SIMB Annual Meeting and Exhibition 2019

Sunday, July 21

7:00 AM - 8:00 AM Workshop breakfast

Coolidge, Mezzanine

7:00 AM - 8:00 AM Workshop registration

Coolidge, Mezzanine

8:00 AM - 3:00 PM SIMB Board of Directors

Madison B, Mezzanine

8:00 AM - 3:30 PM Workshop: Fermentation Basics

Hoover, Mezzanine

8:00 AM - 3:30 PM Workshop: Industrial Genome Engineering: Modern Tools, Approaches, and Applications

Harding, Mezzanine

9:00 AM - 7:00 PM SIMB Annual Meeting Registration

Atrium, Lower level

11:00 AM - 3:00 PM Poster set up

Exhibit Hall C, Lower level

12:00 PM - 1:00 PM Workshop lunch

Coolidge, Mezzanine

4:00 PM - 5:00 PM Welcome and Keynote Address: Dr. Jo Handelsman, Director of the Wisconsin Institute for Discovery at the University of Wisconsin-Madison, a Vilas Research Professor, and Howard Hughes Medical Institute Professor. SPONSORED BY CARGILL

Marshall Ballroom, Mezzanine

5:00 PM - 6:00 PM Science Slam

Marshall Ballroom, Mezzanine

6:00 PM - 8:00 PM Session: PS1: Poster Session 1/Reception Exhibits open

Exhibit Hall C, Lower level

P1 Bioconversion of acrylonitrile to acrylamide using whole cells, lysate, and purified enzyme of *Rhodococcus rhodochrous* DAP 96253

B. Galbreath, M. de la Croix[*]*, N. Amadason, E. Sanchez and G.E. Pierce, Georgia State University, Atlanta, GA, USA*
Acrylamide (AMD) is an important industrial chemical used in coagulators, water treatment, soil conditioners, mineral refining, paper treatment, adhesives, paints, and petroleum recovering agents. It is typically shipped in an aqueous solution (30-50% w/w acrylamide). To bypass the cost which occurs with shipping a solution that is 70-50% w/w water, one can locally produce AMD or poly-AMD using microorganisms to convert acrylonitrile (AN) to AMD in a small-scale bioreactor. Induced cells of *Rhodococcus rhodochrous* DAP 96253 produce high levels of the enzyme nitrile hydratase, which is capable of converting acrylonitrile to acrylamide. Whole cells, immobilized whole cells, lysate, and free purified enzyme derived from induced cells of *R. rhodochrous* DAP 96253 were used to compare the production of 20% to >40% AMD from AN. Analysis of the production of acrylamide and the existence of by-products and remaining substrate was performed using GC-MS.

P3 Beneath the surface: evolution of methane activity in the Bacterial Multicomponent Monooxygenases

C. Osborne and V. Haritos[*]*, Monash University, Clayton, VIC, Australia*
The bacterial multicomponent monooxygenase (BMM) enzyme family has evolved to oxidise a wide array of hydrocarbon substrates of importance to biotechnology; foremost of these is methane which requires the most powerful oxidant in biology to activate. To understand how the BMM evolved methane oxidation activity in methanotrophs, we investigated the changes in the enzyme family at different levels: operonic, phylogenetic analysis of the catalytic hydroxylase, subunit or folding factor presence, and sequence-function analysis across the entirety of the BMM phylogeny. Our results show that the BMM evolved with new activities coinciding with incremental increases in oxidative power of the active site, and these occur in multiple branches of the hydroxylase phylogenetic tree. While the hydroxylase primary sequence changes that resulted in increased oxidative power of the enzyme appear to be minor, the principle evolutionary advances enabling methane activity occurred in the other components of the BMM complex and in the recruitment of stability proteins. These included an additional sub-unit in the hydroxylase complex, an assembly protein, and a specialised chaperonin. We propose that enzyme assembly and stabilization factors have independently-evolved multiple times in the BMM family to support enzymes that oxidise increasingly difficult substrates. We show an important example of evolution of catalytic function where modifications to the active site and substrate accessibility, which are the usual focus of enzyme evolution, are overshadowed by broader scale changes to structural stabilization and non-catalytic unit development. Retracing macroscale changes during enzyme evolution, as demonstrated here, should find ready application to other enzyme systems and in protein design.

P5 Exploring microbial biodiversity to expand the molecular toolkit for biological lignin valorization

G.N. Presley, O.N. Cannon, J.K. Michener and J.G. Elkins[*]*, Oak Ridge National Laboratory, Oak Ridge, TN, USA*
Lignin-rich waste streams from lignocellulosic biorefineries could potentially be upgraded to valuable products by using engineered microorganisms that are capable of metabolizing aromatic compounds and producing various chemicals. However, known biological decomposition pathways are primarily limited to a few well-studied model organisms. This work describes efforts to isolate novel environmental bacterial strains with the capacity to degrade lignin-based aromatic compounds and determine the genetic basis of these decomposition pathways. To date, we have isolated over 200 bacterial strains from soils and river sediments using enrichment techniques with model lignin monomers (*p*-coumarate, ferulate, syringate, and guaiacol) and dimers (β-O-4 and 5-5 bonds). Strains within the following genera have been identified: *Burkholderia* (28), *Paraburkholderia* (18), *Pseudomonas* (13), *Pandorea* (13), *Sphingomonas* (11), *Rhodococcus* (7), *Novosphingobium* (4), *Bacillus* (2), *Agromyces* (1), *Chryseobacterium* (1), *Methylobacterium* (1), *Methylorubrum* (1), *Mesorhizobium* (1), *Serratia* (1), *Streptomyces* (1), and *Variovorax* (1). Isolates were screened against a panel of aromatic compounds for their capacity to utilize various lignin-based aromatics as a sole carbon and energy source. Several isolates were shown to degrade model lignin dimers (47), G-lignin (113), and/or S-lignin (91) monomers. Growth rates on *p*-coumarate, ferulate, and syringate were measured and at least one isolated *Pseudomonas* spp. grew at 1.7X the rate of *P. putida* KT2440 on *p*-coumarate or ferulate. Strains with select phenotypes are being submitted to the Joint Genome Institute for whole genome sequencing and to date 24 strains are in progress. Comparative genomics will be used to determine the genetic basis of lignin-degrading phenotypes.

P7 Whole-genome sequence and variant analysis of *Saccharomyces cerevisiae* strains evolved for high solids pine fermentations.

O.A. Thompson[*]*, University of Georgia, Athens, GA, USA*
The potential of lignocellulosic biomass as a sustainable substrate for bioethanol production is limited by the pretreatment process that generates inhibitory compounds impairing the growth and performance of fermenting microorganisms. Development of strains with increased tolerance to a range of inhibitors is necessary as methods of inhibitor abatement are economically and environmentally unfavorable. *S. cerevisiae* strain XR122N was subjected to directed evolution and adaptation in pretreated pine fermentations and resultant strains, GHP1 and GHP4, demonstrated improved fermentative ability with GHP4 exhibiting constitutive tolerance and GHP1 exhibiting conditional tolerance dependent on the selective pressure of inhibitory media. Previously reported findings identified 52 differentially expressed genes that may account for improved tolerance to multiple

inhibitors simultaneously and revealed improved robustness of mitochondria of evolved strains that were resistant to the damaging effects of inhibitors in contrast to the parent. Presently, whole genome sequencing of evolved strains and the parent was employed to identify genomic changes that have facilitated adaptation to inhibitors for improved stress tolerance. Variant analysis of all three strains will potentially reveal structural variations in the genome including chromosomal rearrangements, copy number changes, as well as SNP/indel mutations in key genes. Variants will be further characterized to evaluate functional importance in inhibitor tolerance. The results of this study are important for determining key mechanisms of tolerance to biomass derived inhibitory compounds and broadening the general understanding of stress tolerance of *S. cerevisiae*. This study also has direct implications for further development of robust yeast strains for multiple industrial applications.

P9 One-Pot biocombinatorial synthesis of herbicidal thaxtomins and substituted aromatic 2,5-diketopiperazines-

G. Jiang, R. Zuo, M. Powell, Y. Zhang, P. Zhang, S. Hylton, N. Hiller and Y. Ding, University of Florida, Gainesville, FL, USA; R. Loria, University of Florida, Department of Plant Pathology, Institute of Food and Agricultural Sciences, Gainesville, FL, USA*

Thaxtomins are a group of phytotoxic diketopiperazines produced by tens of plant pathogenic *Streptomyces* strains and have received considerable attention as bioherbicide. To synthesize thaxtomin analogue libraries for herbicide development, we here develop an *in vitro* one-pot biocombinatorial approach using four recombinant thaxtomin biosynthetic enzymes including two nonribosomal peptide synthetases TxtA and TxtB and two distinct P450s TxtE and TxtC. The combination of these enzymes led to the synthesis of 124 thaxtomin analogues with verified structures from unnatural amino acid building blocks. Of note, some unnatural thaxtomin analogues possessed potent herbicidal activities. Furthermore, we provided the detailed characterization of substrate requirement of TxtC that sequentially catalyzes both aliphatic and aromatic hydroxylation, a unique combination of P450 reactions. Our results revealed the importance of *N*-CH_3 of thaxtomin diketopiperazine core to TxtC and demonstrated the enzyme tolerance to modifications on the indole and phenyl moieties of its substrates. Importantly, we employed TxtC along with TxtA and TxtB or one promiscuous *N*-methyltransferase Amir_4628 from the actinobacterium *Actinosynnema mirum* to produce over 40 novel hydroxylated, methylated aromatic DKPs. These studies demonstrated the feasibility of *in vitro* synthetic biology approaches for the generation of natural product-like libraries covering broad and diverse chemical spaces.

P11 The polysaccharide deconstruction capabilities of *Paenibacillus amylolyticus* 27C64

C. Keggi and J.D. Peterson, University of Georgia, Athens, GA, USA*

Paenibacillus amylolyticus 27C64 is a Gram-positive bacterium that was previously isolated from the hindgut of a *Tipula abdominalis* (giant cranefly) larvae where it is a part of the microbial community responsible for deconstructing the lignocellulosic leaf litter the insect eats. Previous work identified *P. amylolyticus* 27C64 as the isolate from this community with the broadest array of plant cell wall polysaccharide deconstruction capabilities. In this work the genome sequence of *P. amylolyticus* was mined for potentially useful enzymes and a diverse set of 193 glycoside hydrolases and polysaccharide lyases were identified including cellulases, pectinases, and xylanases. The genomic analysis also revealed that this bacterium likely specializes in deconstruction of non-cellulosic polysaccharides, and growth experiments confirmed that cellulose degradation occurs slowly whereas xylan and various pectins are rapidly broken down and utilized as sole carbon sources. The complexity of the pectinolytic system in particular, paired with the previous identification of two interesting pectate lyases, made these enzymes a target for further study. The differential regulation of each putative pectinase was evaluated on various substrates using RT-qPCR, and a model of the system is described. Elements of this system are novel and have the potential to improve the repertoire of known pectin-degrading enzymes, including one bifunctional rhamnogalacturonan acetylesterase (RGAE) and rhamnogalacturonan lyase (RGL) which has eight non-catalytic domains. The importance of this multi-domain structure to the function and potential application of the enzyme is being actively explored.

P13 Stuck on you: *Caldicellulosiruptor changbaiensis* uses alternate cellulose attachment mechanisms

S.E. Blumer-Schuette, A.M. Khan, C. Mendoza and V. Hauk, Oakland University, Rochester, MI, USA*

Past pangenome analyses of the genus *Caldicellulosiruptor* have highlighted the vast biodiversity of this genus, and the unique mechanisms used to attach to and degrade plant biomass. *Caldicellulosiruptor changbaiensis*, representing the 14th genome added to the *Caldicellulosiruptor* pangenome, is a strongly cellulolytic species isolated from China. The *C. changbaiensis* genome was assembled as a single contig from both long- and short sequence reads. Similar to previous observations of biodiversity within this genus, updating the *Caldicellulosiruptor* pangenome with *C. changbaiensis* increased the pangenome size (3,791) and determined that the pangenome remains open. In comparison to other available *Caldicellulosiruptor* genome sequences, the size of the *C. changbaiensis* genome (2.91 Mb) is larger than the average (2.69 Mb), and encodes for 120 genes not observed in prior pangenome analyses. Among these, the majority were annotated as hypothetical proteins, underscoring the “known unknowns” of the genus *Caldicellulosiruptor* that require further study, others were annotated as ABC transporter loci, response regulators, transcriptional regulators and metabolism-related genes. Interestingly, notable physiological differences were observed when *C. changbaiensis* was compared against *C. bescii*, the benchmark plant biomass degrading species. While *C. changbaiensis* possesses a glucan degradation locus (GDL) similar to *C. bescii*, it does not grow as fast on microcrystalline cellulose, nor solubilizes it to the extent that *C. bescii* can. Furthermore, *C. changbaiensis* is able to metabolize pectin and glucomannan more efficiently than *C. bescii*. Most surprisingly was the noted absence of classical tāpirin

genes in the genome of *C. changbaiensis*, and instead atypical tāpirins are encoded for. *C. changbaiensis* is as capable of attaching to cellulose as *C. bescii*, and may represent a new alternative to non-cellulosomal polysaccharide attachment.

P15 Nitroaromatic Reduction in *Clostridium acetobutylicum* Affected by Oxidation State

S. Liu, E. Gerlach, M. Servinsky, C. Sund and K. Akingbade, CCDC US Army Research Laboratory, Adelphi, MD, USA*

Nitro containing compounds are ubiquitous and can be found in a range of molecules from antibiotics to energetic material. *Clostridium acetobutylicum* has been studied for its ability to biodegrade TNT, which is an effective method of anaerobic TNT reduction. Conversely, it is also widely known that *C. acetobutylicum* as well as other clostridium species are sensitive to nitro containing antibiotics, such as nitroimidazoles and amphenicols. Both TNT and nitro containing antibiotics are thought to be reduced via nitroreductases and ferredoxins by actively growing *Clostridium*. This process requires the input of NADH/NADPH, which concentrations are affected by the oxidation state of available feedstock. In this presentation we studied the reduction of TNT and nitro-containing antibiotics by C. acetobutylicum during growth on hexose derivatives with different oxidation states. The metabolites of the fermentation products and the TNT reduction products were characterized by HPLC/MS and GC/MS. The minimal growth inhibition of *C. acetobutylicum* by metronidazole and chloramphenicol was studied during growth on the different hexose derivatives. The results showed that when the substrate switched to a more oxidized state, i.e., from glucose to gluconate to galacturonate, the nitro- reduction rate was decreased by gradations. When TNT was fermented with galacturonate, only minimum amount of TNT reduction could be observed and growth was significantly inhibited in the presence of antibiotics. Oxidized sugars sensitized the bacteria to the nitro containing compounds.

P17 Caldicellulosiruptor species use surface (s)-layer glycoside hydrolases and novel binding proteins (tāpirins) for plant cell wall deconstruction

T. Laemthong and R.M. Kelly, North Carolina State University, Raleigh, NC, USA; V. Lunin, Y.J. Bomble and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO, USA; M.W.W. Adams, University of Georgia, Athens, GA, USA*

The genus *Caldicellulosiruptor* consists of extremely thermophilic, Gram-positive, fermentative anaerobic bacteria (T_{opt}> 70^{o}C), typically isolated from terrestrial thermal features. All species with available genome sequence information to date degrade hemicellulose, whereas only a subset degrade microcrystalline cellulose. *Caldicellulosiruptor* species physically associate with the substrate during growth on the plant biomass. One mechanism for this association is through multi-domain hemicellulases, which are anchored to the cell surface through S-layer homology (SLH) domains. Engineered strains of *Caldicellulosiruptor bescii* in which an SLH-domain hemicellulose from *Caldicellulosiruptor kronotskyensis* was inserted bound more tightly to certain xylans. These bacteria also deploy novel binding proteins, called tāpirins, which are encoded in proximity to the region of the genome referred to the Glucan Degradation Locus (GDL), which contains up to six multi-domain cellulases. Tāpirins specifically interact with microcrystalline cellulose to various extents. For example, the tāpirin (Calkro_0844) from *Caldicellulosiruptor kronotskyensis* has a cellulose binding affinity comparable to family 3 carbohydrate binding modules (CBM3). The tāpirins from *Caldicellulosiruptor hydrothermalis* (Calhy_0908) and *Caldicellulosiruptor kristjianssonii* (Calkr_0826) bind to cellulose to a greater extent compared to other tāpirins, although these species are not prolific cellulose degraders. Furthermore, *Caldicellulosiruptor bescii* mutants lacking the tāpirin genes did not bind to the cellulose.

Discussed here are current efforts to engineer the surface of *C. bescii* to improve its capacity for biomass degradation. This includes inserting genes for non-native SLH-domain hemicellulases from other *Caldicellulosiruptor* species into the *C. bescii* genome. Furthermore, we are investigating the tāpirin cellulose binding mechanism through site-directed mutations in Calhy_0908, Calkr_0826, and Calkro_0844 in which cysteine residues are being inserted to strategically enable disulfide bridges within the protein to form. Our goal is to assess the significance of flexibility in hydrophobic binding pocket in the tāpirins as this relates to cellulose affinity.

P19 Enzymatic deconstruction of oligosaccharides in black liquor from deacetylation process of corn stover

W. Wang, X. Chen, R. Katahira and M.P. Tucker, National Renewable Energy Laboratory, Golden, CO, USA*

The Deacetylation and Mechanical Refining (DMR) process developed by National Renewable Energy Laboratory has proved to be a very successful pretreatment process that generates a highly fermentable and less toxic sugar syrups. Meanwhile, the deacetylation step to remove acetyl groups also generates the so called “black liquor” mainly containing acetic acids, oligosaccharides and lignin. Characterization of black liquor revealed that the oligosaccharides in black liquor were mainly trimers and dimers and more xylooligomers than glucooligomers were released into black liquor. In this work, for better hydrolysis of the valuable polysaccharides, chemical and spectral analysis for components in the black liquor in order to collect compositional and structural information, was performed. Enzymatic breakdown of oligosaccharides in black liquor was conducted based on the structural information collected. By loading commercial enzyme Htec2 and accessary enzymes, 57% of xylooligosaccharides in black liquor were hydrolyzed into monomeric sugars, which could be used as carbon sources in biological fermentation. All these results will help to understand the potential value of black liquor. This presented work could serve as a baseline for potential utilization of black liquor from DMR process.

P23 Co-production industrial enzymes by thermotolerant soil bacteria and potential application for cleaning

T. Thinh, F. Toussaint, R. Loomis, D. Warren, C. Woods and B. Okeke, Auburn University at Montgomery, Montgomery, AL, USA*

Microbial biocatalysts such as protease, amylase, lipase, cellulase and xylanase have several industrial applications including production of cleaning products. In the past decade there has been increasing application of microbial biocatalysts as cleaning agents for breakdown of macromolecules. Microbial biocatalysts can be used for removal of aggregates of lipids, proteins, and starch that clog sinks, sewer pipes and septic tanks; muck and odor in recreational ponds and lakes. The overall objective was to select thermotolerant soil bacteria for low cost co-production of enzymes for cleaning products. Over 50 soil microbial isolates were examined for production of lipase, protease, and amylase. Nine isolates co-producing relevant enzymes were identified by 16S rRNA gene sequence analysis using universal bacterial primers 27F and 1492R. One isolate, *Bacillus* species M13, is unique in that it produces amylase, cellulase, lipase, protease and xylanase; and is a potential organism for low cost co-production of industrially important enzymes. Potential reduction of viscosity of macromolecules present in liquid waste is under study.

P25 Unique asparaginase produced by induced cells of Rhodococcus rhodochrous DAP 96253 can serve as a therapeutic agent against acute lymphoblastic leukemia.

N. Amadasun, E. Sanchez, K. Cannon, M. de la Croix and G.E. Pierce, Georgia State University, Atlanta, GA, USA*

Nitrile hydratase (Nhase) is an industrially relevant enzyme utilized in bioconversion of acrylonitrile to acrylamide. When produced from induced cells of the Gram-positive bacterium *Rhodococcus rhodochrous* strain DAP 96253, purified Nhase preparations have been shown to exhibit asparaginase activity which is comparable in activity to Asparaginase expressed in recombinant *Escherichia coli* and *Erwinia chrysanthemi*. Asparaginase has been included in the cocktail utilized for treating acute lymphoblastic leukemia (ALL).

There are a host of problems associated with expressed asparaginases including endotoxin associated hypersensitivity, protein stability and in some cases, lymphoblasts express asparagine synthetase rendering the treatment process ineffective. Utilization of an asparaginase sourced from a Gram-positive organism, if properly purified will circumvent the challenges associated with Gram negative cell proteins. This study focuses on process development for the purification as well as characterization of the protein of interest. This protein has been shown to be cytotoxic at a relatively low dose to Jurkat leukemia cells; T lymphocytes expressing acute T cell leukemia.

P27 Reveal cell wall integrity signaling pathway of the tolerant industrial yeast *Saccharomyces cerevisiae*

Z.L. Liu, USDA-ARS, Peoria, IL, USA*

Well characterized haploid laboratory strains of *Saccharomyces cerevisiae* are popularly used for investigations in the field of lignocellulose-to-biofuels conversion due to the readily available genetic tools for these strains. The industrial yeast, often diploid, is a workhorse widely applied in fermentation-based industries including for production of advanced biofuels and chemicals. Performance response between the industrial yeast and the laboratory strains is not always consistent although they fall into the same species specifications. Significant differences have been observed in gene expression response to environmental stimuli, heterologous gene expression, constitutive genetic engineering performance, plasticity of the genomic structure, and the rate of genome evolution. Cell wall integrity signaling pathway in *S. cerevisiae* is a conserved function for detecting and responding to cell stress conditions but little is known for the industrial yeast. This research reports quantitative gene expression dynamics for a tolerant industrial yeast strain NRRL Y-50049 against synergistic challenge of 2-furaldehyde (furfural) and 5-(hydroxymethyl)-2-furaldehyde (HMF) compared with a laboratory strain BY4741 using qRT-PCR array assays. While both strains showed resistant response, strain Y-50049 displayed significantly overall higher increased gene expressions than the laboratory strain after the furfural-HMF treatment. Furthermore, *WSC3* from Y-50049 demonstrated the most increased expression over time in both strains among all five activated sensor genes of *WSC1, WSC2, WSC3, MID2* and *MTL1*. DNA sequence of *WSC3* from Y-50049 was also found to have five single nucleotide variations and three of them were non-synonymous mutations resulting in amino acid alterations of Ser_{158} ->Tyr_{158}, Val_{186} -> Ile_{186}, and Glu_{430} -> Asp430. Results of this study suggest a more robust and effective cell wall integrity signaling pathway against furfural-HMF for the tolerant industrial yeast.

P29 Discovery of a novel β-glucosidase from *Saccharophagus degradans* 2-40^T capable of efficiently hydrolyzing laminarin from brown macroalgae

D.H. Kim and K.H. Kim, Korea University, Seoul, Korea, Republic of (South)*

With increasing concern global warming, macroalgae has received much attention owing to its sustainability. Laminarin as one of the major polysaccharides from brown macroalgae is a potential biomass feedstock for the production of glucose. Here, we discovered novel β-glucosidase from *Saccharophagus degradans* 2-40^T, which can effectively hydrolyze laminarin from brown. First, to characterize the activity of Bgl1B, the *bgl1B* gene encoding β-glucosidase was cloned and overexpressed in *Escherichia coli* BL21 (DE3). The molecular weight of Bgl1B was 49.8 kDa on SDS-PAGE. The optimal pH and temperature of Bgl1B were pH 6.0 and 40^{o}C, respectively. Interestingly, the activities of Bgl1B were maintained to be about 59-85% of its

maximum activity even at low and moderate temperatures of 2-20^{o}C. Similar to most of β-glucosidases in GH1, Bgl1B hydrolyzed a variety of disaccharides having different β-linkages such as laminaribiose, cellobiose, and gentiobiose as well as lactose and agarobiose. In particular, it showed significantly high substrate specificity for laminaribiose when compared to other disaccharides. In addition, it was able to hydrolyze laminarin, which is one of major polysaccharides in brown macroalgae, to glucose with the conversion yield of 75%. Bgl1B also produced oligosaccharides from laminarin and laminaribiose by transglycosylation under the low enzyme concentration. These results indicate that Bgl1B is a unique β-glucosidase capable of efficiently producing glucose and the functional oligosaccharides from brown macroalgae for industrial purposes.

P31 A data-driven approach for exploiting enzyme promiscuity as a means to predict novel biochemical reactions

S.T. Gupta, P. Ramanathan and J.L. Reed, University of Wisconsin Madison, Madison, WI, USA*

Systems metabolic engineering has been widely used to produce chemicals of high commercial value from low cost substrates. But this process has challenges for some applications, such as harnessing lignocellulosic biomass for biofuel and biochemical production, due to our limited metabolic knowledgebase. With current advances in protein engineering, it is possible to exploit substrate promiscuity of enzymes to enable novel biochemical reactions. Nevertheless, performing experiments to determine what substrates an enzyme can act on can be time consuming and it is not always clear what potential substrates to test. So, the current work aims to employ machine learning approaches for identifying novel substrates and in turn, predicting novel reactions that are more promising than the putative reactions predicted simply based on compound similarity measures (e.g., Tanimoto coefficient). A highly accurate (up to 88.3%) machine learning model was developed to identify candidate substrates for *alcohol dehydrogenase* (ADH) using a dataset consisting of 23 metabolites (with 8 of them being known positives) and 46 chemo-informatics based molecular descriptors (e.g., topology, stereochemistry, and electronic features). In addition, support vector regression proved to be a useful method for estimating enzyme kinetics (characterized by Michaelis-Menten constants, Km and Vmax) for a variety of oxidoreductases that are typically found in biofuel biosynthesis pathways. Such machine learning methods can be applied to other classes of enzymes and hence, used as a tool to expand the knowledgebase of metabolic reactions paving the way for next generation of metabolic/pathway engineering.

P33 Cellulase recycling at high-solids enzymatic hydrolysis of lignocellulosic biomass

J.K. Kim, J. Yang and K.H. Kim, Korea University, Seoul, Korea, Republic of (South)*

Cellulase recycling is a key to reduce the cost of producing sugars from lignocellulose. Herein, high-solids (20%) enzymatic hydrolysis of hydrothermally-pretreated empty fruit bunches was performed for cellulase recycling with 40 FPU of Cellic CTec3/g glucan. In the second round of hydrolysis using a recycled enzyme, only 19.3% of glucose yield was obtained. The limiting factors for cellulase recycling were enzyme inhibition by glucose, loss of enzyme activities, and binding of enzymes to insoluble biomass solids. To overcome these limitations, glucose was removed from the enzyme fraction, PEG was added prior to the first-round hydrolysis to reduce unproductive enzyme binding, and EFBs solids from the first-round hydrolysis was used in the second-round hydrolysis. These measures resulted in a 3.5 times higher glucose yield (i.e., 68.0%) than that of the control (19.3%). These results underline the positive steps for improving the process involving cellulase recycling.

P35 Unique asparaginase produced by induced cells of *Rhodococcus rhodochrous* DAP 96253 can serve as a therapeutic agent in the treatment of acute lymphoblastic leukemia

N. Amadasun, E. Sanchez, K. Cannon, M. de la Croix and G.E. Pierce, Georgia State University, Atlanta, GA, USA*

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P37 Efficient production of itaconic acid by *Aspergillus terreus* overcoming the strong inhibitory effect of manganese

B.C. Saha and G.J. Kennedy, Bioenergy Research Unit, National Center for Agricultural Utilization Research, USDA-ARS, Peoria, IL, USA*

Itaconic acid (a building block platform chemical) is currently produced industrially from glucose by fermentation with *Aspergillus*

terreus. In order to expand the use of itaconic acid, its production cost must be lowered. Lignocellulosic biomass has the potential to serve as a low cost source of sugars for itaconic acid production. Previously, we found that the fungus could not produce itaconic acid from dilute acid pretreated and enzymatically saccharified wheat straw hydrolyzate even at 100 fold dilution. In addition to typical fermentation inhibitory compounds such as furfural, HMF and acetic acid, Mn^{2+} present in the hydrolyzate was found to be a strong inhibitor of itaconic acid production by the fungus. Mn^{2+} at 50 ppb inhibited the itaconic acid production by 97%. Recently, we showed that phosphate limitation alleviated the inhibitory effect of Mn^{2+} on itaconic acid production. We have developed a novel medium for production of itaconic acid using response surface methodology which alleviated the strong inhibitory effect of Mn^{2+}. The tolerance level of Mn^{2+} in optimized medium for itaconic acid production by the fungus was also established. The fungus was able to tolerate up to 100 ppm Mn^{2+} very well in the newly developed medium.

P39 Fed-batch cultivation to increase yields of polyhydroxybutyrate production by *Cupriavidus necator* from corn stover alkaline pretreatment liquor

M. Li[*] and M. Wilkins, University of Nebraska-Lincoln, Lincoln, NE, USA

In a lignocellulosic biorefinery, lignin is a waste material that is burned to generate heat and power. Valorization of lignin into valuable products such as polyhydroxybutyrate will profit and make lignocellulosic biorefineries more sustainable. Using corn stover as an example, alkaline pretreatment liquor from corn stover is an example of a lignin-enriched stream. An earlier study at 250 mL shake flask scale in our lab reported PHB production from APL by *C. necator* 545 reached 2.1 g/L with supplements (laccase 2 U/mL, AAO 20 U/mL, silica nanoparticle Aerosol R816 0.02%, ABTS 5 mM and Tween 80 2%) (Li et al., 2019). In this study, APL conversion into PHB under the same conditions was performed at 1.5 L bioreactor scale and PHB production reached 3.3 g/L. Fed-batch cultivation with two different feeding strategies were applied. Under single pulse feeding with 300 mL, PHB production reached 4.0 g/L. Under 4 times pulse feeding with 75 mL media each time, PHB production reached 4.5 g/L. This is the highest PHB production from lignin that the authors have been aware of in literature. Gel permeation chromatography will be applied to study the molecular change during lignin bioconversion by *C. necator* 545 with the aforementioned supplement system.

P41 Biobased xylitol production by *Candida* species grown on prairie cordgrass hydrolysates

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Xylitol has commercial applications as a sweetener and potential biomedical application. Xylitol could be produced more economically if a biobased process could be developed for its production. A possible biobased approach to synthesize xylitol could involve the microbial bioconversion of a grass hydrolysate to the sugar alcohol. It has previously been demonstrated that species of *Candida*, such as *Candida mogii* ATCC 18364, *Candida guilliermondii* ATCC 20216 or *Candida guilliermondii* ATCC 201935, can produce xylitol on a xylose-containing grass hydrolysate. In this study, prairie cordgrass served as the biomass to be hydrolyzed. The grass was initially suspended in phosphate buffer pH 4.5, 5.0, 5.5 or 6.0 and then subjected to high temperature and pressure using an autoclave. After cooling, the buffered grass solids were treated with cellulase and glucosidase to enzymatically remove the cellulose fraction from the grass. The grass solids representing the hemicellulose fraction were resuspended in phosphate buffer pH 5.0 and treated with a thermostable xylanase at 50^{o}C for 48 h on a rotary shaker (100 revolutions/min). The buffered liquid hydrolysate was subsequently used in the supplemented medium to grow the yeast species to measure xylitol production. Each *Candida* strain was grown in the hydrolysate-containing, supplemented medium for 120 h or 168 at 30^{o}C in a rotary shaker (150 revolutions/min). After collecting the yeast cells by centrifugation, xylitol levels in the supernatant were measured enzymatically. It was found that all three *Candida* strains produced the highest xylitol concentration when the growth medium was buffered to pH 5.5 independent of whether the strain was grown for 120 or 168 h. In summary, it was concluded that it is feasible to produce xylitol by yeast bioconversion of a hydrolysate derived from the hemicellulose fraction of prairie cordgrass.

P43 Potential for using sweet sorghum and beet syrups for production of the industrial precursor 3-hydroxybutanone via fermentation

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Acetoin (3-hydroxybutanone) is a four-carbon ketone-alcohol used in the food industry and is also a precursor to important industrial chemicals such as butanediols and butanols but the nutrient requirements when grown on complex sugar sources such as sweet sorghum and beet syrups are relatively unknown. Therefore, we tested the growth on the complex sugar sources and investigated the potential for replacing glucose as the carbon source.

This is part of our preliminary work with sweet sorghum and sugar beet as starting materials for production of acetoin using the bacterium *Bacillus subtilis.* A large number of strains were screened and the results show that acetoin was easily produced at concentrations up to 6% (weight/volume) from diluted sugar crop syrups with some strains.

P45 Upstream microbial process characterization with single-use bioreactors from 15 mL to 50L

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Developing biological and industrial molecules derived from microbial fermentation relies upon performant bioreactors to allow a rapid scale up to commercial batches. For this it is relevant to minimize any possible risks while developing a process that fits the industry quality standards. The choice of a well characterized system plays an important role from R&D through to production stages. The aim of this poster is to provide evidence to demonstrate the benefits of a microbial process developed using single-use, high throughput, and scalable upstream solutions. The method chosen to showcase this consistency is based on the DECHEMA Guidelines for Engineering Characterization principles and with the Zurich University of Applied Sciences, ZHAW. DECHEMA guidelines include a set of standard conditions for bioreactor characterization. By using process development and pilot scale bioreactors like the ambr 15f, ambr 250, and BIOSTAT STR 50, it is possible to accelerate development timelines and ensure process success.

P47 Pre-pilot fed-batch fermentation of *Rhodococcus rhodochrous* DAP 96253 with emphasis on production of therapeutic enzymes, post-harvest agricultural applications, and microbial bioconversion

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Rhodococcus rhodochrous is a Gram-positive, aerobic, non-pathogenic bacterium ubiquitous in soil. The bacteria exhibit pleomorphic growth, forming branched rod-cocci. Environmentally derived *Rhodococcus* strains have been used for over 70 years in industrial fermentation to produce pharmaceutical grade products. The bacterium has a broad metabolic and physiological diversity that allows for numerous practical applications such as biocatalysis and biotransformation. Our strain *R. rhodochrous* DAP 96253 when grown with an induced medium, over-expresses many enzymes of industrial and medical importance that can be isolated and purified. Current research, in our laboratory, has shown that under specific inducing conditions, *R. rhodochrous* DAP 96253 in pre-pilot fed-batch fermentations can produce therapeutic enzymes, aid in the delay of fruit ripening of climacteric fruit, act as an antifungal catalyst, and be utilized for the bioconversion of acrylonitrile to acrylamide.

Glycerol stocks of *R. rhodochrous* DAP 96253 were revived and propagated in a nutrient medium and supplemented with inducers to prepare a seed inoculum which was used to initiate a two-stage fed-batch fermentation process. The fermentation was sampled frequently and analyzed for media composition, morphological characteristics, enzyme activity, volatile analysis to determine the mode of action for delayed fruit ripening, and the conversion of acrylonitrile to acrylamide was conducted in a conversion bioreactor. Contact-independent delayed fruit ripening, and antifungal catalyst experiments were performed using immobilized whole-cells.

Pre-pilot fed-batch fermentation was employed to confirm and verify smaller scale studies. This process allowed for more significant production output and analysis of cell paste by fine-tuning media concentrations such as glucose and other inducers, and environmental conditions such as dissolved oxygen and pH. Specific induction conditions, coupled with age-related physiological and morphological conditions, during fermentation has been shown to upregulate nitrile hydratase and asparaginase. Reliable, consistent bioconversion of acrylonitrile to acrylamide was achieved with induced immobilized whole cells. Contact-independent delayed fruit ripening, and antifungal setups showed significant efficacy when paired with climacteric fruit.

P49 Fatty Acid Production from an Agricultural Co-Product

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The *ExxonMobil 2018 Outlook for Energy* predicts a 20% increase in demand for transportation liquid fuels by 2040, assuming that all light-duty transportation has been converted to electric vehicles. Biodiesel, an alternative fuel that can be produced from the triacylglycerides (TAGs) of plants and microbes could help offset fossil fuel consumption by the commercial transportation sector.

Microalgal TAG production has the advantage of not competing with food production and a higher productivity per acre. However, microalgae cultivation in open ponds suffer contamination and both water and nutrient loss, while algal growth in photobioreactors can be quite costly. As an alternative to microalgal TAG production, we have developed a system in which *Rhodococcus opacus* PD630 grows on an agricultural co-product in a simple shake flask and accumulates ~40% of the cell dry weight as fatty acids. The fatty acid profile is similar to that of microalgae and plants, indicating that *R. opacus* TAGs could easily be used in biodiesel production. This process does not depend on light, so *R. opacus* can be grown in simple, closed reactors with aeration.

P51 Engineering *Magnetospirillum magneticum* AMB-1 for diagnostic assays

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Magnetic nanoparticles are materials of interest for various biomedical applications, including diagnostic assays. Surface-enhanced Raman scattering (SERS) is a sensitive spectroscopic technique that relies on magnetic particles that have a protein conjugated to the surface. SERS assays are of interest for point-of-care diagnosis with a hand-held Raman spectrometer and

have recently gained attention for the detection of trace quantities of biomolecules. Like other immunological assays, SERS relies on the interaction between an antigen and an antibody, and an antigen conjugated to magnetic particles is used to bind and subsequently detect the presence of an antibody in serum.

Magnetotactic bacteria synthesize intracellular, nanometer-sized particles of magnetite covered by a lipid bilayer. The consistent size and magnetic properties of these magnetic particles make them appealing for medical applications. Due to the tightly bound membrane, genetic engineering can be used to display proteins on these bacterial magnetic nanoparticles (BMNPs). The magnetotactic bacterium Magnetospirillum magneticum AMB-1 has been genetically engineered to express a West Nile virus protein on the surface of BMNPs. These magnetic particles are incorporated into a SERS assay to examine the effects of antigen display versus conjugation. Sensitivity and selectivity of the assay will be tested and compared to that with abiotically prepared magnetic nanoparticles with antigen conjugated to the surface. The oriented antigen may be more likely to be recognized by antibodies as compared to the conjugated antigen so that less antigen would result in the same sensitivity. This work is a proof-of-concept for the use of engineered BMNPs in a diagnostic assay, which would allow the all-in-one production of magnetic particles for diagnostic assays.
Furthermore, a promoter analysis is conducted in Magnetospirillum magneticum AMB-1 to compare gene expression from two Magnetospirillum and two heterologous promoters. Gene expression from the pmms16 and pmsp3 promoters will be compared with expression from the synthetic Tac and the psbA promoters active in gram negative bacteria.

P53 Comparing culture methods in *Escherichia coli*Â fermentation: batch, fed-batch, and continuous

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In this study, three different culture methods, batch, fed-batch, and continuous fermentation, were carried out to grow *E. coli* (ATCC 25922GFP™) in the Eppendorf BioBLU® 3f Single-Use Vessel controlled by the BioFlo® 320 bioprocess control station. The objectives of this study are (1) to show bioprocess parameter setup, vessel preparation, and compare how different culture methods affect microbial growth, nutrient consumption, productivity, and yield; (2) to provide a detailed cost analysis for growing *E. coli* under different fermentation modes and further compare their pros and cons; and (3) to demonstrate the strong performance of BioBLU f Single-Use vessel in high density microbial applications. Batch fermentation is a relatively easy operation with competitive biomass yield on glucose, and it can be applied in early development for process optimization including medium selection. With a feasible feeding strategy, fed-batch fermentation gave the highest cell dry weight at 85 g/L among all culture methods in this study, and it is very cost effective per unit biomass. In continuous fermentation, feeding and harvest take place simultaneously at the same rate. It has the potential to achieve steady state to greatly reduce the downtime, and it is scale-up friendly since the working volume is kept constant. Therefore, depending on the experimental needs and the laboratory settings, and with a brief estimate of the process budget and scheduling, this study can help fermentation scientists to choose the ideal culture method to meet their unique needs.

P55 Molecular identification of the predominant lactic acid bacteria in whole wheat sourdough fermentation

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The sourdough starter is a mixture of flour and water that are spontaneously fermented with lactic acid bacteria and yeasts. In the present study, sourdough production was conducted under laboratory conditions according to the traditional (sourdough Type I) protocol without using a starter culture or baker's yeast. The sourdoughs were prepared by whole wheat flour and propagated over a period of 7 days according to the daily back-slopping (refreshment) procedure. Microbiological and chemical properties of the sourdoughs were investigated at different days. Also, 134 presumptive lactic acid bacteria cultures were isolated from different days of the fermentation. Potential lactic acid bacteria cultures were subjected to genotypic characterization by RAPD-PCR analysis and then identified by sequence analysis of 16S rRNA genes. According to 16S rRNA gene sequencing analyses, strains were grouped into 7 lactic acid bacteria species. Lactic acid bacteria identified at the species level were *Leuconostoc mesenteroides, Lactobacillus curvatus, Lactococcus lactis* subsp. *cremoris* and *Weissella cibaria* in the 1st refreshment of the sourdough. Following refreshment, *Leuconostoc mesenteroides* and *Lactobacillus curvatus* were detected but *Lactococcus lactis* subsp. *cremoris* and *Weissella cibaria* were not isolated. *Pediococcus pentosaceus* was also detected at the 2nd refreshment. At the 4th refreshment, the lactic acid bacteria detected at the species level were *Lactobacillus plantarum, Lactobacillus paralimentarious* and *Lactobacillus curvatus*. On the last day of refreshment, only *Lactobacillus paralimentarious* and *Leuconostoc mesenteroides* were isolated.

P59 Yeasts immobilized in microporous alginate beads display higher productivity than the normal beads in a repeated-batch process for the production of ethanol

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Mass transfers pose significant challenge in fermentation due to wide diffusion gradient occurring between the culture broth and the immobilized cells. In the present study, *Saccharomyces cerevisiae* LC 269108 was immobilized in a recently described

microporous alginate beads and compared with the conventional calcium alginate gel beads in a repeated batch process for the production of ethanol. The fermentation conditions implemented were 110 rpm, pH 5.5 and temperature of 30°C for 60 h. In separate batch experiments conducted by the SSF method, the free cells maintained plateau at peak ethanol concentration of 7.50±0.33% after 36 h. In the conventional alginate (6.51±0.05%) and microporous beads (7.06±0.10%), ethanol dropped in concentration until reaching final volumes of 5.65±0.33 and 6.56±0.64% respectively. In the repeated batch experiments, five fermentation batches or runs were conducted over a 12 h period each. The concentrations of ethanol produced in batches with the cells immobilized in calcium alginate were 2.91±0.34%, 5.80±0.22%, 5.01±0.39%, 4.41±0.14% and 3.77±0.21% respectively. Cells immobilized in microporous beads had higher ethanol output with concentrations 2.33±0.07%, 6.62±0.04%, 6.16±0.32, 5.90±0.2% and 4.70±0.26% obtained after five respective batches. Glucose metabolism was found to be lower with cells immobilized in alginate beads. From initial glucose concentration of 14.30±0.2%, residual glucose was detected after the first (3.61±2.11%), fourth (3.18±0.98%) and fifth (5.30±0.86%) batches of fermentation. In the batches containing microporous beads, residual glucose (5.36±0.29%) was confirmed after the first batch only. The present study demonstrates the feasibility of using microporous beads in the production of ethanol.

P57 Experimental study of Biopharma nutrients available on the market through expression of recombinant GFP in the model organisms *E. coli* and *Pichia pastoris*.

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Developing the most efficient and applicable culture media to ensure optimal performance of microbial strains is one of the main challenges of the fermentation industry, and it is especially true for biopharma applications.

In the interest of creating the most favorable formulation, fermentation scientists are committed to finding the best nutrients to optimize microorganism viability and vitality.

As a key player in the manufacturing of yeast-based nutrients, Procelys has designed the ProCel® product range to meet the demands and requirements of pharmaceutical fermentations. Procelys' yeast-based nutrients provide a comprehensive source of natural, animal-free peptides, free amino acids, nucleotides, vitamins, amino nitrogen, and trace elements.

Procelys has conducted a case study on nutrients currently available in the biopharma market that are designed to support the fermentation industry. Using a bioperformance assay developed in our applications lab, two organisms of biopharma interest, *E. coli* and *Pichia pastoris,* were studied and characterized by analyzing the expression of recombinant GFP. In both hosts, we were able to specifically observe the physiological state and biological activity when grown with varying combinations of yeast-based nutrients. The results indicate that an advantage is obtained by cultivating microbial strains on medium that contains Procelys yeast-based nutrients.

Through collaboration between customers and our technical service, Procelys is committed to improving biopharma media formulations.

P61 Studies on cultural conditions of agricultural products byÂ *Bacillus cereusÂ* S8 for methionine production

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Methionine is nutritionally essential amino acid secreted by various microorganisms that is required in the diet for proper growth and body functions of mammals. Since the discovery of amino acid producing bacteria, emphasis has been on use of fermentation process for producing methionine, as against the chemical and protein hydrolysis approach, so there is a need. This study has shown that agricultural products have great potential to be utilized in methionine production. When compared with previous study, starch hydrolysate, defatted protein and optimization of some cultural conditions parameter was found to accumulate more of methionine than synthetic carbon/nitrogen sources studied. *Bacillus cereus* S8 recovered from Nigerian soil and characterized by 16S rRNA sequencing was used for methionine production in submerged medium employing plantain/groundnut as the carbon/nitrogen sources respectively. The effects of medium/fermenter volume ratio, inoculum size, pH, agitation speed, carbon/ nitrogen varying concentrations, $Caco_2$, growth factors, bivalent metals, surfactants, vitamins and amino acids were studied. In shake flask experiments, a 20% medium/fermenter volume ratio and a 1ml inoculum size increased methionine yield. Plantain(40g/L)/groundnut(20g/L) was the best carbon/nitrogen pH at 7.0, agitation speed of 170rpm, the mixture of yeast extract/peptone and soya enhanced methionine yield. Oleic acid at 0.5µg/ml gave the highest methionine yield of 2.59mg/ml while Pyridoxine at 100.0µg/ml (2.69mg/ml), Zn^{2+}at 5.0µg/ml and DL-leucine stimulated the highest methionine production of 300mg/ml using *B. cereus* S8. Time course experiments for methionine production by *B. cereus* S8 showed that maximum methionine production was obtained after 96h.

