-carbon. We hypothesize that the mutagenesis of these residues will have the greatest impact on the

function of engineered synthases.



10:30 AM S87: Study of showdomycin biosynthesis in *Streptomyces showdoensis*

D. Ren and H.W. Liu, University of Texas at Austin, Austin, TX, USA*

Showdomycin is a *C*-nucleoside bearing an electrophilic maleimide base. We have recently characterized the biosynthetic pathway of showdomycin in *Streptomyces showdoensis*. The initial stages of the pathway involve non-ribosomal peptide synthetase (NRPS) mediated assembly of a 2-amino-1*H*-pyrrole-5-carboxylic acid intermediate. This intermediate is prone to air oxidation whereupon it undergoes oxidative decarboxylation to yield an imine of maleimide, which in turn yields the maleimide upon acidification. It is also shown that this pyrrole intermediate serves as the substrate for the *C*-glycosidase SdmA in the pathway. After coupling with ribose 5-phosphate, the resulting *C*-nucleoside undergoes a similar sequence of oxidation, decarboxylation and deamination to afford showdomcyin after exposure to air. These results suggest that showdomycin could be an artifact due to aerobic isolation; however, the autoxidation may also serve to convert an otherwise inert product of the biosynthetic pathway to an electrophilic *C*-nucleotide thereby endowing showdomycin with its observed bioactivities. The experiments and results of this study will be presented.

11:00 AM S88: High-Throughput RiPP Discovery (R)

*D.A. Mitchell**

Advances in genome sequencing and bioinformatics have catalyzed a resurgence of interest in natural products. Our principal focus has been on the ribosomally synthesized and post-translationally modified peptides (RiPPs), which, despite being comprised of more than 40 known structural classes, share a simplified biosynthetic logic and require a minimal genomic footprint. This talk will describe the challenges we encountered and insights we gained from large-scale RiPP genome mining. New synthetic biology approaches to accessing these compounds to evaluate their suitability in downstream applications such as cellular probes, agriculture, and medicine will also be discussed.

8:00 AM - 11:30 AM Session: 20: Metabolic Engineering and New Tools for Non-Model Organisms

Conveners: **Thomas Mansell**, Iowa State University, Ames, IA, USA

Waller Ballroom - Salon C-D, Level 3

8:00 AM S89: CO_2 to Plastic: Integrating Chemical Catalysis and Biological Conversion of Carbon Intermediates into Polyhydroxyalkanoates

R. Hamilton, B. Marcus and M. Kalyuzhnaya, San Diego State University, San Diego, CA, USA; P. Bohutskyi, Pacific Northwest National Laboratory, Richland, WA, USA; K. Rapp and M.J. Betenbaugh, Johns Hopkins University, Baltimore, MD, USA*

Plastics are an inevitable part of our everyday life. Considering the high environmental cost of petroleum-based polymers, the need for bio-benign materials produced from renewable sources is becoming urgent. Despite the commitment, the transition from petroleum-based polymers to renewable alternatives is challenging. We focus on polyhydroxyalkanoates (PHAs) for two main reasons: PHAs are a large family of natural polyesters often envisioned as a sustainable solution for future polymer manufacturing. The industrial production of polymers requires an abundant, cheap and sustainable carbon feedstock to fulfill the needs. Single carbon (C1)-compounds, such as methanol, methane, and formate, represent an attractive feedstock to induce the transition. C1-substrates are virtually unlimited carbon sources, and they can be readily produced from CO_2 via chemical electrocatalysis.
Many natural microbes can accumulate poly-3-hydroxybutyrate (or PHB); however, the polymer has limited applications due to its poor mechanical properties and low melting temperatures. However, it is well recognized that targeted metabolic engineering can modify the final product PHB to market-attractive, non-natural PHA polymers. Here we demonstrate a new synthetic microbial chassis for the production of medium chain-length PHAs (MCL-PHAs). While MCL-PHAs are typically produced by supplementing microbes with fatty acids, we engineered *Methylomicrobial alcaliphilum* $20Z^R$ to produce MCL-PHAs from methanol. The methylotrophic strain is one of few biological systems that has increased fatty acids levels due to the formation of intracytoplasmic membranes. In our project, heterologous PHA-biosynthesis pathways were integrated into the strain. The fermentation strategies were optimized to coordinated PHA production with the fatty acid turnover. While further optimization is still required, the novel synthetic microbial system can enable PHA synthesis from electrocatalytically produced methanol.

8:30 AM S90: The non-model insect pathogenic fungus *Tolypocladium inflatum* as a platform for discovery of novel pharmaceuticals, agrochemicals, and chitin and alkane degrading enzymes (R)

K. Bushley, United States Department of Agriculture (USDA-ARS), Ithaca, NY, USA and J. Spatafora, Oregon State University, Corvallis, OR, USA*

Insect pathogenic fungi possess unique metabolisms that function in parasitizing and consuming their insect hosts. Recent genome sequencing projects have shown that these fungi contain expansions of genes encoding fungal secondary metabolites as well as novel carbohydrate active enzymes (CAZymes) and oxidative enzymes such as cytochrome P450s. The CAZymes and oxidative enzymes found in insect pathogens differ from those found in lignocellulose degrading fungi and are tailored to break down the chitin as well as long-chain fatty acids and alkanes found in insect cuticle. The genome sequence of the beetle pathogen *Tolypocladium inflatum*, producer of the immunosuppressant drug cyclosporin, and advances in functional ‘omics tools are developing this non-model fungus into a platform for discovery of unique chemistries with uses in agriculture, medicine, and industrial microbiology. We report on transcriptomic studies that identify genes encoding enzymes that specifically degrade insect-specific carbon substrates and secondary metabolites involved in virulence towards insects or modulation of the insect immune response. We also discuss efforts to develop this fungus as a tractable genetic system and heterologous fungal expression systems for efficient production of chemical and enzyme products for industrial uses.

9:00 AM Break

10:00 AM S92: CRISPR-enabled metabolic engineering of the thermotolerance yeast Kluyveromyces marxianus

I. Wheeldon, S. Wei, M. Li, X. Lang, D. Baisya and S. Lonardi, UC Riverside, Riverside, CA, USA; D. Bever-Sneary and N. DaSilva, UC Irvine, Irvine, CA, USA*

Driven by the discovery of CRISPR and CRISPR-associated proteins, the past 5 or more years has seen a dramatic increase in the availability and complexity of genome editing approaches. Engineered CRISPR-Cas systems have been developed that enabled targeted gene knockouts, base-editing, multiplexed gene regulation, and genome-wide screens for functional genomics. The widespread adoption of these systems has rapidly increased access to the genomes of many non-conventional microorganisms, and in doing so has increased the number of hosts that can be used for chemical biosynthesis. In this talk, we present our work in developing CRISPR-based genome editing for the thermotolerant yeast *Kluyveromyces marxianus* and demonstrate that these new tools can rapidly engineer new strains for the biological production of fuels and chemicals. We focused on K. marxianus because various strains have the natural ability to grow at temperatures upward of 50 °C and because *K. marxianus* is commonly described as the fastest growing eukaryote, traits that can benefit high temperature, high rate bioprocessing. The ability to metabolize a range of C5, C6, and C12 sugars, as well as organic acids also makes it well-matched with biomass-derived feedstocks. To better exploit these traits, we have created a set of CRISPR-based genome editing tools as well as developed a series of standardized promoters and inducible gene regulation switches. These new metabolic engineering tools have allowed us to create strains that produce high titers of 2-phenylethanol, a valuable flavor and fragrance compound. We have also understood and enhanced the natural ability of *K. marxianus* to produce grams per liter quantities of the short chain volatile ester ethyl acetate. Lastly, we have developed genome-wide libraries of single guide RNAs that target every gene in the genome, a screening tool for functional genomics and directed evolution of desired phenotypes. Together, these new tools and examples of their application in metabolic engineering demonstrate that *K. marxianus* is a viable host for chemical biosynthesis.