P63 Rapid microbial assay applied to a model biomanufacturing system

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We evaluated a rapid bacterial identification assay for contamination control in mammalian cell culture, a popular biomanufacturing process. Control of bacterial contamination is essential in biomanufacturing, hence a rapid microbial assay is essential. However, the complex background of mammalian cell culture often requires subculture of samples prior to

identification. A sufficiently sensitive test would be able to identify contamination without subculture, thus providing critical speed advantages.

For example, CAR-T therapies culture reinfuse the patient's own serum, so bacterial contamination can infect the patient, who is usually immunocompromised. Rapid microbial ID is thus critical to indicate administration of an appropriate antibiotic in conjunction with treatment.

In this study, we evaluated Fast Lipid Assay Technique (FLAT) against the speed and sensitivity requirements of mammalian cell-culture manufacturing. FLAT comprises a heat-based lipid extraction followed by MALDI-TOF mass spectrometry (MS).

We applied FLAT to HeLa cultures inoculated with *Pseudomonas aeruginosa, Escherichia coli* or methicillin-resistant *Staphylococcus aureus* (MRSA). HeLa cultures are typical of mammalian cultures in their complexity, thus evaluating FLAT against a plausible background. Centrifuged samples were extracted by the FLAT method. Mass spectra were acquired by MALDI-TOF-MS in negative ion mode on a Bruker microflex LRF using the matrix norharmane.

We observed MS peaks that are diagnostic for contaminating bacterial species. Thus, FLAT exhibits promise in detecting and identifying contaminant microorganisms in mammalian cells. These results show that FLAT's speed and sensitivity may offer improved safety for cell-based therapies.

P65 Fermentation and bio-preservation of processed African indigenous vegetables

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In recent years, there is an increasing trend to use fermentation and bio-preservation methods to inhibit or destroy undesirable organisms, increase the shelf-life, palatability and sensory quality of food. In this study, strains of *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus homohiochi*, *Lactobacillus delbrueckii* and *Lactobacillus leichmannii* from spontaneous fermentation (0-7% NaCl) of onions (*Allium cepa*) were selected to be used as starter cultures for controlled fermentations of processed African tomato (*Solanum lycopersium*), bell pepper (*Capsicum annum l.*) and chili pepper (*Capsicum species*) pastes with the aim to preserve and improve the nutritional qualities, as well as secure the vegetables. Proximate analysis was carried out on the samples to determine their nutrient profile and the paste samples were stored at both ambient (25 ± 2^0C) and refrigeration temperatures (4 ± 1^0C) for a period of 7 days. The microbial load and some organoleptic properties were ascertained. Antimicrobial effect of the lactic acid bacterial isolates on *Escherichia coli* and *Salmonella typhi* isolated from spoiled foods was also determined using agar well diffusion. The results for the proximate analysis revealed the crude fiber of the three samples to be from 1.25 to 2.55%; moisture content: 42.62 to 53.80%; dry matter: 38.2 to 59.0%; ash content: 0.88 to 2.52%, crude protein: 2.28 to 5.42%; carbohydrate: 2.58 to 5.56% and ether extract of 0.64 to 2.62%. There were little changes in the taste, firmness, color and odor of the pastes. The viable counts of the lactic acid bacteria increased during the preservation. This results show that the lactic acid bacteria were effective against the food spoilage organisms analyzed and could be employed as potential starter cultures for fermentation and preservation of African indigenous vegetable foods.

P69 Electric and magnetic fields and their influence upon microbe DNA expression and industrial fermentation efficiency

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For over 4 billion years, life has evolved upon Earth in the constant presence of Electric and Magnetic Fields, "EMF". Industrial bio-reactors presently do not employ the use of either Electric or Magnetic field stimulation of microbial metabolism.

Industrial bio-reactors have significant and complex productivity challenges. Since the late 80's hundreds of electric and magnetic field EMF experiments have been conducted and published in respected journals. The introduction of a variety of EMF methods has established a notable positive influence. However, they have primarily concluded that promising initial results have not been sustainable or scale-able.

The Electrified Earth and Microbial Evolution

All emerging and evolving life forms were and are today immersed in constant electric and magnetic fields, which continue to influence their evolution and DNA expression. Microorganism metabolism has many inter connected influence factors that direct gene expression. E & M Fields are intimately part of its adaptation to subtle changes in its environment as well as providing vital bio available electrons for its metabolic processes.

By following the complex intricate metabolic processes that microorganisms employ to move electrons from one molecular path to another offer an opportunity to imagine what would happen if they were exposed to the intense and essential electric and magnetic field conditions that exist upon earth.

Since 2014 my ongoing research and discoveries have inspired continuous exploration and an ever growing confidence that EMF system integration with existing industrial bio-reactors can be demonstrated providing additional means of control, efficiency and productivity.

P71 Mutation of *Aspergillus niger* for the production of amylase from banana peels

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Strain improvement through mutagenesis plays a critical role in the commercial development of microbial fermentation process. This investigation was aimed at the production of amylase by mutant strain of *Aspergillus niger* using Banana peels. The fungus spores were treated with ultra violet radiation (UV) and nitrous acid (HNO_2) for strain improvement. Acid pretreated Banana peels and mutated strains of *A. niger* were evaluated for amylase production via fermentation of substrate. Fungal biomass and amylase activity were analyzed in the course of fermentation using standard procedures. The results revealed that banana peels pretreated with 0.8 N hydrochloric acid had the highest biomass and amylase activity of 3.02 ±0.07 g/L and 2.81 ± 0.07 U/L respectively. From the UV mutants, strain AnUV1 had the highest biomass and amylase activity of 4.50 ± 0.21 g/L and 3.46 ± 0.14 U/L respectively. Subsequently, HNO_2 mutants showed that strain AnNA6 had a 30.64 % improvement in amylase activity over AnUV1 strain and 60.85 % improvement over the wild *A. niger*. The study revealed that Banana peels can be utilized for amylase production however, UV and HNO_2 were effective mutagens for *A. niger* strain improvement for the enhancement of amylase productivity.

Keywords: Amylases, Banana peels, Nitrous acid, Strain, Fermentation

P73 About International Commission on Yeasts (ICY)

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The International Commission on Yeasts (ICY) was established in Bratislava in 1966 as the Council for Yeast Research, composed of prominent specialists in the field of yeasts. In 1971 the Council was transferred into the International Association of Microbiological Societies (IAMS), now International Union of Microbiological Societies (IUMS). ICY is now a commission under the Division of Mycology and Eukaryotic Microbiology. See https://www.iums.org/index.php/home-icy

ICY publishes the Yeast Newsletter by courtesy by Dr. Marc-André Lachance. See also https://www.uwo.ca/biology/YeastNewsletter/Index.html

The general objectives of ICY shall be: To establish an effective liaison between persons and organizations concerned with yeast investigations, and between them and the practical users of results of investigations including yeast culture collections. ICY will use the "Yeast Newsletter" as a means of communication. ICY shall sponsor conferences and symposia on topics and problems of common interest. Every four years a General Symposium (International Congress on Yeasts; ICY) and if possible each year in the meantime a Specialized Symposium (International Specialized Symposium on Yeasts; ISSY) shall be held. Members of ICY shall be informed about regional conferences of yeasts.

In 2019 the 35th International Specialized Symposium on Yeasts (ISSY35) will be held in Antalya, Turkey, on October 21-25, 2019, organized by Dr. Huseyin Erten (Faculty of Agriculture, Cukurova University, Turkey), under the auspices of ICY. The title of ISSY35 is gYeast Cornucopia: Yeast for health and wellbeingh, which will encompass the use of yeasts in fermented foods and beverages, the role of spoilage yeasts and their control, yeasts as sources of ingredients and additives, yeasts as biocontrol agents, yeast taxonomy, ecology and biodiversity, yeasts in health and probiotics, and yeast genetics and genomics. See also http://www.issy35.com/en/

P75 Converting the yeast *Starmerella bombicola* from a natural glycolipid producerto a free fatty acid production platform

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Free fatty acids are basic oleochemicals implemented in a range of applications including surfactants, lubricants, paints, surface coatings, plastics, and cosmetics. Microbial fatty acid biosynthesis has gained much attention as it provides a sustainable alternative for petrol- and plant oil- derived chemicals. Beside oleaginous microorganisms, *Starmerella bombicola* is another microbial cell-factory that possesses a powerful lipid metabolism. Yet, this yeast species deploys its capacities for the production of industrially relevant compounds such as the biodetergent sophorolipids (>300 g/L).

We aimed to exploit the lipidic potential of *S. bombicola* and converted it from the glycolipid production platform into a free fatty acid cell factory. We used several metabolic engineering strategies to promote extracellular fatty acid synthesis which include blocking competing pathways (glycolipid biosynthesis and β-oxidation), preventing free fatty acid activation, and increasing the cytosolic acetyl-CoA pool. The best producing mutant secreted 0.933g/L (±0.04) free fatty acids with a majority of C18:1 (43.8%) followed by C18:0 and C16:0 (40.0 and 13.2% respectively). Interestingly, some of the modifications applied to create a free fatty acid producing cell-factory proved to be useful as well to increase *de novo* sophorolipid synthesis. The overall concentration of glycolipids secreted by the mutant during the shake flask experiment was over 20% higher as compared to the control strain ($p = 0.0054$).

We believe that our work is pivotal for the further development and exploration of *S. bombicola* as a platform for synthesis of environmentally friendly oleochemicals.

P77 Effect of over expressing protective antigen on global gene transcription inÂ *Bacillus anthracis*Â BH500

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Protective antigen (PA) of *Bacillus anthracis* is being considered as a vaccine candidate against anthrax and its production has been explored in several heterologous host systems. Since the systems tested introduced adverse issues such as inclusion body formation and endotoxin contamination, the production from B. anthracis is considered as a preferred method. The present study examines the effect of PA expression on the metabolism of *B. anthracis* producing strain, BH500, by comparing it with a control strain carrying an empty plasmid. The strains were grown in a bioreactor and RNA-seq analysis of the producing and non-producing strain was conducted. Among the observed differences, the strain expressing rPA had increased transcription of *sigL,* the gene encoding RNA polymerase σ^{54}, *sigB*, the general stress transcription factor gene and its regulators *rsbW and rsbV*, as well as the global regulatory repressor *ctsR.* There were also decreased expression of intracellular heat stress related genes such as *groL, groES, hslO, dnaJ* and *dnaK* and increased expression of extracellular chaperons *csaA* and *prsA2.* Also major central metabolism genes belonging to TCA, glycolysis, PPP and amino acids biosynthesis were up-regulated in the PA-producing strain during the lag phase, and down-regulated in the log and late-log phases, which was associated with decreased specific growth rates. The information obtained from this study may guide genetic modification of *B. anthracis* to improve PA production.

P79 Fermentation Optimization by Understanding the Yeast Gene Transcriptomics in Cellulosic Ethanol Production in Bench-top Bioreactors

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The United States is the world's largest producer of corn ethanol and produced 15.8 billion gallons in 2017 reaching the mandated target outlined in the Energy Independence and Security Act (EISA). According to EISA's. Renewable Fuel Standard, the target cellulosic ethanol production for 2018 was 7 billion gallons and 16 billion gallons by 2022. However, the cellulosic ethanol production for 2018 is around 15 million gallons which is far from the target. Unlike corn ethanol production, several critical challenges like enzyme cost, biomass pretreatment, conversion efficiency, presence of inhibitors etc. have hindered efficient cellulosic ethanol production. Understanding yeast transcriptomic signatures during cellulosic ethanol fermentation of different types of biomass like corn stover, miscanthus, switchgrass, and poplar may provide insights into calibrating the process. Accordingly, the goal of this study was to evaluate and compare the yeast gene regulation at 24h, 48h and 72h of yeast inoculation for poplar bioethanol production in three 7L bench-top bioreactors. Mild acid-pretreated poplar feedstock was used for separate hydrolysis and fermentation using yeast, *Saccharomyces cerevisiae* under high solids medium. The saccharification at 48 hours resulted in 8g/l of glucose and 1.87g/l of xylose. Fermentation with yeast resulted in 4.04g/l of ethanol at 24h and thereafter the ethanol production slightly reduced. The overall enzyme and fermentation kinetics along with the regulation of select yeast genes involved in fermentation will be presented. Further, the impact of mass transfer factors such as aeration and temperature on the behavior of yeast will be discussed.

P81 Polyhydroxyalkanoate production from biodiesel resources by *Burkholderia glumae*

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Polyhydroxyalkanoates (PHAs) are bacterial polyesters produced generally under excess carbon source and nutrient limitation, whose properties can be modified according to their monomeric constitution. Although poly(3-hydroxybutyrate) [P(3HB)] exhibits physico-chemical properties similar to polypropylene (PP) of petrochemical origin, its use is limited to industrial processes due to its high crystallinity. On the other hand, the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] exhibits suitable properties such as greater flexibility due to the incorporation of 3HV monomers. Several oils from biodiesel industry have become an attractive carbon source for PHA production, generating an ecologically friendly production set based on biofuels and bioplastics as a sustainable alternative to petrochemical compounds. In this work, *Burkholderia glumae*MA 13 was cultivated in mineral salts medium containing 1 $g.L^{-1}(NH4)_2SO_4$) and 10 $g.L^{-1}$carbon source (soybean, rapeseed, sunflower and castor oils) for 96 h, at 34 °C and 150 rpm. Cells were harvested by centrifugation and lyophilized to constant weight. The residual oil was determined gravimetrically as hexane solubles. Lyophilized cells (5 to 10 mg) were subjected to methanolysis reaction to obtain methyl esters which were analyzed by GC-MS. The PHA production by *B. glumae*MA13 showed intracellular polymer accumulation values of 55-75 %CDW. 3HV fractions were obtained without addition of precursors with a P(3HB-*co*-3HV) production from castor oil constituted of 23.4 mol% of 3HV. Therefore, *B. glumae*MA13 can be considered a bacterial strain with high potential for PHA production from vegetable oils associated with the biodiesel industry.

P83 Influence of the gene *rhlG* on rhamnolipids production using hydrophobic or hydrophilic carbon sources

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Surfactants are amphipathic molecules, which due to the presence of regions with distinct polarities present unique properties such as the ability to reduce surface and interfacial tension. Biosurfactants are biological molecules that can be synthesized by

a wide range of living beings, however the most studied producers are microorganisms. Despite the numerous advantages of biological surfactants, such as low toxicity, biodegradability and high stability, these compounds are not widely used because of the production costs. For this reason, it is mandatory to study the genes involved in this microbial metabolite biosynthesis, the present study carried out the construction of a mutant strain, with a deletion of the gene *rhlG*. Subsequently, bioprocesses were performed in bioreactor, using sunflower oil and glycerol as carbon source using this mutant and the wild type strain *P. aeruginosa* MPAO1 to verify how this deletion affected the rhamnolipids production, once there are some divergences about this gene rule on this biosurfactant synthesis. These cultures allowed to verify the influence of the genes in the rhamnolipids biosynthesis. The results showed that the mutant strain can produce the same amount of rhamnolipids as the wild type when the carbon source is sunflower oil, but it produce less than the wild type when the carbon source used was glycerol. These results showed that*rhlG*gene is related with the biosurfactants productions.

P85 Overexpression of recombinant RNA in *Corynebacterium glutamicum* using a strong promoter derived from corynephage

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Polymeric nucleic acid RNA molecules are expected to be applied in a wide range of industrial fields. For example, in agriculture, there are applications as nucleic acid pesticides (RNA insecticides) to replace conventional chemical pesticides. On the other hand, in the pharmaceutical field, there are also expectations for the creation of new mRNA pharmaceuticals. Therefore, we first examined the mass production of recombinant RNA of such a target nucleotide sequence using *Corynebacterium glutamicum* as a cell-factory. As a model RNA for the expression, a recombinant RNA (about 160 nt in total length) including a part of U1A-RNA involved in mRNA splicing process was designed and was designated U1A*-RNA. As a promoter and a terminator for the expression, those derived from the genome DNA of a corynephage which infects *C. glutamicum* were adopted. In order to increase the gene dosage of the target RNA expression unit, two new high copy number plasmids[1,2)] were constructed and used as the vector for the high-level RNA expression. Furthermore, as a host strain into which the expression system was introduced, a mutant strain lacking an RNase gene in *C. glutamicum* was employed. As a result, we were able to construct recombinant RNA-overproducing microbe capable of accumulating a target recombinant RNA in amount of about several hundreds of mg per L of medium[3)]. *C. glutamicum* is an industrial microbe used for amino acid fermentation at commercial level, and have a history of stable fermentation at low cost. Therefore, mass production of various recombinant RNAs will be expected in this host strain.

1) *Biosci. Biotechnol. Biochem.*, vol. 82, 2212-2224 (2018)
2) *J. Biosci. Bioeng.*, vol. 127, 529-538 (2019)
3) *J. Biosci. Bioeng.*, In press

P87 Applications of Foodomics in Food Science and Technology

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Foodomics is a new and comprehensive approach for the investigation of food science based on information related to human nutrition. It is defined as a discipline that studies the Food and Nutrition domains through the application of omics technologies (Cifuentes, 2009). Food science is a multi-disciplinary field including chemistry, biology, nutrition, engineering and processing. Innovations and researches in food science are very important since this field is directly related to human health. The progression of analytical technologies in food science and technology has greatly developed especially in the past 20 years and this enabled to detect many compounds at various levels. In addition, high-throughput omics technologies together with bioinformatics tools produce large scale molecular-level data in many fields including food science and nutrition. These improvements and technologies allowed the birth of foodomics in which the collected information from different fields mainly food technology, microbiology, nutrition, genomics, proteomics and metabolomics are combined to promote human health, to improve food quality, safety, traceability and sustainability, to prevent food adulteration, to evaluate dietary and toxic compounds, to develop functional foods and nutraceuticals, to investigate the bioactivity of consumed food ingredients in the body at a molecular level and also to produce new approaches in food science, technology and nutrition. A few examples of the studies on the applications of omics in food science and technology include the application of genomics and proteomics in food microbiology and biotechnology for the identification of microorganisms. Also, proteomic studies are applied for protein studies in milk products adulteration and gluten protein detection in cereal grains. In oil adulteration, lipidomics can be applied. Metabolomics approaches are used to study the gut microbiota due to the relation of diet and microbial composition in the gut. Also, metabolomics studies investigate compound identification, sensorial evaluation and authentication of foods. As a result, foodomics is a new field with huge application in food science and this field is evolving rapidly.

P89 *In vivo*Â amelioration of gut-brain signaling and testicular abnormalities by a synbiotic combination of *Lactobacillus gasseri* 505 and *Cudrania tricuspidata*Â leaf extract

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The primary role of chronic psychological stress in triggering or aggravating the development of disease has recently become a

topic of intense research. While one's body evokes a central neuroendocrine response to stress via the activation of the hypothalamic pituitary adrenal (HPA) axis, overexposure to stress hormones can predispose to gut-brain-endocrine alteration. Despite evidence that the pernicious effects of stress on these biological contexts are on the rise, little is known about the impact of stress on reproductive health. The discovery of crosstalk on brain-testis axis have provided novel insights for the understanding of psychological stress-related testicular conditions. Noteworthy, these testicular conditions may resist conventional treatment of male infertility. Interestingly, there has been a shift in recent years toward discovering the therapeutic effects of probiotics, as well as their fermented products beyond the gut, such as the testicles. *L. gasseri* 505-fermented *C.tricuspidata* leaf extracts (505-CT) have been previously shown to exert potent *in vivo* anti-oxidant and anti-inflammatory activities. However, their effect on psychological stress-related diseases remains unknown. The study was thus performed in stressed mice to investigate the potential of 505-CT in improving gut-brain signaling and testicular abnormalities. The results revealed that eight-week administration of 505-CT, not only significantly reduced serum levels of stress hormone (corticosterone) and pro-inflammatory cytokine (IL-6), but also modulated brain endothelial tight junction and neurotransmitter receptors (GABA, BDNF) in the stressed mice, compared to the negative control. 505-CT treatment is also capable of improving colonic function by up-regulating expression levels of zonula occludens and IL-10. Moreover, 505-CT was also shown to ameliorate testicular structural and functions in the stressed mice, by normalizing expression levels of genes related to testicular development, steroid biosynthesis, hormone and growth factors. Mechanistic data further suggested that testicular-health modulating potential of 505-CT could be attributed to the elevated level of brain-testis crosstalk in the stressed mice. Altogether, the results in this study demonstrate that 505-CT may represent promising intervention options for the treatment of stress-related disorders.

P91 Methane bioreforming for the production of ammonia: a microbial consortia "bio Haber-Bosch"

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Fixed nitrogen is required for protein biosynthesis and growth, but fixed nitrogen (NH_3, NO_3, urea) is considered a limiting resource. Traditional industry employs the Haber-Bosch process to synthetically produce the fixed nitrogen product ammonia (NH_3), however, the process requires 1-2% of the annual global energy supply. Substantial capital investments are required to construct the high-pressure and temperature (300°C, 200 atm) Haber-Bosch reactors, logistically constraining NH_3 manufacturing to centralized locations near feedstock streams and thereby limiting opportunities to capitalize on remote resources. With nitrogen demand anticipated to expand during the next century, the availability of biological enzymatic methods for ammonia (NH_3) synthesis and methane energy capture that operate at ambient temperatures and pressures (30°C, 1 atm), and the recent identification of remote waste methane (CH_4) resources, development of a one-pot biomanufacturing system was explored which utilizes CH_4 gas and nitrogen gas (N_2) in air for the biological production of NH_3. The system contains a microbial consortium of *Azotobacter vinelandii* M5l3 and *Methylomicrobium buryatense* 5GB1 pAMR4-dtom1 which performs methane bioreforming (MBR) and biological nitrogen fixation to generate NH_3. Proof-of-concept experiments were carried out to identify the fate of carbon and nitrogen products in the system and optimize co-culture growth conditions for maximum NH_3 generation. Fermentation products and NH_3 concentrations were determined by HPLC and enzymatic assay. This contribution discusses the development of an ambient CH_4-driven NH_3 biosynthesis system, the products of which could be either recovered for direct external application or utilized as a tool to drive higher-value N-based bio-product formation.

P93 Recycling of yeast biomass in lactic acid production at low pH

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Lactic acid is an organic acid that is used in food, cosmetic and pharmaceutical industries and that serves as building block for polylactic acid (PLA), a biodegradable and biocompatible polymer. The global lactic acid market is estimated to increase from 714.2 kilo tons in 2013 to 1,960.1 kilo tons by 2020 with a revenue of USD 4.3 billion [1]. Currently, optically pure lactic acid is mainly produced by sugar fermentation of lactic acid bacteria. However, this production process has many drawbacks. Particularly, lactic acid bacteria are sensitive to low pH. Therefore, neutralizing agents have to be added to the production medium making the separation and purification of lactic acid very costly. In the presented project, we use *S. cerevisiae* as production organism for lactic acid. Budding yeast has many advantages over *Lactobacilli* such as the simple nutritional requirements or the tolerance to low pH, making it an excellent cell factory for producing organic acids. Since *S. cerevisiae* cells do not naturally produce lactic acid, metabolic engineering is required in the first place to develop a lactic acid producing strain. Initially, the genes coding for pyruvate decarboxylases involved in the reduction of pyruvate to ethanol were deleted. By overexpressing a heterologous lactate dehydrogenase, pyruvate can then be converted into lactic acid. By applying the powerful adaptive laboratory evolution, we could further increase the tolerance of the yeast cells to high lactic acid concentrations and low pH values. Due to this robustness, we were able to reuse the biomass in many consecutive lactic acid production rounds. This recycling of the cells and further process engineering will increase the lactic acid yield and titer and thus significantly lower the costs of the whole lactic acid production process.

P95 A microbial consortium for enhanced metabolite production from an artificial garbage slurry

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Food waste is a readily available source of abundant nutrients for microbial growth. Microbial consortia are often better able to break down complex growth substrates than individual bacterial species. Here, we leverage genome scale metabolic modelling (GSMM) of bacterial species from the human gut microbiome provided a synthetic "Western" diet to predict the optimal hydrogen production from a two member bacterial consortium. GSMM predicts a consortium of *Clostridium beijerinckii* and *Yokenella regensburgei* to produce the highest amount of hydrogen over any individual specie. We constructed this consortium and measured the metabolites and gases produced from it and the individual species that comprise it when provided an artificial garbage slurry. Individual cultures suggest that *C. beijerinckii* is the primary hydrogen producer, while also producing butyrate and acetate. *Y. regensburgei* produces a small amount of hydrogen, but primarily produces lactate, which accumulates to lower levels in the consortium. Cross-feeding of spent laboratory media indicates that lactate and a second unknown metabolite produced by *Y. regensburgei* provide an additional nutrient source to *C. beijerinckii*, which converts a portion of these substrates to butyrate. Lactate utilization by *C. beijerinckii* appears to be enhanced by the presence of other carbon substrates. This designed microbial consortia has similar hydrogen production capacity to *C. beijerinckii* and could potentially be further improved by the addition an organism capable of breaking down the complex substrates found in food waste.

P97 Microbial upgrading of a side stream from cellulose fiber production

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On our way to a zero-waste-economy, valorisation of side-streams from industrial processes becomes increasingly important. Biotechnological approaches are among the key-technologies for conversion of low value compounds derived from renewable resources. Naturally occurring or metabolically engineered organisms are used to produce valuable products like enzymes, chemicals or biofuels.

The aim of the study is to valorise a side stream of cellulose fiber production. One of the remains of the sulphite process for dissolving wood pulp, the precursor material for cellulosic fibers, is spent sulphite liquor (SSL). SSL has a complex composition and is rich in various organic carbon compounds like monomeric, hemicellulose-derived sugars and lignosulfonates. Some of them have potential as carbon sources for microorganisms, while others are quite toxic. A proprietary separation procedure yields a carbon stream, containing a mixture of substrates, which should ideally all be consumed in a single process.
To this end, a database and literature search was performed to identify microorganisms capable to use most of the carbon sources in question. A selection of enterobacteriaceae, lactobacillaceae, pseudomonadaceae and bacillaceae was then tested on their ability to grow on different components as single carbon source. The most promising organisms, regarding growth rate, substrate flexibility, production potential as well as the availability of a genetic toolbox were subsequently fermented on media, supplemented with the solution separated from real SSL. These fermentations were compared to fermentations, where medium was supplemented with equal amounts of sugars present in the purified side stream. The results revealed that for three organisms the lag phase was prolonged for maximal 2 hours by components, which were not removed by the initial cleaning step of the SSL. In addition to similar growth, it was observed that substrates others than sugars where partly metabolized within the SSL media, resulting in a higher biomass formation. In a further step these organisms will now be genetically engineered to catabolize all relevant substrates to gain a higher biomass yield in SSL supplemented media.

P99 Effect of mutations in citrate synthase on acetate flux in *Escherichia coli*

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Altering central carbon metabolism (CCM) of microorganisms offers the possibility of impacting a wide range of biochemical products. Acetyl-CoA is a key precursor to a variety of industrially relevant compounds, and its availability is thought to limit the formation of biochemicals derived from this intermediate. The largest metabolic drain of acetyl-CoA in microbes such as *Escherichia coli* is to citrate via citrate synthase (coded by the *gltA* gene). Unfortunately, merely knocking out citrate synthase would eliminate growth of *E. coli* using glucose or glycerol as a sole carbon source. This study examines the effect of reducing the catalytic activity of citrate synthase on the conversion of acetyl-CoA to acetate, which serves as a model product derived from acetyl-CoA. Activity was reduced by generating ~30 point mutations on chromosomal citrate synthase. These variants were assessed in shake flask, batch and continuous experiments using *E. coli* having the *poxB* background. Shake flask studies of strains having point mutations in the residues W260, A267 and V361 resulted in the greatest acetate yields (0.20-0.25 g/g glucose) compared to the wild-type citrate synthase (0.05 g/g), suggesting these mutations led to an increased pool of acetyl-CoA. Controlled batch and C-limited and N-limited chemostats further showed the effect of point mutations in citrate synthase. The results provide important insights on improving the production of compounds derived from acetyl-CoA.

P101 Microbial 2-butanol production with *Lactobacillus diolivorans*

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Petroleum based products are indispensable for human daily life. The increasing awareness about environmental problems due to this petroleum-based lifestyle and the increasing scarcity of fossil oil leads to a rethinking of this way of life. Especially, in the transport sector, where most of the produced fossil oil is consumed, renewable alternatives gain more importance. Biobutanol

with its higher similarity to gasoline gains increased attention and is predicted to be the biofuel of the future. Butanol occurs in four different isomers, with 1-butanol and isobutanol most commonly used as biofuels. The benchmark for 1-butanol production are solventogenic *Clostridium* spp. through ABE fermentation. For the second isomer, isobutanol, engineered *Escherichia coli* and *Saccharomyces cerevisiae* strains are used as production hosts. Interestingly, for 2-butanol no production process is described in literature. *Lactobacillus* spp. are known to produce 2-butanol at low levels, as an unwanted by-product during beverage fermentations. In this case, 2-butanol is produced from meso-2,3-butanediol via a two-step metabolic pathway. The same pathway is used by *Lactobacillus* spp. for the conversion of glycerol to 1,3-propanediol. *Lactobacillus diolivorans* is known to be a very efficient producer of 1,3-propanediol. In this study we proved that *L. diolivorans* is also very efficient in producing 2-butanol from meso-2,3-butanediol. The production of 2-butanol at high level was achieved by the development of a two-step production process. In the first step meso-2,3-butanediol is produced from glucose by *Serratia marcescens*. The formed meso-2,3-butanediol is than converted to 2-butanol reaching titers of 10 g/L with the *L. diolivorans* wild type strain. During the fermentation the accumulation of 2-butanone, the intermediate product of the pathway, was observed. Until the end of the fermentation the accumulated 2-butanone was entirely converted to 2-butanol. Using an engineered strain overexpressing the alcohol dehydrogenase from the two-step metabolic pathway, the reutilization of accumulated 2-butanone was faster and the 2-butanol titer was increased to 13,4 g/L.

P103 Novel approaches to bio-production via inducible asymmetric cell division and programmable cell differentiation'

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Genetic engineering of microbial strains has enabled large-scale production of some biopharmaceuticals and other materials in bio-factories. However, high level of heterologous enzymes expression puts a burden on cellular metabolism, causing slower culture growth. Proper balance between growth and production rate is a key to increase product yields, but is hard to achieve in isogenic cultures. Differentiation of cells into multiple types may be beneficial for bioprocess optimization. For example, one type of cells can be completely devoted to the production, as “factory” cells, while another is free of production and is responsible for the culture regeneration. We have designed a genetic circuit to control differentiation of cells into different types, as an outcome of induced asymmetric cell division. The circuit involves a bacterial scaffolding protein that is stably maintained at a single cell pole. The protein scaffold was functionalized to degrade the signaling molecule c-di-GMP. By transiently regulating synthesis of the functionalized scaffold via small molecules or light, we can chemically or optogenetically control production of two distinct cell types characterized by either low or high c-di-GMP levels. We employed c-di-GMP downstream effectors to control differences in protein complex assembly or gene expression, which in turn produce differential cellular behavior or biosynthetic activities. Differentiation may also involve a mechanism to restrict a replication potential of factory cells, in order to reduce a chance of them to mutate, lose productivity and jeopardizing the whole bioprocess through outgrowing producers. Our strategy can also be used for improving the production rate of complex biosynthetic pathways. Programmable differentiation of cells can generate multiple cell types, responsible for separate biosynthetic pathway segments, thus, dividing the metabolic burden. It is advantageous comparing to co-culturing of several strains, each expressing partial set of biosynthetic enzymes, because it simplifies simultaneous culturing of different cell types, and facilitate facilitate maintenance of their ratios over time. Complex architecture of microbial communities used in bioprocess is an attractive tool to improve their efficiency.

P105 Distinct metabolic pathways in yeast required for tolerance to phenolic fermentation inhibitors identified using a chemogenomic screen

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The conversion of agricultural and forestry waste into biofuels and high value products is a two-step process of hydrolysing plant lignocellulose and next fermenting the sugars produced. However, lignocellulosic hydrolysis does not only free sugars for fermentation but it simultaneously generates toxic chemicals, including phenolic compounds which severely inhibit yeast growth and fermentation. To understand the molecular basis of phenolic compound toxicity, we performed genome-wide high-throughput chemogenomic screens using baker’s yeast Saccharomyces cerevisiae to identify deletion mutants that were either hypersensitive or resistant to three common phenolic compounds found in plant hydrolysates: coniferyl aldehyde, ferulic acid and 4-hydroxybenzoic acid. Despite being similar in structure, our screen revealed that yeast utilizes distinct pathways to tolerate phenolic compound exposure. Furthermore, although each phenolic compound induced reactive oxygen species (ROS), ferulic acid and 4-hydroxybenzoic acid-induced a general cytoplasmic ROS distribution while coniferyl aldehyde-induced ROS partially localized to the mitochondria and to a lesser extent, the endoplasmic reticulum. Intriguingly, we found that the glucose-6-phosphate dehydrogenase enzyme Zwf1, which catalyzes the rate limiting step of the pentose phosphate pathway, migrates from the cytosol to sites of ROS accumulation during coniferyl aldehyde treatment. This change in localization is potentially required for reducing the level of coniferyl aldehyde-induced ROS at the sites of ROS accumulation. Our novel insights into biological impact of three common phenolic inhibitors will inform the engineering of yeast strains with improved efficiency of biofuel and biochemical production in the presence hydrolysate-derived phenolic compounds.

P107 Engineering a synthetic, catabolically-orthogonal co-culture system for enhanced conversion of lignocellulose-derived sugars to bioproducts

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Fermentation of lignocellulosic sugar mixtures is often suboptimal due to inefficient xylose catabolism and sequential sugar utilization caused by carbon catabolite repression. Unlike in conventional applications employing a single engineered strain, the alternative development of synthetic microbial communities facilitates the execution of complex metabolic tasks by exploiting the unique community features, including modularity, division of labor and facile tunability. A series of synthetic, catabolically-orthogonal co-culture systems were systematically engineered, as derived from either wild-type *Escherichia coli* W or ethanologenic LY180. Net catabolic activities were effectively balanced by simple tuning of the inoculum ratio between specialist strains, which enabled co-utilization (98% of 100 g L^{-1} total sugars) of glucose-xylose mixtures (2:1 by mass) for both culture systems in simple batch fermentations. The engineered ethanologenic co-cultures achieved ethanol titer (46 g L^{-1}), productivity (488 mg L^{-1} h^{-1}) and yield (~90% of theoretical maximum), which were all significantly increased compared to LY180 monocultures.

P109 New Tools for Targeted Cloning and Over Expression of Biosynthetic Gene Clusters

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Millions of biosynthetic gene clusters (BGC) are predicted from genome sequencing efforts. Isolating a physical DNA clone for refactoring or expression analysis can be slow and expensive due to the large size of BGCs. Classical methods of cloning can take months to complete, and gene synthesis is expensive and can be stymied by GC-rich and/or repetitive sequence issues. Here we describe direct cloning of large BGCs from genomic DNA without using gels or agarose plugs. CRISPR-Cas9 targeted cuts flanking a BGC are combined with *Streptomyces* or fungal BAC shuttle vectors with overlaps matching the BGC cut sites, and the vector and restricted DNA are assembled and transformed. 31 BGCs from multiple *Streptomyces* and fungal strains have been successfully captured, ranging from 12 to 96 kb (median = 49) in size. Starting with genomic DNA isolation from a cell pellet takes ~5 days to generate a BGC clone, which is directly ready for heterologous expression studies. To improve heterologous expression a new BGC vector was developed which uniquely includes *two* inducible promoter elements, one flanking each side of the cloning site. The blue and red colored antibiotic compounds from *Streptomyces coelicolor* ACT (21 kb) and RED (33 kb) BGCs were cloned in both orientations and integrated into *S. lividans* ΔredΔact. Qualitative inducible production of the blue actinorhodin product of the ACT cluster and the red undecylprodigiosin product of the RED cluster was demonstrated in the inducible promoter system but not from the native promoters in this heterologous expression experiment.

P111 Dissecting polyunsaturated fatty acid synthases for product control

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Polyunsaturated fatty acids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) are essential fatty acids for human. Some microorganisms biosynthesize theses PUFAs by PUFA synthase composed of huge enzyme complexes with acyltransferase, acyl carrier protein, malonyl CoA transacylase, β-ketoacyl synthase (KS), ketoacyl reductase, dehydratase (DH), and enoyl reductase domains. DHA, EPA, and ARA synthases identified in marine bacteria create the specific PUFA even though the multiple catalytic domains in each subunit are similar to each other. Although functional analyses of some domains were investigated, the detailed biosynthetic machinery are still obscure. Here, we examined the control mechanism of PUFA profiles by *in vivo* and *in vitro* experiments. Consequently, ARA and EPA synthases utilized polyketide synthase-type DH and FabA-type DH depending on the carbon chain length for introduction of saturation or cis double bonds on growing acyl chains[1]. Furthermore, KS_A domain in “A” subunit accepted short to medium chain substrates while KS_C in “C” subunit accepted medium to long chain substrates. However, the last elongation steps in EPA (C_{18} to C_{20}) and DHA (C_{20} to C_{22}) biosynthesis were unexpectedly catalyzed by KS_A and KS_C, respectively[2]. Based on the results, we converted a practical microalgal DHA synthase into an EPA synthase[2].

1) S. Hayashi *et al., Angew. Chem. Int. Ed.* **58**. 2326–2330 (2019)
2) S. Hayashi *et al., Angew. Chem. Int. Ed.* just accepted

P113 Investigations of the Pseudomonad-active natural product, Bulgecin A

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Lytic transglycosidases catalyze the breakdown of glycosidic linkages (MurNAc to GlcNAc) of peptidoglycan in species of *Pseudomonas*. The natural product bulgecin A has been shown to inhibit lytic transglycosidase activity as well as potentiate the activity of b-lactam antibiotics. Bulgecin A has been isolated from two ATCC strains (31433 and 31363), previously phenotypically characterized as taxonomically distinct Pseudomonads. Yet, the lack of a robust synthetic route or a bacterial over-producing strain has limited further study.

In this work, culturing of both strains produced detectable levels of bulgecin A. The assembled genomes provides a detailed genetic understanding of the isolates’ taxonomy, operon regulation elements, and proteins coded in putative natural-product pathways. Comparative bioinformatic analysis of the shared secondary metabolite pathways revealed a conserved NRPS and a poorly annotated glycoamino acid pathway. Transposon mutagenesis of *bulE*, a putative NADH-dependent dehydrogenase,

disrupted bulgecin A production. These results enable pathway biochemical characterization.

P115 Quillaic acid biosynthesis in *Saccharomyces cerevisiae* through combinatorial studies of heterologous proteins

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QS-21 is a purified plant extract that enhances the ability of the immune system to respond to vaccine antigens. As such it is a component of the FDA-approved Shingrix shingles vaccine and currently under clinical evaluation as an additive for various trial vaccines, including those for HIV, malaria and cancer. However, the isolation of QS-21 destroys the soap bark trees and therefore, has resulted in regulation of the trees by the local government.

We investigated the production of the QS-21 triterpene core, quillaic acid, by fermentation from engineered *Saccharomyces cerevisiae* using a combinatorial strategy of co-expressing heterologous proteins in the strain that has a high production yield of beta-amyrin. Combinations of cytochrome P450 reductases and P450s from various plant origins are investigated. Because of enzymes' high specificity, P450s can selectively functionalize carbon positions of choice, forgoing the need to detour synthetic steps to ensure stereo- and chemoselectivity.
Since triterpenes constitute a large and structurally diverse class of natural products with various industrial and pharmaceutical applications, and P450-catalyzed structural modification is crucial for the diversification and functionalization of the triterpene scaffolds, our strategy provides a simple yet versatile platform to provide a renewable supply of triterpenes and their P450-functionalized products in engineered yeast.

P117 IndigoidineÂ biosynthesis comprises oxidation of glutaminyl on nonribosomal peptide synthetase

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Indigoidine synthetases are nonribosomal peptide synthetase (NRPS) homologs that produce indigoidine, a natural blue pigment. Because of the colorful product, these synthetases have been engineered toward diverse biotechnology applications, including L-glutamine measurement, substrate-free blue/white screening, and flow cytometry. While previous research has demonstrated that the phosphopantetheinylated (holo) indigoidine synthetases convert two molecules of L-glutamine to one molecule of indigoidine, the biosynthetic pathway has not been fully elucidated. It was suggested that two key steps, oxidation and cyclization, are involved in the pathway, but neither the reaction sequence nor the substrate states have been revealed. Here, we present that indigoidine biosynthesis comprises the oxidation of L-glutaminyl on the peptidyl carrier (PCP) domain. The purified holo-blue pigment synthetase A (BpsA), an indigoidine synthetase from *Streptomyces lavendulae*, can convert L-glutamine to indigoidine, whereas its thioesterase (TE) domain inactive mutant, holo-BpsA-ΔTE, lost indigoidine biosynthesis activity. After the reaction, we analyzed the PCP bounded products on holo-BpsA-ΔTE via 4′-phosphopantetheine (PPant) ejection assay and found an intermediate, 5-amino-2,5-dioxopentanoyl-PCP, which did not exist in the negative control reaction or holo-BpsA catalyzed reaction. This intermediate indicates that the oxidation occurred on L-glutaminyl-PCP and resulted in dehydroglutaminyl-PCP, which tend to be unstable and readily hydrolyzes to produce 5-amino-2,5-dioxopentanoyl-PCP. These results showed that the indigoidine biosynthetic pathway is in the sequence of on-line oxidation and then cyclization.

P119 Identification of biocatalysts from marine aqueous extracts

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Natural products extracts represent a substantial repository of bioactive compounds that have resulted in treatments currently used in numerous cancer chemotherapy regimens. These drugs have largely been discovered by natural product chemists investigating the small organic solvent-soluble components of these extracts. The protein components of crude natural product extracts have been discarded by natural product chemists due to the long-held opinion that proteins were unlikely to be useful drug candidates. However, clinically approved biologics from natural sources including exenatide, a Type II diabetes drug derived from a peptide originally found in the saliva of Gila monsters; miacalcin, a protein from the brain of salmon used to treat osteoporosis; and, botox, a protein toxin from *Clostridium botulinum*, provide proof that the discovery of novel bioactive proteins from nature can lead to viable, clinically relevant therapeutics. We examined the proteinaceous components of aqueous marine extracts from the Natural Products Branch repository for entities with potent anticancer activities using the NCI-60 cancer-cell panel. Extracts with activities concentrated in the proteinaceous fraction were subjected to biochemical analyses, which identified a subset of extracts that exhibited cell-free reduction of XTT in a dose-dependent manner with enzyme-like kinetics. Further isolation of one extract revealed a protein that is responsible for the cell-free reduction of XTT and microarray analysis supported that the enzyme mediates cytotoxicity against HCT-116 through the generation of Reactive Oxygen Species. This surprising identification of ROS-generating enzymes highlights the potential of biocatalysts and biologics discovery from natural product aqueous extracts. Here we report the methodology of isolation and characterization of proteinaceous extracts that exhibited cell-free reduction of XTT with enzyme-like kinetics.

P121 Engineering chaperone network to improve yield of natural products in yeast biofactories'

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Normally, when it comes to production of non-native compounds in yeast biofactories, the most common strategies employed to improve yield include overexpression of genes necessary to produce the new product and blocking of seemingly competing native pathways. However, when the product is a natural product, the challenges are more complex. First, for polyketides and non-ribosomal peptides, the first enzymes in the pathway are often multi-domain megaenzymes consisting of > 2000 amino acid residues, and they may fold inefficiently in yeast. In turn, this may lead to intracellular stress and catalytically inactive aggregates. Second, the requirement to modify the precursor compound through oxidative chemistry might impose elevated oxidative stress that may also negatively impact productivity.

Chaperones are a set of proteins that act as protein folding catalysts. However, some of them are also involved in response to various cellular stresses. We hypothesize that engineering of chaperone networks in yeast biofactories not only allows for higher success rate in producing various heterologous synthases, but also improves tolerance of the cells to oxidative stresses generated during the biosynthesis. We tested this hypothesis by first generating a library of chaperones of varying doses and combination. We then employed a high-throughput method to create combinations of strains expressing both the model synthase and the chaperone combination in question. The success of the engineering is measured through improvement of specific product yield. Here we are reporting outcomes of the study using three fungal natural products as the models.

P123 Characterization and fermentation optimization of a natural product antimicrobial peptide

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Naturally occurring antimicrobial peptides (AMPs) have been shown to be effective against a wide spectrum of targets including viruses, bacteria, fungi, and parasites. AMPs typically have a net positive charge, allowing them to selectively interact with anionic bacterial membranes and with other negatively charged cell structures. In this study, we will present preliminary evaluation of a natural product that we have isolated from soil. Initial characterization includes the general characteristics of AMP compound CMB001, its antimicrobial properties towards clinically relevant bacterial strains and biofilms, evaluation of producer strain culturing conditions, and morphological studies of AMP treated bacterial cultures by Scanning electron Microscopy (SEM). Our preliminary study suggests that CMB001 is stable over a wide range of conditions and retains its full antimicrobial activity in various conditions, including in the presence of serum proteins. It is a ribosomally synthesized peptide containing unusual amino acids, such as dehydrated and lanthionine residues. These residues likely to contribute to its selectivity and exceptional stability. The *in vitro* evaluation demonstrates that CMB001 is active against a range of gram-positive bacteria (including MRSA and VRE), as well as some gram-negative bacteria pathogens. SEM imaging analysis shows that this AMP acts on cell membrane integrity. Further analysis will reveal its structure and mode of action (MOA).

P125 Involvement of peptide epimerization in poly-γ-glutamic acid biosynthesis

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Poly-γ-glutamic acid (PGA) is a naturally occurring linear polymer that contains both enantiomers of glutamic acid (Glu). Because PGA exhibits promising features such as being non-toxic, biodegradable, water soluble, and edible, PGA and its derivatives are used in a wide range of applications including as humectants, thickeners, cryoprotectants, drug carriers, and heavy metal absorbers. PGA is produced mostly by *Bacillus* strains and its biosynthesis requires a minimum of three proteins, PgsBCA. However, PgsBCA constitute membrane-anchored complex and the detailed biosynthesis, especially the mechanism for introducing D-Glu residues into PGA, remains unknown. We recently discovered a novel peptide epimerase, MslH, responsible for the biosynthesis of a D-tryptophan (Trp)-containing lasso peptide natural product, MS-271.[1] Because PgsA has homology to the epimerase, we hypothesized that PgsA catalyzes epimerization of L-Glu residues in the growing PGA chain.

This hypothesis can be examined by identifying whether the direct biosynthetic origin of D-Glu residue in PGA is D- or L-Glu. If peptide epimerization is operating in PGA biosynthesis, the origin of D-Glu residue in PGA would be L-Glu. However, PGA-producing bacteria have glutamate racemases to catalyze interconversion between D-Glu and L-Glu. To overcome this problem, we heterologously expressed *pgsBCA* in the glutamate racemase-defective *Escherichia coli* mutant, WM335, and carried out feeding experiments with deuterated Glu. Chiral LC-MS analysis after enzymatic hydrolysis of the PGA products revealed deuterium incorporation into D-Glu residues in PGA when deuterated L-Glu, but not D-Glu, was supplied. These results clearly indicated that D-Glu units in PGA are biosynthesized from L-Glu and that peptide epimerization is involved in the biosynthesis of PGA.
[1] Zhi Feng et al. *ChemBioChem* **2018**, *19*, 2045.

P127 Production of eps fromÂ *Lactobacillus Paracasei*Â andÂ *Lactobacillus Casei*

G. Deomedesse Minari, Universidade Estadual Paulista UNESP, Rio Claro, Brazil and J. Contiero, UNESP, Rio Claro, Brazil*
Exopolysaccharides (EPS) from lactic acid bacteria (LAB), strains generally recognized as safe (GRAS), are natural molecules with beneficial physiological effects. They can be antitumor agents, show immunomodulating activities, are capable of reducing cholesterol, have antioxidants properties (for example, comparable antioxidant properties to the potent antioxidant ascorbic

acid), and besides this, can be used in the food and environmental industries. The objective of this study was to evaluate EPS production of two bacterial genera in a growth medium with different carbon sources. Strains of Lactobacillus paracasei (Ke7) and Lactobacillus casei (Ke8) were grown in the selection medium with glucose and glycerol as the only carbon source (SDM, modified) for 80h at 37º C, continuously stirred. Samples were withdrawn every 8h, and the growth of bacteria was analyzed by colony forming units and absorbance at 600nn. The crude EPS was precipitated from the supernatant by adding 2 volumes of cold ethanol stored at 4º C for 24 h, and then it was collected by centrifugation at 10,000 ×g for 20 min. The pellet was dried at 40º C and dissolved in deionized water, lyophilized and then weighed. The amount of EPS produced was determined by weight, and the total sugar of EPS was available by using the phenol sulfuric method at 495 nm of the lyophilized samples. The highest EPS production was between 30-46h of fermentation for both, and for Ke7, the values of pH remained at 6.6 with decreasing cell growth, while for the Ke8 isolate, pH values were reduced from 6.5 to 5.7 and showed constant cell growth. The weight (g) of the EPS samples had no relation to the sugar quantification.

P129 A new bioinformatic search strategy expands the chemistry of radical SAM metalloenzymes in natural product biosynthesis

L. Bushin, Princeton University, PRINCETON, NJ, USA*

Radical SAM enzymes are known to catalyze some of the most complex transformations known in nature. Recently, their roles as tailoring enzymes in the biosynthesis of ribosomally synthesized and post-translationally modified peptide (RiPPs) have emerged, expanding the diversity of post-translational modifications known to adorn peptide natural products. In this talk, I will present a bioinformatic search strategy in which we targeted radical SAM enzymes in the context of quorum sensing control to uncover an array of uncharacterized families of RiPPs encoded in the genomes of streptococcus bacteria. Investigations into the structural motifs that define these new families revealed several unprecedented post-translational modifications, including a tetrahydro[5,6] cyclization motif between the side chains of lysine and tryptophan residues. I will highlight the utility of our approach in uncovering nature's untapped biosynthetic potential.

P131 Otic microbiome engineering for prevention of middle ear infections

K. Jacob and G. Reguera, Michigan State University, East Lansing, MI, USA*

For long assumed to be sterile, the middle ear mucosa harbors a rich microbial community. *Streptococcus* sequences are highly represented in the otic microbiome and are predicted to sustain syntrophic interactions critical for mucosal nutrition and health. Further, members of the genus isolated from otic secretions have antimicrobial activity against common otopathogens and can be reintroduced as nasal aerosols to alleviate the frequency of middle ear infections in pediatric patients. Here we show that the production of surfactant is prevalent in otic isolates of *Streptococcus* and used to swarm on viscous environments. Many of the isolates also have antimicrobial activity against common otopathogens such us *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, and *Moraxella catarrhalis*. These findings highlight the recolonization potential and antimicrobial activity of otic *Streptococcus* that is of interest for the development of bacterial replacement therapies, which boost the natural protective role of the otic microbiome to prevent middle ear infections.

P133 Targeting microbial biofilms using naturally occurring and combinatorial-engineered natural products against Gram-positive and Gram-negative biofilm forming marine and pathogenic bacteria

M.A.R. Khan and C.C. Liaw, National Sun Yat-sen University, Kaohsiung, Taiwan*

Marine organisms are taxonomically diverse yet unique, hence they are considered as a reservoir of potential bioactive secondary metabolites. Many of these secondary metabolites have already been successfully developed for medicinal usages, based on the initial knowledge about their chemical interactions among marine organisms. Bacterial biofilm is regarded as one of the causes that pathogens could resist the effects of antibiotics. Given escalating evolution of bacterial resistance coupled with a diminished antibiotic pipeline, we need to discover and develop effective antimicrobial drugs and bacterial biofilm inhibitors. In our ongoing research for new secondary metabolites from marine resources that exhibit antimicrobial and anti-biofilm formation activities, we have screened 65 marine crude extracts that exhibited antimicrobial activities against *Staphylococcus aureus*. Among them, 18 extracts showed positive inhibitory effects against *S. aureus*. Furthermore, by the bioassay-guided fractionation isolation, we obtained ten bromopyrrole alkaloids including four new and six known compounds from EtOAc extract of *Agelas* sp. and four known compounds from EtOAc extract of *Spongia* sp. The bioactivity results of the compounds from *Spongia* sp. demonstrate that compound **4** (Metachromin C) showed a wide range of inhibition activities that includes against planktonic and biofilm growing Gram-positive and Gram-negative bacteria, while compounds 1-3 possess weak activities. However, bioactivities of new and known metabolites from *Agelas* sp. are under-investigation. Using combinatorial engineering strategies, we synthesized six α-pyrones analogues based on preliminary results of one of the naturally obtained α-pyrones that exhibited excellent anti-microbial and anti-biofilm activities. The next-generation of α-pyrones derivatives showed potent inhibitory activities against Gram-positive and Gram-negative bacterial pathogens in both planktonic and biofilm stages. These novel chemical entities are now being developed as potential therapeutic and anti-biofilm agents.

P135 Conversion of blackwater COD in the anode chamber of microbial electrochemical cells for production of useful chemicals

J.A. Deaver, K. Diviesti and S.C. Popat, Clemson University, Anderson, SC, USA*
Microbial electrochemical cells (MECs) are a potentially useful technology for the conversion of blackwater to useful chemicals, especially in remote areas where resources are limited. Anaerobic biodegradation of blackwater organics produces short-chain fatty acids, allowing anode respiring bacteria (ARB) to oxidize these and respire electrons to the anode. The electrons then move through an external circuit to the cathode where they may be used to produce useful chemicals, such as hydrogen peroxide. To better understand the function of the anode microbiome in the degradation of blackwater, a complex waste that requires hydrolysis, we are studying the overall conversion of blackwater chemical oxygen demand (COD). In this study, two two-chamber MECs fed with a primary sludge-based synthetic blackwater are operated with varying parameters, such as solids retention time. Samples for volatile fatty acids are taken and current is constantly recorded. The goal of this study is to present an overall electron balance demonstrating the conversion of complex blackwater components to simpler substrates, and ultimately to the production of electrical current at the anode, as well as to characterize the associated microbial communities. Variation of operational parameters will help determine which conditions are optimal for maximal recovery of electrons at the anode, and thus greatest product formation potential at the cathode. Understanding how operational parameters affect the conversion of organics to electrons for production of useful chemicals will bolster the development of this technology for operation in resource limited locations.

P137 *In situ* Clostridium dry cell weight monitoring in complex cellulosic feedstocks

M. Debreczeny, BugLab LLC, Concord, CA, USA; Y. Behl, D. Vaidya and H. Smith, Green Biologics Ltd, Abingdon, Oxfordshire, United Kingdom*
The economically competitive bioproduction of specialty chemicals using microbial cultures often demands the use of complex feedstocks that have undergone minimal processing and purification. Such feedstocks may be high in cellulosic content, have dark color, contain a wide mixture of particle sizes, and exhibit substantial batch-to-batch variation. The real-time monitoring of cell density (or cell biomass) is essential to maintaining the health of the microbial culture and for optimizing the yield of bioproducts, but has been a long-standing challenge within such complex feedstocks. The traditional method of monitoring microbial cultures by optical absorbance (“OD”) is hampered by strong absorbance and scattering from the feedstock itself, and by the limited linear range of response to changes in cell biomass. In this study, optical reflectance in the near infrared is demonstrated to accurately predict (9.7% standard error of prediction, r^2=0.985) *in situ* Clostridium dry cell weight grown in two different types of complex corn-based cellulosic feedstocks during exponential growth across multiple batch fermentations. The same algorithm that has previously been demonstrated to effectively compensate for the effects of bubbles, is shown to also be effective at compensating for the influence of insoluble feedstock particles on the dry cell weight prediction. The effects of and compensation for variable Clostridium cell flocculation will be discussed. Successful application of the *in situ* predicted dry cell weight as the feedback for a Clostridial fermentation using complex feedstock will also be presented.

P139 Bioinformatics-guided Discovery of Novel Ergosterol-binding Polyene Macrolides

X. Guo, A. Lin, A. Lewandowska, C. Price, C. Rienstra and M. Burke, University of Illinois Urbana-Champaign; D. Mitchell, University of Illinois at Urbana-Champaign, Urbana, IL, USA*
Fungal infections pose an elevated risk for all individuals who are immunocompromised. Current standard-of-care antifungal treatments include small molecule azoles and amphotericin B (amB), both of which display off-target toxicity upon administration. AmB is especially attractive due to its resistance-evasive mechanism of action, but its use is severely limited by nephrotoxicity derived from nonspecific binding of both fungal ergosterol and mammalian cholesterol. This sterol-binding is linked to a portion of the highly conserved carbon framework found in amphotericin-like polyene macrolides. The discovery and development of polyenes of diverse structures will give greater understanding into the structural drivers of ergosterol- vs cholesterol-binding. To this end, we have bioinformatically characterized the entire family of amphotericin-like polyene biosynthetic gene clusters. This analysis revealed more 111 polyene gene clusters, of which 79 were unannotated, with many clusters predicted to produce structurally distinct tetraene through heptaene macrolides. The producing organisms of these unique clusters were screened and we report the discovery of a novel tetraene, kineosporicin, with unprecedented polyene methyl-substitution, hydroxylation and diepoxidation. The ergosterol-binding ability of kineosporicin, along with 6 other representative glycosylated polyenes was then probed by both MICs with polyene-ergosterol and –cholesterol precomplexation as well as paramagnetic relaxation enhancement NMR. The results of these experiments will give precious atomic-level insight toward the mechanism of sterol-binding and potentially aid in the design of a less toxic antifungal.

Monday, July 22

7:00 AM - 8:00 AM SIMB News Editorial Board - Stone's Throw, Lobby

7:00 AM - 5:00 PM Registration

Atrium, Lower level

8:00 AM - 11:30 AM Session: 1: Extremophilic biocatalysis in bioprocessing

Conveners: Dr. Rajesh Sani, South Dakota School of Mines and Technology, RAPID CITY, SD, USA and **Sara E. Blumer-Schuette**, Oakland University, Rochester, MI, USA

Madison B, Mezzanine

8:00 AM S1: Biocommodity Engineering:Â Roles of Extremophilic Deep Biosphere Microbes - REPLACEMENT

R. Sani, Department of Chemical and Biological Engineering And Applied Biological Sciences, Rapid City, SD, USA*

This talk will describe the limitations in exiting solid waste (e.g., lignocellulose) conversion technologies and possible ways to overcome those limitations using extremophiles and their enzymes. The influence of high temperatures on various existing solid waste conversion processes and those that are under development, including extremophilic consolidated bioprocess will be discussed. Integrated decentralized thermophilic biofuel production employing lignocellulolytic and fermentative thermophiles in a single step consolidated process will also be discussed. Roles of deep-biosphere extremophiles in generating fuels and value-added products from organic waste in space will also be presented. In addition, this talk will introduce the current status of BioGTL (Biogas to Liquid Fuel) technology in bioenergy sectors.

8:30 AM S2: Role of extremophilic enzymes in improving bioprocesses

M. Bibra, Green Biologics, LLC., Little Falls, MN, USA*

The enzyme market and its applications are on rise, as the world eyes to replace chemical products and processes with bio-based products and processes. The enzymes are one of the critical components in the industrial bioprocesses with a direct effect on the finished product. However, the effect of the enzymes on the downstream processing had not been studied in detail.

The presentation will focus on the application of enzymes in the industrial bioprocesses that produce biofuels and bioproducts, utilizing both the conventional and non-conventional substrates. It will be described how the upstream enzyme usage have an impact on the downstream processing and finished product. The advantages of using the extremophilic enzymes in the industrial bioprocesses will be highlighted due to their ability to withstand industrial bioprocessing conditions such as high temperature, high pH, high substrate concentration, high inhibitor tolerance, shearing forces etc. Further, the impact of using extremophilic enzymes on the CAPEX and OPEX in the industrial bioprocesses will also be presented.

9:00 AM S3: Extremophiles as Electrocatalysts for Microbial Electrochemical Interface Technologies - REPLACEMENT

N.K. Rathinam, South Dakota School of Mines and Technology, Rapid City, South Dakota. USA., SD, USA, R. Sani, Department of Chemical and Biological Engineering And Applied Biological Sciences, Rapid City, SD, USA and D. Salem, South Dakota School of Mines & Technology, Rapid City, SD, USA*

Bioelectrochemical systems are realized as promising technologies for a wide range of applications such as bioremediation, powering implantable devices, electrosynthesis of value-added products, and biosensing in the deep and extreme environments. Electroactive microorganisms are key players in bioelectrochemical systems. Good selection of electroactive microorganisms is vital for improved biofilm formation and electrocatalysis. Use of extremophiles will help in using a wide range of substrates including recalcitrant wastes as feedstocks in bioelectrochemical systems. In this presentation, different engineering strategies for enhancing the performance of extremophiles in bioelectrochemical systems will be discussed. Finally, the presentation will cover the effects of cold plasma treatment on extremophiles and electrocatalysis.

Keywords: Bioelectrochemical systems, extremophiles, electrocatalysis, recalcitrant wastes

9:30 AM Break

10:00 AM S4: Haloarchaea as a cell factory for production of recombinant proteins: a mutagenic study of enzyme activity in cold temperature and high salinity

V. Laye, P. DasSarma and S. DasSarma, University of Maryland School of Medicine, Baltimore, Baltimore, MD, USA*

Halophilic Archaea (Haloarchaea) are found in extremely saline environments and survive saturating salt, intense solar radiation, and temperature extremes. They use the “salt-in” strategy to maintain osmotic equilibrium, with cytoplasmic components easily released by hypotonic lysis, making them ideal candidate cell factories for overexpression of recombinant proteins (DasSarma and Fleischman, Halophiles, Cold Spring Harbor Laboratory Press, 1995). We designed a series of haloarchaeal overexpression plasmids, pDRK and pARK, to express unique haloarchaeal proteins and diverse foreign proteins (DasSarma et al. *BMC Biotechnology* 13:112, 2013). These plasmids utilize either the *gvp*A or *csp*D2 promoter for gene expression, both of which were found to be cold-inducible in transcriptomic studies (Coker et al. *Saline Systems* 3:6, 2007). The expression system allows

directed protein overproduction after growth of haloarchaeal cultures to high density at optimal temperature by induction at low temperature, thereby lowering other metabolic activities and stabilizing overexpressed proteins. Addition of a His-6 tag allows large scale single-step purification of overexpressed proteins. We have used this expression system to overproduce and characterize a cold-active β-galactosidase from *Halorubrum lacusprofundi*, a haloarchaeon from Deep Lake, Antarctica (Laye et al. *Proceedings of the National Academy of Sciences USA* 114: 12530, 2017; Laye and DasSarma *Astrobiology* 18:412, 2018), a single-stranded DNA binding protein conferring increased radioresistance (Karan et al. *Applied Microbiology and Biotechnology* 98:1737, 2014), and foreign bacterial or eukaryotic proteins in either free form or displayed on haloarchaeal gas vesicle nanoparticles (GVNPs). The latter include the *Gaussia princeps* luciferase in enzymatically active forms, a mouse immune system bactericidal protein which can rescue animals from endotoxic shock, and a variety of antigens for vaccine development (DasSarma and DasSarma *Vaccines* 3:686, 2015; Balakrishnan et al. *Scientific Reports* 6:33679, 2016). In this presentation, I will provide an overview of our versatile haloarchaeal expression system showing great promise for industrial, environmental, and biomedical applications, and detail a β-galactosidase mutagenesis and steady-state kinetic study aimed at understanding catalysis in cold temperature and high salt conditions.

10:30 AM S5: Metabolic processes preserved as biosignatures in iron-oxidizing microorganisms: implications for biosignature detection on Mars

M. Floyd[*] and A. Grubisic, NASA Goddard Space Flight Center, Greenbelt, MD, USA; A. Williams, University of Florida, Gainesville, FL, USA; D. Emerson, Bigelow Laboratory for Ocean Science, East Boothbay, ME, USA

Iron-oxidizing bacteria occupy a distinct environmental niche. These chemolithoautotrophic organisms require very little oxygen (when neutrophilic), or outcompete oxygen for access to Fe(II) (when acidophilic). The utilization of Fe(II) as an electron donor makes them strong analog organisms for any potential life that could be found on Mars. Using on-line thermochemolysis and GC-MS, a strong, replicable signal of small molecular weight, doublet peaks was discovered in multiple iron-oxidizing isolates as well as several iron-dominated environmental samples, from both freshwater and marine environments, and in both modern and older iron rock samples. This GC-MS signal was not detected in bacterial isolates grown aerobically on complex media. Mass spectral analysis indicates that the molecule bears the hallmarks of a pterin molecule. The rock samples indicate the possibility that the molecule can be preserved. The specificity of the signal to organisms requiring iron in their metabolism make this a novel biosignature to investigate both the evolution of life on ancient Earth and potential life on Mars.

11:00 AM S6: Efficient microbial degradation of organic pollutants from industrial saline process streams using extreme halophilic *Haloferax mediterranei*

T. Mainka, C. Herwig and S. Pflügl[*], TU Wien, Vienna, Austria

The aim of this work is the development of a microbial treatment process for removal of organic pollutants from industrial water streams. The organic load originated from pollutants such as organic acids and aromatic compounds which are accumulated during chemical plastic production have to be redcued in order to recycle and dispose of these streams. To achieve this goal, first different halophilic and extreme halophilic strains have been evaluated for their capacity to degrade the organic pollutants contained in real industrial brine in shake flask experiments. The most promising strain, *Haloferax mediterranei*, was selected for further characterization and process development. A continuous process using cell retention was established to decouple liquid dilution rates from growth rates of *H. mediterranei* in order to achieve high biomass concentrations and thus, cultures with high degradation rates. The mixed feed process uses low concentrations of glycerol as the carbon and energy source for biomass propagation and to maintain catalytic activity. That way, an overall reduction of 85 % of the total organic carbon content was achieved in lab-scale bioreactors to levels below 15 ppm. A custom corrosion-resistant airlift bioreactor was designed and built for pre-piloting at the production facility of the industrial partner. Continuous remote operation for > 4 weeks using real brine substrate from the production facility showed stable reduction of organic pollutants in an industrial environment with fluctuating composition of the stream using extreme halophilic cultures. This work nicely demonstrates how the remarkable capabilities of extremophilic organisms can be utilized for industrial applications when combined with systematic and quantitative bioprocess development.

8:00 AM - 11:30 AM Session: 2: Women in environmental science Sponsored by US DOE-BER

Conveners: Gemma Reguera, Michigan State University, East Lansing, MI, USA and **Arpita Bose**, Washington University in St. Louis, St. Louis, MO, USA

Madison A, Mezzanine

8:00 AM S7: Environmental science goes electric

G. Reguera[*], Michigan State University, East Lansing, MI, USA

Electrically active microorganisms are widespread in Nature and rely on electrical signals to couple their metabolism and drive geochemical processes of global significance. This talk will describe seminal studies of electric microbes led by women in the

Reguera lab that have established the foundation of electromicrobiology as a new subfield in microbiology. The work includes basic research to elucidate the complex respiratory machinery that wires cells in the genus *Geobacter* to minerals, toxic metals and radionuclides and that allows these bacteria to intertwine the cycles of carbon, nitrogen and other essential elementals. The talk will also describe work in the lab that harnesses the activities of electric microbes and their conductive biomaterials in nanotechnology, bioremediation, and bioenergy applications.

8:30 AM S8: Solar Energy to Fuels – A New Vision using Electricity Consuming Phototrophic Microbes

A. Bose[*]*, Washington University in St. Louis, St. Louis, MO, USA*

Solar energy is our most abundant renewable energy resource. Solar energy can be converted to electrical energy effectively. However, going from solar energy to usable biochemicals like biofuels still remains a challenge. Although natural photosynthesis has been leveraged in this regard, the efficiency of these processes is very low. Each photosynthetic organism that can be used to produce biofuels also poses unique challenges. In comparison, solar cell technology is very efficient at solar energy capture into electrical energy. How can we leverage this to create biofuels? The answer might be electricity consuming microbes that can be engineered to make designer biochemicals such as biofuels. We have isolated and engineered phototrophic microbes that can consume electricity to convert carbon dioxide into bioplastics and biofuels such as *n*-butanol using the energy of light. We get a high electron yield for these products and have created many different configurations to explore that possibility of using electricity off the grid to create biochemicals. This paves the future for improved utilization of solar energy while creating carbon-neutral biochemicals particularly biofuels.

9:00 AM S9: Commercializing bioelectrochemical wastewater treatment systems for breweries

S. Babanova, O. Bretschger[*]*, J. Jones, J. Soles, J. Cornejo, J. Garcia and D. Barocio, Aquacycl LLC, San Diego, CA, USA*

Wastewater management for breweries located in small municipalities can become a cost-prohibitive operational expense. Breweries that are not connected to a sewer system have the option to hold and haul their wastewater to a treatment facility or invest in an onsite treatment solution. Brewery wastewater contains high concentrations of chemical oxygen demand (COD), which is a measure of total oxidizable carbon. The composition of COD from a brewery will include spent yeast, unfermented sugars and trace amounts of hops and/or other flavoring compounds. A brewery that produces 120 barrels of beer per day may generate 150,000 gpd of wastewater with a COD that is 50x higher than what is measured in a typical domestic sewer line. The management of this waste stream may cost a brewery nearly $2M per year in operational expenses alone.

Aquacycl has a developed a product called the BioElectrochemical Treatment Technology (BETT™), which can provide energy-neutral wastewater treatment for high-strength waste streams like brewery wastewater. Here we present a subset of results from a BETT™ system piloted at a small brewery to treat concentrated wastewater at a scale of 150 gallons per day (0.6 m^3/day). The system was operated as fed batch having a COD of approximately 12,000 mg-COD/L, with small tests executed under continuous flow. The organic removal of the system varied during operation with results between 20%-40% removal of total COD in a 4-hour hydraulic retention time. Overall, these data suggest that BETT™ systems can be practically scaled for cost-effective wastewater treatment in breweries.

9:30 AM Break

10:00 AM S10: The facts about lead in drinking water

E. Betanzo[*]*, Safe Water Engineering, LLC, Royal Oak, MI, USA*

Convincing decision makers that the Flint Water Crisis was happening was a challenge, but improving requirements to protect drinking water is a long-term process with even more trials along the way. In this presentation I will share my experience as an environmental engineer in uncovering the Flint Water Crisis, and how I use my work in science and policy to protect public health.

10:30 AM S11: The genetic diversity of environmental, uncultivated Beggiatoa

J. Biddle[*] *and K. Bousses, Univ of Delaware, Lewes, DE, USA*

The large, filamentous, freshwater sulfur-oxidizing bacteria, *Beggiatoa*, have been cultivated and widely used in wastewater treatment. Yet their marine relatives are part of the ultra-rare biosphere in the ocean, are poorly understood and uncultivated. The goal of our study was to broaden the knowledge on *Beggiatoa* diversity by finding strains in Atlantic Ocean waters and comparing them to other sequenced relatives. To investigate Atlantic-based strains of *Beggiatoa*, we placed a pork bone in flowing seawater and grew a sulfur-oxidizing biofilm, which contains members of the *Beggiatoacaea*, the sulfur-oxidizing Gammaproteobacteria, and eventually developed Epsilonproteobacteria. Through a metagenomic-enabled perspective, we were able to assemble multiple genomes of *Beggiatoacaea*, and compare them to other deep-sea strains and cultured freshwater relatives. The genome comparisons suggest that there is an open genome of marine *Beggiatoacaea* globally, and many more genomes will be needed to fully understand the complete metabolic capability within this group. Within the *Beggiatoacaea* of the

high salinity water, we see that there are drastically different abundances in genes for regulation and community coordination, which lends some suggestions to why these organisms are so difficult to cultivate and also creates some targets for industrial investigation. With this backyard approach to understanding these extremophiles, we can better prepare ourselves to understand unique strains in the environment, and potentially bring more organisms into cultivation in the laboratory.

11:00 AM S12: Environmental Science Research at the Department of Energy, Office of Biological and Environmental Research

D. Adin, U.S. Department of Energy, Washington, DC, USA*

The Department of Energy's Office of Science is the lead federal agency supporting fundamental scientific research for energy and the Nation's largest supporter of basic research in the physical sciences delivering scientific discoveries and major scientific tools to transform our understanding of nature and advance the energy, economic, and national security of the United States. As a program office within the Office of Science, Biological and Environmental Research (BER) supports transformative science and scientific user facilities to achieve a predictive understanding of complex biological, earth, and environmental systems for energy and infrastructure security, independence, and prosperity. Research funded includes understanding the underlying biology of plants, microbes, and interactive biological communities as they respond to and modify their environments, enabling the re-engineering of microbes and plants for energy and other applications, and advancing understanding of the dynamic processes needed to model the Earth system, including atmospheric, land masses, ocean, sea ice, and subsurface processes. By developing an understanding of the relationships between molecular-scale functional biology and ecosystem-scale environmental processes, we can illuminate the basic mechanisms that drive biogeochemical cycling of metals and nutrients, large scale carbon cycle processes, and greenhouse gas emissions in terrestrial ecosystems or bioenergy landscapes. This research spans across spatial and temporal scales ranging from sub-micron to global, from individual molecules to ecosystems, and from nanoseconds to millennia. Information on BER, funding opportunities in environmental sciences, and a general perspective of federal program management will be discussed.

8:00 AM - 11:30 AM Session: 3: From zero to hero - Integrated approach for strains, fermentation and DSP developments Sponsored by BASF

Conveners: Paul Handke, BASF Enzymes, San Diego, CA, USA and **Susanne Kleff**, Michigan State University, East Lansing, MI, USA

Marshall Ballroom Southeast, Mezzanine

8:00 AM S13: A high-throughput genome engineering platform to optimize a variety of microbes for industrial fermentation

M. Schwartz, Zymergen, Emeryville, CA, USA*

We have established a platform that leverages the latest innovations in computational and manufacturing technology and applies them to genetic engineering. Our microbial strain engineering platform integrates several core technologies including custom software, high-throughput laboratory automation, machine learning algorithms and genome engineering. High-throughput laboratory automation empowers more robust and predictable DNA assembly and microbial gene editing as well as high-throughput assays with exceptional precision for the accurate measurement of desired fermentation metrics. These HTP processes complement and exceed the capabilities of manual laboratory work. We have developed custom scientific computing tools for specifying and tracking the creation of engineered microbes. Our machine learning algorithms enable efficient navigation of the immensely vast biological search space. We describe how we are applying this modular and iterative approach to engineer a diverse set of microbes with improved performance for desired traits.

8:30 AM S14: Integrated microbial product development at AgBiome

A. Smith, AgBiome Inc., Research Triangle Park, NC, USA*

Modern crop protection practices employ high-quality agronomics, chemistries, and germplasm to control damage due to pests and diseases. Despite these efforts, growers continue to experience crop yield losses of approximately 30% per year, and are faced with the rapid development of resistant pests and diseases after the introduction of each new tool. At AgBiome, we streamline complex processes between microbiology, fermentation, and downstream processing to address these significant crop challenges. We leverage our collection of more than 60,000 fully sequenced microbes to inform our decisions in microbiology and fermentation, therefore aligning efforts to maximize success of our products. Access to such a large sequenced collection allows us to identify, develop, register, and commercialize the most highly effective microbial pesticides. Our unique company organization and culture empowers teams and experts to make quick decisions and to ensure troubleshooting happens early in the workflow, thereby avoiding costly mistakes further downstream. Our iterative workflows are constantly receiving necessary feedback both upstream and downstream to improve our processes in real time, allowing us to be adaptive and nimble. This agility ultimately enables AgBiome to rapidly deliver new products and the most effective solutions to growers to address today's, and tomorrow's, crop challenges.

9:00 AM S15: Holistic process design for conversion of biomass to advanced biofuels with engineered, non-canonical hosts

E. Sundstrom, C. Araujo Barcelos, I. Wolf, J.P. Prahl, E. Oksen, O. Jacobson and D. Tanjore, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Continued advances in strain development have enabled increased usage of non-canonical organisms as platform hosts for biochemical production. Leveraging a poorly characterized host challenges both strain engineering and process development, but when applied to a well-designed process, a properly selected host strain can enable consolidation of previously incompatible unit operations, utilization of highly specialized native metabolic pathways, consumption of lower-cost heterogenous feedstocks, and operation under conditions better suited for in-situ product recovery. At the Advanced Biofuels and Bioproducts Process Demonstration Unit (ABPDU), we work to integrate, consolidate, and intensify a variety of novel bioconversion processes to improve the end-to-end economics of advanced biofuel and biochemical production. Key examples include process development with *Rhodosporidium toruloides* as a platform host featuring high osmotic tolerance and metabolic flexibility, use of *Streptomyces albus* for production of ketones generated via engineered polyketide synthase (PKS) pathways, and holistic process design for effective production and recovery of volatile fermentation products. Lessons learned from integrated scale-up of these processes - coupled with insights from our industrial partners - help to de-risk these emerging technologies, reducing time to market for future practitioners and creating potential long-term reductions in CAPEX and OPEX for biomanufacturing of fuels and commodity chemicals.

9:30 AM Break

10:00 AM S16: Industrial bioprocess intensification

P. Wagner, DMC Biotechnologies, USA*

A major barrier to the commercialization of new industrial biotechnology processes is the total capital investment required for development and plant construction. Large capital investments increase the feasible selling price for a product due to the need to achieve a competitive return on the investment. One route to minimize plant capital costs is to intensify the fermentation process to increase the efficiency of the equipment being used. High volumetric productivities are an essential feature of intensified bioprocesses; however, any approach to achieve increased productivity must be evaluated against costs inherent to intensification such as increased oxygen demand or heat removal. The goal must be to optimize economics, rather than to maximize any specific technical metric. This talk will provide an overview of DMC's approach to process intensification which includes 1) The decoupling of cell growth from product formation to maximize productivity and robustness to the process environment, 2) increasing the specific, or per-cell, productivity, and 3) continuous removal of product from bioreactors with cell retention. The benefits of this integration of strain development, upstream, and downstream operations will be described in the presentation.

10:30 AM S17: How one flavor can save the world: productionÂ of a commercially viable supply of hemoprotein for the replacement of animal agriculture

M. Speer, S. Balatskaya, B. RoyChaudhuri, E. Colasante, C. Cox, X. Guo, M. Hoyt and R. Maples, Impossible Foods, Redwood City, CA, USA; S. Shankar, Impossible Foods, Inc., Redwood City, CA, USA*

Early work at Impossible Foods identified hemoproteins as a key driver of the taste and aroma associated with beef. Impossible Foods uses Leghemoglobin, a hemoprotein found naturally in soybean root nodules, to generate these meaty qualities without the use of animals. The Leghemoglobin used in the Impossible Burger is heterologously produced by the yeast Pichia pastoris using a combination of novel processes which greatly decrease the cost and impact of producing this critical flavor compound. The molecular-biology team at Impossible Foods has developed novel genetic approaches which have led to a dramatic departure from the traditional approach to heterologous protein production in Pichia pastoris. This has necessitated concurrent developments in fermentation and purification scale-up, which has generated a commercially viable supply of Leghemoglobin, enabling the production of more than a million pounds of Impossible Burger every week.

11:00 AM S18: Better materials for a better world

A. Echaniz, Bolt Threads, Emeryville, CA, USA*

Nature provides an infinite source of inspiration for structural proteins and polysaccharides from which we can design novel biomaterials for use in textiles and personal care products. Bolt Threads has developed a molecular biology platform to mimic and tune the mechanical properties and inherent environmental compatibility of these materials. Our protein platform includes genetic-level control over the amino acid sequence, allowing us to fine tune the polymer processing windows and functional material properties. Today, Bolt Threads is capable of producing a recombinant spider silk protein at commercial scale and spin this material into filament and staple yarns. This first fiber demonstrates the baselined capability of Bolt Thread's designer protein polymer pipeline.

The mission of Process Development is to continue to optimize and deploy scalable, economically viable processes for the

production of the natively-inspired bio-derived materials with sustainability, performance, and market advantages. This session will describe our approach used to accelerate the launch of our first commercial products: 1. A recombinant spider silk protein and 2. Mylo™ a mycelium leather.

A little more about Bolt Threads: Bolt Threads is a vertically integrated company driven by 100+ dynamic scientists, engineers, artists, and operations specialists. At Bolt, our endeavors include molecular biology and materials science R&D, polymer production, fiber manufacturing, product development, marketing, merchandising, and direct to consumer sales. Bolt Threads operates direct to consumer through Best Made Co. and partners with well-known brands such as Stella McCartney and Patagonia.

8:00 AM - 11:30 AM Session: 4: Metabolic Engineering and Products from New Model Organisms Sponsored by: Pivot Bio

Conveners: Gayle Bentley, National Renewable Energy Laboratory, Golden, CO, USA and **Joe Shaw**, Novogy Inc., Cambridge, MA, USA

Marshall Ballroom West, Mezzanine

8:00 AM S19: Alterations to pyruvate metabolism in *Clostridium thermocellum*Â LL1210 reduce amino acid secretion and improve biofuel yields

H. Schindel, K. O'Dell, D. Cowan, A. Steiner and S. Thurmon, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Clostridium thermocellum is an attractive organism for consolidated bioprocessing of cellulose to fuels due to its native abilities to metabolize cellulose and produce biofuels such as ethanol and isobutanol. Previous engineering efforts have resulted in ethanol production up to 76% of the maximum theoretical yield, achieved by removing competing fermentation pathways for hydrogen, lactate, acetate, and formate production. The resulting strain, LL1210, secretes amino acids in amounts equal to roughly 10% of input carbon. Recent efforts have focused on fine-tuning the carbon and electron metabolism of *C. thermocellum* to further increase biofuel yields by reducing amino acid production and secretion. Specifically, deletion of the PPDK enzyme and subsequent replacement with a heterologous pyruvate kinase (pyk) has been shown in wild-type strains to reduce amino acid production, likely due to less generation of NADPH for biosynthesis. We found that replacing PPDK with pyk in LL1210 also results in lower amino acid secretion, but also decreases growth rate. Adaptive laboratory evolution of the DPPDK::pyk strain (AG3364) produced multiple strains with faster growth and increased ethanol production relative to strain LL1210. Genome resequencing has identified multiple mutations that could account for the improved phenotype. Further efforts to engineer *C. thermocellum* for cellulosic isobutanol production will be discussed.

8:30 AM S20: Rewiring the Regulation of Diazotrophic Nitrogen Metabolism for Agricultural Benefits

B. Ozaydin, Pivot Bio, Berkeley, CA, USA*

Cereals make up more than two thirds of the human food supply and their efficient production depends on a steady supply of nitrogen. In its most abundant form, molecular nitrogen is inaccessible to plants and can only be utilized by nitrogen-fixing, diazotrophic bacteria and archaea. Therefore, to accommodate the ever-rising demands of crop production, nitrogenous fertilizers are routinely applied in agriculture. However, more than half of the applied fertilizers are lost to denitrification, evaporation, surface runoff and leaching, leading to greenhouse gas emissions and aquatic dead zones. Utilizing diazotrophic bacteria as an environmentally-friendly alternative to synthetic fertilizers has been a long sought-after dream. With the increased understanding of nitrogen fixation mechanisms and the advancement in molecular biology tools, we are now better situated to engineer microbes that can establish successful associations with crops' roots to supply the much-needed nitrogen in a more efficient way.

At Pivot Bio, we are discovering new microbes that can colonize corn roots effectively and fix nitrogen naturally. Due to high energetic demands of the nitrogenase enzyme, nitrogen fixation is tightly regulated within the microbe in response to available nitrogenous compounds and, as a result, it is repressed in fertilized fields. We bypassed the microbe's regulation through non-transgenic approaches and developed our first-generation microbial product, PIVOT BIO PROVEN™, which has demonstrated efficacy across various weather and soil conditions. As we design our next-generation microbes, we are focusing our efforts on further improving nitrogen fixation, ammonia excretion, and establishing efficient symbiotic relationships with corn roots.

9:00 AM S21: Synthetic biology and metabolic engineering in *Yarrowia lipolytica*: towards an affordable bioproduction

R. Ledesma-Amaro, Imperial College London, London, United Kingdom*

Synthetic biology has emerged as a powerful discipline that facilitates the manipulation of cells in a more reliable, predictable and standardized manner. Metabolic engineering aims to modify metabolic pathways to manufacture chemicals and fuels in an environmentally friendly way. In combination, synthetic biology tools can boost the development of metabolic engineering

strategies and can help to conventionalise non-conventional organisms.

In this talk we will see several examples on the synergies between metabolic engineering and synthetic biology, not only to maximize production yields but also to diminish the cost of the upstream and downstream parts of the process. In particular, we have developed synthetic biology tools to conventionalise the oleaginous yeast Yarrowia lipolytica and we have used it to reduce the overall cost of different bioprocess. This has been achieved by 1) expanding the substrate range to cope with low-cost substrates such as raw lignocellulosic hydrolysate, 2) maximizing lipid production to reach the theoretical maximum yield and uncoupling biomass and product formation allowing up to 120% of Cell Dry Weight as lipids, 3) facilitating the recovery of the lipids by engineering the secretion of those to the culture media, and 4) producing higher-value lipid-derived compounds such as beta-carotene, where we obtained the highest titers described so far in microbes.

9:30 AM Break

10:00 AM S22: Metabolic engineering as a tool to improve growth rate and polyhydroxyalkanoate production in Burkholderia sacchari and Pseudomonas sp. strains

L. Guaman, Universidad UTE, Quito, Ecuador, E. Ramos Oliveira, Sao Paulo University, Sao Paulo, Brazil and C. Barba, Yachay Tech., Quito, Ecuador*

Despite its ability to grow and produce high-value molecules using renewable carbon sources, two main factors must be improved to use Burkholderia and Pseudomonas as a chassis for bioproduction at an industrial scale of polyhydroxyalkanoates: first, the lack of molecular tools to engineer these organisms and second, the inherently slow growth rate and polyhydroxyalkanoates production using alternative carbon sources. Using metabolic engineering approaches we have successfully improved both specific growth rate and P(3HB) production achieving the highest P(3HB) yield and μmax from xylose.

10:30 AM S23: Simultaneous catabolism of all major components of lignocellulosic biomass by engineered Pseudomonas putida

J. Elmore, Pacific Northwest National Laboratory, Richland, WA, USA; D. Salvachúa, M. O'Brien, D.J. Peterson and G.T. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; G. Peabody, G. Dexter, D.M. Klingeman, K. Gorday and A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA; R. Jha and T. Dale, Los Alamos National Laboratory, Los Alamos, NM, USA*

Valorization of all major lignocellulose components, including lignin, cellulose, and hemicellulose is critical for an economically viable bioeconomy. In most biochemical conversion approaches, the standard process separately upgrades sugar hydrolysates and lignin. Here, we present a new process concept based on an engineered microbe that could enable simultaneously upgrading of all lignocellulose streams, which has the ultimate potential to reduce capital cost and enable new metabolic engineering strategies. Specifically, we engineered *Pseudomonas putida*, a robust microorganism capable of catabolizing aromatic compounds, organic acids, and D-glucose, to utilize D-xylose and L-arabinose by tuning D-xylose transport and pentose phosphate pathway flux. Distinct L-arabinose pathways enabled D-glucose, D-xylose, and L-arabinose co-utilization in minimal medium using model compounds as well as corn stover hydrolysate. After modifying catabolite repression, our engineered *P. putida* simultaneously co-utilized five representative compounds from cellulose, hemicellulose, and lignin, demonstrating the feasibility of simultaneous total lignocellulosic biomass upgrading. Furthermore, simultaneous catabolism of multiple carbon sources also offers the potential for novel metabolic engineering strategies. We demonstrate this by modifying a *P. putida* strain previously engineered to produce muconic acid from glucose to catabolize L-arabinose and/or D-xylose. Co-utilization of glucose with either pentose had a synergistic effect on growth rate, improving volumetric productivity by ~60%. Finally, we developed a D-xylose biosensor to enable dynamic regulation of the D-xylose catabolic pathway and transporter. Utilizing dynamic pathway regulation, we decreased the metabolic burden of the D-xylose catabolic pathway, substantially improving the fitness of this engineered *P. putida* strain in the absence of xylose.

11:00 AM S24: Metabolic Engineering of *Corynebacterium glutamicum* for conversion of biomass-based diverse carbon substrates into D-lactate

A. Jha, A. Mhatre and A.M. Varman, Arizona State University, Tempe, AZ, USA; S. Shinde, DOE Great Lakes Bioenergy Research Center, Michigan State University, Lansing, MI, USA; Z. Wardak, J.M. Gladden and R.W. Davis, Sandia National Laboratories, Livermore, CA, USA

D-lactate, the primary precursor of Polylactic acid (PLA) which is a bio-based polymer, an economically feasible packaging material like fresh fruit container, drinking cups, lamination films, and many more. Although glucose as the sole carbon source in d lactate biosynthesis is reported, utilization of xylose and arabinose as a sole carbon source with overexpression of xylose and arabinose pathway D- lactate biosynthesis has not been elucidated in *Corynebacterium glutamicum* till date. In present study, various heterologous strains of *C. glutamicum* were generated to produce D-lactate by exploiting the L-lactate production pathway and followed by overexpression of different D-lactate dehydrogenase (*ldhA*, *gldA101* and *gldA101**) which was further encoded with overexpression of xylose (*xylA* and *xylB*), and arabinose (*araB, araA, and araD*) biosynthetic pathway.

Heterologous strains with the constitutive promoter were grown in minimal medium BTM2 supplemented with different level of various carbon sources. The recombinant strain SSL02 harboring *gldA101** produced 11530.83 mg/L, SSL03 harboring *xylA* and *xylB* produced 133.05 mg/L and SSL08 harboring (*araB, araA, and araD*) produced 49.6 mg/L of highest D-lactate from a minimal medium BTM2 supplemented with glucose 4%, xylose 2%, and arabinose 2%, respectively. The highest D-lactate producing strain SSL02 was also cultured in different aromatics (benzoic acid, cinnamic acid, vanillic acid, and coumaric acid) and found to be growing well with different levels of D-lactate production without any sugar supplementation in BTM2. U-13C fingerprinting study carried out by supplementing different aromatics and U-13C glucose to the cells, exhibited significant assimilation of aromatics especially in case of coumaric acid and cinnamic acid. Finally, the strain SSL02 modified by overexpressing the xylose and arabinose biosynthetic pathways to develop new strain SSL09 to utilize various carbon sources from biomass hydrolysates together at the time to produce D-lactate. Strains were also grown in fed batch culture wherein biomass hydrolysate was subsequently utilized in optimized fermentation trial to increase D-lactate production to 30g/L. These results demonstrate efficient use of biomass hydrolysate (Corn Stover) to produce D-lactate.