10:30 AM S93: Domesticating anaerobic fungi for direct biomanufacturing from renewable plant biomass

K. Solomon, University of Delaware, Newark, DE, USA*

Microbial chemical factories are sustainable biomanufacturing platforms that complement traditional petrochemical industries by using renewable feedstocks. However, there are few economical methods to convert plant biomass into sugars that can be more readily used by existing microbial platforms without the production of toxic byproducts. An alternative strategy is the use of non-model microbes that are adapted to the direct use of crude, untreated biomass and engineer these microbes for biomanufacturing. In my lab, we focus on one such class of organisms, early-diverging anaerobic fungi (phylum Neocallimastigomycota), due to their robust degradation of untreated plant feedstocks in the herbivore digestive tract and their novel biosynthetic capabilities. Here, I highlight progress towards genetic and epigenetic tools for control and optimization of anaerobic fungal function and describe approaches to deploy anaerobic fungi today for direct production of fragrances and solvents from untreated feedstocks.

11:00 AM S94: Conamax™ - A microbial platform for the rapid production of high-quality, fully humanized monoclonal antibody therapeutics at low cost

C. Lippmeier, O. Yu and G. Mao, Conagen*

Therapeutic monoclonal antibodies (mAbs) are expensive, block-buster drugs, which are only practically available in first-world countries. Globally, the tolerance for the high prices of pharmaceuticals has waned and thus alternative methods for their manufacture have gained in interest. Monoclonal antibodies are complex molecules which require precise cellular assembly and post-translational modifications - primarily glycosylation. Several non-glycosylated mAbs are commercially made in low-cost microbial systems, but no microbe-derived, glycosylated mAb has successfully advanced through the clinical pipeline to date. For this reason, the Conamax™ system was developed to express pharmaceutically important glycoproteins with humanized glycosylation patterns, in less time, and at lower cost than can be attained by established systems. The Conamax system is based on an organism from a heterotrophic

genus of protists in the family Thraustochytriaceae, aka the “Thraustochytrids”, which in turn is a member of the phylum of Heterokonta. This phylogenetic classification makes this genus the only one in its phylum to be used for the purposes of biopharmaceutical production. The commercial application of Thraustochytrids was first developed by a number of companies which used it as an excellent source of docosahexaenoic acid; a nutritionally important polyunsaturated omega-3 fat. For this reason, it is well known that these organisms are robustly fermentable - capable of rapid, reproducible growth in scales up to 250,000 liters, at costs which are compatible with the pricing pressures of nutritional food ingredient markets. However any effort to translate the cost-driven lessons of scaled processes for food ingredient production to biopharmaceutical manufacturing will require a microbial host organism which readily tolerates the “humanization” of its glycosylation machinery as well the optimization of secreted mAb titers. In this talk we will describe the tools and methods used to establish the Conamax host as a platform for the next generation of fully humanized mAb therapeutics.

8:00 AM - 11:30 AM Session: 21: Biocatalysis in Bioprocessing: Extremophiles and Bio electrocatalysis

Conveners: **Dr. Rajesh Sani, Professor**, South Dakota School of Mines and Technology, Rapid City, SD, USA and **Brian Hedlund**, UNLV

Waller Ballroom - Salon B, Level 3

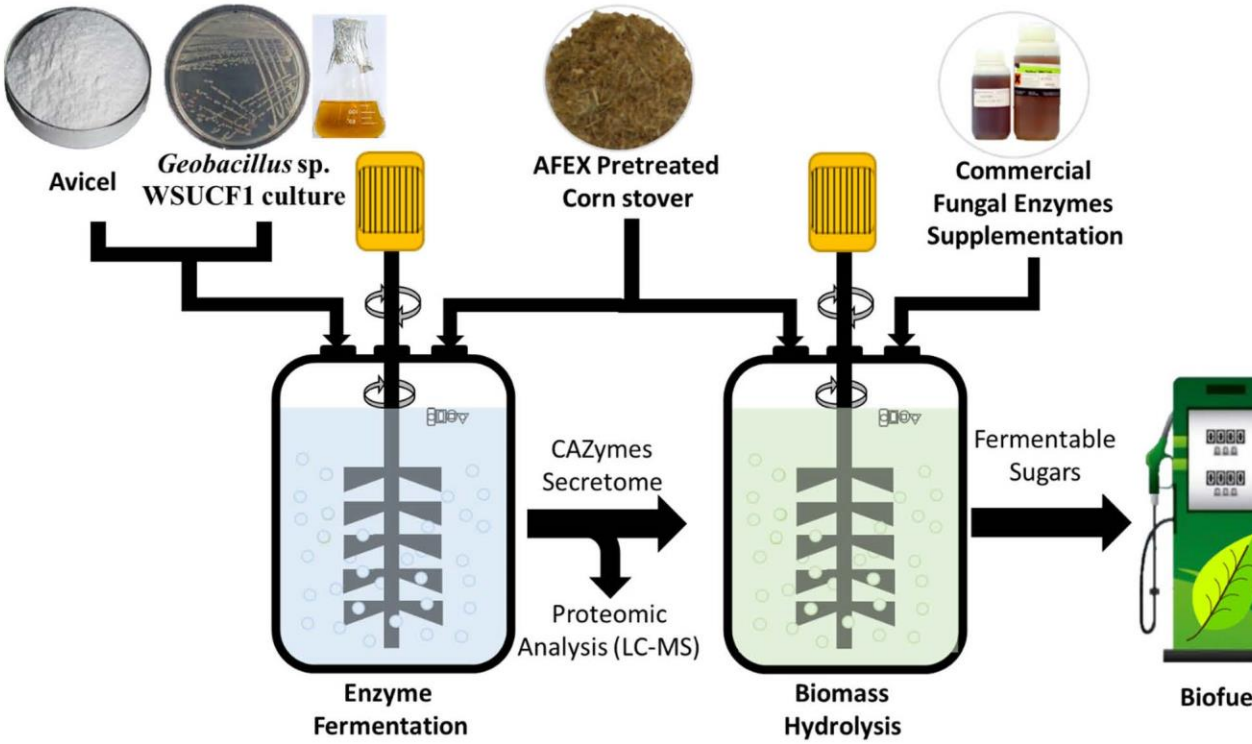
8:00 AM S95: Thermophilic *Geobacillus* sp. WSUCF1 secretome for saccharification of Lignocellulosic Biomass

A. Bhalla, South Dakota Mines, Rapid City, SD, USA; J. Arce, B. Ubanwa and V. Balan, Assistant Professor, University of Houston, Sugarland, TX, USA; R. Sani, Professor, South Dakota School of Mines and Technology, Rapid City, SD, USA*

Geobacillus sp. strain WSUCF1 is a thermophilic bacterium and has been reported to secrete thermostable cellulases and hemicellulases when grown on lignocellulosic substrates Rastogi. The genome of this microbe was also sequenced and annotated before. We have re-annotated the genome and identified some novel enzymes that were not identified before. Subsequently, we produced secretomes using different substrates Avicel, Xylan, Untreated Corn Stover (UT-CS), Ammonia Fiber Expansion pretreated corn stover (AFEX-CS) and Extractive Ammonia pretreated corn stover (EA-CS) for the first time using established methods. The respective secretome (AVI-S, XYL-S, UTC-S, AFC-S and EAC-S) produced using above mentioned substrate were subjected to proteomic analysis using established protocols. Two secretomes (AVI-S and AFC-S) that gave the highest enzyme activities were used to hydrolyze AFEX-CS and EA-CS by combining with commercial enzymes. Since WSUCF1 bacterial secretome lack endo-cellulase activities, we doped the secretome with purified fugal cellobiohydrolase I and II enzymes (CBHI and CBHII). Since the whole fermentation broth can be used directly for enzymatic hydrolysis, this will help to reduce the cost associated with adding stabilizers, formulation, concentration, refrigeration, and transportation as reported before. Our study has confirmed the synergy operating between *Geobacillus* sp. WSUCF1 bacterial and commercial fungal enzymes. Adding a small portion of commercial enzymes to compensate the missing enzyme activities in bacterial secretome will produce comparable sugar yield to using commercial enzymes alone in a biorefinery. This

will reduce the overall biofuel and biochemical production cost.

Abstract Graphic



8:30 AM S96: Bio-electrochemical interactions of extremophiles with nanoscale coatings on metal surfaces

P.K. Obulisamy, C. J Allen, J. D. Zaug, D. Saurabh, B. Jasthi, R. Sani, Professor and V. Gadhamshetty, South Dakota School of Mines and Technology, Rapid City, SD, USA; N. K. Singh and V. J Kasthuri, California Institute of Technology, Pasadena, SD, USA*

Abstract: Extremophiles can be metabolically active and abundant even under the unusual environments on the earth and outer space. They are relatively interconnected and contribute to the productivity and stability of the ecosystem. The biofilms based on extremophiles can be extremely beneficial and sometimes detrimental to metal structures. Here we address the knowledge gaps regarding the interactions of environmental microbiomes, especially extremophiles, with technologically relevant metal surfaces. Our current study focuses on the elucidation of the interaction of sulphate reducing bacteria (SRB) as well as novel isolates from extreme environments. Rhodobacter sp was isolated from the Sanford Underground Research Facility, an abandoned gold mine in South Dakota, that was grown on methane to produce value-added products (e.g., bioplastics). Methylobacterium Ajmali, a novel isolate from the International Space Station (NASA Mars Mission) that was grown on methanol was also tested for its ability to grow as biofilm on metal surfaces. Here we explore phenotypical and genotypical responses of these isolates with an emphasis on their exoelectrogenic properties that are relevant to futuristic bioprospecting applications. Specifically, we study their interactions with the metal surfaces modified with nanoscale coatings, using advanced electrochemical methods including scanning electrochemical microscopy (SECM) techniques. Irrespective of the extremophiles tested, the nanoscale coatings were found to greatly influence the surface properties and subsequently metal-microbe interactions. However, the key proteins involved in sensing the metal surface interfaces are different for these extremophiles. Isolation and characterization of such a unique proteins and electron conductive molecules will pave a way in reversing the detrimental effects caused by these unexplored strains (and other extremophiles) on metals and useful in other bioprospecting applications. Keywords: 2D materials, archaea, methanotroph, methanol, metal electrodes, scanning electrochemical microscopy,

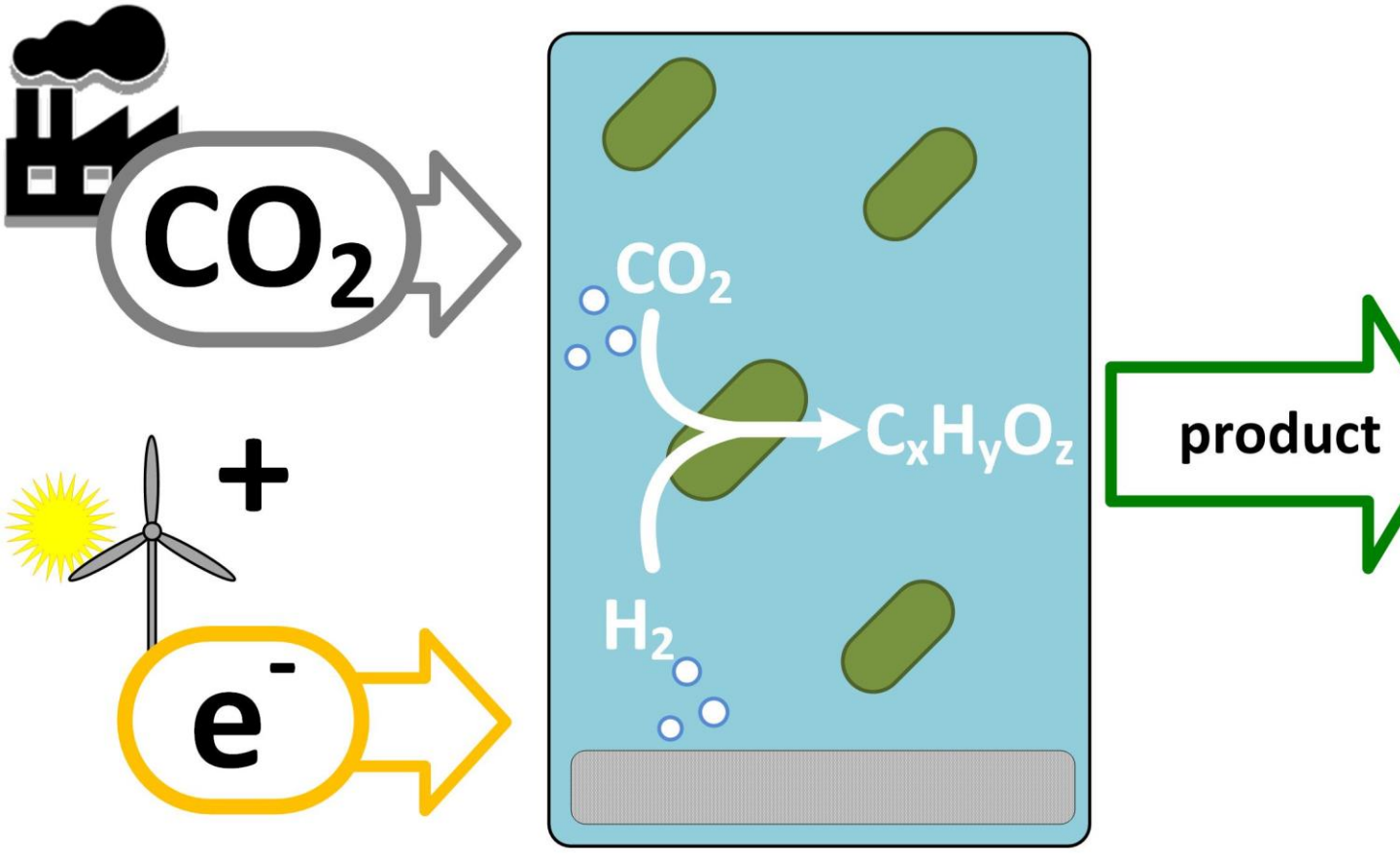
9:00 AM S97: Efficient microbial electrosynthesis from CO_2 via electrochemical *in situ* production of H_2

F. Kracke, J.S. Deutzmann and A.M. Spormann, Stanford University, Stanford, CA, USA; B.S. Jayathilake, S. Chandrasekaran, S.H. Pang and S.E. Baker, Lawrence Livermore National Laboratory, Livermore, CA, USA*

Microbial electrosynthesis is a biotechnology in which microorganisms utilize electrons supplied via a solid electrode for CO_2 reduction, thus enabling the sustainable production of chemicals from CO_2 and electricity. The efficient delivery of electrochemically *in situ* produced H_2 could present a key advantage of microbial electrosynthesis over traditional gas fermentation. However, the technical details of how to supply large amounts of electric current per volume in a biocompatible manner remain unresolved. Here, we investigated for the first time in-depth the effect of electrochemical *in situ* H_2-production on microbial metabolism.

From several non-precious-metal materials, a Nickel-Molybdenum alloy was identified as particularly suitable to perform durable hydrogen evolution under biologically relevant conditions, while avoiding catalyst deactivation and toxicity effects. Individual experiments using NiMo-cathodes and pure cultures, demonstrated the production of acetate and methane at coulombic efficiencies close to 100%, respectively. A physiological analysis of cells under electrosynthesis-conditions revealed robustly-growing cultures with nearly identical protein expression patterns compared to controls grown on gaseous H_2/CO_2. However, we found a small but noticeable fraction of cell lysis specific to the electrochemical-cultures and hypothesize the origin to be local pH conditions at the cathode surface during H_2 evolution. We manufactured 3D-printed electrodes with varying ratios of surface area to volume and used these to systematically study the effect of *in situ* H_2 supply as a function of current density on microbial electrosynthesis. We found that at identical total current supplied, larger surface area cathodes enabled higher volumetric production and minimized escape of H_2. At high current densities, the formation of

hydrogen gas bubbles and locally alkaline pH on the cathode surface prevented efficient microbial catalysis. Our data show however, that high surface area cathodes can mitigate these effects by keeping the specific current density below a critical threshold (here ≤ 1 mA/cm^2), and, thus, resolve key critical limitations for *in situ* electron delivery in microbial electrosynthesis.