8:00 AM - 11:30 AM Session: 5: Peptidic Natural Products

Conveners: A. James Link, Princeton University, Princeton, NJ, USA and **Albert Bowers**, UNC Chapel Hill, Chapel Hill, NC, USA

Marshall Ballroom North, Mezzanine

8:00 AM S25: Engineering multifunctional enzymes and investigating versatility of enzymatic halogenation for nonribosomal peptide synthesis

S. Garneau-Tsodikova[*]*, University of Kentucky, Lexington, KY, USA*

Nonribosomal peptides are natural products biosynthesized by multi-modular enzymatic assembly-lines comprised of domains performing varied activities. Adenylating enzymes play a key role in dictating the identity of building blocks to be incorporated in growing peptides during nonribosomal peptide biosynthesis. To increase the structural diversity of the products it generates, Nature has evolved unique interrupted adenylating enzymes capable of performing both adenylation and methylation reactions. We will present our biochemical and structural work towards understanding the mechanism by which these unique enzymes function and our efforts towards engineering novel interrupted enzymes with adenylating and methylating activities. Additionally, we will discuss halogenation, an important biotransformation and a highly promising transformation in medicinal chemistry, which could lead to improvement in pharmacological and pharmaceutical properties of compounds or enabling further modifications by using the halogen as a reactive handle. In the last decade, halogenases have emerged as highly promising tools that may serve as an alternative or the only route to halogenating some molecules, especially natural products. Our biochemical and structural study of two halogenases will be presented.

8:30 AM S26: Improved preparation of substrate-like peptide fluorophosphonate inactivators and their application to reveal the bifunctional thioesterase mechanism acting in monocyclic beta-lactam antibiotic biosynthesis

C. Townsend[*]*, Johns Hopkins University, Baltimore, MD, USA*

The human genome encodes >200 serine hydrolases, a ubiquitous superfamily of enzymes of central importance in biology and medicine. The subset of thioesterases (TEs) play prominent roles in natural product biosynthetic pathways, yet remarkably few x-ray structures exist of these enzymes with either their native substrate bound or a structurally accurate mimic at usefully high resolution. A number of confounding technical factors have led to this state of affairs. One strategy to overcome them is to prepare diphenylphosphonate substrate analogs in the hope they will form a covalent adduct, a tetrahedral transition state mimetic, with the catalytic seryl residue. Often, however, no reaction occurs owing to steric bulk and/or intrinsically poor reactivity. Resort to the more electrophilic fluorophosphonates has been long known, but harsh reagents are required (DAST, for example), which limit the substrate authenticity of the inactivators that can be synthesized. Reported in this lecture will be a simple, mild, two-step protocol to convert readily synthesized diphenylphosphonates to their corresponding smaller and potently reactive methylfluorophosphonates. This advance is applied to investigate the unusual dual epimerase/hydrolase activity of NocTE, which carries out critical late transformations in the biosynthesis of the beta-lactam antibiotic Nocardicin A. High-resolution crystal structures have been obtained of the unliganded enzyme and reacted with a structurally faithful fluorophosphonate substrate mimic that identify the full peptide binding pocket that accommodates both diastereomers. These structures and biochemical data combine to give detailed mechanistic insight into this unique NRPS domain.

9:00 AM S27: Dissecting biosynthetic logic of modular production of tricyclic peptides possessing anti-serine protease activity

Y. Zhang, S. Bruner and Y. Ding[*]*, University of Florida, Gainesville, FL, USA*

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are an important family of natural products. Their

biosynthesis follows a common scheme in which the leader peptide at the N-terminus of a precursor peptide guides the modifications on the single C-terminal core peptide (CP). Microviridins are a unique family of RiPPs that are featured with an unprecedented tricyclic cage-like architecture formed by two ester and one amide linkages by two ATP grasp ligases. The 3-D structure of microviridin represents a novel chemical scaffold of serine protease inhibitors with high specificity and potency. Misregulation of serine proteases plays a crucial role in many life-threatening human diseases, making serine proteases as proven drug targets. Our genome mining efforts identified one microviridin cluster from the filamentous cyanobacterium *Anabaena* sp. PCC 7120, where the precursor peptide AMdnA harbors three core peptides. Modular biosynthesis of RiPPs represent a genetic cost-saving strategy to explore broader chemical space. However, the enzymology of these systems has not been sufficiently characterized. Here we report the reconstitution of the modular processing of AMdnA by two pathway-specific ATP grasp ligases AMdnC and AMdnB. Our biochemical and kinetic studies revealed the catalytic functions of AMdnC and AMdnB and characterized their unique enzymology features. In addition, we developed two chemical biology strategies to synthesize multiple microviridin analogs and biologically evaluated their inhibitors toward serine proteases. These results provide important, new understanding of the biosynthetic processing of multi-cassette configuration of microviridin.

9:30 AM S28: Flexizyme-Enabled Benchtop Biosynthesis of Thiopeptides

S. Fleming, T. Bartges, C. Kirkpatrick, L. Hicks and A. Bowers, UNC Chapel Hill, Chapel Hill, NC, USA; A. Vinogradov, Y. Goto and H. Suga, Graduate School of Science, The University of Tokyo, Tokyo, Japan*

Thiopeptides are natural antibiotics that are fashioned from short peptides by multiple layers of post-translational modification. Their biosynthesis, in particular the pyridine synthases that form the macrocyclic antibiotic core, has attracted intensive research but is complicated by the challenges of reconstituting multiple- pathway enzymes. By combining select RiPP enzymes with cell free expression and flexizyme-based codon reprogramming, we have developed a benchtop biosynthesis of thiopeptide scaffolds. This strategy side-steps several challenges related to the investigation of thiopeptide enzymes and allows access to analytical quantities of new thiopeptide analogs. We further demonstrate that this strategy can be used to validate the activity of new pyridine synthases without the need to reconstitute the cognate prior pathway enzymes.

10:00 AM Break

10:30 AM S29: Citrocin, a novel RNA polymerase-targeting antimicrobial lasso peptide

W.L. Cheung and A.J. Link, Princeton University, Princeton, NJ, USA*

Lasso peptides are a class of ribosomally-derived natural products (RiPPs) typified by their lasso or slipknot structure. The chiral 1-rotaxane structure of lasso peptides comes about via installation of an isopeptide bond between the N-terminus of the peptide and a Glu or Asp sidechain. This curious structure begets an array of different therapeutically relevant functions, the most well-studied of which is antimicrobial activity. In this talk I will describe a new antimicrobial lasso peptide, citrocin, which is found in two sequenced *Citrobacter* genomes, *C. pasteurii* and *C. braakii*. Citrocin can be produced at 0.7 mg/L yield from *C. braakii*. We improved this titer further to 2.7 mg/L by refactoring the low-GC gene cluster and producing citrocin heterologously in *E. coli*. The structure of citrocin was determined by NMR. Citrocin, like the well-studied lasso peptide microcin J25, is an inhibitor of RNA polymerase (RNAP), though citrocin is ~100-fold more potent than microcin J25 in *in vitro* assays of RNAP inhibition. Despite this potent activity *in vitro*, citrocin has only moderate antimicrobial activity against a panel of enterobacteria, suggest that its antimicrobial activity is dictated by its uptake into susceptible cells. Since microcin J25 enters susceptible cells via the ferrichrome receptor FhuA, we were surprised that citrocin still killed an *fhuA* knockout of *E. coli*. Citrocin was active against all TonB-dependent receptor knockouts in *E. coli*. Furthermore, citrocin was active against *E. coli* knocked out in either the Ton and Tol/Pal systems, suggesting that it may cross the outer membrane of susceptible bacteria in an energy-independent fashion. Mutagenesis of citrocin demonstrated the importance of an Arg residue within the peptide for maintenance of both the structure and antimicrobial activity of citrocin.

11:00 AM S30: Discovery and biosynthesis of hybrid polyketide-nonribosomal peptides in nematodes

R. Butcher, University of Florida, Gainesville, FL, USA*

The nemamides are the only polyketide-nonribosomal peptides produced by an animal system in an assembly-line process. These natural products are biosynthesized in the nematode *C. elegans* by two megaenzymes, PKS-1 and NRPS-1, in the canal-associated (CAN) neurons and promote survival during starvation-induced larval arrest. Here, we use CRISPR-Cas9 to sequentially inactivate enzymatic domains in PKS-1 and NRPS-1 in order to trap biosynthetic intermediates and map the assembly-line process that leads to the nemamides. Furthermore, we identify multiple additional enzymes, including a methyltransferase and a CoA ligase, that function *in trans* in nemamide biosynthesis. Surprisingly, we show that a high percentage of genes that are expressed in the CAN neurons are devoted to nemamide production. Our work reveals a number of noncanonical features to nemamide biosynthesis and provides new insights into the trafficking and role of polyketide-nonribosomal peptides the context of an animal system.

11:30 AM - 12:45 PM International Outreach Committee

Marshall Ballroom Southeast, Mezzanine

11:30 AM - 1:00 PM Diversity Committee meeting

Taylor, Mezzanine

11:30 AM - 1:00 PM Membership Committee meeting

Taft, Mezzanine

11:30 AM - 1:00 PM New SIMB Members-Meetup at coffee break

Exhibit Hall C, Lower level

11:30 AM - 1:00 PM Quarter Century Club Luncheon - members only

Balcony A, Mezzanine level

12:15 PM - 12:25 PM Exhibitor Showcase: Distek, Inc.

Exhibit Hall C, Lower level

12:25 PM - 12:35 PM Exhibitor Showcase: Process Analyzer Schneider Electric

Exhibit Hall C, Lower level

12:35 PM - 12:45 PM Exhibitor Showcase - Bug Lab, LLC.

1:00 PM - 3:30 PM Session: 6: Student Oral Presentations

Conveners: Stephanie Gleason, Dupont Nutrition and Biosciences, Grand Rapids, MI, USA and **Katherine Chou**, Dupont Industrial Biosciences, N. Liberty, IA, USA

Madison A, Mezzanine

1:00 PM S31: Whole-genome sequence and variant analysis of *Saccharomyces cerevisiae* strains evolved for high solids pine fermentations

O.A. Thompson[*] and J.D. Peterson, University of Georgia, Athens, GA, USA

The potential of lignocellulosic biomass as a sustainable substrate for bioethanol production is limited by the pretreatment process that generates inhibitory compounds impairing the growth and performance of fermenting microorganisms. Development of strains with increased tolerance to a range of inhibitors is necessary as methods of inhibitor abatement are economically and environmentally unfavorable. *S. cerevisiae* strain XR122N was subjected to directed evolution and adaptation in pretreated pine fermentations and resultant strains, GHP1 and GHP4, demonstrated improved fermentative ability with GHP4 exhibiting constitutive tolerance and GHP1 exhibiting conditional tolerance dependent on the selective pressure of inhibitory media. Previously reported findings identified 52 differentially expressed genes that may account for improved tolerance to multiple inhibitors simultaneously and revealed improved robustness of mitochondria of evolved strains that were resistant to the damaging effects of inhibitors in contrast to the parent. Presently, whole genome sequencing of evolved strains and the parent was employed to identify genomic changes that have facilitated adaptation to inhibitors for improved stress tolerance. Variant analysis of all three strains will potentially reveal structural variations in the genome including chromosomal rearrangements, copy number changes, as well as SNP/indel mutations in key genes. Variants will be further characterized to evaluate functional importance in inhibitor tolerance. The results of this study are important for determining key mechanisms of tolerance to biomass derived inhibitory compounds and broadening the general understanding of stress tolerance of *S. cerevisiae*. This study also has direct implications for further development of robust yeast strains for multiple industrial applications.

1:20 PM S32: One-Pot biocombinatorial synthesis of herbicidal thaxtomins and substituted aromatic 2,5-diketopiperazines

G. Jiang, R. Zuo, Y. Zhang, M. Powell, P. Zhang, S. Hylton, N. Hiller and Y. Ding, University of Florida, Gainesville, FL, USA; R. Loria, University of Florida, Department of Plant Pathology, Institute of Food and Agricultural Sciences, Gainesville, FL, USA*
Thaxtomins are a group of phytotoxic diketopiperazines produced by tens of plant pathogenic *Streptomyces* strains and have received considerable attention as bioherbicide. To synthesize thaxtomin analogue libraries for herbicide development, we here develop an *in vitro* one-pot biocombinatorial approach using four recombinant thaxtomin biosynthetic enzymes including two nonribosomal peptide synthetases TxtA and TxtB and two distinct P450s TxtE and TxtC. The combination of these enzymes led to the synthesis of 124 thaxtomin analogues with verified structures from unnatural amino acid building blocks. Of note, some unnatural thaxtomin analogues possessed potent herbicidal activities. Furthermore, we provided the detailed characterization of substrate requirement of TxtC that sequentially catalyzes both aliphatic and aromatic hydroxylation, a unique combination of P450 reactions. Our results revealed the importance of *N*-CH_3 of thaxtomin diketopiperazine core to TxtC and demonstrated the enzyme tolerance to modifications on the indole and phenyl moieties of its substrates. Importantly, we employed TxtC along with TxtA and TxtB or one promiscuous *N*-methyltransferase Amir_4628 from the actinobacterium *Actinosynnema mirum* to produce over 40 novel hydroxylated, methylated aromatic DKPs. These studies demonstrated the feasibility of *in vitro* synthetic biology approaches for the generation of natural product-like libraries covering broad and diverse chemical spaces.

1:40 PM S33: Metabolic engineering of *Escherichia coli* for direct fermentative production of lactate esters

J.W. Lee and C.T. Trinh, University of Tennessee, Knoxville, TN, USA*
Green organic solvents such as lactate esters have broad industrial applications and favorable environmental profiles. However, lactate esters are currently produced by esterification of lactic acid with alcohols in an eco-unfriendly condition using toxic catalysts such as sulfuric acid, hydrogen chloride, and/or phosphoric acid. Alternatively, a microbial platform can be harnessed to produce these esters from renewable feedstocks in an eco-friendly environment. In this study, we present a microbial platform for direct fermentative production of lactate esters from fermentable sugars. To enable the microbial biosynthesis of lactate esters, we first designed a pyruvate-to-lactate ester module that consists of a lactate dehydrogenase (*ldhA*) to convert pyruvate to lactate, a propionate CoA-transferase (*pct*) to convert lactate to lactyl CoA, and an alcohol acyltransferase (*AAT*) to condense lactyl-CoA and alcohol(s) to produce lactate ester(s). Next, we screened the efficient alcohol acyltransferase (AAT) for lactate ester production by generating and characterizing a library of five pyruvate-to-lactate ester modules carrying divergent AATs with an exogenous alcohol supply. Finally, we demonstrated for the first time the direct fermentative production of ethyl and isobutyl lactate esters from glucose by co-introducing a pyruvate-to-lactate ester module and an alcohol module into a modular *Escherichia coli* (chassis) cell. Further, with efforts in probing and alleviating the metabolic bottlenecks, we were able to identify that AAT is the most rate limiting step in biosynthesis of lactate esters and achieved 4.96-fold improved ethyl lactate production.

2:00 PM S34: Novel approaches to bio-production via inducible asymmetric cell division and programmable cell differentiation

N. Mushnikov, AsimicA LLC, Laramie, WY, USA*
Genetic engineering of microbial strains has enabled large-scale production of some biopharmaceuticals and other materials in bio-factories. However, high level of heterologous enzymes expression puts a burden on cellular metabolism, causing slower culture growth. Proper balance between growth and production rate is a key to increase product yields, but is hard to achieve in isogenic cultures. Differentiation of cells into multiple types may be beneficial for bioprocess optimization. For example, one type of cells can be completely devoted to the production, as “factory” cells, while another is free of production and is responsible for the culture regeneration. We have designed a genetic circuit to control differentiation of cells into different types, as an outcome of induced asymmetric cell division. The circuit involves a bacterial scaffolding protein that is stably maintained at a single cell pole. The protein scaffold was functionalized to degrade the signaling molecule c-di-GMP. By transiently regulating synthesis of the functionalized scaffold via small molecules or light, we can chemically or optogenetically control production of two distinct cell types characterized by either low or high c-di-GMP levels. We employed c-di-GMP downstream effectors to control differences in protein complex assembly or gene expression, which in turn produce differential cellular behavior or biosynthetic activities. Differentiation may also involve a mechanism to restrict a replication potential of factory cells, in order to reduce a chance of them to mutate, lose productivity and jeopardizing the whole bioprocess through outgrowing producers. Our strategy can also be used for improving the production rate of complex biosynthetic pathways. Programmable differentiation of cells can generate multiple cell types, responsible for separate biosynthetic pathway segments, thus, dividing the metabolic burden. It is advantageous comparing to co-culturing of several strains, each expressing partial set of biosynthetic enzymes, because it simplifies simultaneous culturing of different cell types, and facilitate facilitate maintenance of their ratios over time. Complex architecture of microbial communities used in bioprocess is an attractive tool to improve their efficiency.

2:20 PM S35: Engineering *E. coli* for methylotrophy – insights from ^{13}C-isotope tracing

J.R.G. Har, K. Bennett, J. Rohlhill, T. Papoutsakis and M. Antoniewicz, University of Delaware, Newark, DE, USA*
Methanol is an attractive substrate for fermentations due to its high energy content and the presence of large supplies of natural gas that can be cheaply converted to methanol. Different metabolic challenges must be overcome in engineering a purely methylotrophic *E. coli* – an engineered strain that can metabolize and grow exclusively on methanol (MeOH), a non-natural substrate. One key challenge that must be overcome in constructing a purely methylotrophic *E. coli* is to improve methanol

assimilation. We demonstrate that by employing ^{13}C-MeOH, we can comprehensively analyze the extent of MeOH assimilation into engineered methylotrophic *E. coli* strains that we have constructed. Across various engineered strains grown in minimal media supplemented with ^{13}C-MeOH (and a limited amount of yeast extract), we observe that while the MeOH carbon is well-assimilated into intracellular central carbon metabolites, it is not the case for amino acids (AAs). Additionally, only a small amount of AAs synthesized from MeOH is assimilated into the biomass.

In the present work, we have identified methods to improve the assimilation of MeOH carbon into biomass components, especially all proteinogenic amino acids. To this end, we have focused on identifying culture conditions that would impact the strain's ability to assimilate carbon from MeOH. Various media formulations that were supplemented with different combinations of AAs have improved the assimilation of MeOH into biomass components, especially proteinogenic AAs. Furthermore, these experiments have revealed regulatory mechanisms in *E. coli* that should be engineered to improve synthesis of biomass components from metabolites synthesized from methanol.

2:40 PM S36: Engineering chaperone network to improve yield of natural products in yeast biofactories

W. Nurani, DTU Biosustain (Novo Nordisk Foundation Center for Sustainability), Kgs. Lyngby, Denmark and U.H. Mortensen, DTU Bioengineering, Kgs. Lyngby, Denmark*

Normally, when it comes to production of non-native compounds in yeast biofactories, the most common strategies employed to improve yield include overexpression of genes necessary to produce the new product and blocking of seemingly competing native pathways. However, when the product is a natural product, the challenges are more complex. First, for polyketides and non-ribosomal peptides, the first enzymes in the pathway are often multi-domain megaenzymes consisting of > 2000 amino acid residues, and they may fold inefficiently in yeast. In turn, this may lead to intracellular stress and catalytically inactive aggregates. Second, the requirement to modify the precursor compound through oxidative chemistry might impose elevated oxidative stress that may also negatively impact productivity.

Chaperones are a set of proteins that act as protein folding catalysts. However, some of them are also involved in response to various cellular stresses. We hypothesize that engineering of chaperone networks in yeast biofactories not only allows for higher success rate in producing various heterologous synthases, but also improves tolerance of the cells to oxidative stresses generated during the biosynthesis. We tested this hypothesis by first generating a library of chaperones of varying doses and combination. We then employed a high-throughput method to create combinations of strains expressing both the model synthase and the chaperone combination in question. The success of the engineering is measured through improvement of specific product yield. Here we are reporting outcomes of the study using three fungal natural products as the models.

1:00 PM - 4:30 PM Session: 10: Automation, Modeling & Machine Learning in Metabolic Engineering

Conveners: Peter L. Lee[1];**Dr. Benjamin Kaufmann-Malaga**[2] and **Dr. Amoolya Singh**[2], (1)Transcriptic Inc., Menlo Park, CA, USA(2)Amyris, Inc, Emeryville, CA, USA

Marshall Ballroom North, Mezzanine

1:00 PM S55: Automating Bioengineering - First the Hands, Then the Head

B. Kaufmann-Malaga, Amyris, Inc, Emeryville, CA, USA*

Until 2015 Amyris primarily aimed to replace the "hands" in the lab, using automation & computing to perform routine calculations and liquid manipulations for the reproducible construction of engineered microbes. Since then we have focused on replacing the "head" in the lab by implementing a suite of computational algorithms to design metabolic pathways, direct their synthesis and phenotyping, learn from the data, and iterate on the design for improved target molecule production. In this presentation I describe our progress on joining the hands to the head by attempting to create discrete strains that produce 450 novel molecules in 24 months.

1:30 PM S56: Culture Biosciences: high-throughput, automated bioreactor infrastructure for the biotechnology industry

W. Patrick, Culture Biosciences, Inc, SOUTH SAN FRANCISCO, CA, USA*

Culture Biosciences develops high-throughput, automated benchtop bioreactors and then runs them as a service for the biotechnology industry. Culture Biosciences was founded in 2016 and now operates a high-throughput fermentation facility with over 100 bioreactors. Culture Biosciences' technology development has three key goals. First, Culture Biosciences enables fermentations scientists to run tens to hundreds of bioreactor experiments in Culture's laboratory, enabling broader access to high-throughput bioreactor experiments. Second, Culture Biosciences develops new bioreactor hardware, software, and automation tools that enable rapid bioreactor facility scale-out and efficient operation. Finally, Culture Biosciences designs cloud-based software tools that enable customers to plan and visualize high-throughput benchtop bioreactor experiments. In this

talk, Will Patrick, Culture Biosciences' CEO and Co-Founder, will give an overview of the company's business strategy and company history, discuss current and planned technology development, and talk about computational opportunities to mine high-throughput fermentation data sets for new insights.

2:00 PM S57: Data-driven steady-state programming in genetic networks

E. Yeung[*]*, A. Hasnain and N. Boddupalli, University of California Santa Barbara, Santa Barbara, CA, USA*

A central challenge in industrial microbiological applications is engineering of genetic networks to achieve a target yield or steady state concentration. This is a particularly challenging problem when optimizing under novel reaction conditions where canonical models of metabolic pathways are no longer valid. In these scenarios, the biological systems are entirely represented by data for which no direct methods exist to optimize the reaction output. We introduce a deep learning model discovery approach that leverages Koopman operator theory and time-series expression measurements to discover models that predict untested reaction outcomes. We introduce the deep Koopman controllability gramian, an empirical construct that allows us to estimate the controllability of a given biochemical state. Spectral analysis of these gramian models allows us to determine the reachable states of the system that maximize the reaction yield subject to the constraints imposed by novel reaction conditions. We illustrate this framework on a simulated metabolic network. We show how machine learning and Koopman operator theory can thus be used program the steady state of a genetic network in a data-driven context.

2:30 PM Break

3:00 PM S58: Context and Connection: Preparing Data for Maximum Analytical Return

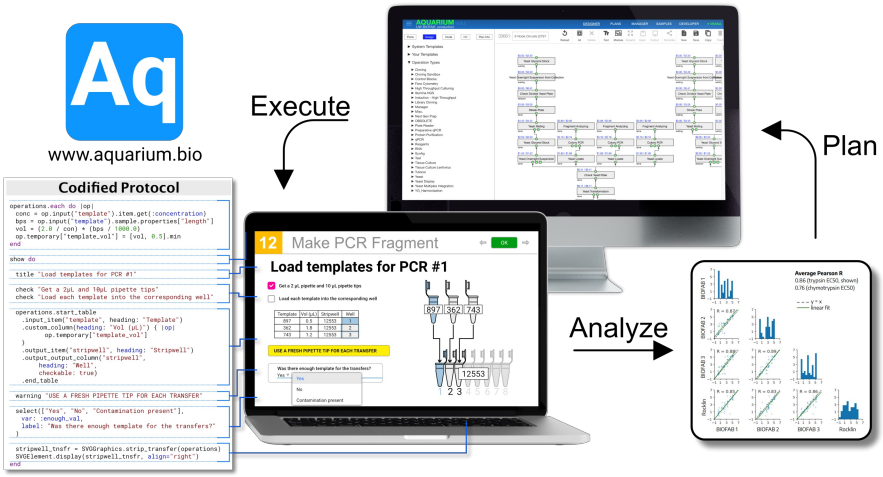
L. Perelman[*]*, Riffyn, Oakland, CA, USA*

Industrial microbiology breakthroughs live in a complex matrix of multivariate data sets across scientific disciplines that evolve with time. Making meaningful discoveries requires breaking down barriers between silos, pulling data from uninterpretable spreadsheets while giving it context, and creating a holistic statistical data framework. Riffyn, a cloud-based solution, links experimental designs and measurement data for analysis within moments post-collection. This talk will discuss examples of how bioprocessing groups use these capabilities to integrate data, identify unexpected correlations, uncover root causes of error, improve process quality, and deliver right-first-time technology scale-up and technology transfer.

3:30 PM S59: Aquarium: a laboratory operating system for reproducible experimental design and execution

J. Vrana[*]*, D. Strickland, B. Keller, E. Lopez, S. Halabiya, C. Corday and E. Klavins, UW-Seattle, Seattle, WA, USA*

Industrial scale engineering of organisms is becoming increasingly data-driven, often requiring thousands of genetic variants. To operate at large scales many labs employ robotics and instrumentation automation that perform assembly-line type workflows on well-validated techniques. However, many biological engineering projects stagnant during the exploratory phase when design parameters are unknown and failure rates are high. Because of this, many experiments are still performed by hand in a time-consuming, expensive, and unstandardized way. To address unique challenges posed by automating exploratory research, we present Aquarium, a open-source human-in-the-loop laboratory automation system that enables rapid, flexible, and reproducible workflow development and execution. Unlike most other automation systems, Aquarium embraces the practicality of humans in the laboratory, using codified human-readable instructions to automate lab operations. Using our Python API, we have coupled Aquarium with other software tools to enable automated planning and management of multi-week experimental workflows. We are currently using our software systems as part of a design-build-test loop to engineer highly-stable computationally designed proteins and have experimentally measured ~300 such proteins using our automated yeast surface display assay. Recently, we have also deployed machine-learning to automate *S. cerevisiae* strain construction and are currently using the software to engineer new cell behaviors in yeast using CRISPR dCas9 gene circuits. Overall, our software provides an interface for algorithms and computerized-agents to interact the scientific laboratory, enticing the possibility of engineering systems that can not only learn from experimental data, but can actually schedule and run its own experiments with little human intervention.



4:00 PM S60: Hallucinating Proteins with Generative Models

Z. Costello[*]*, Lawrence Berkeley National Laboratory, Emeryville, CA, USA*

Understanding the relationship between protein sequence and function is one of the fundamental challenges in biology. As a result of our inability to predict how changes in sequence affect protein phenotype, metabolic engineering has been largely focused on finding natural enzyme homologs and balancing gene expression to improve titer, rate, and yield of valuable bioproducts. However, abundant sequence data and rapid development of unsupervised machine learning present a timely and significant opportunity.

Recently, deep generative models have produced impressive results allowing for the generation of seemingly authentic examples of human faces, music, and text. With approximately 140 million protein sequences available on the UniProt database, similar techniques can be applied to great effect to generate novel proteins. Protein sequences are blueprints for powerful nanomachines capable of a diverse array of functions including catalysis, mechanical transduction, and sensing. Therefore, generating valid protein sequences could be even more impactful than creating sound or images.

I will present a novel approach to protein design and phenotypic inference using a generative model for protein sequences. This model, a variational autoencoder variant, can hallucinate syntactically valid protein sequences that are likely to fold and function.

1:00 PM - 4:30 PM Session: 7: Advances in Biocatalysts for Lignocellulose Conversion Sponsored by Novozymes

Conveners: Elena Y. Vlasenko, Novozymes, Inc., Davis, CA, USA

Madison B, Mezzanine

1:00 PM S37: The role of lignin modifying enzymes in deacetylation and mechanical refining of corn stover

J.M. Yarbrough[*]*, V. Subramanian, J.G. Linger, T.A. Vander Wall, T. Vinzant, X. Chen, M.P. Tucker, M.E. Himmel and S.R. Decker, National Renewable Energy Laboratory, Golden, CO, USA*

Alkali-catalyzed Deacetylation followed by Mechanical Refining (DMR) of corn stover has potential as a pretreatment process to enable the production of value-added products such as biofuels from biomass. However, the economic viability of this process requires glucan and xylan conversions after enzymatic hydrolysis of DMR treated corn stover to exceed 85% of theoretical limits. A primary obstacle to achieving this level of conversion is the presence of lignin which restricts cellulase and hemicellulase access to the structural polysaccharides remaining in pretreated biomass. Lignin modifying enzymes (LMEs) are a promising tool in lignin depolymerization and modification. Here, we demonstrate the utility of LMEs to improve cellulose and hemicellulose conversion rates and extents of DMR corn stover. In this study, we explore several commercially available LME's and introduce them during various steps of the DMR process to determine at which steps the LME's have the most impact on the improvement of cellulose conversion rates and extents using commercial cellulases and hemicellulase.

1:30 PM S38: Novel, multidomain glycoside hydrolases from the extremely thermophilic *Caldicellulosiruptor*: physiological and biochemical perspectives

J. Crosby[*]*, J.M. Conway, T. Laemthong and R.M. Kelly, North Carolina State University, Raleigh, NC, USA*

Extremely thermophilic bacteria in the genus *Caldicellulosiruptor* utilize the carbohydrate content of lignocellulose as a carbon and energy source. Some, but not all, species are capable of microcrystalline cellulose hydrolysis, while all species can degrade hemicelluloses. A number of novel, multi-domain glycoside hydrolases (GH) are localized to the secretome or S-layer

and act synergistically to recruit C5 and C6 sugars, ultimately for fermentation to organic acids, $H_2$and CO_2. The GH inventory varies across the genus, but key to the most cellulosic species is the Glucan Degradation Locus (GDL) identifiable in *Caldicellulosiruptor* genomes that encodes up to six GHs, not all of which are essential for cellulose hydrolysis. *In vivo*and *in vitro* studies have shed considerable light on how *Caldicellulosiruptor*species deploy GHs and how their natural secretome compares to optimized enzyme cocktails for complex polysaccharide hydrolysis. Discussed here will be recent developments along these lines and the potential for *Caldicellulosiruptor* as a metabolic engineering platform for biotechnological applications that involve renewable feedstocks.

2:00 PM S39: Pectic polymers – bioactive glycans for improved growth and saccharification of bioenergy feedstock

A. Biswal, M. Atmodjo, S. Mohanty and D. Ryno, The University of Georgia, Athens, GA, USA; M. Li, Oak Ridge National Lab (ORNL), Oak Ridge, TN, USA; C. Yoo, State University of New York College of Environmental Science and Forestry, Syracuse, NY, USA; Y. Pu, Oak Ridge National Laboratory, Oak Ridge, TN, USA; A. Tolbert, Georgia Institute of Technology, Atlanta, GA, USA; A. Ragauskas, The University of Tennessee - Knoxville, and Oak Ridge National Laboratory, Knoxville, TN, USA; D. Mohnen, University of Georgia, Athens, GA, USA*

The recalcitrance of plant biomass to enzymatic and/or microbial deconstruction of the cell wall polymers in bioenergy feedstocks is a major challenge for the use of this abundant carbon resource for generation of biofuels. Plant cell walls are rich in cellulose, xylan, lignin, and pectin. The structural diversity of these polymers, their architectural arrangement in the wall, and the degree of their covalent and non-covalent cross-linking and interactions contribute to their recalcitrance to bioconversion into ethanol, other chemicals, fuels, and bio-based materials. It is therefore crucial that we gain an understanding of the structural complexity and interactions between the different cell wall polymers to support the engineering of less recalcitrant bioenergy feedstocks with improved functional properties. The pectic polysaccharides are the most structurally complex of the plant cell wall glycans, consisting of the polysaccharides homogalacturonan (HG) and rhamnogalacturonan I (RGI) and II (RGII). Here we show that genetic manipulation of a pectin biosynthetic gene (*Galacturonosyltransferase 4*, *GAUT4*) by RNA silencing in poplar, switchgrass and rice results in up to 49%-190% increased dry biomass and 7-17% increased total sugar release. Importantly, this trait was maintained in a 3-year field-trial study (Biswal et al. 2018 *Nature Biotechnology,* 36: 249-257). Mechanistic studies show that downregulation of *GAUT4* reduces cell wall HG and rhamnogalacturonan II (RGII) content and their associated polymer crosslinking. More recently, we have shown that the transgenic switchgrass also has a lower abundance of ferulate and lignin-carbohydrate complex cross-linkages than controls, and a greater coalescence and migration of lignin after hydrothermal pretreatment (*Li et al. 2018 Communication Biology, 2:22*). These results indicate that the modification of a relatively minor component in plant cell walls, pectin, can lead to a simultaneous improvement in cell wall deconstruction and greater plant growth and saccharification yield. This project was funded by the Center for Bioenergy Innovation in the Department of Energy's Office of Science.

2:30 PM Break

3:00 PM S40: Mining marine and terrestrial environments for new enzymes to overcome obstacles in lignocellulose valorization

N.C. Bruce, University of York, York, United Kingdom*

The long-term success of biorefining is dependent on the development of effective low-cost enzyme cocktails for processing plant biomass to exploit the energy rich polysaccharides and the aryl-aromatic polymer lignin in plant cell walls for fermentation and conversion into commodity chemicals, respectively. One of the major challenge in identifying new enzymes involved in lignocellulose digestion in the natural environment for use as biocatalysts lies in the complexity of the process itself.

We have focused our efforts on discovering new lignocellulose degrading enzymes and associated proteins using a multi 'omics approach, combining the power of extracellular proteomics and transcriptomics, to identify proteins critical for lignocellulose deconstruction from microbial communities and animals obtained from marine and terrestrial environments. This approach is allowing us to identify novel enzymes, both broadening our fundamental understanding of this process, as well as providing new activities for the generation of renewable fuels from biomass-derived sugars but also for the production of chemicals from lignin.

3:30 PM S41: General relationship between binding strength and surface attack for fungal cellulases

T. Sørensen, Novozymes A/S, Protein Diversity, Copenhagen, Denmark and P. Westh, DTU BIOENGINEERING, 2800 Kgs. Lyngby, Denmark*

We have characterized several fungal cellulases from different Glycoside Hydrolase (GH) families with respect to kinetic- and adsorption parameters. The substrate was pure cellulose (Avicel). The enzymes represented a wide range of hydrolytic cellulases. Despite this variability among the investigated enzymes, we found distinct interdependences between functional parameters. For example, the turnover number (k_{cat}) scaled inversely with substrate binding strength regardless of GH family and catalytic mechanism. Furthermore, the density of attack sites that a given enzyme could recognize on the substrate surface increased commensurate with the substrate binding strength throughout the dataset. These relationships of binding strength

functional parameters are discussed along the lines of the century-old Sabatier principle for interfacial catalysis. We propose that the observed scaling relationships reflect limited functional plasticity of cellulases and discuss consequences of this for the design of enzymes for technical applications.

4:00 PM S42: Leveraging the oxidative power of 'LPMOs' in enzymatic processing of lignocellulosic biomass: Principles, advances and perspectives

A. Várnai[*]*, Norwegian University of Life Sciences, Ås, Norway*

The discovery of lytic polysaccharide monooxygenases (LPMOs) has revolutionized enzymatic processing of lignocellulosic biomass to fuels and chemicals [1-2]. With unprecedented catalytic chemistry, LPMOs depolymerize polysaccharides oxidatively, using a single copper as only co-factor. To date, LPMOs are part of modern commercial cellulase cocktails for biomass processing, boosting saccharification of biomass by hydrolytic enzymes. While being highly valuable in industrial bioprocessing, the powerful oxidative chemistry LPMOs catalyze generates considerable challenges in terms of scientific understanding and optimal implementation. One issue of particular interest relates to the catalytic mechanism of LPMOs and the fact that both molecular oxygen and hydrogen peroxide can drive LPMO action on lignocellulosic biomass [3-4]. In my talk, I will review recent insights into the catalytic mechanism and oxidative inactivation of LPMOs. Then, I will give examples from recent, applied studies that address the impact of process conditions on LPMO performance. Finally, I will discuss opportunities how the current knowledge on the catalytic mechanism can be used to optimize the way we harness the potential of LPMOs in biomass processing today.

References:

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2. Chylenski P et al. (2019) Lytic polysaccharide monooxygenases in enzymatic processing of lignocellulosic biomass. *ACS Catal* 9:4970-4991.
3. Walton PH & Davies GJ (2016) On the catalytic mechanisms of lytic polysaccharide monooxygenases. *Curr Opin Chem Biol* 31:195-207.
4. Bissaro B et al. (2017) Oxidative cleavage of polysaccharides by monocopper enzymes depends on H_2O_2. *Nat Chem Biol* 13:1123-1128.

1:00 PM - 4:30 PM Session: 8: Advances in fermentation of microbial communities/mixed cultures

Conveners: Michelle O'Malley and **Sean Gilmore**

Marshall Ballroom Southeast, Mezzanine

1:00 PM S43: Cultivating microbiomes to understand the deconstruction of plant biomass

S.W. Singer[*]*, Joint BioEnergy Institute, Emeryville, CA, USA*

In natural systems, plant biomass is deconstructed by complex microbiomes that deploy powerful degradative systems to digest plant biomass. These microbiomes are central to human health, soil health and agricultural productivity. The complexity of these microbiomes complicates the assignment of specific enzymatic roles to individual microbial community members. Microbiomes with simplified community compositions obtained by cultivation have been identified as important systems to develop a mechanistic understanding of microbial community function that may be generalized to more complex microbiomes. In this talk, I will describe studies of cultivated microbiomes for biomass deconstruction that have been used to identify new cellulase enzymes, to elucidate new mechanisms for lignin deconstruction and to demonstrate the importance of strain variation in microbiome function.

1:30 PM S44: Engineering modular microbial communities for cellulosic biomass degradation and high-value chemical production

C.H. Collins[*]*, Rensselaer Polytechnic Institute, Troy, NY, USA*

Lignocellulosic biomass is an abundant waste material that can serve as an ideal carbon source to generate high-value bioproducts from microbial systems. Biochemical approaches to biomass degradation rely on expensive enzyme purification steps to produce the required cellulases. In addition, the hydrolysis of lignocellulose yields mixtures of glucose and xylose, which are inefficiently used in combination due to carbon catabolite repression. To address these challenges, we have engineered synthetic communities to streamline both cellulose degradation and sugar co-utilization. Our division of labor approach involves the engineering of specialized co-culture modules, with the first module dedicated to cellulose degradation and the second module to bioproduct synthesis. To construct the cellulose degradation module, EGI1, an endoglucanase, and Cel9AT, a multimodular cellulase, were targeted for secretion from *B. megaterium*. A small library of signal peptides (SPs) and

linkers was screened for secretion. The best SP constructs were identified for the two cellulases and activity against amorphous cellulose was confirmed. We observed synergistic cellulolytic degradation from mixtures of the two enzymes and, importantly, from cocultures of the two cellulose secreting strains. To enable co-utilization of glucose and xylose, we built and characterized microbial co-cultures of *E. coli* strains express the pathway for the production of a target biomolecule and are specialists for either glucose or xylose consumption. We have identified co-culture conditions that result in higher biomolecule production than either strain in mono-culture. Future efforts will combine the degradation and biosynthesis modules to enable a platform for improved production of biomolecules from lignocellulosic biomass.

2:00 PM S45: *Rationally-Selected Microbiota*™ for Treatment of Dysbiosis Associated Diseases

J. Henske, Finch Therapeutics, Somerville, MA, USA*

Microbiota and their human hosts interact in extensive ways that can dictate health and disease throughout the body. The human gastrointestinal tract harbors a dense and diverse population of microbes that serve a wide variety of functions involved in facilitating metabolism and regulating physiology of the host. Diseases associated with microbial dysbiosis, such as recurrent Clostridium difficile infections (rCDI) and inflammatory bowel disease (IBD), enable treatments to mediate patient health by specifically targeting the microbiome composition. For diseases such as rCDI, fecal microbiota transplants have shown a high rate of success in a donor-independent manner suggesting that a *Full-Spectrum Microbiota*® treatment is applicable. However, successful treatment of IBD can be donor-dependent, suggesting that success of the treatment is tied to the presence of specific components in that donor material. Finch Therapeutics uses a data-driven human first approach to identify bacteria using cross-sectional datasets to identify bacteria specifically associated with healthy populations and interventional datasets to identify efficacious strains associated with successful treatment of disease. These beneficial bacteria are then isolated using targeted approaches to enrich and culture them from human stool samples. Isolated strains are characterized based on mechanisms associated with the disease. In the case of IBD, short chain fatty acids represent one well known mechanism of disease mediation. SCFA production profiles of isolated bacteria can be determined such that strains with favorable profiles are selected for inclusion in a *Rationally-Selected Microbiota* (*RSM*™) treatment. In this manner, treatments for dysbiosis associated diseases can be designed by targeting mechanisms that will improve patient health.

2:30 PM Break

3:00 PM S46: Desiccation resistant Pseudomonads isolated by directed evolution in microplate format for biological control of potato diseases

P.J. Slininger, D.A. Schisler and M.A. Shea-Andersh, National Center for Agricultural Utilization Research, Peoria, IL, USA*

Thiabendazole (TBZ) fungicide, traditionally used to control post harvest diseases of table stock potatoes, is now of little use because of genetic resistance developed by causative pathogens. Additionally, the most common sprout inhibitor on the market CIPC (chlorpropham) is facing stricter regulation in many countries due to public health and safety concerns. Three beneficial bacteria, originally found in potato field soils, have been shown to grow together in liquid co-culture in a variety of culture media (including lignocellulosic hydrolyzates) and to be biological alternatives to chemicals to control late blight, pink rot, and dry rot diseases, as well as to inhibit sprouting. However, numerous stress factors associated with large scale production are known to reduce biological control agent performance. To improve these strains for economical commercial use, a targeted evolution process was applied in an automatable micro-plate format to directly select for variants of parent strain populations able to thrive despite various hurdles imposed by economical manufacturing, including growth on a low cost culture medium, survival of a rapid air drying process, survival of extended dry storage at room temperature, and rapid growth recovery from a dry state by rehydration on a minimal nutrient solution. Using this approach, biological control strains were improved in their tolerance to conditions of manufacturing, especially in their resilience to drying and extended storage. These findings can potentially impact the potato industry by adding key technology needed to successfully manufacture and apply this new biological control strategy to protect potatoes in storage as an alternative to chemical tools which have lost efficacy.

3:30 PM S47: Preservation by vaporization enables long-term stabilization of non-sporulating, anaerobic microorganisms for scalable deployment of live microbiome products

S. Gilmore, R. La, C. Dodge and M. Embree, Ascus Biosciences, San Diego, CA, USA; V. Bronshtein, Universal Stabilization Technologies, Inc., San Diego, CA, USA*

The microbiome has a direct impact on host health and performance through conversion of feed and production of nutrients. Ascus Biosciences identifies key microbes native to the host microbiome that help to facilitate increased performance in areas like milk production in dairy cows or feed efficiency in beef cattle. In order to distribute these microbes and deliver at biologically-relevant doses, efficient and scalable methods are needed to preserve them, so that they retain viability when delivered into the animal. Preservation by vaporization is a foam drying process, alternative to lyophilization, which results in high viability through drying, formulation, and distribution. By immobilizing microbes within a sugar glass matrix, cell processes involving diffusion and reaction occur at much longer time scales, essentially suspending all cell activity. Consistent viability is achieved from small scale drying in vials through production scale drying in trays. Efficient preservation allows for deployment of microbes previously difficult to utilize at scale, such as non-sporulating and strict anaerobes, which allows for selection of the most effective

microbes for reintroduction into the host.

4:00 PM S48: Adhesion of Bacteria at Surfactant-Decorate Oil/Water Interfaces

N. Dewangan and J. Conrad, University of Houston, Houston, TX, USA*

Interactions between bacteria and oil/water interfaces underpin technologies in wastewater treatment, in bioremediation, and in droplet microfluidics for pathogen detection, antibiotic susceptibility, and biotechnological selection. How the presence of surfactants, used to stabilize droplets or alter their size and interfacial tension, influence adhesion remains incompletely understood. Here, I will describe experiments in which we use microfluidics and microscopy to characterize adhesion of bacteria at the interface of dispersed hydrocarbons. We use as model organisms several species of marine bacteria that are found near oil spills and can degrade hydrocarbons, including *Halomonas titanicae*, *Shewanella haliotis*, *Marinobacter hydrocarbonoclasticus*. Specifically, I will show that adhesion of non-motile *M. hydrocarbonoclasticus* depends on droplet radius and surfactant type and concentration, and can be modeled using Langmuir adsorption, and that (chemotactic) motility enhances the accumulation of bacteria on the interface. These results indicate that surfactants can strongly alter bacterial interactions with and accumulation on dispersed hydrocarbons.