9:30 AM Break

10:00 AM S98: A Biocatalyst- based CO_2- utilization platform for applications on earth and ISRU in space

T. Karimi, T.K, Cemvita Factory Inc, Houston, TX, USA*

Cemvita Factory Inc., is a Houston- based Biotech startup with the focus of CO_2 utilization. Cemvita applies biocatalysts (enzymes) for conversion of CO_2 to value-added products. In with partnership Occidental Petroleum, Cemvita developed an enzymatic pathway which utilizes CO_2, water and light to produce Bioethylene. Ethylene is the most produced petrochemical worldwide and its production via steam cracking is the most CO_2 emitting process in the chemical industry. An alternative process is to apply a bio-catalyst- based approach to decrease the energy consumption rate and consequently lowering the CO_2 emission rate. Unlike the chemical-based reactions with high energy consumption rate, the biocatalyst reactions normally happen at ambient temperature and pressure which leads to less CO_2 emission. In partnership with Occidental Petroleum, Cemvita Factory developed a Biocatalyst process using Ethylene Forming Enzyme (EFE) which converts CO_2 to Bioethylene. The pilot project will scale up the process that has been successful in laboratory tests. In another project Cemvita applied a biologically-inspired method (which mimics photosynthetic) to convert CO_2 to glucose for In situ resource utilization

(ISRU) of human life supplies in long-duration space missions. In summary, this biomimetic CO_2 utilization platform can be adapted for the sustainability on earth and future of human explorations in deep space.

10:30 AM S99: CO_2 Solutions - Driven by enzyme-enabled carbon capture

T. Govil, M. Vaughn and R. Sani, Professor, Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, Rapid City, SD, USA; D. Shroeder, Department of Geology & Geography, West Virginia University, Morgantown, West Virginia, WV, USA; G. Ustunisik, Geology and Geological Engineering, South Dakota School of Mines and Technology, Rapid City, SD, USA; B. Lingwall, Civil and Environmental Engineering (CEE), South Dakota School of Mines and Technology, Rapid City, SD, USA*

One approach for mitigating excessive carbon dioxide (CO_2) in the atmosphere, a factor that contributes to extreme weather and wildfires, is to reduce excessive CO_2 levels by capture and storage. However, storage runs the risk that CO_2 injected underground as fluid can migrate and escape. Fortunately, nature has provided a solution through carbonate mineralization in deep rock, which stores the carbon in the rock as part of the rock itself. Since biology accelerates carbon mineralization in basalts, therefore, in this talk, I will discuss the systems available to increase the sequestration of CO_2 via carbonate mineralization from either bioaugmentation or bio stimulation in deep rock. One amongst this system is the use of microbial carbonic anhydrase that catalyzes reversible CO_2 hydration and forms metal carbonates that mimic the natural phenomenon of weathering/carbonation. However, little is known if this process could be made more effective with the assistance of extremophiles. This work aims to collect the pilot data needed to begin developing an extremophile mediated carbon mineralization sequestration system that can be deployed at industrial to community scales. For this purpose, we are using microbes isolated from on the 4100 feet level and sediments from the 4850 feet level of Sanford underground research facility (SURF), Lead, SD and other locations, 65% of which are unique and can grow in conditions as varied as at high temperatures (thermophiles), low pH (acidophiles) or salinity (halophiles). Further, work is in progress to understand life rules in the rocks of core samples drilled from the 4100 feet level of SURF at high temperatures and pressures.

11:00 AM S100: Bacterial exopolysaccharides from extreme environments of Chile: Realms of possibilities in food industry

A. Banerjee, A. Gómez and P. González-Faune, Universidad Católica del Maule, Talca, Chile; G. Cabrera-Barjas, Universidad de Concepción, Coronel, Chile; R. Bandopadhyay, The University of Burdwan, Burdwan, India*

Microbes in extreme environment are uniquely adapted to great temperature variations by different cellular and molecular modifications, such as exopolysaccharide production, genomic changes, regulation of gene expression or production of pigments and many more. In an era of ever increasing climate change impacts, it is more important to address the extremophiles before we loss them. Extremophiles often harbour industrially important secondary metabolites like enzymes, pigments or polysaccharides that have promising industrial applicability. In the southern hemisphere, especially in Chile, polyextremophiles thrive in hot springs of the Andes Mountains. The main shared feature of these extremophiles is production exopolysaccharides (EPS) as a part of protection strategy. The thermotolerant microbes thriving in the natural, slightly acidic, volcanic hot springs of the Transitional Southern Volcanic Zone of Maule region, Chile show the presence of complex α- and β-glycosidic bonds in it, with notably high thermal stability. They are forming a complex network of EPS that additionally capable of different free radical scavenging property and emulsification of an array of vegetative oils, indicating its probable application in food industry. In conclusion, our study highlights the occurrence of highly thermostable polysaccharides that may be used as antioxidant, emulsifiers or viscosifiers in the food industry. Overall, the compositional dynamics of polyextremophiles reveals excellent potential for industrial applications.

Keywords: Extreme environment, Hot springs, Exopolysaccharides, Microbial diversity, Microbial conservation

8:00 AM - 11:30 AM Session: 22: Developing engineered functions: uses in resource extraction and recovery, biodegradation, and low-waste materials development

Conveners: **Fiona Crocker**, US Army Engineer Research and Development Center, Vicksburg, MS, USA and **Kate Elson**, University of Texas, TX, USA

Waller Ballroom - Salon E, Level 3

8:00 AM S101: Engineering a culturable *Serratia symbiotica* strain for aphid paratransgenesis

K. Elston, J. Perreau, G. Maeda, N. Moran and J. Barrick, University of Texas at Austin, Austin, TX, USA*

Insects play incredibly diverse roles in ecosystems. Some are beneficial pollinators and decomposers, while others spread human pathogens and destroy crops. Despite the impact insects have on our lives, researchers have engineered very few of them. The diversity of diets, reproductive modes, and lifestyles can make it challenging to genetically engineer some insects. A way around these challenges is to instead engineer the bacteria that live inside insects in what is known as paratransgenesis. This technique can be especially effective because some microbes are embedded in insect tissues and cells and inherited through insect generations. Our lab has developed a toolkit for engineering symbiotic bacteria from honey bees, aphids, and other insects. Typical applications of paratransgenesis include studying the symbiotic relationship between bacteria and host, expressing a toxin from the bacteria to destroy pathogens vectored by the host, and controlling insect gene expression. Here, we describe the pursuit of some of these applications in aphids, a sap-sucking insect and common agricultural pest. We engineered an aphid gut bacterium isolated from the black bean aphid called *Serratia symbiotica* CWBI-2.3^T. This strain is closely related to other *S. symbiotica* strains that are vertically transmitted from mother to daughter during asexual aphid reproduction. We engineered this strain with diverse genetic parts, integrated genes into the genome, and delivered the engineered strain to aphids through feeding. By engineering the strain with fluorescent genes, we could then visually track the colonization patterns of the strain inside living aphids and more easily assay its effect on aphid fitness. Finally, we showed that we could induce heterologous gene expression from engineered CWBI-2.3^T in living aphids. The capabilities for engineering S. symbiotica CWBI-2.3^T that we have demonstrated may help researchers build a better understanding of how this symbiosis evolves and provide a foundation for new approaches for pest management.

8:30 AM S102: Engineering bacterial vesicles for gene delivery

J. Boedicker, M. Gangan, C. Vidal Silva and F. Tran, University of Southern California, Los Angeles, CA, USA*

Bacteria naturally produce extracellular vesicles. These vesicles are packaged with a variety of biomolecules, including genetic material. Uptake of vesicles containing DNA leads to horizontal gene transfer and is a mechanism for gene transfer between even distantly related bacteria. Because vesicle production and uptake does not involve any specialized molecular machinery, it may be a universal route of gene transfer between bacteria. Our recent work has engineered vesicle-mediated gene transfer to improve the rate of gene exchange and the specificity of DNA packaging, focusing mainly on plasmid exchange between *Escherichia coli* and a few other Gram-negative bacteria. The rate of gene exchange depends on both the production and uptake of vesicles, both processes which involve membrane deformation. Membrane deformation can be assisted by the presence of molecules that bind to and restructure membranes. Several such molecules also increase the rate of the both vesicle production and uptake. Many membrane-structuring molecules are naturally produced by bacteria, some of them with

antibiotic activity at high concentrations. Another key step in vesicle-mediated gene transfer is the loading of double-stranded DNA, such as plasmids, into vesicles. Plasmid loading into vesicles depends on factors such as DNA origin and plasmid copy number. Tethering specific sequences of DNA to the inner membrane of a cell also resulted in increased loading into vesicles and a subsequent increases in the gene transfer rate. These results indicate the potential for modifications of both target DNA and the vesicle-producing donor cells to control the rate and specificity of gene exchange between bacteria.

9:00 AM S103: Preventing unwanted and unintentional evolution of engineered bacteria

J. Barrick, The University of Texas at Austin, Austin, TX, USA*

Directed evolution is a powerful tool for biotechnology. It can be used to create enzymes with new activities and to improve the stress tolerance of cells used in bioproduction, for example. However, evolution will also inevitably degrade the performance of engineered microbes, particularly when these “living factories” must be cultured in large volumes and/or on long timescales, because malfunctioning mutants that disrupt an engineered function typically have a growth advantage. We examined how hundreds of plasmids from the iGEM Registry affect *E. coli* growth rates to define a limit on how much burden can be tolerated before it becomes impossible to construct a plasmid. A related analysis of plasmid sequences from Addgene suggests that unintentional evolution of lower plasmid copy number may have reduced the burden of some of these plasmids. We have developed several strategies that can be used to reinforce engineered bacteria against unwanted evolution. They include computationally screening DNA sequences with negative design tools that detect hypermutable DNA sequences and cryptic gene expression, genome-scale deletion or silencing of selfish DNA elements, and a directed evolution procedure for isolating strains with reduced point mutation rates. Unwanted evolution is a foundational challenge that is unique to synthetic biology. It must be understood and confronted before the engineering of living systems can become as reliable and predictable as traditional engineering disciplines.

9:30 AM Break

10:00 AM S104: Real-time environmental monitoring of chemicals using living electronic sensors

J. Atkinson, L. Su, X. Zhang, G. Bennett, C.M. Ajo-Franklin and J. Silberg, Rice University, Houston, TX, USA*

Real-time chemical sensing is needed to counter the global threats posed by pollution. Synthetic biology holds great promise for engineering cells as bioelectronic sensors that response to diverse chemical and physical stimuli by producing extracellular electrical signals. It can be challenging to interface engineered cells with electronics to maintain a high signal-to-noise ratio in diverse environmental settings. Here, we combine synthetic biology and materials engineering approaches to develop a living bioelectronic sensor platform with minute detection times in environmental samples. *Escherichia coli* was programmed to reduce an electrode in a chemical-dependent manner using a modular, eight-component, synthetic electron transport chain. Encapsulation of the engineered cells with electrodes and conductive nanomaterials yielded a living bioelectronic sensor that produced significantly more current upon exposure to thiosulfate, an anion that causes microbial blooms. To diversify the ligands that can be sensed to include chemicals that affect vertebrate reproduction and health, we incorporated a protein switch into the synthetic pathway that enabled the detection of an endocrine disruptor within two minutes in riverine water, implicating the signal as mass transfer limited. These findings provide a new platform for miniature, low-power sensors that can be used to safeguard ecological and human health.

1:00 PM - 5:00 PM Session: 23: New Natural Products and New Sources

Conveners: **Jaclyn Winter**, University of Utah, UT, USA and **Dr. Xuejun Zhu**, Texas A&M University, College Station, TX, USA

Waller Ballroom - Salon A, Level 3

1:00 PM S105: Bacterial symbionts as sources for new bioactive small molecules

E. Mevers, Virginia Tech, Blacksburg, VA, USA*

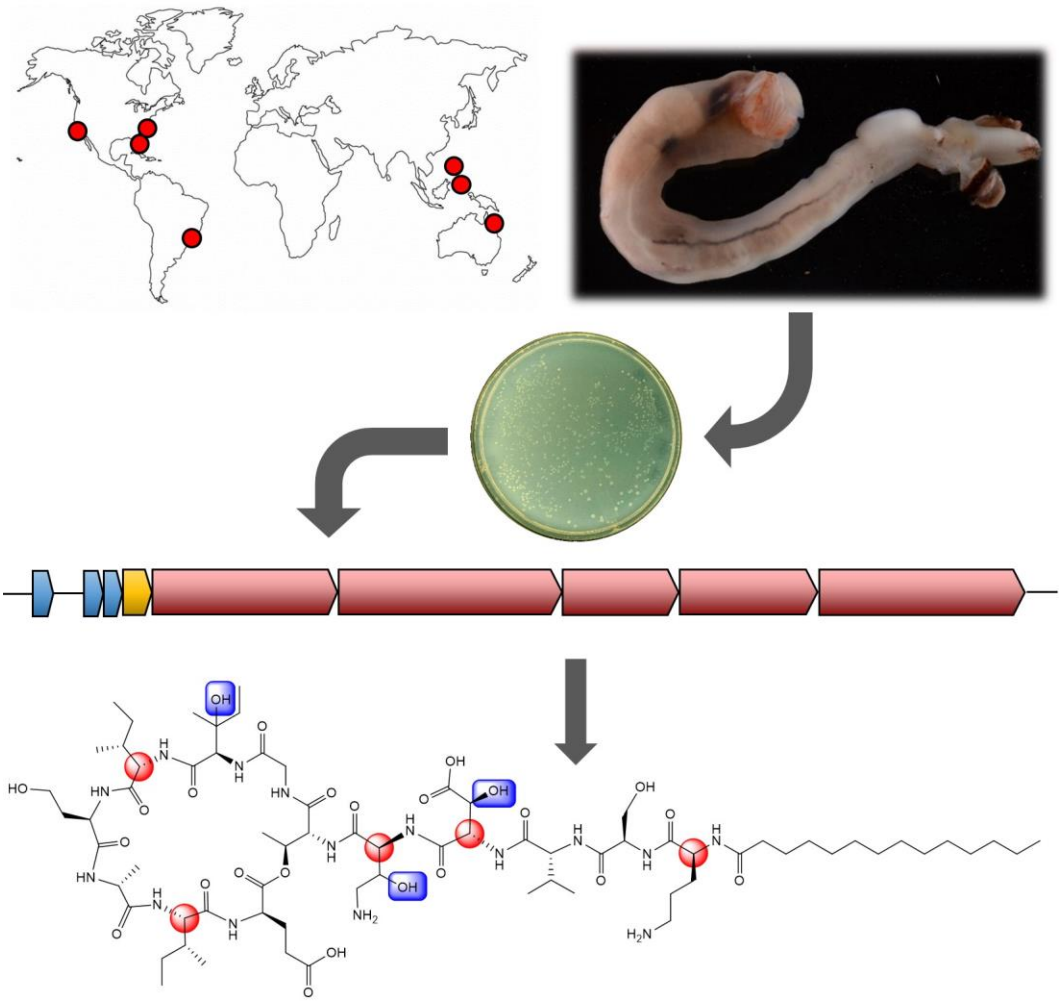
Natural products have played a critical role in drug discovery and innovation for many decades - roughly 65% of all approved small molecule drugs are either natural products, derivatives, or embody a natural product pharmacophore. This is especially true for the treatment of infectious diseases, including viral and fungal pathogens, both of which have significant and growing unmet needs for new therapeutic agents. The success of natural products in the clinic is due to their evolutionary history, their structures and functions having evolved over millions of years of selective pressures to carry out an essential role for the producing organism. One particularly important role is the production of defensive metabolites by symbiotic microorganisms to protect their eukaryotic host. Recent investigations into two bacterial symbionts from distinct ecosystems – moon snail egg masses and fungus-growing ants – has led to the discovery of two new bacterial-derived metabolites that likely defend the host from pathogens and predation. Both bacterial strains showed impressive antifungal activity in binary intruder assays and were prioritized for mass spectrometry-based metabolomics analysis using the Global Natural Products Social Molecular Networking (GNPS). GNPS utilizes tandem mass spectrometry to cluster putative metabolites based on relatedness of the observed fragmentation pattern. Two intriguing clusters were identified as they appeared to contain new chemistry. Isolation and structure elucidation of one of the metabolites has revealed a novel class of highly modified terpene, including a C35 carbon backbone, high degree of oxidation, and new sugar moiety. This unprecedented structure raises additional unanswered biosynthetic questions and potential biomedical applications.