1:00 PM - 4:30 PM Session: 9: Genome Editing & Genetic tools for Non-Model Microorganisms

Conveners: Carrie A. Eckert, National Renewable Energy Laboratory, Golden, CO, USA and **Dr. Rajib Saha**, University of Nebraska-Lincoln, Lincoln, NE, USA

Marshall Ballroom West, Mezzanine

1:00 PM S49: Genetic transformation and tool development for domestication of diverse non-model microbes

L. Riley, N. Wood, J. Huenemann, G. Peabody and A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA; J. Elmore, Pacific Northwest National Laboratory, Richland, WA, USA; J. Westpheling, University of Georgia, Athens, GA, USA*

Many non-model microbes have native phenotypic advantages over model organisms like *E. coli* and *S. cerevisiae*, such as the ability to catabolize polymeric biomass feedstocks, extreme tolerance to various stressors, or high flux through complex metabolic pathways. Unfortunately, these non-model microbes typically lack the genetic tools that would enable further modifications and metabolic engineering. Our pipeline for achieving transformation in diverse bacteria will be discussed, with a focus on libraries of genetic parts and rationally avoiding restriction-modification systems. This approach has enabled first-of-kind or increased efficiency of transformation in numerous phylogenetically diverse bacteria. Beyond development of initial transformation technologies, we have also developed high efficiency DNA integration tools using site-specific DNA recombinases. This system allows for rapid and stable insertion of DNA into the chromosome of the target host, enabling rapid screening of both genetic parts (e.g., promoters, terminators, reporter genes) and metabolic pathways. Because of the high efficiency, these recombinases also enable combinatorial libraries to be constructed in vivo for pathway optimization. Together, these approaches allow the rapid development of non-model microbes into bioengineering platforms.

1:30 PM S50: Development of an Emerging Model Microorganism *Megasphaera elsdenii* for the Conversion of Biomass and Organic Acids to Fuels and Chemicals

J. Westpheling, University of Georgia, Athens, GA, USA*

Development of an Emerging Model Microorganism *Megasphaera elsdenii* for the Conversion of Biomass and Organic Acids to Fuels and Chemicals

Lauren Riley[1], Neely Wood[1], Matthew Russo[2], Melissa Tumen-Velasquez[2], Adam Guss[1] and Janet Westpheling[2]

[1]Oak Ridge National Laboratory, Oak Ridge, Tn, [2]University of Georgia, Athens, GA

The metabolic diversity of microorganisms in nature is largely an untapped source of important compounds that are difficult or impossible to engineer in the few model systems available. The native ability to condense acetyl-CoA groups to efficiently generate C4 to C8 compounds makes *Megasphaera elsdenii* a compelling platform for the production of fuels and chemicals from lactate and plant carbohydrates. *M. elsdenii* produces organic acids as fermentation products when growing on lactate and glucose, including formation of butyric (four carbon), hexanoic (six carbon), and in some cases octanoic (eight carbon) acids as major fermentation products, likely via a chain elongation pathway using acetyl-CoA. Our focus is to engineer *M. elsdenii* to efficiently produce next-generation, drop-in lignocellulosic fuels such as hexanol at high yield and titer. As with most non-model organisms there are no methods of genetic analysis of any member of this genus. We have used metholome analysis to identify restriction systems that present a barrier to DNA transformation, cloned the cognate methytransferases into *E. coli* for plasmid preparation and successfully transformed both the ATCC type species as well as a related NCIMB strain at high frequency. The introduction of an *adhE* gene from *Clostridium acetobutylicum* resulted in detectable amounts of butanol from lactate.

Engineering of the strain to eliminate propionate production from lactate, a competing pathway, enhancing alcohol production and introducing pathways for xylose and arabinose utilization are in progress.

2:00 PM S51: CRISPR-assisted genome engineering in Lactococcus lactis

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

The lactic acid bacterium *Lactococcus lactis* has various industrial uses, most notably in dairy fermentation and lactic acid production. In addition, it has recently been engineered as a live biotherapeutic capable of delivering small molecules, peptides, and proteins *in situ* in the human gut. Complex phenotypes such as survival and proliferation in the GI tract; protein secretion; and thermal, acid and phage tolerance are often governed by genetic contributions that are uncharacterized or unknown. Thus, the time is right for the development of tools that enable high-throughput genome engineering in this organism to explore these phenotypes. Here we present a CRISPR-assisted technique to ensure scarless double-crossover-mediated insertion of a double-stranded DNA donor cassette. We have leveraged this technique to enable inducible protein expression in environmental isolate strains with interesting properties, e.g., xylose metabolism. We will further describe how our lab uses these techniques to endow *L. lactis* with industrially relevant phenotypes such as inducible protein expression, expanded carbon source usage, and production of high-value chemicals.

2:30 PM Break

3:00 PM S52: Systematic analysis on lignin degradation mechanisms and metabolic pathways in marine protist

Y. Zheng and X. Li, Kansas state university, Manhattan, KS, USA*

The conversion of lignin through biological platforms can upgrade lignin into high value-added products such as lipids, polyhydroxyalknoate and carotenoids. Based on lignin metabolism process, strategies such as genetic modification, protein engineering and metabolic engineering can either improve lignin conversion efficiency or establish novel metabolic pathways for production of various bioproducts. Marine protist has been proven to accumulate fatty acids and carotenoids with lignin as a sole carbon source. However, the mechanisms of lignin degradation and the specific metabolic pathways remain unknown. This study aims at investigating the mechanism of lignin degradation and construction of lignin degradation pathways. The black liquor from alkaline pretreatment of corn stover was used as a carbon source as it contains multiple aromatic compounds and short lignin fragments, which makes it a good candidate for the study of breakdown of lignin linkages, aromatic compound degradation, and interactions between different processes. An integration of omics analysis at different levels were applied during black liquor fermentation. Genomics analysis was conducted to identify genes in marine protist responsible for lignin degradation. Transcriptomics and proteomics analysis were used to explore gene expression pattern and identify critical enzymes involved in this process. The profile of metabolites from diverse metabolic process were be determined by metabolomics analysis. With all the results, the metabolic pathways of lignin degradation was constructed. This is the first study to investigate lignin degradation by marine protist the fundamental aspect. The results will bridge the knowledge gap on lignin degradation mechanisms and provide theoretical basis for the development of marine protist as a novel platform for lignin bioconversion.

3:30 PM S53: Metabolic Engineering of a Non-model Alga, Desmodesmus armatus, to Rewire Algal Carbon Energetics

L.M.L. Laurens, D. Douchi and E. Knoshaug, National Renewable Energy Laboratory, Golden, CO, USA*

Photosynthetic microbes such as algae, are promising candidates to contribute to the fuel and bioproduct portfolio of the future. Even though naturally efficient at photosynthesis and carbon capture, improvements along the fundamental growth paradigm can make dramatic impacts in the cost and sustainability parameters of the production value chain. A DOE-funded Rewiring Algal Carbon Energetics for Renewables (RACER) consortium focuses on addressing photosynthetic carbon conversion inefficiencies in a pathway from algae to a trifecta of fuel intermediates in a coordinated and integrated manner. A single, commercially-relevant algae species, Desmodesmus armatus (SE00107) is developed as a genetic chassis to build on to increase transitory carbon storage in the cells through targeted metabolic engineering. We have dedicated a concerted effort to the establishment of nuclear engineering tools and have built a well annotated and covered version of the D. armatus genome. Because of unique challenges associated with over-expressing metabolic targets in native pathways of carbon assimilation and thus no new product is created as evidence of successful engineering, we have initially used reporter fluorescence in parallel to ‘genes of interest’ to tailor promotor, terminator combinations and help ascertain robust expression. This presentation will highlight our approach to bring a demonstrated successful outdoor organism into the laboratory to achieve improvements targeted to the central carbon metabolism pathways.

4:00 PM S54: Host onboarding for Yarrowia lipolytica - trials and tribulations in the development of genomic and genetic engineering tools.

D. Gao, V. Ganesen, M. Spagnuolo, A. Beitz and M. Blenner, Clemson University, Clemson, SC, USA*

Oleaginous yeast have been successfully engineered to produce a wide variety of lipids, and oleochemicals. Their success is tied to the native high flux pathways producing malonyl-CoA and NADPH that serve as better starting points for metabolic engineering than *S. cerevisiae*. A rapidly improving understanding of *Y. lipolytica*genetics and metabolism, coupled with increasing tools to engineer *Y. lipolytica*have opened up myriad engineering opportunities. In this talk, we'll discuss the numerous promoters systems, including endogenous promoter, hybrid promoters, inducible promoters and tunable promoters. We characterize properties beyond the strength of the promoter and address dynamics and modularity in promoters. Another enabling technology we've developed is CRISPR-Cas9 gene editing for creating indels, targeted integration, gene excision, and simultaneous excision and integration. Last, we'll discuss the development and use of CRISPR-Cas9 genome wide screens.

3:30 PM - 4:30 PM Career Workshop Agenda: Resume review-Themes. Verbal-Engage audience, structure presentation. Visual-Use poster/slide to augment message. Handouts on resume preparation; 50 questions for a face to face interview. Sell yourself, Know your audience

Conveners: Sheena Becker, Corteva, Brownsburg, IN, USA;**Noel Fong**, Nucelis LLC, San Diego, CA, USA and **Bob Berger**, BB & Associates, Sarasota, FL, USA

Madison A, Mezzanine

4:30 PM - 5:30 PM General Session: Korean Society for Microbiology and Biotechnology (KMB) Lecture: Prof. Jeong Kug Lee, President, KMB

Marshall Ballroom Southeast, Mezzanine

5:30 PM - 7:30 PM Session: PS2: Poster Session 2/Exhibits open

Exhibit Hall C, Lower level

P2 Optimization of different organic amendments during the bioremediation of kerosene polluted soils using Box Behnken design

S.C. Onuoha, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria, Abakaliki, Nigeria and C.O. Onwosi, University of Nigeria, Nsukka, Nigeria*

Kerosene polluted sites are difficult to remediate because they are more viscous and have less biodegradable characteristics. However, the addition of organic nutrients, as a biodegradative measure, has received wide acknowledgement as an effective way of restoring soil fertility and promote plants' growth. The goal of this study was to evaluate the optimization of total petroleum hydrocarbon removal (TPH) removal and lipase activity by the addition of organic amendments (sawdust, SW; grass trimmings, GT; and poultry dropping, PD) using the Box Behnken Design (BBD) as well as the relationship between the independent variables. The result revealed a high significance of the model for TPH removal with R^2 value of 0.9776 while the R^2 value of the lipase activity was 0.9953, thus showing a highly significant model. The two responses were shown to follow a non linear pattern. According to the response optimizer, 170 g GT, 585 g SW and 40 g PD was revealed to be optimal for stimulating polluted soil indigenous microflora and hence enhance TPH removal and lipase activity.

P4 Lanthanide effects on methylotrophic metabolism and plant growth promotion

C. Friel and N.C. Martinez-Gomez, Michigan State University, East Lansing, MI, USA

Increasing agricultural productivity is vital due to climate change and population growth. Beneficial plant-microbe interactions are promising for increasing yields, but the biochemistry of these interactions is frequently poorly understood. One example is the interaction between plants and methylotrophs that colonize plant leaves. Rare earth elements called lanthanides were considered biologically inert, but were recently discovered to be enzymatic cofactors in the oxidation of methanol. We have recently shown that lanthanide biochemistry affects methylotrophic metabolism to promote plant growth. Here, we describe the methylotrophic community of soybean leaves. We isolated 500 strains and identified 22 methylotrophic strains with unusual metabolic functions. These strains have 99-100% identical 16S rRNA sequences but are able to grow on diverse carbon substrates including vanillic acid, fructose, and oxalate. 5 strains increased final yield 10-50% when grown with lanthanides, while 1 other strain decreased growth rate 50% and yield 40% when grown with lanthanides. These results indicate that the metabolic diversity of methylotrophs in the phyllosphere is poorly represented by 16S rRNA analysis and that lanthanide availability affects the metabolism of this community. Further, 4 novel strains previously grown on methanol and lanthanides were inoculated in the phyllosphere of soybean increasing plant yield by 18-21%. We are currently comparing the metabolomic and transcript profile of the methylotrophic community during plant growth when acclimated or not to lanthanides. The results of

this study will allow us to understand how lanthanides affect the metabolism of methylotrophic communities, and what bacterial traits are important for maximizing plant benefit.

P6 Marine cyanobacteria growth dynamics from an automated, in situ flow cytometer

K. Hunter-Cevera, Marine Biological Laboratory, Woods Hole, MA, USA; M. Neubert, R. Olson, A. Shalapyonok, A. Solow and H. Sosik, Woods Hole Oceanographic Institution, Woods Hole, MA, USA*

Understanding changes in plankton abundances requires observation at the timescales relevant to their physiology and ecology (hours-days) and for extended periods of time to separate sub-seasonal, seasonal, and interannual variability. From long-term deployments of a custom-built, automated, in situ flow cytometer (FlowCytobot), we have collected 16 years of high-resolution (hourly) observations of the marine cyanobacterium *Synechococcus* population on the New England Shelf. We use these data to characterize and explain the annual seasonal cycle of cell abundance, which ranges from a 10^2 to 10^5 cells per mL. With hourly observations of cell size, we are able to estimate an in situ, daily population division rate from a matrix model that represents diel changes in cell size. This method accurately estimates division rate of both cultured and wild *Synechococcus* populations. From these time series, we find that temperature limits division rate in winter and spring, but light limits division rate in fall. Back-calculated loss rates closely follow division rates throughout the entire year. Changing controls on division rate, coupled to small, but systematic, offsets between loss and division produce the large, seasonal abundance changes that we observe.

P8 The role of malleilactone, a *Burkholderia pseudomallei* cytotoxic polyketide, in bacterial iron acquisition

J. Klaus and J. Chandler, University of Kansas, Lawrence, KS, USA; A. Soldano and M. Rivera, Louisiana State University, Baton Rouge, LA, USA*

The two bacterial species *Burkholderia pseudomallei* and *Burkholderia thailandensis* are closely-related soil saprophytes. In addition, *B. pseudomallei* is the causative agent of the often-fatal human disease melioidosis. These two species encode almost a dozen predicted polyketide (PK) biosynthetic gene clusters, many of which are highly conserved. Many PK products are being recognized for their contributions to virulence and potential for therapeutic development; however, for many that are characterized, their functional role and survival benefits are generally not well understood. We are interested in the PK gene cluster involved in biosynthesis of malleilactone in *B. pseudomallei* and *B. thailandensis*. Our studies have focused on understanding the genetic regulation and biological function(s) of malleilactone. Previously, we showed that malleilactone is cytotoxic and contributes to the ability of *B. pseudomallei* to kill the soil nematode *Caenorhabditis elegans*. The conservation of this gene cluster *in B. thailandensis* suggests malleilactone is also important for survival in the environment. Here, we describe evidence linking malleilactone with iron acquisition. Iron is a required growth nutrient for bacteria, and it is of especially low bioavailability within eukaryotic hosts and microbial communities of the soil. Thus, malleilactone might be important for growth and survival in iron-limited conditions where these two species are commonly found. Our results show that malleilactone production is activated during growth in low iron, and that purified malleilactone coordinates with iron molecules. We also show that malleilactone is important for *B. pseudomallei* growth in iron-limited conditions, and that this effect requires production of two other siderophores, pyochelin and malleobactin. Altogether, our data support the idea that malleilactone is a previously unrecognized player in the important process of acquiring iron from limited-resource environments, and may function as a 'siderophore helper' in soil and host environments.

P10 *Geobacter sulfurreducens* requires the inner membrane *b*-type *c*-type cytochrome CbcBA for reduction of electron acceptors below -0.2 V vs SHE

K. Joshi, C.H. Chan and D. R. Bond, University of Minnesota Twin Cities, Saint Paul, MN, USA*

G. sulfurreducens can transfer electrons to many electron acceptors that cannot penetrate the cell membrane, such as chelated Fe(III), insoluble Fe(III)-oxyhydroxides, and poised electrodes. The ability of *G. sulfurreducens* to perform this extracellular electron transfer is useful in biotechnology applications such microbial fuel cells, bioelectrochemical desalination, and transformation of toxic metals. Despite this importance, key steps in this extracellular electron transfer pathway remain unknown. The *G. sulfurreducens* genome encodes multiple putative inner membrane quinone oxidoreductase-like cytochromes predicted to be involved in transfer of electrons out of the inner membrane quinone pool. Prior work found that the inner membrane *c*-type cytochrome ImcH is essential for reduction of electron acceptors above - 0.1 V vs SHE, while a *bc*-type cytochrome CbcL is required for electron transfer at redox potentials below -0.1 V vs SHE. Recent work found a previously uncharacterized *b*-type *c*-type cytochrome complex CbcBA is required for reduction of electron acceptors at redox potentials below -0.2 V vs SHE. As members of *Geobacteraceae* are often enriched on low potential microbial fuel cell anodes operated at a working redox potential <-0.2 V vs SHE, and the *cbcBA* genes are among the most conserved cytochromes across all *Geobacter* species, this new low potential system may represent a common trait among highly competitive *Geobacter* strains.

P12 The potential for fungal biotransformation of reactive amorphous silica as a means to beneficiate indigenous materials for improved performance.

K. Indest, C. Jung, C. Strack, K. Klaus, C. Weiss, K. Pokrzywinski and R. Moser, US Army Engineer Research & Development Center, Vicksburg, MS, USA*

Recent advancements in bio-enabled synthesis of materials and the use of bio-engineering approaches have tremendous potential for military benefit. Of specific interest to the Army is the design of biological systems to produce high performance materials and manipulate / beneficiate materials. The goal of this research is to develop a method for reduction of harmful expansion in concrete due to alkali-silica reaction (ASR) through fungal biotransformation of amorphous (i.e., reactive) silica to crystalline (i.e., non-reactive) silica, reducing is potential for deleterious reactivity in the alkaline environment within concrete. Five fungal strains of the *Fusarium* genus were evaluated for their ability to transform various silica containing substrates in liquid media over a 72 hour period. Based on data from preliminary SEM analysis, opal substrates were selected for further evaluation due to their high reactivity and amorphous structure. Opal thin sections and blocks were exposed to three fungi for one week and four weeks and transformation was determined visually implementing optical and electron microscopy. Textural changes were apparent within the samples, affecting the opal and suggesting potential changes within the crystalline matrix of the amorphous material. The research will enable new pathways to produce high performance materials and better utilize marginal indigenous materials in support of military operations both forward on the battlefield and fixed facilities and infrastructure on installations.

P14 Biological and synthetic platforms for cobalt biomineralization using microbial nanowires

M. Dulay, M. Tabares, K. Kashefi and G. Reguera, Michigan State University, East Lansing, MI, USA; K. Cosert, Georgia Institute of Technology, Atlanta, GA, USA*

Geobacter bacteria inhabit environments impacted by metal contamination and can be stimulated *in situ* to promote their extracellular reductive precipitation. Their ability to bind and reduce toxic metals requires the expression of protein nanowires (pili) that are decorated with specialized sites for metal binding and reduction. Computational models identified ligands in the nanowire traps for the coordination and reductive precipitation of divalent cobalt, a mechanism that could provide energy generation and protection from metal toxicity. Given the wide industrial use of cobalt and economic, socio-political and environmental costs associated with its mining and purification, we investigated the suitability of *Geobacter*-based platforms for cobalt immobilization. We first demonstrate the ability of the model representative *Geobacter sulfurreducens* to grow in the presence of concentrations of Co^{2+} previously considered to be too toxic to sustain microbial metabolism and the cell's ability to remove the soluble metal from solution, consistent with a mechanism involving the nanowires. In support of this, Co^{2+} was reductively precipitated as Co^{0} nanoparticles by recombinant pilins assembled on electrodes as monolayers, which concentrate the nanowire metal traps on its surface. The results provide evidence in support of a biological mechanism for the reduction of Co^{2+} via pilus nanowires and highlight material properties that can be harnessed to develop platforms for the bioremediation of Co^{2+} and the biological synthesis of cobalt nanoparticles.

P16 Identification of the dynamic otic microbiome in healthy adults

J.Y. Lee, K. Kashefi and G. Reguera, Michigan State University, East Lansing, MI, USA*

The physical isolation of the middle ear, essential for hearing, has been assumed to prevent the establishment of a natural microflora. Yet the otic mucosa extends into the naso- and oropharynx via the Eustachian tube, which is closed at rest but opens when we swallow to aerate the middle ear, depressurize the eardrum and drain excess mucus in the back of the throat. Because otic ventilation introduces microorganisms that could colonize the otic mucosa, we sequenced 16S-V4 rRNA amplicons from otic secretions and neighboring mucosae (oropharyngeal and buccal) of 19 healthy young adults, including 9 recreational divers trained in middle ear ventilation. The study identified a diverse and robust otic microbiome in healthy young adults comprised of mostly oropharyngeal and buccal genera. Yet in contrast with the communities from more aerated oral mucosae, the otic microbiome was dominated by obligate anaerobes (Bacteroidetes and Fusobacteria) and, to a lesser extent, facultative anaerobes in the Proteobacteria and Firmicutes. Furthermore, the structure of the otic community was responsive to episodic aeration as indicated by the lower relative abundance of anaerobic taxa in the diver's group. Despite these differences, the metabolic structure of the otic communities was similar to the oral microbiomes and suggestive of a role of otic microorganisms in mucosal health. These results challenge the long held view of a sterile middle ear mucosa and reveal instead a diverse otic community adapted to the redox fluctuations of the otic cavity and the host's mucosa.

P18 Environmental control of nitrate ammonifying microorganisms for sustainable management of nitrogen in soils

M. Tabares and G. Reguera, Michigan State University, East Lansing, MI, USA*

Sustainable management of nitrogen (N) in food, energy and water systems requires innovative solutions to retain nitrate fertilizers as ammonia, a process that nitrate ammonifying microorganisms carry out naturally (dissimilatory nitrate reduction to ammonia [DNRA]). *Geobacter lovleyi*-like organisms are key drivers of DNRA in nature, but the regulatory, signaling and metabolic networks behind these activities remain largely unknown. Here we show that low nitrate induces DNRA in *G. lovleyi* independently of the availability of electron donor, challenging the prevailing view that high carbon-to-nitrogen ratio is the main trigger of DNRA. The nitrate transcriptome revealed a complex metabolic network of periplasmic (Nap) and cytoplasmic (Nar) nitrate reductase systems for nitrite production. It also identified 3 *Geobacter*-specific nitrite reductases (NrfA) for nitrite ammonification and a membrane-bound NrfH cytochrome, which electronically connects NrfA to the menaquinone pool. Flagellar motility and chemotaxis proteins were also among the most upregulated genes in the nitrate cultures, consistent with an

adaptive response that allows cells to sense and access the limited supply of nitrate in anaerobic zones of the soils and sediments. These results highlight the role of nitrate limitation in the regulation of DNRA and the complex adaptive responses that nitrate ammonifiers undergo to access the limited supply of electron acceptor. Of special significance is the identification of molecular markers to monitor the activity of DNRA bacteria in nature and the regulatory networks that can be manipulated *in situ* to partition DNRA from denitrification and minimize N losses as greenhouse gases and leachates.

P20 Comparative proteomic and transcriptomic analyses of *Moraxella osloensis*, an alkylphenol polyethoxylate degrader, grown on pyruvate and *t*-octylphenol polyethoxylates

M. Ganzorig and K. Lee[*]*, Changwon National University, Changwon, Korea, Republic of (South)*

Alkylphenol polyethoxylates (APEOn), with varying lengths of ethoxyl units (n= 9~40), are massively used as non-ionic surfactants for emulsification and wetting agents of industrial products. The recalcitrant metabolites, alkylphenol and its mono- or diethoxylates, formed by bacteria degradation are demonstrated to play as an endocrine disruptor in experimental animal cell lines. The objective of this study is to identify the genes and enzymes involved in the bacterial degradation pathway, which has not been firmly established. Because APEOn and their intermediate metabolites consist of an array of various molecular weights, there are still unsolved questions on enzymes involved in the sequential cleavage reactions of the terminal ethoxylate group. One enzyme or different enzymes can catalyze the same reaction on different molecular weights. In this study, from human skins, we isolated several *M. osloensis* strains that could use APEOn such as *t*-octylphenol polyethoxylates (OPEOn) as a sole source of carbon and energy. Of the *M. osloensis* strains, strain TT16 is most resistance to OPEOn and can rapidly grow up to 5% OPEOn by accumulating low molecular weight *t*-octylphenol ethoxylates. The proteomic analysis result showed that especially dehydrogenases with varying molecular weights, autotransporter domain-containing protein, isocitrate lyase, 50S ribosomal protein L9, and cold-shock protein are highly expressed when OPEOn is used as sole carbon source. In addition, RNAseq results enabled quantification of gene expression and provided *in silico* modeling for OPEOn degradation by *M. osloensis*.

P22 Effect of Silver Oxide Nanoparticle on the sediment bacteria of Gulf of Mexico in Removing Carbon and Nitrogen

C. Oubre[*] *and R. Boopathy, Nicholls State University, Thibodaux, LA, USA*

Nanoparticle use in engineering, medicine, cosmetics, personal care products, and manufacturing is becoming more common. Nanoparticles are incredibly useful because of their ability to change the properties of the compound they are made of, but unfortunately have been reported to be toxic to microbes including bacteria. Because nanoparticles are becoming commonplace, it is likely that they are being disposed of improperly and will inevitably end up in the coastal waters of South Louisiana because of drainage of Mississippi watershed into this coastal ecosystem. Nanoparticles in coastal waters should have an impact on the bacteria that play a key role in biogeochemical cycles such as carbon, nitrogen, and phosphorous cycles, but to our knowledge no study on this subject has been reported.

In this study, sediment samples were collected from Grand Isle and Cocodrie of coastal Louisiana and were enriched for common heterotrophic bacteria that carry out carbon and nitrogen cycles in coastal waters. These enriched bacteria were exposed to various concentrations of silver oxide nanoparticle. The results showed that the nanoparticle at concentrations 10 mg/L or above were lethal to the bacteria and the bacterial growth was inhibited resulting in no loss of carbon and nitrogen from the media. Concentration of silver oxide nanoparticle lower than 2 mg/L were able to be tolerated by the bacteria with little effect on the growth and carbon and nitrogen cycles. The lethal concentration 50 (LC50) of silver oxide for the coastal bacteria will be studied and the affect that silver oxide nanoparticles have on the carbon and nitrogen cycle in the natural water systems will be reported in this paper.

P24 Microbial bioconversion of thermally depolymerized polypropylene by *Yarrowia lipolytica* for fatty acid production

M. Mihreteab[*]*, B. Stubblefield and E. Gilbert, Georgia State University, Atlanta, GA, USA*

Plastic production and waste generation will continue to rise as nations worldwide grow economically. In this work, we detail a pyrolysis-based bioconversion process for polypropylene (PP) to produce value-added fatty acids (FAs). PP pellets were depolymerized by pyrolysis, generating an oil that consisted of mainly branched chain fatty alcohols and alkenes. The oil was mixed with biodegradable surfactants and trace nutrients and mechanically homogenized. The resulting medium, OP4, was used for fermentation by *Yarrowia lipolytica* strain 78-003. *Y. lipolytica* assimilated > 80% of the substrate over 312 h, including 86% of the fatty alcohols. *Y. lipolytica* produced up to 492 mg L^{-1} lipids, compared to 216 mg L^{-1} during growth in a surfactant-only control medium. C-18 compounds, including oleic acid, linoleic acid, and stearic acid were the predominant products, followed by C-16 compounds palmitic and palmitoleic acids. Two percent of the products were C-20 compounds. The majority of the products were unsaturated FAs. Growth on hydrophobic substrates (OP4 medium, hexadecane) was compared with growth on hydrophilic substrates (glucose, starch). The resulting FA profiles revealed an absence of short-chain fatty acids during growth on hydrophobic media, findings consistent with *ex novo* FA biosynthesis. Overall, FA profiles by *Y. lipolytica* during growth in OP4 medium were similar to FA profiles while growing on natural substrates. The process described here can help

reduce the amount of plastic waste entering the environment.

P26 Sustainability considerations in feedstock chemistry improvement efforts: research insights from studies of *Populus*- beneficial microbe interactions

A. Matthiadis, M. Clark, A. Veach, S. Jawdy, N.L. Engle, J. Labbe, C. Schadt, D. Pelletier, T.J. Tschaplinski and U. Kalluri, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

The goal of the present research is to understand the implications of *Populus* cell wall chemistry variation on interactions with beneficial microbes, both bacterial and fungal isolates. We are taking a multipronged approach that spans from micro-scale to field-scale, under two studies, to understand the core and divergent components of the complexity in plant-microbe interactions. The first study seeks to understand the molecular, biochemical and physiological processes underlying host specificity, in tissue culture as well as under field settings. The goal of this larger Plant-Microbe Interfaces (PMI-SFA) project is to understand the genome-dependent molecular and cellular events involved in establishing and maintaining beneficial interactions between plants and microbes. The second study seeks to understand effects of longer-term co-culture on aboveground growth and biomass properties in greenhouse settings. The goal of this latter Center for Bioenergy Innovation (CBI) project is to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain. Results obtained from co-culture experiments using host cell wall variants comprised of transgenic as well as naturally occurring variants of *Populus* and beneficial microbes (single or mixed microbe cultures of bacterial and fungal isolates) showed that the functional outcomes of plant-microbe interaction are strongly host genotype-dependent. Furthermore, our results show that changes in host secondary metabolome composition has a greater implication on interactions with beneficial microbes than changes in host cell wall chemistry alone.

P28 Increased concentration of diesel in soil has varying impacts on different soil biological activities

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In this study, soils polluted with varying concentrations of diesel (IC 1, IC 2, IC 3) at initial TPH concentrations of 14785.84 mg/kg, 23859.52 mg/kg and 42134.96 mg/kg, respectively, were bioremediated using rice husk as biostimulant. Different soil biological parameters namely soil enzyme activities (catalase and β-glucosidase), soil microbial biomass carbon, nitrogen and phosphorus, soil microbial respiration as well as the soil phytotoxicity were used to monitor the bioremediation process. At the end of 56-day study, the degradation rate for IC 1, IC 2 and IC 3 were recorded at 99.1%, 98% and 97.6% respectively. The nth-order kinetic equations were used in determining the efficiency of the treatment for the increasing concentrations of diesel polluted soils. The results of nth order kinetics were: IC 1 (R^2= 0.9492, k= 4.472 d^{-1}, n= 0.7919); IC 2 (R^2= 0.9394, k= 6.239 d^{-1}, n= 0.7517); IC 3 (R^2= 0.9882, k= 0.0028 d^{-1}, n= 0.3082). From the results, it could be deduced that IC 1 and IC 2 followed first order degradation pattern while the IC 3 followed zero kinetic order. All biological activities for IC 1 except microbial biomass nitrogen were most responsive to the rice husk treatment than those of the IC 2 and IC 3. Improved plant growth was also observed in IC 1 and IC 2, as compared to IC 3, towards the end of the bioremediation study. These outcomes showed that the use of biological parameters is indispensible in monitoring the efficacy of a bioremediation process on contaminated soil.

P30 Engineering Bacterial Biofilms for Environmental Biotechnological Applications

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Biofilm represents the prevalent mode of microbial growth in natural, engineered, and medical settings. Advancement in biofilm research in recent years has enabled designing and improving biofilm bioprocesses, harnessing the power of beneficial biofilms and combating detrimental biofilms, for desirable performance in various industrial and environmental applications. Here, we describe various strategies to engineer biofilms by manipulating potential biofilm targets. The matrix-targeted approach has demonstrated engineered biofilms which surface displayed functional proteins in the biofilm matrices. Specifically, we displayed a redox sensitive fluorescence protein roGFP onto the cell surface of *Shewanella oneidensis* by fusing it to the C-terminus of BpfA, a large surface protein, to quantify the extracellular redox status in the matrix of *S. oneidensis* biofilms. This study exhibits a non-invasive approach to monitor extracellular redox status in microenvironments within biofilms, which can be used to understand redox responses of biofilms to environmental perturbations. The c-di-GMP–targeted optogenetic approaches have demonstrated controllable biofilm development by modulating the intracellular c-di-GMP level. Firstly, we constructed a synthetic c-di-GMP gene circuit in *S. oneidensis* that responds to the near infrared (NIR) light and demonstrated in a bioelectrochemical device the possibility of controlling biofilm development using light. Furthermore, we engineered a light-responsive, quorum quenching (QQ) bacterial biofilm whose growth and dispersal can be modulated by light through a dichromatic, optogenetic c-di-GMP gene circuit in which the bacterial cells sense NIR light and blue light to tune its biofilm formation by regulating the c-di-GMP level. We also demonstrated the potential application of the engineered light-responsive QQ biofilm in mitigating biofouling of water purification forward osmosis membranes.

P32 Biodegradation of metribuzin, an herbicide used in the sugarcane farms in Louisiana

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Metribuzin (4-amino-6-tert-butyl-3-(methylithio)-1,2,4-triazin-5(4H)-one) is a photosystem II inhibiting herbicide currently being used as a substitute for atrazine. Metribuzin is a triazinone class herbicide and known endocrine toxin like atrazine though approximately 1% as toxic. Biodegradation of metribuzin by bacteria from the soil of the USDA farm in Houma, LA, which has been exposed to metribuzin for a few years, will be evaluated under aerobic and anaerobic conditions. Anaerobic conditions will include fermentative, nitrate reductive, sulfate reductive, and mixed reductive environments. Once a condition under which metribuzin is degraded is found, the bacteria from that triplicate will be streaked for isolation and identified using BioLog. The concentration of metribuzin will be analyzed by HPLC, while the byproducts of degradation will be evaluated by GC/MS. COD and OD indicate that nitrate reducers can degrade metribuzin, though only about 30% is degraded before the bacterial curve enters death phase. Bacteria from Houma sugarcane farm soil have only been exposed to metribuzin for a few years, but acclimation is already occurring.

P34 Characterization of RarA, a rare and unusual redox active protein

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The metal and metalloid reducing bacterium *Sulfurospirillum barnesii* SES-3 has a unique redox active protein that when provided an artificial electron donor (i.e., reduced methyl viologen, FMN, or NADH) can reduce a variety of other substrates including arsenate, selenate, selenite, nitrite and even phosphate. The enzyme, RarA, has an inferred amino acid sequence that has no significant similarity to any known protein. Further, it does not contain any cysteine-residues or metal-binding motifs and is only found in certain species of *Sulfurospirillum*. The purpose of this project is to express RarA in *E. coli* and demonstrate it has the same properties as the native protein in *S. barnesii* conferring general metal/metalloid reductase activity to *E. coli*. This study shows the activity assays and kinetics of heterologously expressed RarA in *E. coli* by different substrates such as arsenate, selenite, nitrate and nitrite.

P36 Identification of phage encoded receptor binding proteins in Agrobacterium phage 7-7-1

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In efforts to combat the rising number of difficult to treat bacterial infections, the use of phages in various industries has increased in recent years. Accordingly, research centered on studying how phages successfully infect their bacterial hosts has also boomed. Phages initiate the infection of their bacterial hosts by interacting with different cell surface components using receptor binding proteins (RBPs). These proteins are responsible for the targeted recognition of host bacteria and can therefore be used as molecular tools for evaluating the presence of pathogenic bacteria in crops, food, and in clinical settings. Due to enzymatic properties of some of these proteins, they can also be used as bactericidal agents. To date, the RBPs for numerous phages have been identified. However, no such protein has been discovered in a flagellotropic phage. This type of phage must bind to the bacterial flagellar filament, an appendage used for cell motility, to initiate infection. Thus, the RBPs for these types of phages are likely different from those found in other systems and warrants exploration. Our studies are focused on identifying the RBP(s) in the flagella-dependent phage 7-7-1, which infects soil-dwelling *Agrobacterium* spp. Although the genome for this phage is published and annotated, there is little sequence similarity to other well-characterized phages. This has hindered our ability to pinpoint an RBP for this phage using an *in silico* approach. To circumvent this problem, we have employed the use of an *in vivo* binding approach designed to identify RBPs and present our findings.

P38 Ultrafast One-step Coating of Antimicrobial Peptides via DOPA Incorporation

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Effective antimicrobial surface coatings are critical from both economic and health perspectives. In the present study, we aimed to design novel adhesive antimicrobial peptides (AMPs), based on adhesive proteins in mussels, for one-step coating without the need for prior surface functionalization. In the system, we modified the sequence of a potent synthetic AMP, NKC (APKAMKLLKKLLKLQKKGI), with additional repeats of an unnatural amino acid, 3,4-dihydroxy-L-phenylalanine (DOPA), at the C-terminus and evaluated their adhesiveness and antibacterial properties depending on the number of DOPA moieties, using the bicinchoninic acid assay and minimum inhibitory concentration (MIC) test. Surface characterization assays such as X-ray photoelectron spectroscopy and atomic force microscopy confirmed that NKC-$DOPA_5$ was successfully immobilized onto the surfaces of polystyrene, titanium, and silicone, when treated with NKC-$DOPA_5$ solution for 10 min. The antibacterial effect of NKC-$DOPA_5$ coating was evaluated via a colony formation assay and the peptide-coated surfaces revealed 99.999% growth inhibition of 10^6 cells of *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* within 2 h. In addition, NKC-$DOPA_5$-immobilized surfaces retained their antimicrobial activity for at least 84 days. NKC-$DOPA_5$-coated polystyrene, titanium, and silicone catheters displayed no cytotoxicity on a human keratinocyte HaCaT cell line, on an MTT assay, thereby indicating a promising biocompatibility. Furthermore, this coating method involves water-based solutions, which are compatible with most surfaces and are environmentally compatible. Overall, the adhesive AMPs developed in this study have high potential for their use as antimicrobial coating agents to prevent material surfaces from bacterial colonization.

P40 Improving cellulosic ethanol production by an engineered yeast consortium displaying a pentafunctional

mini-cellulosome

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Due to concerns about energy security, sustainability, and global climate change, microorganisms have been increasingly used as cell factories for the production of biofuels. Consolidated bioprocessing (CBP), combining cellulase production, saccharification, and fermentation into one step, has been proposed as the most efficient way to reduce the production cost of cellulosic bioethanol. When a large number of cellulase genes are expressed in *Saccharomyces cerevisiae*, the overexpression of heterologous proteins may result in a metabolic burden on the cells. Here, we developed a consortium of yeast strains capable of secreting and assembling five cellulases on yeast surface. This consortium consists of one yeast strain displaying a scaffoldin on the surface and five yeast strains that produce and secrete each of the five target cellulases. These secreted dockerin-containing target enzymes including cellobiohydrolase, endoglucanase, β-glucosidase, cellobiose dehydrogenase, and lytic polysaccharide monooxygenase, were randomly assembled to the surface-displayed scaffoldin (mini CipA) via cohesin-dockerin interactions to generate a pentafunctional minicellulosome. The cellulosome activity and ethanol production using Avicel and phosphoric acid swollen cellulose (PASC) as substrates were optimized by controlling the mixing ratio among the six yeast strains using Design of Experiments (DOE). One of the advantages of this system is that it relieves the metabolic burden placed on the engineered single yeast strain displaying all the components of the five-enzyme minicellulosome. In addition, it is more likely to achieve increased enzyme production, higher assembly efficiency, and enhanced ethanol production. Most of all, optimization can be easily carried out in this system by altering the ratio of each component so that it will more likely be able to achieve maximal cellulose to ethanol conversion. This work will significantly increase our knowledge on the design and engineering of optimal yeast strains for production of fuels and chemicals from cellulosic biomass.

P42 *Acinetobacter baylyi*: a model organism for biofuel production

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With dwindling supplies of oil, the humanity is actively seeking alternative sources of transportation fuel. *Acinetobacter baylyi* naturally produces waxes and triglyceride. In our hands, its wild-type strain produces about 25% of non-polar fats (wax and triglycerides combined). It also has the ability to utilize aromatic compound produced by lignocellulose hydrolysis. The analyses of the *A. baylyi* genome and lipid biosynthesis/degradation pathways revealed a number of potential targets for mutagenesis or cloning. Genes of interest were mutated using the *cre*/*loxP* site specific recombination system. To improve lipid productivity, the genes for two putative lipases (*lip1* and *lip2*) and several putative acyl-CoA dehydrogenase genes (*fadE)* were consecutively inactivated. For the next step, the genes for phosphate acetyl transferase (*pta*), the product of which is involved in acetate production/utilization, as well as genes for several additional putative acyl-CoA dehydrogenases, the products of which are involved in β-oxidation of fatty acids, were also knocked out. The mutation in the *pta* gene resulted in reduced growth on acetate, whereas only one the *fadE*::*loxP* mutation resulted in the lack of growth on oleic acid. These mutations are now being combined with the lipase and the *pta* mutations. The combined effect of the mutations (*lip1*, *lip2* and *fadE)* resulted in a four-fold increase in the total lipid production compared to the wild type. Despite a multiplicity of fatty acid degradation pathways, *A. baylyi* remains one of the most attractive bacterial models for production of triacylglycerides and waxes.

P44 Metabolic engineering of yeast for increased production of cyclopropane fatty acids

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Bioproduction of chemicals and fuels is an important and growing segment of manufacturing and yeasts such as *Saccharomyces cerevisiae* are both widely used as production organisms and as metabolic models. Fatty acid-containing lipids are one example of moderate value, highly versatile chemicals produced by yeasts and used in a broad range of industries. Production levels of standard fatty acids by yeasts has increased enormously through metabolic engineering to being close to commercial reality. Functionalised fatty acids such as cyclopropane fatty acids that possess a strained 3-membered ring in a saturated chain, are also attractive targets for bioproduction as they have application in cosmetics and speciality lubrication. However, bioproduction of cyclopropyl fatty acids present greater challenges as they are not naturally present in yeast.

When we expressed the *Escherichia coli* cyclopropane fatty acid synthetase (EcCFAS) in *S. cerevisiae,* both *cis*-9,10-methylene-hexadecanoic and -octadecanoic acids were identified in the phospholipid and triacylglycerol fractions of the cell. Furthermore, EcCFAS expressed in cells engineered for increased lipid production had cyclopropyl fatty acid content increased 4-fold in triglyceride. There is great potential to improve content further as the triacylglycerol fraction had just 16% cyclopropyl fatty acid whereas phospholipid had 40%. To further improve yield and purity of this fatty acid in yeast we have undertaken a systematic analysis of cyclopropane fatty acid synthesis, assessed native and introduced lipid handling genes and pathway modifications and examined potential substrate limitations. This investigation shows how to improve cyclopropyl and other similarly synthesised high value exotic fatty acid production in yeasts.

P46 Genetic toolkit for *Zymomonas mobilis* and other industrially-relevant host organisms

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Many non-model organisms are well suited for industrial processes due to unique metabolic capabilities and innate tolerance to conditions commonly found in industrial bioreactors. However, optimization of product titer, rate, and yield, or introduction of heterologous biosynthetic pathways for alternate fuel and chemical production is frequently limited by inefficient or non-existent genetic engineering tools. A primary objective of ours is to establish genetic engineering systems in organisms with promising metabolic capabilities. One such organism, *Zymomonas mobilis*, is an attractive candidate due to its naturally high glycolytic flux via the Entner-Doudoroff pathway. *Z. mobilis* is an obligate fermentative organism that is also aerotolerant, exhibits tolerance to relatively high concentrations of ethanol and sugars, and is capable of growth at pHs as low as 3.5. Despite these advantages, advancement with this organism has been stymied by low transformation efficiencies and limited genetic tools. We hypothesized that inefficient transformation was likely due to DNA restriction-modification systems that target and degrade foreign DNA. To address this, we developed an *E. coli* strain that expresses the DNA specificity and methyltransferase subunits of the two Type I modification systems found in *Z. mobilis* ZM4. Transformation of plasmids that were passaged through the *E. coli* methylating strain prior to moving them into *Z. mobilis* led to a *ca.* 70-fold increase in transformation efficiency relative to plasmids extracted from a standard laboratory strain of *E. coli*. With an enhanced ability to move exogenous DNA into *Z. mobilis*, we have begun generating more advanced genetic tools, such as a system for high-efficiency genome integration via phage integrase-mediated recombination and a library of promoters of varying strengths, similar to a system we previously demonstrated in *Pseudomonas putida*. The success of this project has led us to employ similar tactics to develop and improve genetic tools in *Clostridium tyrobutyricum*, an efficient butyric acid producer, and *Acidothermus cellulolyticus*, a thermophilic and acidophilic cellulose degrader.

P48 Quantifying metabolite cross-feeding through ^{13}C metabolic flux analysis: A case study using *E. coli* and *S. enterica* grown in coculture

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Engineering microbial consortia for fermentation allows for pathway compartmentalization, broadened metabolic capability, and improved robustness. However, tools for modeling consortia are underdeveloped, limiting predictability compared to monocultures. Identifying and quantifying interspecies cross-feeding and its effect on each species' metabolism is crucial to understanding cooperation and exploitation in consortia. In this work, we develop techniques for conducting ^{13}C metabolic flux analysis on consortia using a model coculture of *S. enterica* and *E. coli*. The full metabolic flux profiles of an auxotrophic *E. coli* and a cooperative *S. enterica* were determined, both in isolation and in coculture. In this model coculture, a methionine-auxotrophic *E. coli* cleaves lactose, providing glucose and galactose to *S. enterica*. In turn, *S. enterica* excretes excess methionine that is taken up by *E. coli*. Metabolic flux analysis using ^{13}C-labeled tracers identified and quantified metabolite exchange. We show that both species prefer glucose consumption at most concentrations. However, the rate of lactase cleavage keeps the glucose and galactose concentrations in the co-utilization range. Cocultures were grown in two different experimental setups: 1) bioreactors and 2) transwell plates. The latter setup allowed us to elucidate the effect of diffusion on coculture performance. Lastly, we compared metabolic profiles for each monoculture grown on glucose or galactose to the metabolic profiles in coculture. Knowing only the biomass composition of each species and the combined biomass ^{13}C-labeling, we used our recently developed methodology to simultaneously resolve the ratio of *S. enterica* to *E. coli* and each species' full metabolic profile.