1:30 PM S106: Shipworm symbiosis ecology-guided discovery of an antibiotic that kills colistin-resistant Acinetobacter

B. Miller, Ph.D., USA; A. Lim, Z. Lin, Ph.D., K. Aoyagi, M. Fisher, Ph.D., L. Barrows, Ph.D., E. Schmidt, Ph.D. and M. Haygood, Ph.D., University of Utah, Salt Lake City, UT, USA; J. Bailey and C. Manoil, Ph.D., University of Washington, Seattle, UT, USA*

Teredinibacter turnerae is an intracellular bacterial symbiont in the gills of wood-eating shipworms, where it is proposed to use antibiotics to defend itself and its animal host. Several biosynthetic gene clusters are conserved across numerous *T. turnerae* genomes and detected in the metagenomes of their host shipworms around the world. This high degree of conservation, even over vast geographic ranges and across multiple genera of shipworm hosts, implies that they encode compounds integral to the symbiotic association. Here, we describe turnercyclamycins, lipopeptide antibiotics encoded in the genomes of all sequenced *T. turnerae* strains. Turnercyclamycins are bactericidal against challenging Gram-negative pathogens, including colistin-resistant *Acinetobacter baumannii*. Comparison of the terminal morphology of *Acinetobacter* cells exposed to turnercyclamycins with the growth characteristics of mutant strains deficient in a range of essential proteins identified the outer membrane as the likely target. Turnercyclamycins and colistin operate by similar cellular, although not necessarily molecular, mechanisms, but turnercyclamycins kill colistin-resistant *A. baumannii*, potentially filling an urgent clinical need. Thus, by exploring environments that select for the properties we require, we harvested the fruits of evolution to discover compounds with potential to target unmet health needs. Investigating the symbionts

of shipworms is a powerful example of this principle.



2:00 PM S107: Culturing the unculturable?

W. Fenical, E.J. Choi, S.J. Nam, D. Beatty, L. Paul, C. Kauffman and P. Jensen, UC-San Diego, La Jolla, CA, USA*

Beginning with the publication of the “Great Plate Count Anomoly,” it has been widely accepted that the vast diversity of procaryotic microbes were either difficult or impossible to culture outside their natural environments. Culture experiments, in comparison to direct microscopic observation led to the conclusion that less than 5% of the bacteria present grew on agar plates. This observation has frequently been cited and often used to discourage researchers from continued exploration of specific habitats. The marine environment is a classic example in which the pessimism of cultivation has discouraged comprehensive exploration. Exploring this concept, we examined various aspects of the basis of cultivation. Our study showed that relying on the standardized culture methods used for more than 100 years did indeed result in low yields of cultured bacteria. However, when a diversity of modifications were employed, unique strains that composed the great uncultured majority were successfully cultured.

2:30 PM Break

3:00 PM S108: Mining plant genomes for new cyclic peptide chemistry

R. Kersten, University of Michigan, Ann Arbor, Ann Arbor, MI, USA*

Systematic omics-based discovery of new natural product classes from plants is challenging due to the chemical complexity of plant extracts, the large size of plant genomes, the limited predictability of plant metabolic pathways on a genomic level and knowledge gaps in plant metabolism. Here, we present an approach to systematically discover several classes of side-chain-macrocyclic plant peptides, which are structurally defined by macrocyclizations via tryptophan or tyrosine residues. To overcome the mentioned bottlenecks in plant natural product discovery, we combine peptide-targeted metabolomics and genome mining of candidate precursor peptides of plant ribosomal peptides (RiPPs). Our study reveals a RiPP biosynthetic pathway, which involves a new type of autocatalytic peptide cyclase and yields macrocycles similar to microbial RiPPs derived from radical SAM Fe-S-cluster cyclases, in diverse plant lineages.

3:30 PM S109: Use of MALDI-TOF MS and IDBac to assess patterns in phylogenetic and natural product diversity within the culturable freshwater sponge microbiome

A. Hernandez, C. Clark, R. Dhakal, N. Krull, M. Mullowney, S. Green and B. Murphy, University of Illinois at Chicago, Chicago, IL, USA*

Marine sponges have a rich history as a source of natural product (NP) therapeutics, though their freshwater counterparts remain relatively understudied. Our program focuses on developing the freshwater sponge microbiome from the Great Lakes region as a potential source of bioactive NPs. However, it is difficult to design an effective sample collection strategy, particularly since there are limited studies that document the diversity and variability of this microbiome as a function of sponge type and location. To address this, we assessed patterns in bacterial phylogenetic and NP diversity using two *Eunapius fragilis* var *minuta* freshwater sponges collected 1.5 km apart in the St. Lawrence River, and fifteen sponges of three genera collected within a 1200 km^2 region in Lake Superior. We employed gene sequencing techniques and a MALDI-TOF MS / IDBac bioinformatics pipeline to examine 1) the extent to which culturable bacterial populations within the sponges varied as a function of collection location and sponge taxa; and 2) patterns of NP variation within groups of phylogenetically related bacterial isolates. Results of these studies will be presented in the context of designing a more efficient sample collection strategy so that maximum bacterial and NP diversity can be captured using minimal resources.

4:00 PM S110: Harnessing the Chemical Potential of Unprecedented Microbes from the Great Salt Lake

J. Winter, University of Utah, UT, USA and W. Fenical, UC-San Diego, La Jolla, CA, USA*

Environmental pressures have been shown to influence the structural diversity of compounds produced in Nature, and microorganisms thriving in extreme environment often produce chemical agents not observed in their terrestrial counterparts. The Great Salt Lake, also recognized as “America’s Dead Sea”, is an endorheic hypersaline lake located near the University of Utah. While seawater has an average salinity of ~3.3%, the Great Salt Lake ranges between 8-28%. Recently, our lab started a natural products drug discovery campaign aimed at interrogating halophilic bacteria isolated from this unique environment. Our preliminary data demonstrate that the unexplored hypersaline microorganisms of the Great Salt Lake produce metabolites containing molecular scaffolds never before observed, and their genomes contain unprecedented biosynthetic machinery. Thus, these microbes serve as an ideal resource for the discovery of new therapeutic agents. Fermentation studies with two of these strains led to the isolation and elucidation of the bonnevillamides and salinipeptins, which are new classes of linear nonribosomal heptapeptides and ribosomally synthesized and post-translationally modified peptides, respectively. The discovery and characterization of these new chemical entities as well as their corresponding biosynthetic machinery will be discussed.

1:00 PM - 5:00 PM Session: 24: Metabolic Engineering for Fuels and Chemicals II (specialty chemicals)

Conveners: **James Elkins** and **Sun Qing**, TAMU

Waller Ballroom - Salon C-D, Level 3

1:00 PM S111: Engineering of Microbial Cell Factories for High-Performance Polyesters from CO_2

N. Averesch, V. Pane, R. Waymouth and C. Criddle, Stanford University, Stanford, CA, USA*

Synthetic materials are integral components of consumables and durable goods and indispensable in the modern world. Polyesters are among the most versatile bulk- as well as specialty-polymers and their sustainable production, as well as fate at end-of-life are of great environmental concern. Polyhydroxyalkanoates (PHAs), a class of biological thermoplastic polyesters, are potential bio-degradable replacements for these materials. The most common natural bio-polyesters, poly(3-hydroxybutyrate), can be produced outgoing from non-edible carbon-sources such as carbon dioxide and methane. However, commercial competitiveness with synthetic plastics and shortcomings of the materials properties, have so far hampered its success on global market scale. Enabling bio-production of advanced PHAs with superior properties could change this. Especially materials that can directly replace industrial (petrochemistry-based) polymers are needed, to make PHAs not only economically viable, but commercially attractive, without the need for extensive modifications to the existing processing- and recycling-infrastructure.

We have created microbial cell factories that allow the formation of a wide spectrum of aliphatic and aromatic polyesters. Specifically, a Δ*phaC1* mutant of the lithoautotroph *Cupriavidus necator* was complemented with an engineered PHA synthase (*phaC1437*) from *Pseudomonas* sp. MBEL 6-19, in combination with a promiscuous isocaprenoyl-CoA:2-hydroxyisocaproate CoA-transferase (*hadA*) from *Clostridium difficile*. Expression of the heterologous genes allowed the incorporation of various non-natural monomers into the polymer: co-polymers of 3-hydroxybutyrate with straight-chain hydroxyalkanoates like 4-hydroxybutyrate and 6-hydroxycaproate could be obtained. The aromatic hydroxyalkanoate 3-phenyllactate yielded a co-polymer with an approx. 2:1 ration of 3-hydroxybutyrate to 3-phenyllactate, which significantly altered material properties. Further, we were able to obtain a co-polymer that contained phloretate, for the first time showing incorporation of the aromatic ring in the backbone of a biological polyester. Polymers of phloretic acid have structural analogy with industrial grade high-strength synthetic polyesters and “liquid-crystal” polymers like polyarylates. This opens the door to the bio-production of thermoplastics and thermosets from CO_2. Synthetic biochemical pathways for *de-novo* production of the novel PHAs are under development.

1:30 PM S112: Transcription Regulation Integrated with Metabolic Regulation for Metabolic Engineering

X. Qian, P. Niu, B.J. Yoon and E. Dougherty, Texas A&M University, College Station, TX, USA; M. Soto and I. Blaby, US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; F. Alexander, Brookhaven National Laboratory, Upton, NY, USA*

There has been extensive research in modeling and prediction of genome-scale metabolic reaction networks. However, living systems involve complex and often stochastic processes arising from interactions between different types of biomolecules. For more accurate and robust prediction of target metabolic behavior under different conditions or contexts, not only metabolic reactions, but also the integration of genetic regulatory relationships involving transcription factors (TFs) that may regulate metabolic reactions, should be appropriately modeled. We have developed a pipeline enabling analysis of

Transcription Regulation Integrated with Metabolic Regulation (TRIMER). TRIMER utilizes a Bayesian network (BN) inferred from transcriptomes to model transcription factor regulatory networks. TRIMER then infers the probabilities of gene states of relevance to the metabolism of interest, and predicts metabolic fluxes resulting from deletion of transcription factors at the genome scale. We demonstrate TRIMER's applicability to both simulated and experimental data and provide performance comparison with other existing approaches. In particular, we show that TRIMER can reliably predict indole production for both wild-type *E. coli* and corresponding TF knockout strains.

2:00 PM S113: Cell-free glycomolecule construction enabled by a universal glycoenzyme biosynthesis pipeline

T. Jaroentomeechai[*] and M. DeLisa, Cornell University, Ithaca, NY, USA

Cell-free synthetic glycobiology has emerged as a simplified and highly modular framework to investigate, prototype, and engineer pathways for glycan biosynthesis and biomolecule glycosylation outside the confines of living cells. These developments notwithstanding, constructing *de novo* glycan biosynthesis pathways remains a significant challenge due to limited accessibility to functionally characterized glycoenzymes. Glycosyltransferases (GTs) represent an essential class of glycoenzymes that catalyze glycosidic bond formation on aglycone and glycone within cellular glycan assembly lines. Many GTs are difficult to express recombinantly owing to their membrane-associated nature, making it difficult to obtain sufficient amounts of soluble enzyme for structural and functional studies. To address these challenges, we describe a novel glycoenzyme biosynthesis pipeline that facilitates high-level, soluble production of functional GTs across multiple expression platforms. This technology leverages a protein engineering strategy to remodel GTs with an N-terminal decoy protein that prevents membrane insertion and a C-terminal amphipathic protein that effectively shields hydrophobic surfaces from the aqueous environment. We demonstrate the modularity and universality of the approach through the facile production of more than 100 difficult-to-express GTs across several diverse expression platforms including mammalian, yeast and bacterial cells, as well as cell-free protein synthesis. The utility of this pipeline was further revealed by using these engineered GTs to construct cell-free biosynthesis pathways that furnished several human *N*-glycans including sialylated complex-type biantennary structures. Overall, the platform described here provides a simplified yet highly effective strategy for producing large quantities of diverse, enzymatically active GTs that are expected to find use in structure-function studies as well as applications in biomanufacturing of complex glycans and glycomolecules.

2:30 PM Break

3:00 PM S114: The road to animal-free glycosaminoglycans: complete biosynthesis of chondroitin sulfate in Escherichia coli

M. Koffas, Professor[*], Rensselaer Polytechnic Institute, Troy, NY, USA

Sulfated glycosaminoglycans (sGAGs) are linear polysaccharides that have important applications in the medical and food industries. Engineering bacteria for microbial production of sGAGs will facilitate the one-step, scalable production with good control over sulfation levels and positions in contrast to extraction from animal sources.

Advancing towards this goal, we engineered *E. coli* to accumulate 3'-phosphoadenosine-5'-phosphosulfate (PAPS), the universal sulfate donor, using traditional metabolic engineering approaches. PAPS is one of the least explored components required for biosynthesis of sGAGs. The major intervention for PAPS improvement involved the deletion of PAPS reductase. The resulting engineered *E. coli* strain shows a ~1000-fold increase in intracellular PAPS concentrations.

In a similar fashion, we were able to identify a number of chondroitin sulfotransferases with enhanced activities when provided unsulfated chondroitin and PAPS. These mutants were identified using PROS, a computational platform that allows the identification of point mutations that improve enzyme stability. Mutations were identified based on a homology-based structure model of chondroitin sulfotransferases.

By combining the PAPS produced in *E.coli* with the functionally expressed sulfotransferases, we were able to show for the first time the production of animal-free bioengineered CS with impressive sulfation yields that exceeded 90% of the polysaccharide chain. A similar approach was followed for the production of deuterated heparin, another sulfated GAG, also for the first time.

3:30 PM S115: Model-guided Design Strategies for Bioplastic Overproduction in *Rhodopseudomonas palustris*

A. Alsiyabi and R. Saha, University of Nebraska-Lincoln, Lincoln, NE, USA*

Rhodopseudomonas Palustris is a metabolically versatile Purple Non-Sulfur Bacterium (PNSB). Depending on growth conditions, *R. palustris* can operate on either one of the four different forms of metabolism: photoautotrophic, photoheterotrophic, chemoautotrophic, and chemoheterotrophic. *R. palustris* is also a facultative anaerobe, meaning it can operate both aerobically and anaerobically. Furthermore, the organism is capable of fixing nitrogen and subsequently producing hydrogen and the bioplastic precursor polyhydroxybuyrate (PHB). Recent experimental findings revealed that PHB yields in *R. palustris* were highly dependent on the characteristics of the utilized carbon source. PHB production significantly increased when grown on the carbon- and electron-rich lignin breakdown product *p*-coumarate ($C_9H_8O_3$) compared to acetate when the same amount of carbon was supplied. However, the maximum yield did not improve further when grown on coniferyl alcohol ($C_{10}H_{12}O_3$). To obtain a systems-level understanding of factors driving PHB yield, a model-driven investigation was performed. A thermo-kinetic analysis of the PHB synthesis pathway identified how the relative concentration of various metabolites in the pathway influenced overall productivity. These findings were incorporated into a recently constructed genome-scale metabolic model of the bacterium to understand how characteristics of the utilized carbon substrate affected PHB productivity. This model-guided approach yielded several engineering design strategies for PHB over-production, including utilizing reduced, high molecular weight substrates that bypass the thiolase reaction. Overall, these findings uncover key thermodynamic and enzyme saturation limitations controlling PHB production and lead to design strategies that can potentially be transferrable to other PHB producing bacteria.