P50 ^{13}C-Flux analysis in complex media: quantifying intracellular fluxes for the anaerobic fermenters *Clostridium acetobutylicum* and *Clostridium ljungdahlii*

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In this study, ^{13}C-Metabolic Flux Analysis (^{13}C-MFA) was performed on the ABE fermenting bacterium *Clostridium acetobutylicum* ATCC 824 in the presence of glucose and yeast extract to quantify metabolic fluxes during both acidogenesis and solventogenesis. Historically, ^{13}C-MFA has been difficult to perform in poorly defined media due to the presence of unknown carbon sources in unknown concentrations. Here, we describe a pioneering new technique that was developed to circumvent the complications of undefined media, including: a) calculations to differentiate biomass yielded from yeast extract versus biomass yielded from glucose; b) using algorithms that are based on co-culture ^{13}C-MFA to discretize metabolism into different feeding phases; and c) using isotopomer spectral analysis on cell membrane components (i.e. fatty acids) to calculate marginal growth during solventogenesis. These calculations were shown to be possible in the presence of a shifting labeling pattern for acetate in the medium. The ^{13}C-labeling data for biomass amino acids, intracellular metabolites, glycogen, RNA, fatty acids, and excreted solvents were used in our comprehensive model. We found that 30% of *C. acetobutylicum* biomass was derived from glucose and the remainder was derived from yeast extract. The majority of components from yeast extract proceeded directly into protein synthesis, with only aspartate and glycine entering central carbon metabolism. The new methods developed in this work were subsequently applied to the CO_2-fixing bacterium *Clostridium ljungdahlii*.

P52 Yeast engineering for utilization of pectin-rich biomass.

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Biomass-based renewable energy of Korea is currently concentrated on wood chip boilers of rural areas installed through subsidies. However, due to toxic gases generated during combustion of wood chip wastes and increasing concerns with air pollution in Korea, alternative biomass and its use through an environment-friendly process are required to be developed. In this presentation, we will introduce fruit processing wastes as an alternative biomass as well as an urgent waste problem to be solved, especially in Jeju. A series of metabolic engineering strategies to fuel microbial cells with fruit processing wastes will be presented. Also, we will discuss current limitations of the developed strain and our future plans for biofuel production and industrial applications.

P54 Construction of 2,3-butanediol production pathway in *Eubacterium limosum* KIST612 *via*Â metabolic engineering

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The petroleum refineries are traditional processes for producing chemicals and fuels. There are problematic issues such as the depletion of fossil fuels and negative environmental impacts. As a result, the paradigm has been shifted from petroleum refineries to alternatives including bio-refineries. The bacteria such as acetogen are utilized in the biological conversion process due to the high potential of chemical productions from the source of low carbon materials including CO and CO_2. The Gram-positive, obligately anaerobic, and acetogenic bacterium, *Eubacterium limosum* (Elm) KIST612 can produce mainly acetate, lactate, and butyrate. The 2,3-butanediol (BDO) is a useful chemical with a variety of applications for industrial needs that can be used as a precursor in manufactures for chemical products including methyl ethyl ketone, butadiene, *etc*. Based on the genomic sequence analysis, there is a missing enzyme to produce the BDO that is a 2,3-butanediol dehydrogenase (BDH). To construct the complete metabolic pathway for BDO production, the putative BDH-encoding genes from *Clostridium* spp. were heterologously expressed in Elm KIST612, respectively. It is also necessary to gain reducing power for the synthesis of 2,3-BDO. By deletion of the lactate production pathway, the flow of carbon and reducing power will be redirected to produce the BDO. To compare the product yields, the mutant strains of Elm KIST612 including wild-type were grown on glucose as substrate.

P56 Genome editing using CRISPR/Cas for syngas-utilizing acetogen, *Eubacterium limosum* KIST612

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Increasing interest in acetogens for the production of valuable chemicals (*e.g.*, butanol, ethanol, and 2,3-butanediol) requires the genetic tools for these atypical producing organisms. However, genetic engineering tools for acetogens are usually inefficient due to organisms handling difficulty and inherent characteristics. They are strict anaerobic and Gram positive organisms. Herein, we develop CRISPR (clustered regularly interspaced short palindromic repeats) system for acetogen, *Eubacterium limosum* (Elm) KIST612 that can metabolize synthesis gas (syn-gas) components, H_2, CO, and CO_2 *via* Wood-Ljungdahl pathway, producing acetate and butyrate as end-products. In this study, both Cas9 and Cas9 nickase (Cas9n) were used for the CRISPR system, and target genes (e.g., *pyrF*, *ldh*, *cooS*, and *adh*) were selected to confirm the efficiency of the new genetic engineering system. As a result, it is clearly found that CRISPR-based genome editing was faster and more efficient than a classical homologous recombination-based method. This study not only makes an efficient way of metabolic engineering for producing biochemical and biofuels but also provides valuable guidance and essential references for genome editing in the use of *E. limosum* KIST612 strain that has not been reported on genes editing toolbox.

P58 Metabolic engineering of *Escherichia coli* for direct fermentative production of lactate esters-

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Green organic solvents such as lactate esters have broad industrial applications and favorable environmental profiles. However, lactate esters are currently produced by esterification of lactic acid with alcohols in an eco-unfriendly condition using toxic catalysts such as sulfuric acid, hydrogen chloride, and/or phosphoric acid. Alternatively, a microbial platform can be harnessed to produce these esters from renewable feedstocks in an eco-friendly environment. In this study, we present a microbial platform for direct fermentative production of lactate esters from fermentable sugars. To enable the microbial biosynthesis of lactate esters, we first designed a pyruvate-to-lactate ester module that consists of a lactate dehydrogenase (*ldhA*) to convert pyruvate to lactate, a propionate CoA-transferase (*pct*) to convert lactate to lactyl CoA, and an alcohol acyltransferase (*AAT*) to condense lactyl-CoA and alcohol(s) to produce lactate ester(s). Next, we screened the efficient alcohol acyltransferase (AAT) for lactate ester production by generating and characterizing a library of five pyruvate-to-lactate ester modules carrying divergent AATs with an exogenous alcohol supply. Finally, we demonstrated for the first time the direct fermentative production of ethyl and isobutyl lactate esters from glucose by co-introducing a pyruvate-to-lactate ester module and an alcohol module into a modular *Escherichia coli* (chassis) cell. Further, with efforts in probing and alleviating the metabolic bottlenecks, we were able to identify that AAT is the most rate limiting step in biosynthesis of lactate esters and achieved 4.96-fold improved ethyl lactate production.

P60 Developing tunable, riboswitch-mediated gene regulatory controls in cellulose-degrading bacteria, *Clostridium thermocellum*, engineered to co-ferment cellulosic and hemicellulosic biomass

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The thermophilic and anaerobic bacteria, *Clostridium thermocellum*, capable of effectively hydrolyzing cellulosic biomass is a recognized as a chassis bacterium for consolidated bioprocessing (CBP). CBP is a process through which cellulosic biomass can be directly converted to a target product through fermentation without adding costly hydrolytic enzyme cocktail. While various genetic manipulations have been employed in the metabolic engineering of thermophiles, a robust means to regulate gene expression in these bacteria (~55 °C) is missing.

Riboswitches are mRNA leader sequences that can regulate gene expression upon binding to a small molecule as a ligand. They are composed of two domains, the *aptamer* and the *expression platform*. Our bioinformatic search for various riboswitches in thermophilic bacteria revealed that major classes of riboswitches are present, suggesting riboswitches' regulatory roles in these bacteria. By building synthetic constructs incorporating natural and engineered purine riboswitch sequences originated from foreign species, we quantified respective riboswitches activities in up- and down-regulating gene expression in *Geobacillus thermoglucosidasius* using a green fluorescence protein. The elicited regulatory response was ligand-concentration-dependent. We further demonstrated that riboswitch-mediated gene expression of *adhE* (responsible for ethanol production) in *C. thermocellum* can modulate ethanol production, re-distribute carbon-flux toward different fermentation end-products, and control cell growth in the *adhE* knockout mutant. This work has made tunable gene expression feasible across different thermophiles for broad applications including biofuels production and gene-to-trait mapping. The presentation will also show our current metabolic engineering efforts in enabling *C. thermocellum* to co-utilize cellulose- and hemicellulose-derived hexose and pentose sugars without carbon catabolite repression for improved biohydrogen production.

P62 Development of a CRISPR/Cas9-based tool for gene deletion in *Issatchenkia orientalis*

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Owing to its natural ability to grow in highly acidic conditions, the nonconventional yeast *Issatchenkia orientalis* is a potential platform microorganism for production of organic acids. However, the lack of efficient genetic tools has remained a major bottleneck in metabolic engineering of this organism. Here, we describe our efforts in designing advanced tools for genome engineering. In particular, we develop the first CRISPR/Cas9-based system for gene deletion. The optimized CRISPR/Cas9-based system used a fusion *RPR1'-tRNA* promoter for single guide RNA (sgRNA) expression and could achieve greater than 95% gene disruption efficiency for various gene targets. Additionally, we demonstrated multiplex gene deletion with disruption efficiencies of 89% and 46% for double-gene and triple-gene knockouts, respectively. We will also discuss our attempt to engineer *I. orientalis* for the production of succinic acid. This genome editing tool can be used for rapid strain development and metabolic engineering of this organism for production of biofuels and chemicals.

P64 Multi-strain analysis of the diverse metabolic capabilities of Pseudomonas putida

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Pseudomonas putida has large potential for bio-industrial applications. Part of its potential is due to its broad metabolic abilities towards a variety of different carbon growth substrates, including aromatics and lignin-derived monomers. To date, most research has focused on *Pseudomonas putida* KT2440 and largely ignored other strains of *P. putida.* Using a highly detailed genome scale model (GEM) of *Pseudomonas putida* KT2440 in conjunction with the genomic sequences of numerous other strains has allowed for the construction of GEMs for individual strains that were previously not well characterized. Combining the GEMs with constraint based analytical methods allows for predictions of the metabolic capabilities of the individual strains. This analysis demonstrates the diversity and broad capability of the *P. putida* species and helps identify strains to target for bio-industrial applications.

P66 Synthetic biology parts for control of protein expression and metabolism in *Clostridium*

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Synthetic biology parts for control of protein expression and metabolism in *Clostridium*

The bacterium *Clostridium acetobutylicum* has desirable attributes for military applications, including rapid anaerobic catabolism of multiple C_5 and C_6 sugars, sporulation for ease of transport and storage, and the ability to form productive consortia with lignocellulose-decomposing fungi. However, few truly useful and convenient synthetic biology "parts" have been developed for and characterized within this organism, limiting its "engineerability."

Development of additional tools such as genetic switches, reporters of protein expression, and effective heterologous enzymes would allow us to better engineer *C. acetobutylicum* and, likely, its close relatives, to biosynthesize alternative metabolites and to react to additional applied or environmental stimuli.

Thus far, we have successfully instituted a synthetic riboswitch and are progressing with other regulators for inducible control of protein expression. Additionally, we have tested several reporter proteins and evaluated their suitability with respect to

constraints imposed by *Clostridium*, namely anaerobicity and autofluorescence. Most recently, we compared plasmid replication origins with respect to their effects on protein expression and biosynthesis of phloroglucinol, a model metabolite and precursor to a number of chemicals of military and industrial interest.
Herein, we present the results of our efforts at diversifying the toolkit of *Clostridium*-compatible genetic parts to widen this microbe's repertoire of metabolic and sense-respond capabilities.

P68 Development of CRISPR/Cas9 gene editing tools in *Rhodosporidium toruloides*

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The oleaginous yeast *Rhodosporidium toruloides* is considered a promising candidate for production of chemicals and biofuels thanks to its ability to grow on lignocellulosic biomass, and its high production of lipids and carotenoids. However, efforts to engineer this organism are hindered by a lack of suitable genetic tools. Here we report the development of a CRISPR/Cas9 system for genome editing in *R. toruloides* based on a novel fusion 5S rRNA-tRNA promoter for gRNA expression, capable of greater than 95% gene knockout for various genetic targets. Using the same method, multiplexed double-gene knockout mutants were obtained with an efficiency of 78%. This capability opens the door to performing more advanced CRISPR-based gene-editing techniques in this non-model yeast such as transcriptional reprogramming and site-specific markerless integration.

P70 An orthogonal CRISPR System for multi-dimensional regulation of gene expression in non-conventional yeast *Komagataella phaffii*

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Komagataella phaffii (formerly called *Pichia pastoris*) has been widely used as a platform organism for production of numerous heterologous proteins of medical and industrial interest. In recent years, *K. phaffii* was also established for complex metabolite production. Fine-tuning of gene expression is crucial for protein expression and metabolic engineering. However, there is still lack of efficient genetic manipulation tools for gene regulation in *K. phaffii*. In the present study, we develop an orthogonal tri-functional CRISPR system in *K. phaffii* for transcriptional activation, transcriptional interference, and gene deletion simultaneously. By examining ten CRISPR proteins, three of them were discovered to be functional in *K. phaffii*. All these three CRISPR proteins could efficiently knockout target genes. The orthogonality of these three CRISPR proteins was tested and the results indicate that the CRISPR proteins were only functional when their cognate gRNAs were present. We are currently evaluating the transcriptional activation and transcriptional interference functionalities using deactivated CRISPR proteins fused with different effectors. This work will provide a promising genetic tool for metabolic engineering in *K. phaffii* on a genome-scale.

P72 Pyruvate production by pyruvate dehydrogenase variants in *Escherichia coli*

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Directing metabolic flux from central metabolism to a product of interest commonly involves a competition between native metabolism (i.e., cell growth) and a pathway toward a product of interest. The typical approach to improving the formation of a product is to increase the expression or activity of enzyme(s) leading to that product. An alternate approach to direct metabolic flux preferentially toward a product is by decreasing the activity of the key central metabolic enzyme at that branchpoint. In this study, variants of the Pyruvate Dehydrogenase Complex (PDHc), having reduced activity, were used to accumulate pyruvate from glucose using engineered *Escherichia coli* C having deletions in *ldhA* and *poxB*. We hypothesized that mutations in key residues of PDHc Enzyme I, AceE encoded by *aceE*, would allow for pyruvate accumulation when grown on glucose as a sole carbon source. Chromosomally integrated *aceE* variants were screened for growth rate and pyruvate production in shaking flasks. The highest yielding strain accumulated pyruvate at a yield of 0.48 g pyruvate/g glucose, and showed a 68% decrease in growth rate to 0.34 h^{-1}. These results provide proof-of-concept that point mutated PDHc variants can effectively shift carbon flux away from central carbon metabolism to allow pyruvate accumulation.

P74 Metabolic engineering of *Escherichia coli* for 2,3-butanediol production by a heat inducible expression system

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Escherichia coli MG1655 was metabolic engineered to produce 2,3-butanediol (BDO) using a synthetic promoter and an expression system inducible by heat. To minimize the co-production of fermentative metabolites, the non-fermentative *E. coli* strain AV11 (MG1655 Δ*pflB*, Δ*frdA*, Δ*poxB*, Δ*xylFGH*, Δ*midarpA*, Δreg 27.3 kb, *gatC*S184L, Δ*ldh*, Δ*adhE*) was used. The BDO producing strain EM01 was developed from AV11 by integrating the RNA polymerase T7 gene into the σ^{32} dependent locus HtpG. The synthetic BDO operon, containing $alsS_{Bl}$, $alsD_{Bl}$ and $bdhA_{Bs}$ genes in tandem, was cloned into the vector pAcycDuet-1 to obtain pBDOop. These genes code for acetolactate sintase (from *Bacillus licheniformis*), acetolactate decarboxilase (from *Bacillus licheniformis*), and 2,3-butanediol dehydrogenase (from *Bacillus subtilis*), respectively. The strain EM01/pBDOop was evaluated in 1-L bioreactors using glucose (40 g/L) and mineral media. To promote BDO production and

avoid pyruvate accumulation, an oxygen transfer rate (OTR) study was performed using a biphasic culture strategy. In the first phase at 37 °C, a biomass production stage with dissolved oxygen controlled at 5% was used. In the second phase, the production stage at a low OTR was selected and induction was performed at 41 °C. Under these conditions, pyruvate accumulation was diminished, BDO production reached 4 g/L in 48 hours, and acetoin was obtained at a titer of 3.5 g/L. This metabolic and cultivation strategy allowed to channel approximately 40% of the glucose into the acetoin-BDO pathway.

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P76 Update on the Amyris pipeline for biological production of diverse compounds

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Until recently Amyris engineered strains mainly to produce terpenoid compounds such as artemisinin, farnesene, and various fragrances. We are now nearing the end of a four-year program funded by DARPA named milligrams to kilograms (M2K) with the goal of engineering top quality infrastructure capable of high throughput, high titer, biological production of a diverse array of chemotypes, including flavonoids, polyketides and amino acid-derived compounds. We will provide an update on our progress. We have achieved breakthroughs in the both the throughput and success rate of our Automated Strain Engineering core service. The M2K team was able to make these significant advancements through a combination of new computational tools and the standardization of designs. The throughput and accuracy of the measurement of titers, proteomes, and metabolomes have also improved following the acquisition of state-of-the-art equipment, including QTOF and RapidFire® mass spectrometers. In addition, the automation team has implemented numerous modular systems in support of both analytics and strain improvement. Our automated scientist ("Lila") makes reasonable suggestions about future designs based on a wide range of data streams. Efforts have (rightly) focused on *Saccharomyces cerevisiae* as the host, but other host organisms, particularly *Escherichia coli*, have achieved higher titers than yeast for specific compounds. Bringing *E. coli* into the pipeline that was developed for yeast has presented challenges that will be discussed.

P78 Discovery of a novel P450 fatty acid decarboxylase from *Macrococcus caseolyticus* for biosynthesis of odd chain terminal alkenes

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Alkenes are industrially important platform chemicals with broad applications. To date, a number of different classes of enzymes have been identified and characterized for biosynthesis of terminal alkenes including a P450 fatty acid (FA) decarboxylase/peroxygenase (OleT, CYP152 family), a type-I polyketide synthase-like enzyme (CurM/Ols), a desaturase-like enzyme (UndB), and a non-heme oxidase (UndA). Interestingly, these enzymes exhibit the divergence in substrate specificity resulting in production of alkenes with various chain lengths. Thus, exploring diversity of these enzymes can provide a unique opportunity to develop a microbial platform for tailored production of terminal alkenes. In this study, we present a direct microbial biosynthesis of odd chain terminal alkenes from fermentable sugars by a novel P450 fatty acid decarboxylase from *Macrococcus caseolyticus* ($OleT_{MC}$). We first identified the putative P450 FA decarboxylases from *M. caseolyticus* via genome mining and characterized its FA decarboxylase activities *in vitro* towards linear C10:0-C18:0 FAs. We, next, established the *de novo* biosynthesis pathway of terminal alkenes from glucose by co-expressing $OleT_{MC}$ and an engineered *Escherichia coli* thioesterase (TesA) and demonstrated the direct production of terminal alkenes from glucose in an engineered *E. coli*. Further, we achieved ~2.8-fold improved alkene production by alleviating the electron transfer limitation in $OleT_{MC}$ with a two-component redox partners, a putidaredoxin reductase (CamA) and a putidaredoxin (CamB) from *Pseudomonas putida*. Finally, we highlighted the functional role of FA binding pocket in substrate specificities of $OleT_{MC}$ using *in silico* protein modeling.

P80 Zmo0994, an LEA-like protein from *Zymomonas mobilis*, increases multi-stress tolerance in *Escherichia coli*

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Pretreatment processes and subsequent enzymatic hydrolysis are prerequisites to utilize lignocellulosic sugar for fermentation. However, the resulting hydrolysate frequently hinders fermentation processes due to the presence of inhibitors and toxic products (e.g. ethanol). Thus, it is crucial to develop robust microorganism conferring multi-stress tolerance. In this study, Zmo0994, identified as a novel LEA-like small protein from the bacterium, *Zymomonas mobilis*, had the effect of causing a significant enhancement of tolerance to abiotic stresses such as ethanol, furfural, 5′-hydromethylfurfural (HMF), and high temperature, when expressed in *E. coli*. According to a transcriptome analysis of *E. coli* expressing Zmo0994 by using next generation sequencing (RNA-seq), the cellular mechanism of this protein was revealed as due to its ability to trigger gene clusters, involved in multi-stress tolerance, such as ATP synthesis, cell wall biogenesis, and multi-drug efflux pumps. In conclusion, these findings have significant implications to develop robust microbes for efficient industrial fermentation processes.

P82 Metabolic engineering of *Saccharomyces cerevisiae* for efficient odd-chain fatty acids production

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Odd-chain fatty acids (ocFAs) and their derivatives are valuable products with various applications in chemicals, flavour and fragrance intermediates, pharmaceuticals, cosmetics, plasticizers, coatings, and fuels. However, naturally occurring organisms produce negligible amounts of odd-chain fatty acids. Here, we report the engineering of the budding yeast *Saccharomyces cerevisiae* as a cell factory for ocFAs synthesis. Acetyl-CoA is used as the starting unit for even-chain fatty acid synthesis in both prokaryotes and eukaryotes. In this study, however, *S. cerevisiae* was engineered to produce propionyl-CoA, which is accepted as the starting unit by fatty acid synthetase complex for odd-chain fatty acids production. First, propionyl-CoA was produced by supplementing propionate in the medium, as reported in *Escherichia coli* and *Yarrowia lipolytica*. However, *S. cerevisiae* showed low tolerance to propionate that inhibited cell growth and fatty acid synthesis dramatically. We then sought to construct pathways converting L-threonine to propionyl-CoA to avoid supplementing propionate in the medium. Three pathways have been constructed, including: 1) the PCT pathway consisting of threonine dehydratase (*tdcB*) from *E. coli*, the alpha-ketoisovalerate decarboxylase (*kivD*) from *Lactococcus lactis*, aldehyde dehydrogenase from *S. cerevisiae* (*ScALD4*), and the propionate CoA-transferase (*PCT*) from *Clostridium propionicum*; 2) the PduP pathway consisting of *tdcB*, *kivD* and a coenzyme A-acylating propionaldehyde dehydrogenase (*PduP*) from *Lactobacillus reuteri* or *Klebsiella pneumoniae*; and 3) the prpE pathway consisting of *tdcB*, *kivD*, *ScALD4* and the propionyl-CoA synthase (*prpE*) from *Salmonella enterica*. All these three pathways worked in *S. cerevisiae* and the prpE pathway achieved the highest titer of ocFAs. The highest titer of ocFAs reached 108 mg/L from glucose and L-threonine in batch fermentation, which accounted for 37% of total lipids. The resulting engineered yeast strain represents the first microbial cell factory for *de novo* ocFAs production.

P86 EngineeringÂ *E. coli*Â for methylotrophy – insights fromÂ ^{13}C-isotope tracing'

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Methanol is an attractive substrate for fermentations due to its high energy content and the presence of large supplies of natural gas that can be cheaply converted to methanol. Different metabolic challenges must be overcome in engineering a purely methylotrophic *E. coli* – an engineered strain that can metabolize and grow exclusively on methanol (MeOH), a non-natural substrate. One key challenge that must be overcome in constructing a purely methylotrophic *E. coli* is to improve methanol assimilation. We demonstrate that by employing ^{13}C-MeOH, we can comprehensively analyze the extent of MeOH assimilation into engineered methylotrophic *E. coli* strains that we have constructed. Across various engineered strains grown in minimal media supplemented with ^{13}C-MeOH (and a limited amount of yeast extract), we observe that while the MeOH carbon is well-assimilated into intracellular central carbon metabolites, it is not the case for amino acids (AAs). Additionally, only a small amount of AAs synthesized from MeOH is assimilated into the biomass.

In the present work, we have identified methods to improve the assimilation of MeOH carbon into biomass components, especially all proteinogenic amino acids. To this end, we have focused on identifying culture conditions that would impact the strain's ability to assimilate carbon from MeOH. Various media formulations that were supplemented with different combinations of AAs have improved the assimilation of MeOH into biomass components, especially proteinogenic AAs. Furthermore, these experiments have revealed regulatory mechanisms in *E. coli* that should be engineered to improve synthesis of biomass components from metabolites synthesized from methanol.

P88 Metabolic engineering ofÂ *Escherichia coliÂ* for stereospecific biosynthesis of 1,2-propanediol

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1,2-propanediol (1,2-PDO) is an industrial chemical with a broad range of applications, such as the production of alkyd and unsaturated polyester resins. It is currently produced as a racemic mixture from nonrenewable petroleum-based feedstocks. Here, we demonstrated the engineering of *E. coli* host strains for the *de novo* biosynthesis of 1,2-PDO through a novel artificial pathway via lactic acid isomers as the intermediates from renewable feedstocks. The pathway circumvents the cytotoxicity issue caused by methylglyoxal intermediate in the naturally existing pathway. Under fermenter-controlled conditions, the *R*-1,2-PDO was produced at 17.3 g/L with a molar yield of 42.2% from glucose, while the *S*-isomer was produced at 9.3 g/L with a molar yield of 23.2%. The optical purities of the two isomers were 97.5% *ee* (*R*) and 99.3% *ee* (*S*), respectively. Recent efforts in further optimization of the process will also be discussed.

P90 Development of Met25 in *Y. lipolytica* as a color associated and counter-selectable genetic marker

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Yarrowia lipolytica offers an ideal host for biosynthesis of high value commodity chemicals through recombinant genome engineering, and iterative gene integration techniques have demonstrated abilities to drastically improve microbial yields despite being restricted to a limited number of generic selection markers, and counter-selectability achieved primarily with URA3 (Jang, et al.) (Gao, et al.), (Lv, et al.). We report a versatile, counter-selectable, new marker for this host, met25, as the locus of sulfide housekeeping in the cell which can be exploited in various ways. Divalent lead induces lead(II) sulfide aggregation in Δmet25 cells such that deficient cells grow brown/black, and cells with functional copies of met25 grow white. This gene also confers toxic resistance to methyl-mercury, for met25 deficient cells. The sulfide buildup from met25 deficiency can again be exploited

to neutralize methyl-mercury by mercuric sulfide precipitation and ethanol formation. Met25 was deleted via homologous recombination and restored via plasmid and chromosomal integration. A plasmid was designed utilizing CRISPR-Cas9, Cpf1-rRNA targeted gene disruption (Wong, et. al, 2017) to orthogonally demonstrate met25 as a phenotypic locus and to test transient mutation of this site. Kinetic growth curves were obtained for wildtype, mutant, and plasmid expression of met25 under varying concentrations of methyl-mercury, and the data regressed well to a dose-response curve and Hill-type equation. Similar to findings for met15 in *C. albicans* (Viaene, et al, 2000), and unlike findings for *S. cerevisiae* (Cost, Boeke, 1996) met25 did not induce strict auxotrophic requirements for methionine which provides difficulty when negative selection pressure is required. The value in this selectable marker could be highly utilized with development of cellular biosensors for lead(II), spectrophotometric techniques with sulfide salts, cellular visualization techniques like flow cytometry, high throughput cell imaging, or automation of strain identification.

P92 CRISPR-cas12 mediated genome-editing in oleaginous yeast

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Oleaginous yeast species have been gaining increasing attention due to its capacity to accumulate high titer of lipids, wide range of substrate utilization and generally regarded as safe (GRAS) status. Compared with the baker's yeast *Saccharomyces cerevisiae*, genetic toolbox for oleaginous yeast has been relatively lagging behind due to the limited availability of counter-selection markers and genome integration locus. The clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) has been a powerful tool for precise marker-less genome engineering such as targeted knock-in and knock-out of genes in various hosts. CRISPR-Cas9 has been implemented to perform genome-editing at specific targets in some oleaginous yeasts. Cas12 was classified as a type II endonuclease involved in bacterial immunity. Cas12 is a smaller protein compared with Cas9 and recognizes distinct PAM sequence (TTTN) than that of Cas9 (NGG). A simpler structure of the CRISPR RNA (crRNA) is required for Cas12 targeting. Due to these features, the CRISPR-Cas12 system could be more suitable for the genome-editing particularly in the AT-rich regions. In this work, the CRISPR-Cas12 was harnessed to perform genome-editing in oleaginous yeast with high efficiency. Three counter-selectable markers were successfully disrupted and colony PCR was performed to screen positive colonies. Purified PCR fragments were sequenced to identify in-del mutations. Our redesigned and optimized CRISPR-Cas12 system lead to relatively high genome-targeting efficiency, which could be an invaluable tool to facilitate genome engineering in non-model yeast.

P94 Metabolic engineering of *Yarrowia lipolytica* for *de novo* synthesis of 2-phenylehtanol from sustainable low-cost feedstocks

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2-Phenylethanol (2-PE), as a safe flavoring agent with the rose-like smell, has been widely used in food and cosmetic industries. Currently, 2-PE is primarily produced by chemical synthesis and plant extractions, however, these approaches are often associated with harsh reaction conditions, undesirable by-products formation and costly extraction process. To overcome these drawbacks and achieve sustainable production of 2-PE, *Yarrowia lipolytica*, a native producer of 2-PE, was chosen as the starting strain in this work. First, stepwise pathway engineering was used to identify rate-limiting steps in the 2-PE synthesis pathway, which resulted in a 9.7-fold increase in the 2-PE titer (from 4.8 mg/L to 51.4 mg/L). Then, effective alcohol dehydrogenases and phenylacetaldehyde reductase were screened and determined in *Y. lipolytica*. Competing pathways from endogenous phenylacetaldehyde dehydrogenase were knocked out to improve 2-PE titer. Next, the feedback inhibition caused by L-phenylalanine was relieved by expressing feedback-resistant 3-deoxy-D-arabinoheptulosonate-7-phosphate synthases. Byproducts-forming pathways, including tryptophan, tyrosine and phenylacetic acid, were deleted to increase 2-PE flux. The 2-PE titer was further increased through rewiring the central carbon metabolism by blocking glycolysis pathway and introducing non-oxidative glycolysis pathway (NOG). The redesigned strain was able to produce 2-PE with waste acetic acids or xylose as sole carbon sources. The engineering strategies reported here will be useful for production of other aromatic compounds, including phenylpropanoids, flavonoids and aromatic alkaloids.

P96 Building golden-gate cloning platform for *Yarrowia lipolytica*: a test for violacein production

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Violacein is a naturally-occurring bisindole pigment with a deep purple color. It is produced by various bacterial strains, including *Chromobacterium violaceum*, *Pseudoalteromonas luteoviolacea*, *Alteromonas luteoviolacea*, and *Janthinobacterium lividum*, and shows increasing commercial value, especially for industrial applications in cosmetics, pharmaceuticals and fabrics. In this study, we have developed a powerful and versatile DNA assembly platform for the nonconventional yeast *Yarrowia lipolytica* based on the Golden Gate modular cloning strategy. To achieve this, a set of interchangeable building blocks have been constructed, including 3 promoters (pTEF, pICL1, pZWF1), which allows gene expression to be tuned at strong, medium and low level. Each of the gene fragment was flanked by 4 nt predesigned overhangs as well as a Type IIs restriction enzyme, BsmBI. Gene expression level for the five genes encoding the violacein pathway (vioA, vioB, vioC, vioD, and vioE) was further optimized based on the golden gate cloning platform. A total of 3^5 genetic variants spanning across the design space was used to probe the optimal promoter strength for the five genes. In total, the toolbox comprised 15 golden-gate bricks and its effectiveness had been validated through the violacein pathway by successfully constructing a library containing 243 genetic variants. This

technology greatly enriches the synthetic biology toolbox customized for *Y. lipolytica*, enabling rapid and combinatorial assembly of complex metabolic pathways.

P98 Sequestrating amorphadiene synthase to endoplasmic reticulum improves antimalarial drug production in *Yarrowia lipolytica*

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Artemisinin, an anti-malaria drug, is a sesquiterpene endoperoxide produced by the plant *Artemisia annua*. Due to safety and economic issues, traditional plant extraction or chemical synthesis could not provide a scalable route for large-scale manufacturing of artemisinin. Microbial fermentation was considered as an alternative method. *Yarrowia lipolytica,* is an oleaginous yeast and has proven as a superior platform to produce lipids and oleochemicals. The abundant membrane structure and subcellular organelles provide the ideal environment for plant P450 enzyme functions. The endogenous mevalonate pathway and innate substrates could be harnessed for amorphadiene production, which is the sesquiterpene olefin precursor to artemisinin. HMG1 (HMG-CoA reductase) is the rate-limiting enzyme of sterol biosynthesis pathway and converts HMG-CoA to mevalonate. Squalene synthase (SQS1) converts farnesyl pyrophosphate to squalene. Both HMG1 and SQS1 are localized in endoplasmic reticulum (ER), indicating the sterol biosynthetic pathway is spatially organized and ER may provide the optimal microenvironment for their catalytic efficiency. Biochemical studies and metabolite profiling indicate that squalene synthase is the major competing step for amorphaiene production. To maximize the activity of amorphadiene synthase, we fused the SQS1 ER localization domain with amorphadiene synthase. This chimeric protein maintains ADS activity and effectively competes with squalene synthase for farnesyl pyrophosphate (FPP), leading to about 45 mg/L amorphadiene without dodecane overlay.The localization of ADS to endoplasmic reticulum may serve as a metabolic channel for HMG1-CoA and FPP, which effectively converts HMG-CoA to amorphadiene. Furthermore, the spatially organized protein may prevent intermediates diffusion, concentrate the critical metabolites and increase the catalytic activity.

P100 Large serine recombinase-mediated genome engineering in non-model microbes

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Model microorganisms can be attractive chassis for metabolic engineering to become industrial biocatalysts because they possess a wide range of genetic tools and prior physiological characterization. However, the construction of desirable phenotypes in these model organisms can be hindered by the physiological limitations of the chassis and by the available intellectual property space. Recently, the advent of widespread next generation sequencing has made the genotypes of many organisms with unique and desirable phenotypes available. Capitalizing on this workspace requires operable high throughput genetic tools; unfortunately, traditional techniques of strain engineering are often stymied by host defenses or require intense optimization. One method of genetic manipulation, site specific recombination using large serine recombinases, has been shown to function in all branches of life and is consistently both accurate and efficient. We applied the paradigm of serine recombinases to several different organisms to develop high throughput methods for screening heterologous genes and pathways. When implemented in the hosts *Pseudomonas putida*, *Corynebacterium glutamicum*, and *Cupriavidus necator*, serine integrases were easily functionalized and enabled high throughput, rapid, and stable chromosomal integration of DNA. This integration system is being exploited for the development of additional genetic tools, such as promoter libraries and targeted proteolysis systems, as well as pathway engineering for the conversion of renewable feedstocks to fuels and chemicals.

P102 Beneficial mutations for carotenoids production identified from directed evolution of carotenoid producing yeast

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Metabolic engineering has been used for decades to express of heterologous genes in yeast to produce wide plethora of products. Adaptive laboratory evolution (ALE), a complementary technique to metabolic engineering as it does not require prior knowledge on genetic targets, has been used as a tool to increase the fitness and production of host strains in harsh environmental conditions. Choosing the appropriate selective pressure for ALE experiments is crucial in order to select for the desired phenotype. In our prior work, yeast mutants with increased carotenoids production were successfully selected for in the presence of oxidative stress using ALE by taking advantage of the of antioxidant property of carotenoids. Isolated hyperproducers were subjected to next generation sequencing (NGS) and the results revealed multiple unique mutations in genes which are not known to be related to terpene biosynthesis in yeast. Individual reconstruction of these mutation using CRISPR-Cas9 revealed several to confer significant increase in carotenoid production. Combining various mutations identified from different evolved isolates, we identified combinations of mutations that confer up to ~153% increase in beta-carotene production compared to ancestral strain. Raman spectroscopy analysis of reconstructed mutants revealed an increase in lipid content in the best carotenoids producing strains. In addition, we confirmed that the ALE strategy directly selected for mutants with increased carotenoids production rather than just increased oxidative stress tolerance. Furthermore, the best combinations identified for beta-carotene production also enhanced lycopene production in yeast, suggesting that these mutations may be beneficial for general carotenoids production in yeast.

P104 Enabling Transformation of Non-Model Organisms by Overcoming DNA Restriction-Modification Systems

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Many organisms naturally possess complex physiological traits that are of interest for biotechnology research. These non-model organisms are not routinely used though, due to a lack of foundational knowledge and genetic tools. The development of genetic tools is limited in part by the inability to efficiently transform DNA into these organisms. One of the major barriers to efficient DNA transformation is the native DNA Restriction-Modification systems within bacteria. DNA Restriction-Modification systems act as an immune system to cut heterologous DNA methylated differently than the host. To overcome these systems, DNA needs to be methylated in the same manner as the host organism prior to transformation. In order to determine the sites targeted for restriction we performed methylome analysis for non-model organisms of interest in collaboration with the Department of Energy's Joint Genome Institute. This information was used to choose methyltransferases for expression in *E. coli* to protect DNA for transformation of multiple organisms. Using this approach, we enabled the transformation of eight strains of bacteria including two strains of *Megasphaera elsdenii.* We are now developing *M. elsdenii* into a new bioengineering platform for the production of fuels and chemicals. *M. elsdenii* is a compelling platform organism due to the ability to efficiently produce C-4 and C-6 acids from lactate and glucose. This work acts as a blueprint for the rapid transformation and genetic engineering of non-model organisms.

P106 Lignocellulosic inhibitors, furfural and 5-hydroxymethyl furfural, promote rate of biomass formation in growth deficient ethanologenic *Escherichia coli strain SSK101*

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High temperature and dilute acid treatment of lignocellulosic biomass releases hemicellulose in the form of glucose, xylose and arabinose and other aromatic compounds. However, the treatment also results in breakdown of xylose and glucose into inhibitory compounds furfural and 5-hydroxymethyl furfural (5-HMF) which significantly lower microbial productivity and increase the production cost of compound of interest. Extrachromosomal expression of transhydrogenase *pntAB* involved in coupling oxidation of NADH to reduction of $NADP^+$ rescued growth in *E. coli* in presence of furfural[1]. However, heterologous expression of foreign genes present long-term challenges in terms of plasmid retention and expression of cloned genes.

Without using any foreign gene nor extra-chromosomal DNA, in an ethanologenic *E. coli* strain SSK101 we channeled glycolytic flux towards pentose phosphate pathway. In absence of furfural and using glucose as sole carbon source in AM1 minimal media, the rate of biomass increase in the mutant strain at 3, 6 and 9 hours was 0.014(±0.005), 0.029(±0.003) and 0.047(±0.009) g/L/h, respectively. In **presence of 1g/L furfural** the respective values of biomass increase were 0.025(±0.004), 0.054(±0.003) and 0.071(±0.007) g/L/h which represent **increase in productivity** by 44%, 46.3% and 33.8%, respectively. In presence of 1 g/L 5-HMF respective increases were 57.6%, 68.13% and a decrease of 23.7%.

In the control non-modified strain, under same conditions and time points and absence of furfural, the rates of biomass increase were 0.07(±0.01), 0.201(±0.011) and 0.164(±0.02) g/L/h. In **presence of 1 g/L furfural** the values decreased to 0.013(±0.004), 0.044(±0.005) and 0.11(±0.024) g/L/h, respectively. The latter values represent **decrease in productivity** by 81.4%, 78.1% and 32.9%, respectively. In presence of 1 g/L 5-HMF respective decreases were 41.4%, 27.36% and 3.7%.

These results demonstrate that the common lignocellulosic stress variables - furfural and 5-HMF - subsidize growth of ethanologenic *E. coli* SSK101 strain. The strain needs to be further evaluated at the fermenter level in presence of both furfural and 5-HMF in the growth media.

P108 Minimal modular plasmid vectors for *Cupriavidus necatorÂ* H16

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Minimal modular plasmid vectors for *Cupriavidus necator* H16

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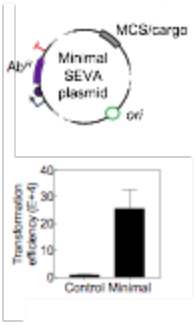

The model chemolithoautotrophic bacteria *Cupriavidus necator* is gaining significant attention in metabolic engineering and synthetic biology applications due to its flexible bioenergetics. Unlike heterotrophic microbial chassis, *C. necator* is more propitious for bioengineering, having the required metabolic pathways for production of high-valued compounds. However, like other chemolithoautotrophs, improving pathway engineering in *C. necator* requires well-characterised tools. The lack of such tools hinders the deployment of *C. necator* as a microbial chassis. Here, we report the design and construction of minimal modular plasmid vectors for *C. necator*. Each biopart of our plasmids was extensively characterised in *C. necator* and can be used for rapid deconstruction and reconstruction for use in other Gram-negative bacteria. We standardised each biopart adopting the Standard European Vector Architecture (SEVA) format. Our minimal plasmids improved transformation efficiency by more than 600-fold in *C. necator*. In the absence of antibiotic pressure, our plasmids can stably be maintained under prolonged cultivation, whilst expressing reporter proteins driven by strong synthetic constitutive promoters. Together, our toolkit will be invaluable for future metabolic engineering of *C. necator*, and will enable efficient, reproducible and high-throughput bioengineering in the bacterium allowing successful deployment of this bacterium as an industrial chassis.

P110 Synthetic mRNA designs for the enhancement of the chaperone-mediated solubilization of recombinant proteins in *Escherichia coli*

T.T. Nguyen, C. Cui, J.H. Lee, M.S. Kim, J.I. Baek and S.C. Kim, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South)*

The formation of insoluble and inactive Inclusion bodies is one of the major obstacles in engineering bacterial hosts for recombinant proteins production. Natural molecular chaperone systems have been widely used to minimize the formation of inclusion bodies, producing soluble and functional recombinant proteins, albeit with limited successes. Here, using synthetic mRNA designs to spatially constrain molecular chaperones of DnaK system next to the location of the translation process producing a protein of interest, the solubility of various aggregation-prone proteins was significantly improved. To this end, we designed two systems, namely chaperone recruiting mRNA scaffold (CRAS) and chaperone-substrate co-localized expression (CLEX) system, in which *Escherichia coli* chaperone DnaJ is anchored to 3' untranslated region of an mRNA encoding a target protein via KH, an RNA binding domain, or coupled the expression of DnaJ and a target recombinant protein using the overlapping stop-start codons 5'-TAATG-3' between the two genes, respectively. By spatially constraining bacterial molecular chaperones to the location of protein translation using the CRAS and CLEX systems, we are able to surpass the in vivo solubilization efficiency of the native chaperone system and to overexpress aggregation-prone recombinant proteins from various organisms, producing up to 95% in functionally active soluble forms.

P112 Production of medium chain length alcohols from lignin-derived aromatic compounds in *Pseudomonas putida*

J. Huenemann, University of Tennessee, Knoxville, Knoxville, TN, USA; J. Elmore, Pacific Northwest National Laboratory, Richland, WA, USA; S. Holtzen and A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Lignin is the second most abundant carbon-based polymer found in nature; however, it is currently underutilized due to its complexity and recalcitrance. The conversion of lignin derived carbon into value-added products is essential to support the economic viability of lignocellulosic refineries targeting the production of biofuels from lignocellulose. *Pseudomonas putida* is a robust host that can catabolize many of the aromatic monomers generated during lignin depolymerization. From these monomers, *P. putida* can funnel carbon into the production of polyhydroxyalkanoates (PHAs) when nitrogen is limited. Taking advantage of this natural carbon flux, we engineered strains of *P. putida*, utilizing heterologous gene expression and deletion of native genes, to produce medium chain length (mcl-) alcohols. Gene deletions targeted pathways that compete with our production pathway for carbon and electrons. For the production pathway, multiple orthologs were explored to tune the pathway for increased production. Using this approach, we were able to demonstrate production of approximately 25 mg/L mcl-alcohols from the model aromatic substrate p-coumarate when expressed from the P_{tac} promoter. Previous work in our lab has demonstrated that the *tac* promoter is poorly expressed during nitrogen starvation conditions in *P. putida*. Therefore, we also utilized a suite of recently developed nitrogen-regulated and hybrid expression promoters to drive the transcription of production pathways. Further optimization with our engineered promoters generated titers that exceeded 70 mg/L total mcl-alcohol production. This work represents the first demonstration of mcl-alcohol production from a model, lignin-derived compound.

Tuesday, July 23

6:15 AM - 7:15 AM Annual 5K Fun Run Walk - Must complete waiver form, available at registration desk. No charge to participate. Tees and refreshments provided.