More details of this work could be found at https://assets.researchsquare.com/files/rs-334477/v1_stamped.pdf?c=1616016069.

4:00 PM S116: Carbon repartition enhances CO_2 to terpene conversion in cyanobacteria

B. Long, M. Li, S. Dai and J. Yuan, Texas A&M University, College Station, TX, USA; J. Golden, University of California San Diego, La Jolla, CA, USA; X. Wang, Miami University, Oxford, OH, USA*

Photosynthetic terpene production represents one of the most carbon and energy-efficient routes for converting CO_2 into hydrocarbon. In cyanobacteria, engineering the terpene biosynthetic pathway has led to limited success in enhancing yield, partially due to the low carbon partition. In this study, we employed systems biology analysis to reveal the strong competition between primary metabolism (e.g., sucrose, glycogen, and protein synthesis) and terpene biosynthesis for carbon substrates. We then engineered key ‘source’ and ‘sink’ enzymes. Carbon repartition by knocking out either sucrose or glycogen biosynthesis significantly enhanced limonene production by overcoming the ‘source’ limitation. Moreover, further optimization of pathway kinetics by fusing geranyl diphosphate synthase (GPPS) and limonene synthase (LS) have synergized the ‘source’ and ‘sink’ strategy to achieve 11 mg/L limonene yield in 5 days. Overall, the study demonstrates that balancing carbon flux between primary and secondary metabolism can be an effective approach to enhance terpene bioproduction in cyanobacteria.

1:00 PM - 5:00 PM Session: 25: Cell-free and Growth-decoupled Biochemical Production

Conveners: **Joe Rollin** and **James Winkler**

Waller Ballroom - Salon B, Level 3

1:00 PM S117: Isobutanol production freed from biological limits using synthetic biochemistry

T. Korman, PhD and H. Yu, PhD, Invizyne Technologies, Monrovia, CA, USA; S. Sherkhanov, PhD and J. Bowie, PhD, UCLA, Los Angeles, CA, USA*

Cost competitive conversion of biomass-derived sugars into biofuel will require high yields, high volumetric productivities and high titers. Suitable production parameters are hard to achieve in cell-based systems because of the need to maintain life processes. Removing the constraints imposed by having to maintain cell viability might facilitate improved production metrics. We have developed a 200 mL scale cell-free system that produces isobutanol from glucose at a maximum productivity of 4 g L^{-1} h^{-1}, a titer of >200 g L^{-1} and >90% yield over the course of nearly 5 days with continuous product removal. These production metrics exceed even the highly developed ethanol fermentation process. We have also demonstrated the synthetic biochemistry system can accommodate cellulosic feedstocks without significant loss in performance. Our results suggest that moving beyond cells has the potential to expand what is possible for bio-based chemical production.

1:30 PM S118: Cell-free synthetic biology platforms to accelerate biomanufacturing

B. Rasor, A. Karim and M. Jewett, Northwestern University, Evanston, IL, USA*

Cell-free systems are rapidly developing as complementary tools for metabolic engineering and biomanufacturing. In the last few years, we have developed cell-free platforms for biosynthetic pathway assembly and demonstrated their use by producing 10 distinct metabolites to date and facilitating the development of industrial metabolite production in non-model organisms. Our approach uses crude cell extracts, which comprise enzymes for catabolism and cofactor regeneration as well as machinery for protein synthesis. Heterologous enzymes can be expressed in crude extracts either during cell growth prior to lysis or through cell-free protein synthesis after lysis to reconstitute complete biosynthetic pathways *in vitro*. These platforms separate catalyst generation from utilization to reduce the need to balance biosynthesis with cell viability, enabling greater volumetric productivities and toxicity thresholds than *in vivo* fermentation. In addition, extract-based systems benefit from rapid design-build-test cycles for pathway prototyping, which we have used to inform strain engineering efforts. In this talk, I will provide an overview of cell-free metabolic systems and discuss how we are developing them for biomanufacturing and cellular design. With a growing list of potential applications, cell-free systems are poised to accelerate the expanding landscape of biomanufacturing.

2:00 PM S119: Lessons in Multi-Step Cell-Free Biocatalysis

N. Brideau and J. Britton, Debut Biotech, San Diego, CA, USA*

Natural product manufacturing via cultivation, chemical synthesis, or engineered cells is subject to complications that can limit the commercial success of specialty chemical production. Cell-free biomanufacturing presents a viable alternative to traditional production methods while providing many benefits. Debut builds cell-free continuous manufacturing systems designed to mimic the natural production of high-value molecules. Using this platform, complex materials are created through multi-step enzyme reactions starting with renewable feedstocks. This session includes critical findings and recent developments.

2:30 PM Break

3:00 PM S120: Use of cell-free systems to prototype the bioconversion of molecules generated through O_2-dependent and O_2-independent activation of methane

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Methane generated from agriculture, landfills, and oil and gas reservoirs is a significant contributor to greenhouse gas emissions. Methane is difficult to transport and store due to its low density and its high C-H bond energy requires high temperature and pressure for chemical activation, making its conversion to value-added products challenging. Biological activation of methane through several known and postulated mechanisms, however, has the potential to solve this problem as it can occur at ambient conditions. Among them, O_2-dependent activation via methane monooxygenase (MMO) and O_2-independent activation via fumarate addition enzymes (FAE) generate activated intermediates methanol and methylsuccinate, respectively. Known metabolic pathways for product synthesis from these activated intermediates are either complex and/or inefficient (methanol) or not known to exist in nature (methylsuccinate).

We have used *E. coli* cell extracts and purified enzymes to prototype product synthesis from methanol and methylsuccinate. Our pathways feature several advantages that make them ideally suited for cell-free bioconversions. For example, they are composed of a small number of reactions catalyzed by simple, oxygen-tolerant enzymes with no requirement for ATP or complex co-factors. The methanol conversion pathway has several advantages over other natural and synthetic routes, including its carbon and energy efficiency, the direct synthesis of multi-carbon products from C1 units, and its high chain length resolution. We have demonstrated the conversion of C1 units to products such as glycolic acid, ethylene glycol and ethanol. Unlike methanol, no pathway has been reported for the conversion of methylsuccinate and hence we designed a new-to-nature route that directly generates pyruvate and acetate. The pathway involves CoA-dependent activation of methylsuccinate, followed by dehydrogenation, hydration and cleavage to pyruvate and acetyl-CoA. In this talk, I will discuss our recent efforts on engineering these pathways along with approaches for their integration with the corresponding methane-activation enzymes.

References: ACS Catalysis, 5396-5404, 2021; Nat. Chem. Biol. 15, 900-906, 2019; Nat. Chem. Biol. 10, 331-339, 2014.

3:30 PM S122: 3D Printing for High Throughput Screening in Cell-Free Biosynthesis

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Cell-free biosynthesis offers the promise of accessing novel small molecules with greener metrics than conventional methods. However, the approach requires tedious optimization of conditions, which is especially problematic for low resource labs, lacking robotics and other technologies. Development and broad deployment of inexpensive 3D printing offer a potential solution to this challenge. Specifically, 3D printing allows the fabrication of arbitrary shapes necessary for high-throughput weighing of solids, such as solid supports for cell-free biosynthesis. For example, a 3D-printed, 96-well pycnometer is open-source for universal applicability and allows measurement of the density for essentially any free-flowing solid. Such density can then be used to program a 3D-printed device for rapidly aliquoting different solid support resins into 96-wells. The format allows optimization of enzyme bioconjugation conditions including the solid supports compositions, the linker, and conditions for combining the two with the enzyme. The approach demonstrates that each enzyme requires its own unique solid support and reaction conditions. Additionally 3D-printed labware to accelerate cell-free development includes simple filter plates and a manifold for transferring reaction products to a conventional 96-well plates for assay. The method opens rapid optimization of cell-free to any lab with a 3D printer.