Lobby level

7:00 AM - 5:00 PM Registration

Atrium, Lower level

8:00 AM - 11:30 AM Session: 11: High added value products from bioelectrocatalysis

Conveners: Dr. Enrico Marsili, Nanyang Technological University, Singapore, Singapore and **Dr. Navanietha Krishnaraj Rathinam**, South Dakota School of Mines and Technology, Rapid City, South Dakota. USA., SD, USA

Madison B, Mezzanine

8:00 AM S61: Control of metabolic state of two *Clostridia* sp. through galacturonate and glucose co-utilization

T. Zu, Army Research Laboratory, Adelphi, MD, USA; C. Sund, S. Liu, E. Gerlach and K. Akingbade, CCDC US Army Research Laboratory, Adelphi, MD, USA*

The use of substrates of different oxidation states by organisms results in different levels of reduced intracellular electron carrying co-factors. In this case-study, we observed that when galacturonate and glucose were simultaneously fed as substrates to *Clostridium acetobutylicum*, both substrates were co-utilized right from the start of the fermentation which is counter intuitive since glucose is thought to be the preferred substrate. We envisage that this finding provides basis for the use of ratios of oxidized and reduced substrates as a control mechanism for cells to regulate intracellular redox environment via controlled production of reduced co-factors. This will allow for fine-tuned control of the metabolic state of the organisms as opposed to simple switching substrate oxidation state which results in step wise control. Applications of this findings are to control metabolism for optimized biological chemical production, increased susceptibility to antibiotics, and modulation of microbial communities for increased performance of organisms and materials.

8:30 AM S62: Continuous caproate (C6) production from CO_2 by microbial electrosynthesis: making feed additive precursor with electricity

L. Jourdin, Delft University of Technology, Delft, Netherlands; C. Buisman and D. Strik, Wageningen University, Wageningen, Netherlands*

Microbial electrosynthesis (MES) is a biotechnology in which microorganisms take up electrons from a solid electrode to reduce CO_2 to organics. Renewable electricity can be used to power MES. A challenge for MES is to produce longer carbon-chain, higher-value, organic compounds than acetate. Here, we report on a MES process achieving elongation of CO_2 to a mixture of acetate (C2), n-butyrate (nC4), and n-caproate (nC6). Moreover, we demonstrate that MES can be tuned from high acetate selectivity (96.4 ± 2.4 %C) to high carbon selectivity into n-butyrate (39.4 ± 6.4 %C) and n-caproate (24.2 ± 3.6 %C). We show that high CO_2 loading rate (173 L d^{-1}) and long hydraulic retention time (14 d HRT) triggers MES to medium-chain fatty acids with high electron recovery into organics, whereas lower CO_2 loading rate (9 L d^{-1}) does not, even with increasing HRT and current density. No other organics were detected. Thick, uniform, and highly electroactive biofilms were developed covering the whole thickness of the 3D cathode (1.2-cm deep). This high microorganisms' density led to high current densities and organics production rates. We report the highest sustained production rates and concentrations of acetate (9.8 g L^{-1} d^{-1}, 17.5 g L^{-1}), nC4 (5.7 g L^{-1} d^{-1}, 9.3 g L^{-1}), and nC6 (2.0 g L^{-1} d^{-1}, 3.1 g L^{-1}) to date in MES from CO_2, at high electron recoveries of 70-100%. MES now offers a unique product spectrum among CO_2 conversion technologies. This work represents a step forward to practical implementation of the technology.

9:00 AM S63: Anaerobic Treatment of High Fat Content Wastewater in Microbial Electrochemical Cells for Peroxide Production

S. Popat, A. Xie and N. Kananizadeh, Clemson University, Anderson, SC, USA*

Microbial electrochemical cells (MECs) are devices in which anaerobic treatment of wastewater can occur in the anode chamber, where anode-respiring bacteria oxidize fatty acids produced from fermentation of waste organics to produce an electrical current. MECs are best suited for high-strength industrial wastewater streams, rather than dilute domestic wastewater streams. We have evaluated the possibility of using MECs for treatment of rendering wastewater, which contains high concentrations of fats (and often also proteins). We first conducted biochemical methane potential (BMP) tests to determine the anaerobic biodegradability of rendering wastewater (COD concentrations of ~7000 mg/L), and to determine optimum organic loading rate (relative to biomass concentration). Using the optimum conditions, we then conducted batch MEC studies to

determine maximum current densities that could be produced, as well as the COD removal and conversion efficiencies. Maximum current densities of up to 2.5 A/m^2 were produced, while COD conversion to electrical current ranged between 70-80%. The eventual goal is to develop MECs for treatment of rendering wastewater with cathodic production of hydrogen peroxide that could be used on site for cleaning. To this end we have tested carbon-based catalysts for peroxide production, as well as evaluated mechanisms that affect peroxide production efficiency. Notably, high cathodic pH at high current densities and/or longer retention times lead to peroxide auto-degradation, resulting in production efficiencies of <50%. We are currently evaluating strategies for alleviating this issue.

9:30 AM Break

10:00 AM S64: Extracellular electron transfer capabilities of phenotypically improved methylotrophs

J. Kalimuthu, V. Gadhamshetty, B. Vemuri and J. Islam, South Dakota School of Mines and Technology, Rapid City, SD, USA; S. Dhiman and R. Sani, South Dakota School of Mines and Technology, RAPID CITY, SD, USA*

Given that typically discarded methane represents an untapped source of energy and that it contributes to radiative forcing of climate change, it is desirable to biologically upcycle methane into electricity under ambient conditions. The use of Microbial fuel cells for harnessing the electrical power from the greenhouse gas methane has been less explored in the recent times. The existing studies on methane microbial fuel cells are either based on mixed microbial populations or synthetic microorganisms. Here we report the use of pure cultures of methylotrophic strain for converting methane into methanol or electricity. We also report the surface modification strategies to promote growth of electrogenic methylotrophs and enhance electricity production. A detailed work based on electrochemistry methods and genome-to-phenome analysis will be presented.

10:30 AM S65: Bacteria-surface interactions and electrochemical signature of microstructured electrodes for early biofilm formation

S.E. Astorga, L.X. Hu and Y. Huang, Nanyang Technological University, Singapore, Singapore; E. Marsili, Nazarbayev University, Nur-Sultan City, Kazakhstan*

Biofilms comprise of microorganisms encased in self-produced extracellular polymeric matrix, which provide mechanical stability, and favours adhesion to nearly any surfaces. When biofilm grows onto an electrode, it is termed electroactive biofilm (EAB). EAB are capable of extracellular electron transfer (EET) to and from solid acceptor, through direct or mediated mechanism. A thorough comprehension of the mechanism underlying EET is needed to develop productive EAB for biosynthetic purposes. The EET mechanisms are investigated through a combination of electrochemical techniques (e.g. CA, PEIS) and microscopy techniques, to determine the optimal condition for biosynthesis or sensing. The electrode material and microstructure play an important role in the characterization, since they both affects the attachment and EET of biofilms. In this work we characterise the EET of early *Escherichia coli* biofilms grown on microfabricated gold and nickel electrodes. Results show that small microfeatures affect transition from planktonic to biofilm and electrochemical impedance spectroscopy is the best method to reveal early biofilm formation, as confirmed by microscopy analysis. Further, the effect of temperature on the biofilm electrochemical signature was investigated. This work contribute to our understanding of bacteria-surface interaction in thin biofilms, which is relevant to develop more effective bioelectrochemical synthesis processes.

8:00 AM - 11:30 AM Session: 12: Bioremediation and Biodegradation: lab and field applications

Conveners: Kevin Finneran, Washington State University, Pullman, WA, USA and **Prof. Haluk Beyenal**, Washington State University, PULLMAN, WA, USA

Madison A, Mezzanine

8:00 AM S66: Microbial Electrochemical Remediation of Estrogens in wastewater and Bioelectricity generation

M. Sogani, A. Dongre and Z. Syed, Manipal University Jaipur, Jaipur, India; A. Fisher, University of Cambridge, Cambridge, United Kingdom*

The cost effective removal of steroid estrogens especially Ethinylestradiol (EE2) micropollutant from municipal wastewaters is recognized as a pivotal technical hurdle that must be overcome due to severe effects on human beings and aquatic organisms. It is able to mimic hormones or interfere with the action of endogenous hormones. The search for new enzymes to degrade EE2 from wastewaters is being pursued aggressively around the world. We have identified a unique bacterium that possibly uses EE2 as a major carbon and energy source and this has raised significant interest in how such an enzymatic mechanism functions with such a highly resistant aromatic substrate that appears to survive for centuries in the environment. Our Research has demonstrated that our strain has the capability to generate power from the synthetic hormone as substrate, which is accomplished by utilizing the organism's different modes of metabolism. Our strain of *R. palustris*, has shown a decrease of

88.45% in EE2 concentration and a power output of 780 μW/m^2 over a period of 14 days. Along with the process of EE2 degradation, this microbe was able to generate hydrogen as part of the process of nitrogen fixation. While more research is required and advances are necessary in modern technologies for *R. palustris* hydrogen production mediated by EE2 to become a viable source of energy, this research provides a better insight into microbial electricity generation and self-sustaining wastewater treatment facilities.

8:30 AM S67: Analysis of termite microbiome and biodegradation of various phenolic compounds by a bacterium isolated from the termite gut

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

The eastern subterranean termite (EST) *Reticulitermes flavipes* is an insect pest in the United States. Like all wood-feeding termites (WFT), EST relies on a complex system of microbes to meet its nutritional requirements. The microbiome of WFT is stable, but the relative abundance of bacteria changes depending on diet. The purpose of this study was to explore the microbial diversity within EST collected in Thibodaux and St. Francisville, LA and detect differences based on diet and location to determine if the microbiome has a strict structure. It was found that taxa did not differ much between nearby colonies, but relative abundance is impacted by the wood in the diet. Half of bacteria from the gut of termites on nuttal oak were Bacteroidales, of which 22.7% were members of the family Porphyromonadaceae. 44% of bacteria from termites on red maple were Spirochaetes. All Spirochaetes were members of the genus *Treponema*. Elusimicrobia, a phylum found exclusively within termites and wood-feeding cockroaches, was not abundant in either St. Francisville colony. Taxa differed more between termite colonies from different locations, but the mircobiome of St. Francisville colonies appeared to begin diverging at the family level. Overall, the microbiome was typical of termites, harboring cellulolytic protozoa, nitrogen fixing bacteria, acetogenic Spirochaetes, and methanogenic archaeans. This has implications in microbial ecology because the organisms are changing, but the function, digestion of lignocellulose, is not. A bacterium isolated and identified from the termite gut as *Acinetobacter tandoii* degraded various phenolics, including phenol, nitrophenol, dinitrophenol, trinitrophenol, and toluene.

9:00 AM S68: PCB Bioremediation: Finally Coming of Age?

U. Ghosh, N. Lombard, T. Needham, R. Payne and K. Sowers, UMBC, Baltimore, MD, USA*

Microbial dechlorination of polychlorinated biphenyls (PCBs) has been reported in numerous studies in the last several decades, but these recalcitrant pollutants have persisted in the environment and continues to impact human health and the ecosystem. Prediction of microbial bioremediation of PCB contaminated sediments has been challenging due the difficulty in measuring the microbial kinetics at environmentally relevant aqueous concentrations and to a lack of understanding of the true kinetic bottlenecks. In our recent work, we report a novel approach to measure the intrinsic dechlorination rate with respect to freely dissolved concentration and show that it is possible to predict the rate of dechlorination in sediments after correctly accounting for the sorption buffering capacity of sediments. The novelty of this approach lies in moving away from past description of microbial kinetics based on PCB concentration in sediment to a freely dissolved concentration basis. We show that for typical PCB-impacted sediments, low abundance of native organisms is the primary bottleneck for PCBs bioremediation which can be circumvented by bioaugmentation. We first validated the proposed approach of bioaugmentation of PCB dechlorinating organisms in laboratory mesocosms with field sediments and followed it with a pilot-scale demonstration at a field site. Treatments with activated carbon (AC) agglomerate bioamended with PCB dechlorinating and oxidizing bacteria decreased the PCB concentration in the top 7.5 cm by up to 52% and the aqueous concentrations of tri- to nonachlorobiphenyl PCB congeners by as much as 95%. Coplanar congeners decreased by up to 80% in sediment and were undetectable in the porewater. This pilot-scale field study shows the promise of bioremediation as a new strategy to reduce contamination of the aquatic food web from exposure to sediment-bound PCBs.

9:30 AM S69: Dechlorinating Activity and Occurrence of *Dehalococcoides*, *Dehalogenimonas*, andÂ *DehalobacterÂ* in the Subterranean Estuary Sediments in San Diego Bay

N. Durant and H. Rectanus, Geosyntec Consultants, Columbia, MD, USA; J. Roberts and M. Healey, SIREM, Guelph, ON, Canada; P. Stang, Trevet Inc., San Diego, CA, USA; M. Pound, NAVFAC Southwest, San Diego, CA, USA*

Groundwater impacted with chlorinated ethenes (CEs) at Naval Air Station North Island IR Site 9 discharges via a subterranean estuary to sediments in San Diego Bay. Field and laboratory studies were performed to assess the role of natural biotic and abiotic processes in attenuating CEs in the sediments, prior to discharge into the Bay. Anaerobic batch microcosms were prepared using sediments and porewater collected from depths between 1- and 8-feet at three offshore locations representing a range of CE porewater concentrations. Microcosms were prepared for each location/depth: at two locations, microcosms were prepared for samples from 1-, 5-, and 8-foot depths, and at the third location, microcosms were prepared for samples from 3.5- and 6.5-foot depths. Microcosms did not receive any amendments and were incubated for up to 39 weeks. CEs [trichloroethene, cis-1,2-dichloroethene, 1,1-dichloroethene, and vinyl chloride] degraded completely at all study location/depths. The fastest CE degradation rates were observed in microcosms for two sample locations at 1-foot depth, with half-lives ranging from 4 to 9 days. qPCR detected *Dehalococcoides* (*Dhc*) bacteria at all study location/depths. *Dhc* containing the vinyl chloride reductase (vcrA) gene were detected at six out of eight location/depths, including location/depths showing the fastest CE degradation rates. Next Generation Sequencing analyses also detected *Dehalogenimonas* and *Dehalobacter* in some locations. Compound specific

isotope analyses indicated that CEs become enriched in ^{13}C as groundwater migrates upward through the Bay sediments. Total organic carbon in six out of eight location/depths was detected in the range of 0.6 to 2.5 g/Kg, levels that have been reported in the literature to support natural reductive dechlorination. Natural abiotic reductants, including magnetite and ferrous sulfide, also were detected in the sediment; however, not at concentrations sufficient to explain the relatively rapid dechlorination rates observed in the microcosms. This study demonstrates that dechlorinating bacteria occur naturally in San Diego Bay sediment and are actively degrading CEs in porewater, in the presence of > 2,000 mg/L sulfate.

10:00 AM Break

10:30 AM S70: Biocorrosion of Mild Steel Coupons was Enhanced during Methanogenic Biodegradation

C. Okoro, Federal University, Ndufu-ALike, Ikwo. Ebonyi State, Nigeria, Abakaliki, Nigeria*

Methanogenic biodegradation of crude oil sludge was investigated using chemical and molecular approaches. 16S rRNA gene sequences recovered from the samples revealed significant presence of *Marinobacterium* (63%), *Pseudomonas* (3%) alongside with acetotrophic *Methanosaeta* (16%) and hydrogenotrophic *Methanobacterium* (5%). The resident microbial community was able to reduce the gravimetric weight of residual oil by 65.5% (with complete degradation of C_5-C_{17} nAlkane fractions) in non-amended samples and 94.13% (with complete degradation of C_5-C_{25} nAlkane fractions) in substrate amended samples during the 60-day incubation period. As biodegradation progressed, acetotrophs consume acetate at the rate of 0.41mM/day^{-1} while hydrogenotrophs consume hydrogen at the rate of 0.59mM/day^{-1}. Respective volume of methane produced and corrosion rates observed were higher in highly biodegraded samples (3.60mmol/0.084 mm/yr) than lesser biodegraded samples (1.64 mmol/0.018 mm/yr). Our results showed that the resident methanogenic archaea were largely responsible for the anaerobic biodegradation of hydrocarbons in crude oil sludge and biodegradation were enhanced with substrate amendment which further accelerated the corrosion rates of mild steel coupons. Considering the relatively high number of facultatively anaerobic *Marinobacterium* and significant presence of *Pseudomonas* in the sequenced data, we speculate that the bacteria were at least partially responsible for biodegradation of crude oil components potentially acting as syntotrophic organisms with methanogens to convert crude oil to methane and subsequently enhance corrosion rates of mild steel coupons.

11:00 AM S71: Grubs and grass roots: Nature's tiny bioremediation factories

C. Jung, M. Carr, R. Lance, D. Lindsay and K. Indest, US Army Engineer Research & Development Center, Vicksburg, MS, USA*

The field of bioremediation is well established and involves the use of biological agents to degrade environmental contaminants. The majority of data collected regarding terrestrial biodegradation of contaminants have focused on soils, microbes, and plants, largely ignoring the arthropod community. However, entomoremediation (insect-driven bioremediation) of priority pollutants has been only sparingly explored. The overall objective of this research was to introduce degradative bacteria into a system in close contact with contaminated soils in order to create a micro-scale, remediation platform with soil-associated insects acting as soil processing reactors. Green June beetles (*Cotinis nitida*) were collected as newly emerged adults and reared in the lab, where resultant larvae were exposed to phenanthrene and RDX contaminated soil with weekly monitoring of survivability and body weight as a range-finding toxicity study. New larvae were inoculated with Phenanthrene or RDX degrading bacteria via feeding with inoculated alfalfa meal and monitored through bacterial DNA sequencing and selective plating of gut contents after 1 month of exposure to ensure uptake. Reduced body weight and survival were observed in the highest dose of Phenanthrene and RDX (25 ppm) but the larvae were still active and feeding regularly after 5 months. This research incorporates new approaches to biodegradation of Army relevant chemicals. Seeding the microbiome of soil-dwelling organisms would be a novel approach to delivering beneficial microbes directly to the site of contamination for "hot spot", low maintenance bioremediation.

8:00 AM - 11:30 AM Session: 13: Frontiers in fermentation: Uncommon microbes and feedstocks

Conveners: Dr. Michael Köpke, LanzaTech Inc., Skokie, IL, USA and **Dr. James Clomburg**

Marshall Ballroom North, Mezzanine

8:00 AM S72: Sustainable bioproduction of the blue pigment indigoidine: Expanding the range of heterologous products in *Rhodosporidium toruloides* to include non-ribosomal peptides

M. Wehrs, J.P. Prahl, D. Tanjore, T.R. Pray, B.A. Simmons and A. Mukhopadhyay, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; J.M. Gladden, Sandia National Laboratories, Livermore, CA, USA*

Non-ribosomal peptides (NRPs) constitute a diverse class of valuable secondary metabolites, with potential industrial applications including use as pharmaceuticals, polymers and dyes. Current industrial production of dyes is predominantly achieved via chemical synthesis, which can involve toxic precursors and generate hazardous byproducts. Thus, alternative routes of dye production are highly desirable for companies seeking to enhance both workplace safety and environmental

sustainability. Biological synthesis of dyes from renewable carbon is an excellent example of green chemistry. Therefore, we engineered the fungal host Rhodosporidium toruloides to produce the blue pigment indigoidine, an NRP with potential applications in the dye industry, using various low-cost carbon and nitrogen sources. We established that the colorimetric features of indigoidine are dependent on the pH of the culture as well as the oxidation state of the molecule. To demonstrate production from renewable carbon sources and assess process scalability we produced indigoidine in 2 L bioreactors, reaching titers of 2.9 ± 0.8 g/L using a sorghum lignocellulosic hydrolysate in a batch process and 86.3 ± 7.4 g/L using glucose in a high-gravity fed-batch process. This study represents the first heterologous production of an NRP in R. toruloides, thus extending the range of microbial hosts that can be used for sustainable, heterologous production of NRPs. In addition, this is the first demonstration of production an NRP using lignocellulose. These results highlight the potential of R. toruloides for the sustainable, and scalable production of NRPs, with the highest reported titer of indigoidine or any heterologously produced NRP to date.

8:30 AM S73: Life on methane: methanotrophy revisited

L. He and M.E. Lidstrom, University of Washington, Seattle, WA, USA; Y. Fu, Nanostring Technologies Inc., Seattle, WA, USA*

Methanotrophs are bacteria capable of growth on methane as sole carbon and energy source. The subset of these bacteria that grow aerobically and use an oxygenase to oxidize methane to methanol are of interest for commercial use of methane as a feedstock to generate chemicals. Recent work involving ^{13}C metabolic flux analysis of wild type and mutant strains of a gamma-proteobacterial aerobic methanotroph, *Methylomicrobium buryatense* has revealed that previous assumptions concerning the metabolic network of this bacterium were incorrect. A full flux map will be presented and notable insights gained from this analysis will be outlined. Accurate depictions of the metabolic network are important for successful metabolic engineering, and examples of how changes in our understanding could impact engineering strategies will be presented. Finally, remaining questions regarding the metabolism of aerobic methanotrophs will be described.

9:00 AM S74: Engineering a methanotroph biocatalyst for natural gas-to-liquids bioconversion

X. Zhao, Intrexon, South San Francisco, CA, USA*

Natural gas is one of the most economical sources of carbon and is therefore an attractive feedstock for microbial bioconversion to industrial products. Methanotrophic bacteria can use methane, the primary component of natural gas, as the sole carbon source to support cellular metabolism and growth. In order to take advantage of the abundant and inexpensive supply of natural gas, Intrexon has developed a methanotroph-based platform technology for the production of higher value industrial products from natural gas. This platform consists of a set of genetic tools that enable facile host modification, a detailed metabolic model, a high throughput strain evaluation platform, and the fermentation infrastructure for lab and pilot scale strain evaluation. This presentation will provide an overview of Intrexon's methanotroph platform and show that strain improvement has enabled fermentation utilizing both the methane and ethane in natural gas as the feedstock, and the 2,3 butanediol (BDO) titer with natural gas is rapidly increasing.

9:30 AM Break

10:00 AM S75: The formate economy: Reprogramming *Escherichia coli* metabolism to use formate

A. Bar-Even, Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany*

The formate economy: Reprogramming *Escherichia coli* metabolism to use formate

Arren Bar-even, Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam, Germany

One-carbon compounds can be efficiently produced from CO_2 using renewable energy and thus provide an alternative microbial feedstock for sustainable and affordable bioproduction. Acetogens support highly efficient anaerobic bioproduction using C_1 feedstocks but are limited by a narrow product spectrum. Hence, in many cases, aerobic C_1-dependent bioproduction provide a favourable alternative. However, aerobic assimilation of C1 compounds into central metabolism is limited to relatively inefficient routes with low biomass and product yields. An effective way to tackle this problem is to design and implement tailor-made pathways that could potentially transform any microbe into an efficient C1-metabolizer. Following this logic, we have designed the reductive glycine pathway – an aerobic 'twin' of the anaerobic reductive acetyl-CoA pathway – which represents the most efficient route for aerobic formate assimilation. In this synthetic pathway, formate is reduced and condensed with CO_2 and ammonia to generate the C_2 amino acid glycine. Glycine is condensed with another reduced formate to produce the C_3 amino acid serine, which is deaminated to provide pyruvate for cellular growth. We are working to establish the reductive glycine

pathway in multiple biotechnological organisms. In this talk I shall focus on our work in *Escherichia coli* and show that a rational integration of native and foreign enzymes enables the THF and glycine cleavage/synthase systems to operate in the reductive direction, satisfying all cellular glycine and serine requirements from the assimilation of formate and CO_2. Our findings assert that the reductive glycine pathway could support highly efficient aerobic assimilation of C_1-feedstocks.

10:30 AM S76: Systems Biology of Acetogenic Bacteria

B.K. Cho, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South)*
Acetogenic bacteria are considered to be the most efficient microorganism for fixing C1 compound as they gain energy from operating the pathway, in contrast to the other C1 compound-fixing bacteria that spend energy during the uptake. Acetogenic bacteria are present in 23 different genera with over a hundred strains isolated from diverse habitats. The Wood-Ljungdahl pathway in the microorganism converts C1 into acetyl-CoA, which is an important cellular precursor that is converted into biochemicals. Despite the potential to reduce C1 compound in the atmosphere and industrial waste gases, lack of a systemic understanding, complex layers of regulation system, and inefficient electron delivery has limited the construction of a cellular factory optimized for producing the desired chemical. To overcome the limitation, molecular level insight has been obtained via multi-layered genome-scale analyses. The results revealed functional genes required for C1 compound fixation and their regulatory systems. Integration of the information-rich data types with genome engineering technologies will facilitate the construction of optimal C1 fixing and biochemical-producing cellular factories.

11:00 AM S77: Harnessing the capabilities of Clostridia strains for industrial biotechnology and nutrition

S. Jones, White Dog Labs, Inc., New Castle, DE, USA*
To help address global challenges, including food sustainability and climate change, White Dog Labs (WDL) is developing technology solutions based upon harnessing the unique abilities of bacteria within the Clostridia class. These microbes can be found in diverse environments from soils to gastrointestinal tracts to extreme environments and have developed exceptional capabilities. One of WDL's foundational technologies is a fermentation platform called MixoFerm, in which specific microbes are used that are able to simultaneously utilize both soluble and gaseous feedstocks. This dual feedstock consumption can improve the efficiency of fermentation systems and help reduce production costs. The first industrial application of this platform will be for a Single Cell Protein (SCP) product called ProTyton and is being targeted as an animal feed ingredient. WDL is also engineering microbes to produce desired biochemicals with the MixoFerm platform at improved mass yields. We have only begun to recognize the full potential of Clostridial organisms, and we will continue applying them to help address food sustainability, animal and human health, and climate change.

8:00 AM - 11:30 AM Session: 14: New Enzymology in Natural Product Biosynthesis

Conveners: Yi Tang, University of California Los Angeles, Los Angeles, CA, USA;**Prof. Wei-chen Chang**, North Carolina State University, Raleigh, NC, USA and **Wenjun Zhang**, UC Berkeley, Berkeley, CA, USA

Marshall Ballroom Southeast, Mezzanine

8:00 AM S78: Non-Heme Iron(II)-Dependent Oxidase/Decarboxylase for Isonitrile Biosynthesis

W. Zhang, UC Berkeley, Berkeley, CA, USA*
The electron-rich functionality of the isonitrile lends itself as a biologically active warhead for naturally derived products and is often exploited for metal acquisition, detoxification, and virulence. Despite the widespread utility of isonitrile in nature, its biosynthesis has long been considered endemic to the IsnA family of isonitrile synthases, which typically convert an amino group to isonitrile on an amino acid and require ribulose-5-phosphate as a co-substrate. In this presentation, our recent efforts in identifying an alternative mechanism for isonitrile formation will be discussed. Specifically, a non-heme iron(II)-dependent enzyme was characterized to promote the conversion of a fatty acid containing an (*R*)-3-carboxymethylamino group to an (*R*)-3-isonitrile group through an oxidative decarboxylation mechanism.

8:30 AM S79: Mechanism elucidation of a dual-functional iron(II)/2-oxoglutarate dependent enzyme in the biosynthesis of quinolone alkaloids

Y. Guo, J. Li, I. Kurnikov and M. Kurnikova, Carnegie Mellon University, Pittsburgh, PA, USA; J.L. Huang and W.C. Chang, North Carolina State University, Raleigh, NC, USA; H.J. Liao and N.L. Chan, National (Taiwan) University, Taipei, Taiwan*
Viriticatin-type alkaloids constitute a sub-family of naturally abundant quinolone alkaloids with antiviral, antibacterial, and anticancer activities. However, the existing synthetic preparations to access viriticatin require cumbersome and lengthy multistep synthesis under harsh chemical conditions. AsqJ, a newly discovered iron(II)/2-oxoglutarate (Fe(II)/2OG) dependent enzyme, is involved in viriticatin-type quinolone biosynthetic pathways. It catalyzes unique sequential desaturation and

epoxidation reactions, which is followed by elimination/rearrangement to construct viriticatin quinolone. Thus, the catalytic function exhibited by AsqJ represents a chemical novel strategy to construct viridicatin. Furthermore, the AsqJ catalyzed desaturation and epoxidation represent two important but mechanistically less explored reactions (compared to the hydroxylation reaction) catalyzed by Fe(II)/2OG enzymes. By using a multi-faceted approach, including mechanistic probe design, crystallography, molecular dynamics simulations, transient kinetics, Mössbauer spectroscopy and liquid chromatography coupled mass spectrometry, we have carried out studies to elucidate catalytic mechanisms of AsqJ. Herein, our recent results in understanding AsqJ catalyzed desatuation and epoxidation, as well as its dual-functionality, will be presetned. The mechanistic implications of these results the overall understanding of Fe(II)/2OG enzyme catalysis will be discussed.

9:00 AM S80: Molecular determinants of substrate promiscuity in biosynthetic enzymes

A. Buller, University of Wisconsin, Madison, WI, USA*

Decades of research have identified enzymes form natural products biosynthetic pathways capable of tantalizingly powerful synthetic transformations. Often, however, the practical implementation of enzymes for the synthesis of natural product analogs is stymied by low fermentation titers of the requisite enzymes, poor stability, and low activity on non-native substrates. I will present our studies on a suite of highly active pyridoxal-phosphate (PLP)-dependent enzymes that can synthesize non-canonical amino acids, which are essential building blocks for diverse natural products. Detailed mechanistic studies of tryptophan (Trp) synthase variants reveal how mutations increase enzyme promiscuity through tuning the conformational ensemble. While the Trp synthase enzymes are relatively well-understood, enzymes that can stereoselectively produce β-hydroxy amino acids are poorly characterized. We have determined the first structure of an l-threonine transaldolase and show that it can synthesize an array of β-hydroxy amino acids. Kinetic and spectroscopic studies reveal the formation of a highly reactive, but persistent glycyl quinonoid nucleophile as the key feature underlying promiscuous activity by this important class biosynthetic enzymes.

9:30 AM Break

10:00 AM S81: Unusual chemistry and protein structure in the biosynthetic pathway of the genotoxic, gut microbiome metaboliteÂ colibactin.

S. Bruner and P. Tripathi, University of Florida, Gainesville, FL, USA*

The human gut microbiome metabolite colibactin is encoded by a polyketide synthase/nonribosomal peptide synthetase gene cluster in certain strains of *E. coli*. The presence of the gene cluster has been associated with DNA damage in eukaryotic cells both *in vitro* and *in vivo* and promotion of tumor formation in mouse models of colorectal cancer. Limited characterization of the gene products and associated function has prevented fundamental knowledge on how this *E. coli* contributes to genotoxicity and the mechanism of action of the mature natural product. We have employed a structural genomics approach, where protein structures of gene products with unassigned function are determined to provide insight colibactin biosynthesis and function. We have targeted gene products in the island that play distinct and key roles in the predicted biosynthesis and function of colibactin. These include an uncommon natural product transporter, ClbM; a self-resistance protein ClbS with novel enzyme chemistry, a synthetase editing enzyme ClbQ and a unique enzyme, ClbL, that functions in the final step of the complex biosynthetic pathway.

10:30 AM S82: Biosynthesis of the Nickel-Pincer Nucleotide Cofactor of Lactate Racemase

R. Hausinger, Michigan State University, East Lansing, MI, USA*

Lactic acid, a central metabolic intermediate of many cells, occurs as L- and D-isomers that are interconverted by lactate racemase. The enzyme from *Lactobacillus plantarum*, LarA, harbors a tethered nickel-pincer nucleotide (NPN) coenzyme derived from niacin [1]. This seminar will summarize recent studies related to NPN synthesis [2,3], a process that requires LarB, a carboxylase/hydrolase of nicotinic acid adenine dinucleotide (NaAD); LarE, a Mg·ATP-dependent sacrificial sulfur insertase; and LarC, a CTP-dependent nickel insertase or cyclometallase.

References

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11:00 AM S83: Heterologous production of a new lasso peptide koreensin that contains two cell adhesion motifs

S. Kodani, H. Fuwa and R. Jain, Shizuoka University, Shizuoka, Japan; H. Hemmi, Food Research Institute, NARO, Tsukuba,*

Japan
Lasso peptide is a unique cyclic peptide with length of normally 15-25 amino acids, possessing common motif of a knot structure in the molecule. Previously, we established a new shuttle vector system for expression of lasso peptide gene cluster (Kodani *et al.* J. Ind. Microbiol. Biotech. 2018). Based on genome-mining, we found a new lasso peptide gene cluster in the genome of *Sphingomonas koreensis*. The gene cluster consisted of a precursor coding gene korA and modification enzyme coding genes korB and korC. Interesting, sequence of the precursor coding gene korA contained two cell adhesion motif KGD and DGR in the core peptide sequence. The gene cluster was amplified and integrated into heterologous expression vector pHSG396Sp. Heterologous production of a new lasso peptide named koreensin was performed successfully with high yield (5.0 mg from 1L culture) in *Sphingomonas subterranea*. The structure was determined by combination of ESI-MS and NMR. As a result of NOE experiment, the three-dimensional structure of koreensin was obtained and it turned out to possess a typical lasso structure. In the “lasso” structure, one cell adhesion motif (KGD) was present at macrolactam ring sequence and the other cell adhesion motif (DGR) located close to the loop, getting through the macrolactam ring. Recombination peptides having RGD or RGE instead of KGD sequence were also obtained by point mutation experiment.

8:00 AM - 11:30 AM Session: 15: Natural Products from Microbiomes

Conveners: Jason Crawford, Yale University, West Haven, CT, USA and **Nina Lin**

Marshall Ballroom West, Mezzanine

8:00 AM S84: Ask first and shoot later - Anticancer natural product discovery from cave actinomycetes using Multiplexed Activity Metabolomics

B.O. Bachmann, Vanderbilt University, Nashville, TN, USA*
Most natural products have been isolated based on properties that bear little or no relationship to their final applications in human medicine. For example, the labor-intensive process of isolation and structure elucidation has typically been guided by unique chromophores, chromatographic properties (isolability), simple cytotoxicity, and more recently a correlated biosynthetic gene cluster of interest. But these properties generally do not predict *a priori* utility in human medicine and these “shoot first ask questions later” approaches result in heavy attrition of natural product lead matriculation into the clinic. In this seminar I will describe methods to assess the activity of secondary metabolites within cave-derived actinomycete metabolomes prior to their isolation. I will discuss the development and application of Multiplexed Activity Metabolomics methods to perform bioactive small molecule discovery using both cell lines and patient-derived cell preparations containing both tumor and functional immune cells directly from patients. These “ask first” methods have the potential to bypass the traditional lead discovery processes using immortalized cell lines and aim to provide lead compounds for chemo and immuno-oncology campaigns with an unprecedented depth of preclinical characterization.

8:30 AM S85: Beta-lactone natural products from the rhizosphere microbiome

T. Wencewicz, Washington University in St. Louis, St. Louis, MO, USA*
Beta-lactones are emerging as an important class of reactive natural products that play facilitating roles in plant-microbe and microbe-microbe interactions within the rhizosphere microbiome. The biosynthetic origins, molecular mechanism of action, and chemoenzymatic synthesis of the non-ribosomal peptide beta-lactone antibiotic obafluorin from common rhizobacteria including *Pseudomonas fluorescens* and *Burkholderia diffusa* will be presented. Special emphasis will be placed on the crystal structure and catalytic domain orientation of the beta-lactone forming non-ribosomal peptide synthetases (NRPSs) from these organisms as a blueprint for mining microbiomes for beta-lactones, rationalizing NRPS catalytic cycles, and developing enzymatic tools for beta-lactone diversification.

9:00 AM S86: A microdroplet co-cultivation and metagenomic analysis pipeline for mining microbiomes

X. Lin, University of Michigan - Ann Arbor, Ann Arbor, MI, USA*
Microbiome study and engineering has been rapidly attracting interest from both academia and industry, due to the tremendous importance and biotechnology potential of diverse microbial communities that drive biogeochemical cycles, promote human health, and produce the world’s repository of natural products. One emerging microbiome biotechnology is the mining of microbiomes for natural products for application in agriculture and medicine. However, efficient screening that can detect the relevant signal in the midst of the overwhelming diversity in natural microbiomes is difficult; many of the bioactive compounds are produced not by single organisms, but require a community of bacteria. Additionally, screening to select for these communities are virtually “needle in a haystack” problems. To address these challenges, we have been developing and applying a technological pipeline, based on nanoliter-scale microfluidic droplets, to co-cultivate and dissect subsets of complex microbial communities in order to elucidate molecular mechanisms. The pipeline consists of droplet generation, co-cultivation, isolation, and metagenomic analysis. We apply this technological framework to the study of a range of microbial communities closely related to human health or the environment. For instance, we demonstrate the anaerobic co-cultivation of diverse sub-communities from a human fecal sample and observe that many of the cultivated bacteria in these encapsulated sub-communities represent the microbial “dark matter”. Furthermore, we are able to reconstruct nearly complete genomes from a

microdroplet and investigate novel metabolic pathways. Extension and application of this pipeline to various environment microbiomes could lead to the discovery of numerous new natural products.

9:30 AM Break

10:00 AM S87: Illuminating the 'dark matter' of the bioactive gut microbiota metabolome

N. Palm, Yale University School of Medicine, New Haven, CT, USA*

The human gut microbiota produces tens of thousands of unique chemicals, yet we only understand the biological functions and physiological effects of a vanishing small fraction of these compounds. However, we know from these few examples that gut microbiota metabolites can impact nearly every aspect of host biology, from regulating gut health all the way to shaping mood and behavior. Thus, understanding the role of the microbiota metabolome in human physiology would unlock myriad opportunities to treat or prevent diverse diseases. However the sheer complexity and diversity of the microbiota metabolome creates a fundamental challenge: How can we identify physiologically relevant gut microbiota metabolites when they are 'hidden' in a sea of potentially irrelevant chemicals? Here, I will introduce our orthogonal approach to this problem where we use host sensing of microbial metabolites as a 'lens' to illuminate physiologically relevant small molecules from complex metabolite mixtures. Using this approach, we have identified dozens of novel microbe-host and microbe-microbe-host axes that affect host physiology both locally in the gut and systemically in distal tissues, including in the brain.

10:30 AM S88: Structure Elucidation of Colibactin and its DNA Interstrand Crosslink Product

M. Xue, C.S. Kim, A. Healy, K. Wernke, Z. Wang, M. Frischling, E. Shine, W. Wang, S. Herzon and J. Crawford, Yale University, West Haven, CT, USA*

Select strains of *Escherichia coli* and *Klebsiella pneumoniae* produce the virulence factor and cytotoxin known as colibactin. Colibactin is derived from its prodrug form known as precolibactin. Pathogenic strains of adherent invasive *E. coli* (AIEC) carrying the colibactin pathway (*clb*+) cause tumor formation in at least four distinct mouse models examined to date and are thought to be responsible for initiating colorectal cancer in humans. Through a combination of genome editing, stable isotope labeling, DNA alkylation, and chemical synthesis studies, we characterized the structures of precolibactin, colibactin, and its DNA interstrand crosslink product from DNA exposed to *clb*+ *E. coli*. The structure of colibactin was confirmed by comparison to a synthetic standard. The standard led to an identical DNA interstrand crosslink as the natural materials. An uncharacterized peptidase ClbL represented the final critical step in precolibactin biosynthesis was responsible for converting DNA alkylators into DNA interstrand crosslinkers. These studies present the structure, mode of action, and biosynthesis of (pre)colibactins that account for all known biosynthesis and cancer cell biology studies for this important pathway.

11:00 AM S89: One small molecule for a microbe, a giant leap for natural products

N. Mouncey, D. Udwary, H. Otani and Y. Yoshikuni, DOE Joint Genome Institute, Walnut Creek, CA, USA*

The galaxy of natural products comprises a large family of diverse and complex chemical entities that have roles in both primary and secondary metabolism, and today >23,000 natural products have been characterized. Secondary metabolites are incredibly important molecules for mankind with uses as antibiotics, antifungals, antitumour and antiparasitic products and in agriculture as products for crop protection and animal health. We are seeing a resurgence of activity in exploring secondary metabolites for a wide range of applications, due to not only increasing antibiotic resistance, but the advent of next-gen genome sequencing and new technologies to investigate natural product biosynthesis. At the JGI, we are developing new tools and processes for identification of novel biosynthetic gene clusters from isolate genomes and metagenomes, and complementing these with a suite of new experimental platforms to access the products of these clusters. We are currently exploring secondary metabolite space by analyzing >100,000 isolate genomes and >65,000 metagenomes and I will share recent results on these analyses, as well as how we are functionally expressing clusters in a range of novel chassis.

11:00 AM - 3:30 PM JIMB Editors Luncheon Meeting

Taylor, Mezzanine

11:30 AM - 1:00 PM Awards and Honors Committee

Cleveland 2, Mezzanine

11:30 AM - 1:00 PM Education and Outreach Committee

Jackson, Mezzanine

12:25 PM - 12:35 PM Exhibitor Showcase: Kuhner Shaker

Exhibit Hall C, Lower level

12:35 PM - 12:45 PM Exhibitor Showcase: M2P Labs

Exhibit Hall C, Lower level

1:00 PM - 4:30 PM Session: 16: Microbial processes for the production of emerging products

Conveners: Daniel Solaiman, Eastern Regional Research Center, Agricultural Research Service, USDA, Wyndmoor, PA, USA and **Inge Van Bogaert**, University of Ghent, Ghent, Belgium

Madison B, Mezzanine

1:00 PM S90: A field of dreams – *Corynebacterium glutamicum* as cell factory for bio-based materials

J. Becker, S. Kind, C. Rohles, M. Kohlstedt and C. Wittmann, Saarbrücken, Germany*

Corynebacterium glutamicum is one of the most important cell factories in industrial biotechnology. Systems metabolic engineering has meanwhile leveraged the product portfolio of *C. glutamicum* far beyond classical products. Within the past five years, the number of products has exploded to approximately 70 natural and non-natural compounds. Of particular relevance are emerging products that serve the bio-plastics market. In this regard, our team has developed tailored strains for the bio-based production of the material monomers diaminopentane, glutarate and aminovalerate.

The genetically defined strains were constructed on basis of the l-lysine-hyperproducing strain *C. glutamicum* LYS-12[1]. Extending the terminal pathway by optimized expression of l-lysine decarboxylase, combined with elimination of by-product formation and transport engineering bred the streamlined *C. glutamicum* DAP-16 strain that produced 88 g/L diaminopentane with a high yield of 0.5 mol/mol during fed-batch fermentation[2]. Diaminopentane was purified to 99% purity by solvent extraction and distillation. Subsequent polymerization with sebacic acid from castor oil produced the 100% bio-based polyamide PA5.10 exhibiting excellent material properties[2].

Aminovalerate and glutarate production recruited a heterologous l-lysine degradation pathway from *Pseudomonas putida*[3]. Elimination of the intrinsic glutarate pathway shifted the product pattern towards aminovalerate with a maximum titer of 28 g/L. In turn, selective production of glutarate was achieved by overexpression of the glutarate pathway and transport engineering[4]. In a fed-batch process, the optimized producer GTA-4 produced more than 90 g/L glutarate. The bio-based glutarate was then purified to 99.9% purity and polymerized with hexamethylenediamine to from the unique bionylon-6,5[4].

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1:30 PM S91: *Corynebacterium* Cell Factory Design and Culture Process Optimization for Muconic Acid Biosynthesis

E.S. Kim, Inha University, Incheon, Korea, Republic of (South)*

Muconic acid (MA) is a valuable compound for adipic acid production, which is a precursor for the synthesis of various polymers such as plastics, coatings, and nylons. Although MA biosynthesis has been previously reported in several bacteria, the engineered strains were not satisfactory due to low MA titers. Here, we generated an engineered *Corynebacterium* cell factory to produce a high titer of MA through 3-dehydroshikimate (DHS) conversion to MA, with heterologous expression of foreign protocatechuate (PCA) decarboxylase genes. To accumulate key intermediates in the MA biosynthetic pathway, *aroE* (shikimate dehydrogenase gene), *pcaG/H* (PCA dioxygenase alpha/beta subunit genes) and *catB* (chloromuconate cycloisomerase gene) were disrupted. To accomplish the conversion of PCA to catechol (CA), a step that is absent in *Corynebacterium*, a codon-optimized heterologous PCA decarboxylase gene was expressed as a single operon under the strong promoter in an *aroE-pcaG/H-catB* triple knock-out *Corynebacterium* strain. This redesigned *Corynebacterium*, grown in an optimized medium, produced about 38 g/L MA and 54 g/L MA in 7-L and 50-L fed-batch fermentations, respectively. These results show highest levels of MA production demonstrated in *Corynebacterium*, suggesting that the rational cell factory design of MA biosynthesis could be an alternative way to complement petrochemical-based chemical processes.

2:00 PM S92: High value products from itaconic acid

J. Shaw[*]*, Itaconix plc, Stratham, NH, USA*

Itaconic acid is a highly-versatile organic acid already produced at commercial scale with well-established fermentation processes. Historically, the barrier for broader use of itaconic acid was the ability to polymerize it at efficient industrial rates. Itaconix operates a commercial itaconic acid polymer plant based on several proprietary process breakthroughs. With commercial polymers currently on the market for use in detergents, odor control, and hair styling, Itaconix is a major user of itaconic acid. Continued success with additional itaconate chemistries will establish Itaconix as a major worldwide user of itaconic acid. With this prospect, the company is mapping out future plans for worldwide growth in itaconic acid production.

2:30 PM Break

3:00 PM S93: Enzymatic Co-production of Hydrogen Peroxide and Oxygenates

S. Hunt[*]*, Solugen, Inc., Houston, TX, USA*

Since the 1940s, hydrogen peroxide has been manufactured from reformed natural gas using the Anthraquinone Autooxidation (AO) Process. Globally, there are around 100 AO plants in operation, and on average, one plant explodes per year. It has become increasingly expensive to build AO plants in the US and EU (>$100M). As such, new plants are built to less stringent standards in the developing world, increasing the global logistical challenges of shipping concentrated peroxide. Although hydrogen peroxide is a green molecule that decomposes into just water and oxygen, the AO process emits more than 2 tons of CO_2 equivalences per ton of peroxide.

Today, millions of tons per year of oxygenates (e.g. alcohols, aldehydes, sugars etc.) are partially oxidized to high value products. During these processes, molecular oxygen undergoes complete four electron reduction to water, resulting in millions of tons per year of wastewater that must be treated. What if instead of making wastewater, each of these processes produced a high-value hydrogen peroxide coproduct? This is Solugen's Partial Oxidation Platform (POP), and this is Bioperoxide^{TM}.
Using a combination of computational chemistry and directed evolution techniques, Solugen engineers highly stable and inexpensive enzymes that co-produce high value partial oxidation products and Bioperoxide^{TM}. These enzymes are incorporated into lean, continuous, and modular chemical process units that enable the onsite production of biobased chemistries, reducing cost and associated carbon emissions. Solugen operates a fully automated, continuous pilot plant 24/7 in Houston, TX and is constructing its first commercial plant in 2019.

3:30 PM S94: Improvement of fermentation ability and product quality in industrial yeast by "functional amino acid engineering"

H. Takagi[*]*, Nara Institute of Science and Technology, Nara, Japan*

There are two major purposes for breeding of industrial yeast: improvement of fermentation ability with enhanced tolerance to environmental stresses during fermentation processes; and diversity of product taste and flavor with modified metabolic pathways. Yeast cells are exposed to various environmental stresses during fermentation, including high ethanol concentration, freezing, drying and high osmotic pressure. Severe stress conditions can reduce the fermentation efficiency of yeast. Thus, in terms of application, stress tolerance is key for yeast cells. Metabolites produced by yeast during fermentation, including amino acids, organic acids, fatty acids, saccharides, alcohol and ester, influence the taste or flavor of fermented products.
In yeast, amino acid metabolism and its regulatory mechanisms vary under different growth environments by regulating anabolic and catabolic processes, including uptake and export, and the metabolic styles form a complicated but robust network. Very few studies, however, have considered the effects of amino acids during fermentation. The control of amino acid composition and content is expected to contribute to an improvement in productivity, and to add to the value of fermented foods, alcoholic beverages, bioethanol and other valuable compounds. Yeast is considered reliable and safe in food production, and thus the development of novel strains that overproduce amino acids would represent a significant contribution to food-related industries.
To improve fermentation ability and product quality in industrial yeast, I will introduce a new breeding technology called "Functional Amino Acid Engineering", focused on the metabolic regulations and physiological roles of amino acids, such as proline, arginine, leucine, valine and cysteine.

4:00 PM S159: Monomers and Polymers derived form Biological Sources: Opportunities and Challenges

M. Terwillegar[*]*, DaniMer Scientific UGA, Athens, GA, USA*

Environmental, political and societal pressure has mandated the reduction and elimination of petroleum-based plastics, especially those plastics that are not bio-degradable regardless of the source. There are several building blocks in the bio-renewable and bio-degradable space giving a formulator, converter or OEM plenty of options to evaluate. The genesis, purification and applications of some common monomers will be described herein. Furthermore, polymers and end use applications derived from these bio-building blocks will be described. Finally, challenges and benefits for the monomers and polymers in question will be illustrated.

1:00 PM - 4:30 PM Session: 17: Process characterization in the manufacture of medications, chemicals and fuels

Conveners: Tiffany D. Rau, Lake Charles, LA, USA and **Frank Agbogbo**, Cytovance Biologics, Oklahoma City, OK, USA
Marshall Ballroom North, Mezzanine

1:00 PM S95: Process Characterization in Biomanufacturing of 'Synbio' products

S. Yadav, Conagen Inc, Bedford, MA, USA*
Process characterization (PC) has played a critical role to validate manufacturing processes and reduce batch failures in the biopharma industry by allowing effective process control strategies. PC is mostly driven by regulatory requirement; however, it can be a good practice from an economic standpoint. Concepts like QbD, implementation of DoE for high throughput PC are applied extensively. Also, guidelines published by the FDA and ICH cover process validation aspects in detail. The presentation will focus on the importance of applying PC and its impact on maintaining a consistent/ desired product quality, in biomanufacturing of synthetic biology products like flavor and fragrance molecules. Being a vertically integrated synthetic biology and fermentation company, how Conagen addresses PC by utilizing Scale down modeling, applying some of the principles like identification of Critical Quality attributes (CQAs), Critical process parameters (CPPs) and Design space, Risk assessment and Process control strategy will be discussed.

1:30 PM S96: Single-use work flow from upstream through production culture and harvest of aggressive microbial cultures

J. Brown and N. Jones, Thermo Fisher Scientific, Logan, UT, USA*
Single-use bioreactors, fermentors, mixers, and other bio-process containers are pre-validated for sterility, function, and integrity. This reduces resources and time put into production culture preparation and allows for more production volume in less space. Facilities can use single-use fermentors instead of traditional stainless steel vessels without modifying their existing culture procedures or reducing product titer. Single-use fermentors are specifically designed to meet the performance requirements of dense, rapidly growing microbial processes while offering the benefits of quick process setup, reduced contamination risk, and high production quality.

2:00 PM S97: Analysis Strategies for Constrained Mixture and Mixture Process Experiments

P. Ramsey, North Haven Group, Brookline, NH, USA*
Although important to process/product development or improvement, constrained mixture and mixture process experiments have been challenging to analyze due to large effect correlations caused by regional and linear constraints. Often small under-fit models with lack of fit or large over-fit models with inflated prediction variance have been selected. With advanced model selection methods it is now possible to fit and evaluate large sets of potential mixture effects. Historically mixture and mixture process experiments have been very difficult to analyze. Model selection techniques such as Pruned Forward, LASSO, or All Possible Models allow one to find a best subset of effects from a large pool of candidates even when the number of potential effects exceeds the number of experimental trials (supersaturation). Using a 10 component mixture experiment with both regional and linear constraints we demonstrate how Generalized Regression can evaluate even large mixture models (e.g., Scheffe full cubic) and identify the important subset of effects. The autovalidation method of Gotwalt and Ramsey (2018) to prevent over fitting is illustrated. We use the same methods to analyze a complicated mixture process factor experiment. The talk will also discuss generating mixture or mixture process experiments in Custom Design platform of the JMP software, which provides a way to create smaller more efficient experiments.

2:30 PM Break

3:00 PM S98: The use of DOE (Design of Experiments) to evaluate *E. coli*Â fermentation under different process conditions

F. Agbogbo, Cytovance Biologics, Oklahoma City, OK, USA and P. Ramsey, North Haven Group, Brookline, NH, USA*
Different process parameters were evaluated with *E. coli* strain in fermentation. Fermentations were performed in 5 L Fermenters with controls for Temperature, pH and DO levels. The process parameters that were evaluated were Temperature, pH, DO level and feed/phosphate ratio. Definitive screening design with the JMP software was used to evaluate the process parameters. The output parameters of OD_{600}, dry cell weight (DCW), product concentration were measured. The results were evaluated in JMP to determine the impact of the various process parameters on cell growth and product concentration. The presentation will cover the results obtained and how process parameters impact cell growth and product concentration.

3:30 PM S99: Production challenges of remodeled nitrogen-producing microbes

F. Rezaei, Pivot Bio, Cary, NC, USA*
Chemical nitrogen fertilizer is responsible for helping produce enough food to feed half of the global population. While it provides abundant food, its use comes at an environmental cost. Chemical fertilizer has disrupted the earth's nitrogen cycle more dramatically that any event in 2.5 billion years. Microbes that once had the capacity to produce ammonia and feed it to plants have stopped this production over time, sensing exogenous nitrogen in the soil (i.e. fertilized field conditions). Using a suite of technologies, Pivot Bio takes these once robust-ammonia producing microbes and remodels them so they produce ammonia again throughout the crop's growing season. U.S. corn farmers are using these microbes now to feed their crops and reduce the use of chemical fertilizer. Although beneficial for increasing nitrogen production, the process of remodeling cells might create challenges in the growth and manufacturing these cells. This talk will focus on our effort from research to scaling up the fermentation and formulation process for Pivot Bio's first generation of remodeled cells.

4:00 PM S100: Applying molecular technology to industrial strain optimization for bio-based chemical fermentation

K. Aeling, Zymergen, Emeryville, CA, USA*
At Zymergen, we have developed a platform to engineer microbes for the manufacture of a variety of chemicals and novel materials with unprecedented flexibility. We have the largest proprietary database of genetic diversity, and we have built rapid prototyping capabilities to explore this diversity and produce a variety of novel molecules at reduced time and cost. Our platform integrates custom software, high throughput laboratory automation, machine learning algorithms and genome engineering tools. We apply custom scientific computing tools to specify and track the creation of designer microbes. Our throughput allows us to test thousands of design ideas, explore perturbations across the genome, and examine the production of bio-based chemicals in different hosts.

1:00 PM - 4:30 PM Session: 18: Genome Mining and Synthetic Biology for Natural Product Discovery

Conveners: Yousong Ding, University of Florida, Gainesville, FL, USA and **Zachary Sun**, Synvitrobio, Inc., San Francisco, CA, USA

Marshall Ballroom Southeast, Mezzanine

1:00 PM S101: Genome analytics elucidates transcriptional and translational regulatory elements encoded in the Streptomyces genomes

B.K. Cho, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South)*
Determining transcriptional and translational regulatory elements in GC-rich Streptomyces genomes is essential to elucidating the complex regulatory networks that govern secondary metabolite biosynthetic gene cluster (BGC) expression. However, information about such regulatory elements has been limited for Streptomyces genomes. To address this limitation, high-quality genome sequences of antibiotic-producing Streptomyces strains were completed, which contains several thousands of newly annotated genes. This genome information provides fundamental references to integrate multiple genome-scale data types, including dRNA-Seq, RNA-Seq, Term-Seq, and ribosome profiling. Data integration results in the precise determination of thousands of transcription start sites which reveal transcriptional and translational regulatory elements, including −10 and −35 promoter components specific to sigma (σ) factors, and 5′-untranslated region as a determinant for translation efficiency regulation. Integrating of transcription start sites and transcript 3′-end positions identified genome-wide TU architecture, in accordance with transcriptome profile. The conserved promoter was found as 5′-TANNNT and 5′-TGAC for the −10 and −35 elements, respectively. In addition, identification of transcription start sites revealed ribosome binding sites, important for translational control of gene expression. This comprehensive genetic information provides a versatile genetic resource for rational engineering of secondary metabolite BGCs in *Streptomyces*.

1:30 PM S102: HEx: A computational and synthetic biology platform for the discovery of bioactive compounds from fungi

P. Wiemann, T. Choera, P. Cordero, A. DeNicola, M. Hillenmeyer, J.E. Jeon, D. Kvitek, B. Naughton, C. Pataki, U. Schlecht, J. Spraker and C. Harvey, Hexagon Bio, Menlo Park, CA, USA*
We present a computational and synthetic biology platform for producing novel compounds with promising bioactivities. Compounds derived from fungi have a long history of therapeutic use, but the wealth of genome data published in the past decade has revealed that known compounds represent a fraction of the medicinal potential that these organisms possess.

We have developed a computational method for mining fungal genome data to identify biosynthetic pathways whose products are likely to have desired bioactivities. We have also developed a synthetic biology pipeline which allows us to engineer chosen pathways into a heterologous host. Our approach allows us to attempt to produce compounds in a tractable organism

regardless of the fungus where the pathway was originally identified.
Here we demonstrate the ability of the HEx platform to produce compounds produced by diverse biosynthetic pathways from across the fungal kingdom. These data demonstrate the potential of approaches such as ours to access the unrealized therapeutic potential present within the growing number of sequenced fungal genomes.

2:00 PM S103: Engineered enzymes, pathways, and tools for the biosynthesis of non-natural polyketides and terpenes

G. Williams, North Carolina State University, Raleigh, NC, USA*
Many clinically used drugs are derived from secondary metabolites that are biosynthesized in a modular fashion by the selection and condensation of small molecule building blocks. Chimeric biosynthetic pathways can be constructed in an attempt to produce analogues for drug discovery. Yet, the scope and utility of this combinatorial approach is limited by the inherent substrate specificity and poor functional modularity of most biosynthetic machinery. Here, our approach to expanding the scope of polyketide and isoprenoid combinatorial biosynthesis by leveraging precursor-directed biosynthesis, enzyme engineering, and synthetic biology will be summarized. Our recent advances that enable the installation of multiple extender units into polyketides by engineered polyketide synthases will be presented. The construction of tailored genetically encoded biosensors for directed evolution of polyketide and terpene biosynthesis will also be described. Furthermore, an artificial biosynthetic pathway for the biosynthesis of non-natural isoprenoids is introduced. Our synthetic biology approach expands the synthetic capabilities of natural product diversification strategies and provides an improved understanding of the molecular basis for specificity in complex molecular assemblies.

2:30 PM Break

3:00 PM S104: Compartmentalized biosynthesis of mycophenolic acid featuring unique cooperation between biosynthetic and β-oxidationÂ catabolic machineries

W. Zhang, X. Zhang and S. Li, Shandong University, Qingdao, China; L. Du, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, China*
The fungal natural product mycophenolic acid (MPA) is a first-line immunosuppressive drug for organ transplantations and autoimmune diseases. However, some key biosynthetic mechanisms for MPA remain a long-standing mystery. Recently, we identified a MPA biosynthetic gene cluster through genome mining of *Penicillium brecompactum* NRRL 864. Here, we elucidate the full MPA biosynthetic pathway for the first time that features both compartmentalized enzymatic steps and unique cooperation between biosynthetic and *β*-oxidation catabolic machineries by targeted gene inactivation, heterologous protein expression, precursor feeding experiments, *in vitro* characterization of enzyme functions, kinetic analysis, and microscopic observation of protein subcellular localization. We reveal the intriguing pattern of compartmentalization for the MPA biosynthetic enzymes, including the cytosolic polyketide synthase MpaC' and *O*-methyltransferase MpaG', the Golgi apparatus-associated prenyltransferase MpaA', the endoplasmic reticulum-bound oxygenase MpaB' and P450-hydrolase fusion enzyme MpaDE', and the peroxisomal acyl-CoA hydrolase MpaH'. The whole pathway is elegantly co-mediated by these compartmentalized biosynthetic enzymes and the peroxisomal *β*-oxidation machinery. Our study not only highlights the importance of considering subcellular contexts and the broader cellular metabolism in natural product biosynthesis, but also will benefit the future efforts for both industrial strain improvement and novel drug development.

3:30 PM S105: Novel natural products from pseudomonads

J. Patteson, A. Krestch, G. Morgan, A. Lescallette and B. Li, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*
The *Pseudomonas* genus of bacteria colonize and infect a broad range of plants and animals and significantly impact agriculture and medicine. They produce many natural products that possess unique structures and functions, including antibiotics, phytotoxins, and signaling molecules. These molecules play essential roles in the life styles of the bacteria, but most remain uncharacterized and thus overlooked. This talk describes the identification of several novel natural products from *Pseudomonas* species, including the opportunistic human pathogen *Pseudomonas aeruginosa*, by means of synthetic biology and chemical elicitation using native signaling molecules. This work reveals new chemistry and sheds light on the signaling and virulence mechanisms of the important genus of bacteria.

4:00 PM S106: Challenges and Opportunities to Activate Silent Biosynthetic Gene Clusters in Native Producers or Heterologous Hosts

B. Shen, Department of Chemistry, Department of Molecular Medicine, Natural Products Library Initiative at The Scripps Research Institute, The Scripps Research Institute, Jupiter, FL, USA*
Natural products continue to inspire novel chemistry, biology, and medicine, but the rate of discovery of novel natural products has slowed significantly as most gene clusters encoding their biosynthesis are silent when the microorganisms are cultured under standard laboratory conditions. By mining the genomes of the actinomycetale strain collection at The Scripps Research

Institute, we have identified potential producers harboring biosynthetic gene clusters (BGCs) that encode several families of natural products with privileged scaffolds, including the enediynes, the leinamycin family of natural products, and thioacid-containing/derived natural products. Selected studies from our current efforts to develop enabling technologies to activate these BGCs, in the native producers or model heterologous *Streptomyces* hosts, will be presented to highlight the challenges and opportunities of natural product discovery in the genomic era.

1:00 PM - 4:30 PM Session: 19: Building Diversity in the Professional World

Conveners: Laura R. Jarboe, Iowa State University, Ames, IA, USA and **Sheena Becker**, Corteva, Brownsburg, IN, USA

Madison A, Mezzanine

1:00 PM S107: Subtle gender bias in science: How do we speak about professionals?

S. Atir, University of Chicago, Chicago, IL, USA*

Despite significant progress, women remain astonishingly underrepresented in many professional fields, especially prestigious and lucrative ones like politics, business, and STEM. Across the board, women continue to hold fewer positions of power and influence. Why is this still the case, given that women perform scholastically similarly or better than men in high school and college? Though blatant discrimination is illegal, men and women are still treated differently in the workplace. These days, many of these differences may be subtle and therefore difficult to identify and eradicate, though they may be just as detrimental to women's professional advancement. One such difference is a gender bias in the way people speak about professionals. It is common to refer to professionals in many fields by surname alone when speaking about them or their work (e.g., *Oppenheimer* was the father of the atomic bomb). We find that, across a variety of domains, people are more likely to refer to male professionals than female professionals in this way. Specifically, analyses of archival data suggest that political pundits on the radio are more likely to refer to male politicians than female politicians by surname alone; similarly, students reviewing their professors online are more likely to refer to male professors than female professors by surname alone. Experimental evidence provides convergent evidence of this gender bias: participants writing about a fictional male scientist were more likely to refer to him by surname alone than participants writing about an otherwise-identical female scientist. We find that, on average, people are over twice as likely to refer to male than female professionals by surname alone. Importantly, the choice to use a surname-only reference is consequential: fictional scientists referred to by surname alone were perceived as being more famous and eminent, and therefore as having higher status and being 14% more deserving of a National Science Foundation career award and its associated funding.

1:30 PM S108: Retention and promotion of women and underrepresented minority faculty in science and engineering at four large land grant institutions

M. Gumpertz, E. Griffith and A. Wilson, North Carolina State University, Raleigh, NC, USA; R. Durodoye, Delaware Department of Education, Dover, DE, USA*

The current climate on college campuses has brought urgency to the need to increase faculty diversity. In STEM fields particularly, the dearth of underrepresented minority (URM) and female faculty is severe. The retention and success of African American, Hispanic/Latino, American Indian and female faculty have direct implications for the quality and diversity of the future scientific workforce. Understanding the ways retention patterns differ by discipline and institution is crucial for developing a diverse faculty. This study investigates tenure attainment, retention, and time to promotion to full professor for women and URM faculty. We analyze personnel records for assistant and associate professors hired or appointed from 1992 to 2015 at four large land grant institutions. Representation of women and URM faculty in STEM disciplines increased substantially from 1992 to 2015, but mostly for women and Hispanic faculty and more slowly for black and American Indian faculty.

2:00 PM S109: Increasing PhD Programs Diversity: From single program change to institutional transformation

A.G. Campbell, Brown University, Providence, RI, USA*

This presentation describes the benefits of implementing a number of best practices on graduate student population compositional diversity and institutional climate. These practices have resulted in significant increases in underrepresented graduate applicants, admitted students and matriculants, and degree recipients. This work, and its achievements, have origins in a faculty-led program designed to increase STEM field diversity, and in a recently launched institution-wide diversity and inclusion action plan, as well as new programming introduced by the Graduate School. The coordinated combined efforts and resulting benefits include: 1) development of relationships with undergraduate institutions serving large numbers of underrepresented students; 2) establishment of a personalized educational program of support and skills-based learning to supplement discipline-based coursework; 3) creation of pre- and post-matriculation programming to support students from the inquiry and pre-application stage through to enrollment and graduation 4) elimination of threshold barriers that make graduate education inaccessible, 5) establishment of an institutional culture that values and celebrates diversity and inclusion, and 6) Investments in faculty diversity training and practices. Though targeted at racial and ethnic minorities, the practices described

here are broadly applicable to the recruitment and retention practices of other under-represented groups.

2:30 PM Enhancing Diversity in the Scientific Workforce: Focusing on Institutional Change

C. Le Fauve[*]*, National Institutes of Health, DC, DC, USA*

Dr. Charlene Le Fauve will describe NIH's current approach and activities related to enhancing scientific workforce diversity. She will highlight four main diversity challenges facing biomedicine: Advancing scholarship of the science of diversity; Using a data-driven scientific approach to understand diversity drivers and outcomes; Studying and mitigating the role of sociocultural factors in recruitment, retention, and career advancement; and Sustaining future workforce diversity. She will also highlight development and use of the NIH Distinguished Scholars Programand the NIH Scientific Workforce Diversity Toolkit, a free, downloadable interactive resource institutions can use to help advance their own faculty diversity. The toolkit guides users through evidence-based interrelated activities that her office is currently using to enhance diversity in the NIH intramural research program. These include expanding diversity of the candidate pool, proactive outreach to diverse talent, mitigating bias in search processes, and developing/sustaining mentoring relationships. Please visit the NIH Scientific Workforce Diversity websitefor more information.

Charlene E. Le Fauve, Ph.D.

Dr. Charlene Le Fauve is the Senior Advisor to the National Institutes of Health (NIH) Chief Officer for Scientific Workforce Diversity (COSWD). She is a public servant with over twenty years as a scientist, administrator, senior manager in program, policy, and legislative arenas. Previously she worked as the Deputy Director of the Office for Research on Disparities & Global Mental Health at NIMH. She served as a Senior Policy Coordinator at the U.S. Department of Health and Human Services. During her time in this role Le Fauve helped implement the Affordable Care Act — broadening access for those on Medicare, improving healthcare quality and reforming private insurance. She was Branch Chief for Co-occurring and Homeless Activities for nearly eight years and was dedicated to behavioral treatment of the homeless. Health Scientist Administrator positions at NIDA and NIAAA in the extramural research program informs her current role at NIH.

1:00 PM - 4:30 PM Session: ST-1: Innovations in agriculture and energy to realize a new carbon economy

Conveners: David Babson, USDA/DOE and **Ian Rowe**, Department of Energy - Energy Efficiency & Renewable Energy, Washington, DC, USA

Marshall Ballroom West, Mezzanine

1:00 PM S159: A sustainable and renewable cycle for food and fuels from sunlight, air and water

D. Nocera[*]*, Harvard University, Cambridge, MA, USA*

Hybrid biological | inorganic (HBI) constructs have been created to use sunlight, air and water (as the only starting materials) to accomplish carbon and nitrogen fixation, thus enabling distributed and renewable fuels and crop production.

The carbon and nitrogen fixation cycles begin with the artificial leaf, which was invented to accomplish the solar fuels process of natural photosynthesis – the splitting of water to hydrogen and oxygen using sunlight – under ambient conditions. The creation of self-healing catalysts for: (1) the artificial leaf allows for the facile interfacing of water splitting catalysis to materials such as silicon and (2) the bionic leaf allows for the facile interfacing of water splitting catalysis to bioorganisms. For the latter, using the tools of synthetic biology, a bio-engineered bacterium has been developed to convert carbon dioxide from air, along with the hydrogen produced from the catalysts of the artificial leaf, into biomass and liquid fuels, thus closing an entire artificial photosynthetic cycle. The HBI, called the bionic leaf, operates at unprecedented solar-to-biomass (10.7%) and solar-to-liquid fuels (6.2%) yields, greatly exceeding the 1% yield of natural photosynthesis.

Extending this approach, a renewable and distributed synthesis of ammonia (and fertilizer) at ambient conditions has been created by coupling solar-based water splitting to a nitrogen fixing bioorganism. Nitrogen is fixed by using the hydrogen produced from water splitting to power a nitrogenase installed in a bioorganism. Nitrogen fixation proceeds at high turnover per cell and operates without the need for a carbon feedstock (other than the CO_2 provided from air), enabling sustainable crop production with a *carbon negative* budget. Increased crop yields of 300% have been achieved.

The science that will be presented will show that using only sunlight, air and water, a distributed system may be established to produce fuel and food. Such science will be particularly useful to the poor of the world, where large infrastructures for fuel and food production are not tenable.

1:45 PM S161: Nitrogen-Fixing Microbes for the Sustainable Intensification of Agriculture

S. Bloch[*]*, Pivot Bio, Berkeley, CA, USA*

Nitrogen is a critical nutrient for crop production, limiting yields in developing regions and requiring large inputs of nitrogen fertilizer in developed regions, much of which is lost to the environment as pollutants and greenhouse gases. The Haber-Bosch

process, a century-old technology for producing synthetic nitrogen fertilizer, requires massive energy inputs and is projected to consume up to 2% of the global energy supply in the coming decades. Meanwhile, a growing human population requires further increases in agricultural productivity, necessitating innovations in nitrogen management for sustainable intensification. Pivot Bio is developing microbial products that provide nitrogen to cereal crops via nitrogen fixation. While synthetic nitrogen fertilizers are subject to leaching and evaporation, root-associated nitrogen-fixing microbes provide an avenue to efficiently provide nitrogen to crops in situ. In on-farm trials, Pivot Bio's first-generation product, PROVEN™, led to yield increases in corn in fully-fertilized conditions, and maintained yield with a reduction in nitrogen applications. At the same time, the production of PROVEN™ used less than 1% of the energy that would be required to produce the equivalent amount of synthetic nitrogen via Haber Bosch. When adopted on a broad scale, this and future microbial nitrogen fixation products will have a significant impact on reducing the need for synthetic nitrogen fertilizers and their energy and environmental implications.

2:30 PM Break

3:00 PM S160: Design and Evolution of Synthetic Pathways in Microorganisms for Carbon Conservation and Fixation

J.C. Liao, Academia Sinica, Taipei, Taiwan*

CO_2 and methane are greenhouse gases that threaten the sustainability of the living condition on earth. Unfortunately, existing solutions based on current science and technology face severe scaling issues. Biological or bio-memetic solutions are promising because of multiple features, including specificity, environmentally friendliness, and amenability to evolutionary approaches. Here we discuss examples of designing and evolving pathway in microorganisms, including *Escherichia coli* and cyanobacteria *Synechococcus elongates*. To improve CO_2 utilization, we showed that a synthetic pathway, termed melonyl-coA-glycerate (MCG) pathway can augment the Calvin–Benson–Bassham (CBB) cycle to improve CO_2 fixation. We also designed an alternative pathway that can potentially replace or enhance CBB cycle for CO_2 fixation. In addition, we designed a non-oxidative glycolysis (NOG) pathway to retain carbon that are already fixed, and evolved an *E. coli* strain that uses NOG exclusively for carbohydrate assimilation. To improve methane utilization, we designed and constructed pathways in *E. coli* to utilize methanol, a common product of methane oxidation.

3:45 PM S162: Synergistic substrate cofeeding enables rapid CO_2 to product conversion

N. Liu, J. Park and G. Stephanopoulos, Massachusetts Institute of Technology, Cambridge, MA, USA*

The continuous rise of atmospheric CO_2 from expanded use of fossil fuels has become a growing concern for the 21st-century. A particularly valuable effort to remedy the imbalance between CO_2 generation and sequestration is the conversion of CO_2 into value-added products, which is beneficial to both the chemical economy and the environment. To achieve this biologically, a balanced supply of carbons, ATP, and reducing agents is required to enable rapid reductive metabolism and efficient CO_2 conversion. However, these components are commonly generated with varying efficiencies depending on metabolic pathways. Here we show that substrate mixtures with concurrent shortcut access to multiple pathways can optimally satisfy the biosynthetic requirements. By controlled cofeeding of superior ATP- and NADPH-generators as "dopant" substrates to cells primarily utilizing inferior substrates, we circumvent catabolite repression and tailor pathway usage to synergistically stimulate CO_2 reduction and its subsequent conversion into products. Glucose doping in *Moorella thermoacetica* CO_2 + H_2 cultures stimulates net carbon reduction (2.3 g-CO_2/g_{cell}/hr) into acetate by augmenting ATP synthesis via pyruvate kinase. Similarly, gluconate doping in *Yarrowia lipolytica* accelerates acetate-driven lipogenesis (0.046 g/g_{cell}/hr) by obligatory NADPH synthesis through the pentose cycle. Together, synergistic cofeeding produces CO_2-derived lipids with 38% energetic efficiency and demonstrates potential to convert CO_2 into advanced bioproducts.

3:45 PM - 4:45 PM Publications Committee

Taylor, Mezzanine

5:00 PM - 6:00 PM Charles Thom Award Lecture Yi Tang, UCLA, 2019 Charles Thom Awardee

Marshall Ballroom Southeast, Mezzanine

6:00 PM - 7:00 PM Banquet Reception

Exhibit Hall C, Lower level

7:00 PM - 8:30 PM Banquet

Marshall Ballroom, Mezzanine

8:30 PM - 9:30 PM Jazz Mixer--all attendees Sponsored by INFORS

Lobby level

Wednesday, July 24

7:00 AM - 3:00 PM Registration

Marshall Ballroom Foyer, Mezzanine

8:00 AM - 11:30 AM Session: 20: Cell-Free Biochemical Production

Conveners: Joseph Rollin, National Renewable Energy Laboratory, Golden, CO, USA and **James Winkler**

Madison A, Mezzanine

8:00 AM S110: The Cell-Free Way to Natural Chemicals

J. Bowie, UCLA/Invizyne Technologies, Los Angeles, CA, USA*

Considerable effort is currently directed to engineer micro-organisms to produce useful chemicals. Yet our needs for commercial viability are often stymied by requirements for maintaining life processes. These problems include product or intermediate toxicities, diversion of input biomass to unwanted side products, cumbersome design-build-test cycles, unpredictability of engineering efforts, and complex downstream processing.

To free us from the constraints of cells, we are building systems to perform biochemical conversions with naked enzymes housed in a bioreactor rather than within cells. Cell-free conversions have many potential advantages: (1) Extremely high conversion yields are possible because non-productive side reactions are essentially absent. (2) Design-Build-Test cycles are much more rapid and flexible. (3) Without cells, there are no product or intermediate toxicity problems. (4) Volumetric productivities can be much higher since the pathway enzymes can be highly concentrated. (5) Downstream processing costs can be greatly lowered because the chemical complexity of the reactor contents is reduced (*e.g.* no membranes, no need to break open cells).

Because the initial staging of a cell free reactor is more complex and resource intensive, it is important that maximum value is extracted from each production run. So far we have built systems that can run up to 7 days and produce titers of monoterpenes, isobutanol and cannabinoids with production parameters that far exceed what has been possible in cells. We are now working toward building large scale cell free bioreactors.

8:30 AM S111: Cell-free enzymatic production of biochemicals

M. Alahuhta, National Renewable Energy Laboratory, Biosciences Center, Golden, CO, USA and Y.J. Bomble, National Renewable Energy Laboratory, Golden, CO, USA*

Several key factors negatively impact the production yield, and thus, cost of biochemicals from renewable sources. Common hindrances in the biological production of chemicals are: 1) microbial toxicity of end-products or intermediates, 2) loss of carbon to microbial biomass formation, 3) co-production of undesired byproducts, and 4) costly or complex product separation steps. Therefore, a possible alternative is to eliminate the microbial biocatalyst entirely and instead operate the desired metabolic pathway in isolation, thus circumventing these issues. However, traditional cell-free technology (CFT) suffers from low productivity and titer. The main limitations are lack of enzyme stability, cofactor cost and the cost of enzyme production. We propose that the second generation CFT platforms can combine flexibility, robustness and high production levels with cost effective enzyme production, high specificity, long enzyme lifetime and total turnover number. To achieve this we are planning to use various immobilization/stabilization methods and pathway optimization through traditional biochemistry and sampling of enzymes from different source organisms. We work with multiple pathways to build a platform that can be used to produce advanced chemicals from different precursor molecules.

9:00 AM S112: Natural Product Discovery or Computational Design? *In-Vitro* Sampling of New Molecular Scaffolds with Computational Enzyme and Pathway Design

A. Zanghellini[*], Arzeda, Seattle, WA, USA
Enzymes are the most versatile and efficient catalysts, able to very selectively and efficiently catalyze difficult chemical reactions. In nature, the combination of enzymes in metabolic pathways has resulted in the diversity of natural products biosynthesized by bacteria and eukaryotes which have a bonanza for pharmacology and other fields. However, the space of molecules that were sampled by evolution is still very limited compared to the estimated $>10^{60}$ small molecules that can theoretically be synthetized.

Utilizing designer enzymes in enzymatic pathways holds great promise to synthetize small molecules of industrial and pharmaceutical interest, beyond what is synthetized in nature or with synthetic chemistry tools. This poses two distinct challenges that need to be solved in an integrated way if we ambition to fully deliver on the promise of synthetic biology. The first challenge deals with the rapid design of novel enzymes with high level of activities for reactions not known to be catalyzed in nature. To this end, Arzeda has developed high-throughput computational enzyme design methodologies (Archytas™). Such approaches are used alongside Arzeda's automated DNA build, protein expression and analytics platforms to rapidly yield the desired active enzymes.
The second challenge is that of finding optimal ways to arrange natural and designed enzymes to biosynthetize new molecular scaffolds. To illustrate our progress towards solving this challenge, we will discuss Scylax™, a software tool for the automated prospection of novel biosynthetic pathways. Scylax™ draws on databases of known natural enzymatic reactions as well as reactions that can be catalyzed by computationally designed enzymes to exhaustively enumerate biosynthetic routes to a specified target molecule. Pathways are ranked based on thermodynamic feasibility and designability of each enzymatic step. We will discuss results obtained during the DARPA 1000 molecules program where precursors to novel butenolides and acrylates were synthetized *in vitro* using Archytas™ and Scylax™. These in-vitro results, when combined with scalable cell-free systems, will open new perspective for synthetic biochemistry.

9:30 AM Break

10:00 AM S113: Cell-free synthetic biology for enzyme and natural product discovery

Z. Sun[*], Synvitrobio, Inc., San Francisco, CA, USA
Cell-free synthetic biology is a promising field for understanding and producing chemicals, fuels, and therapeutics. Tierra Biosciences leverages cell-free systems to explore genetic space for new enzymes and molecules. By utilizing cell-free systems from different hosts, Tierra is able to leverage advances in DNA synthesis, next-generation sequencing, cell-free biochemistry, automation, and protein engineering to conduct high-throughput functional genomics at significantly higher workflow than in cells. Here, we go over the Tierra platform and Tierra findings in the fields of protein engineering and enzyme and natural product discovery.

10:30 AM S114: A cell-free extract (CFE) system in *Clostridium thermocellum* for ethanol production and metabolomics analysis.

J. Cui[*] and D.G. Olson, Dartmouth College, Hanover, NH, USA; L.R. Lynd, Enchi Corporation, Hanover, NH, USA
Clostridium thermocellum is a cellulolytic anaerobic thermophile that has a great potential for lignocellulolytic biofuel production. Over years of genetic engineering in *C. thermocellum*, the yield of bioethanol production has been successfully increased from 35% to 80%, however, there's still a limitation of maximum ethanol titer (about 30 g/L). Cell-free systems is restriction free from cell membrane and genetic regulation, they are more versatile than in vivo systems for metabolic engineering. Here we developed a cell-free extract (CFE) system in *C. thermocellum* to provide an alternative way to study the ethanol production pathway and understand what limits ethanol production. With a few optimizations of growth medium, temperature, substrate, preparation of cell extract and cofactor combination, we established a reproducible CFE assay system that produces about 25 mM ethanol from 14.5 mM cellobiose, comparable to our in vivo control. We took several timepoints in the course of 48 hours of in vitro ethanol fermentation, and measured the concentration of intermediate metabolites and cofactors to better understand the pathway kinetics. To verify potential ethanol bottlenecks, we used the CFE system to test whether we can increase ethanol production by adding the metabolite downstream of the bottleneck reaction. We used 11 other glycolytic intermediates as substrate for ethanol production, in addition to cellobiose. We found that only glucose and fructose 6-phosphate can support similar levels of ethanol production as cellobiose, suggesting this method doesn't work great to identity metabolic bottlenecks for ethanol production, because we can't get rid of all regulations of metabolic pathways in the cell-free system. Next, we used the CFE system to prototype the results of metabolic engineering of the potential bottleneck steps by adding purified enzymes of interest into the CFE system and observe the changes in ethanol production. We found that certain combinations of added enzymes lead to higher ethanol production, suggesting in vivo metabolic engineering of these steps should be our focus in the future.

11:00 AM S115: Functional Biomaterials for Biocatalysis

C. Schmidt-Dannert[*], University of Minnesota, Saint Paul, MN, USA
Intrigued by the spatial organization of biological systems at the subcellular and molecular level, we are investigating, and

repurposing systems involved in self-organization for applications in biocatalysis and for the fabrication of functional biomaterials. In biological systems, proteins, nucleic acids and lipids are precisely organized to form higher ordered structures. Principles underlying the assembly and organization of natural bionanomaterials can be harnessed for the design and fabrication of robust materials for biocatalysis and as functional biomaterials with emergent properties. Self-assembly provides a low-cost approach for bottom-up construction of supramolecular biomaterials from simple building blocks. Peptides and proteins offer the most versatility for the assembly of such designed structures due to the chemical diversity of their amino acid components. Protein building blocks are also genetically encoded, allowing for the genetically programmable production of materials. We are currently designing genetically programmable and self-assembling protein-based nano-architectures for multi-step, *in vitro* biocatalysis and for the fabrication of new types of materials, which will be presented.

8:00 AM - 11:30 AM Session: 21: Biocatalysis for carving better tomorrow

Conveners: Ashish Paradkar, Novozymes, Bangalore, India and **Will Schroeder**, Gingko Bioworks, Boston, MA, USA

Marshall Ballroom North, Mezzanine

8:00 AM S116: Multistate enzyme redesign

R. Chica, University of Ottawa, Ottawa, ON, Canada*

The creation of enzymes displaying desired substrate specificity is an important objective of enzyme engineering. To help achieve this goal, computational protein design (CPD) can be used to identify sequences that can fulfill interactions required to productively bind a desired substrate. Standard CPD protocols find optimal sequences in the context of a single state, for example an enzyme structure with a single substrate bound at its active site. However, many enzymes catalyze reactions requiring them to bind multiple substrates during successive steps of the catalytic cycle. The design of multisubstrate enzyme specificity therefore requires the ability to evaluate sequences in the context of multiple substrate-bound states because mutations designed to enhance activity for one substrate may be detrimental to the binding of a second substrate. Additionally, many enzymes undergo conformational changes throughout their catalytic cycle and equilibrium between these conformations can have an impact on their activity. Here, I will present our development and implementation of two multistate CPD methodologies for the redesign of multisubstrate enzyme specificity and modulation of enzyme conformational equilibrium. Specifically, I will show our redesign of the branched-chain amino acid aminotransferase active site for acceptance of the non-native substrate l-histidine, and our fine-tuning of the aspartate aminotransferase conformational equilibrium for enhanced activity towards the non-native substrate l-phenylalanine. Overall, our approaches open the door to the design of multisubstrate enzymes displaying tailored specificity for target biocatalytic applications.

8:30 AM S117: Amine Dehydrogenase-catalyzed synthesis of chiral amines

R. Franklin, J. Whitley, C. Mount, A. Caparco, J. Robbins, B. Bommarius and A.S. Bommarius, Georgia Institute of Technology, Atlanta, GA, USA*

Chiral amines are often found as precursors to high value active pharmaceutical ingredients, and the need for enantiomeric purity creates a promising opportunity for enzymatic catalysis for their production. Amine dehydrogenases (AmDHs) catalyze the reductive amination of prochiral ketones to chiral amines through the incorporation of aqueous ammonia. During the reaction, hydride transfer occurs to the substrate from NADH, which itself is converted to NAD^+. The enzymes were developed through site-directed mutagenesis of the active site residues of amino acid dehydrogenases (AADHs), resulting in the loss of activity toward keto-acids, and gain of activity toward ketones. Since their introduction in 2012, the family of amine dehydrogenases (AmDHs) has been under continuous development. Improving these enzymes has had three main goals: increasing enzyme activity, broadening substrate scope, and process development toward scale-up. To broaden the substrate specificity of the family, multiple amino acid dehydrogenases, such as leucine dehydrogenase and phenylalanine dehydrogenases, have been used as parent enzymes to create AmDHs. Additionally, a chimeric enzyme consisting of parts of two different AmDHs has been constructed which shows different substrate profile and stability than either of its parents. We also report new work which has used site-directed mutagenesis to increase the activity of leucine amine dehydrogenase (L-AmDH) toward bulky aliphatic ketones. We found surprising synergism across various residues en route to bulkier substrates. Work toward process development began with a detailed kinetic study of one amine dehydrogenase to determine its kinetic mechanism and propose a rate law, a key component of reaction modeling in process design. It was discovered that L-AmDH shows a different kinetic mechanism when compared to leucine dehydrogenase, its parent enzyme. This was surprising, given that the AmDH differs from the AADH by only four residues. A biphasic reaction system was developed to access ketones with low solubility, a common problem for drug compounds. Finally, we report the first continuous application of a packed bed reactor with recycle for the enzymatic production of chiral amines.

9:00 AM S118: An automated data-driven pipeline for improving heterologous enzyme expression

T. Whitehead, University of Colorado, Boulder, Boulder, CO, USA*

A key challenge in biocatalysis and biomanufacturing of fuels, chemicals, and pharmaceuticals is that many pathway enzymes have very low activity, limiting overall titers and productivities. One reason is that enzymes are marginally stable under their native conditions, and expression in a different environment can thermodynamically favor the unfolded state. Additionally, overexpression can result in aggregation because natively expressed proteins are close to their solubility limit.

This challenge suggests an engineering solution: engineer pathways enzymes to be stable in their biomanufacturing chassis. However, this is difficult because: (a.) many enzymes do not have high-throughput activity screens needed for directed evolution; (b.) there are few or no structures available; (c.) there are often multiple limiting enzymes; (d.) most mutations confer small benefits to stability; and (e.) the plurality of stability-enhancing mutations decrease catalytic efficiency.
In this talk I will present a culmination of my group's approach to solve the above challenges, in effect automating the design of stable, active enzymes from limited combinatorial datasets. This engineering strategy involves user-defined precise mutagenesis[1], deep sequencing to evaluate the functional effect of nearly all possible single point mutants on solubility[2], Bayesian methods to discriminate stable, catalytically neutral from deleterious mutations[2], and computational design to combine up to 50 mutations at once[3].I will show show this method can improve the pathway productivity of a medicinal alkaloid pathway in *Saccharomyces cerevisiae*[4], and end with the description of a computational pipeline to automate our process for any enzyme of interest[4].

9:30 AM Break

10:00 AM S119: Identifying and Engineering Halogenases for Selective Catalysis

J. Lewis[*]*, Indiana University, Bloomington, IN, USA*
Halogen substitution has a major impact on the physical and biological properties of organic compounds, and it enables a wealth of additional reactions that can be used to further functionalize those compounds. Flavin dependent halogenases have emerged as useful catalysts for aromatic C-H halogenation. In this talk, I will discuss my group's efforts to engineer halogenase variants with improved stability, expanded substrate scope, and altered site selectivity, all of which are required to establish the utility of these catalysts for C-H functionalization. I will also present recent unpublished studies aimed at establishing the origins of altered site selectivity in evolved variants, computational prediction of site selectivity on non-native substrates, and family-wide genome mining to identify new halogenases for late stage C-H functionalization of complex molecules.

10:30 AM S120: Leveraging Synthetic Biology and Machine Learning to Engineer Better Biocatalysts

S. Govindarajan[*]*, ATUM, Newark, CA, USA*
Synthetic Biology, enabled by advances in oligonucleotide chemistry, gene synthesis and HTP cloning technologies has come to prominence. Machine Learning has become a powerful tool in the information technology era and has found several applications in biology. ATUM combined these two technological advances to create a powerful engineering platform to engineer any biological molecule or system. Biocatalysis is never just about the catalytic prowess of an enzyme, but also involves process and cost consideration. Apart from performing the catalysis, the enzyme must express well, be stable, and amenable to reduced environmental impact and costs considerations. Our approach involves a thorough evaluation of enzyme variants using assays and conditions reflecting the production process, thereby taking into account all aspects of the enzyme that are required to be improved during the course of bioengineering. This is done with a small set of designed synthetic enzyme variants and applying empirical model building based on the data accumulated from the assay. Here we present an overview of our ProteinGPS technology to engineer better enzymes for biocatalysis.

11:00 AM S121: Genomic Mining and Computational Enzyme Design to Build a New Generation of tools to fight mycotoxins

J. Siegel[*]*, UC Davis, Davis, CA, USA*
Roughly 4.5 billion people around the world are exposed to aflatoxin on a daily basis. This toxin is ubiquitous in the food supply chain in developing countries, and is a known cancer causing agents with additional links to stunting and anemia. While a known issue since the 1960's, there is yet to be a widespread tool for detoxification beyond mechanical sorting or harsh chemical treatments. Here I will describe a recent effort utilizing genomic mining, crowd sourcing, and computational enzyme design to develop a customized biocatalyst for this toxin which can be applied in an industrially relevant manner.

8:00 AM - 11:30 AM Session: 22: Antibiotics in the environment

Conveners: Joshua Blodgett, Washington University in St Louis, St Louis, MO, USA and **Josephine Chandler**, University of Kansas, Lawrence, KS, USA

Madison B, Mezzanine

8:00 AM S122: Using comparative metabologenomics in environmental *Streptomyces* spp. to understand polycyclic tetramate macrolactam regulatory control

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

Actinomycete genomes encode far more drug-like compounds than historically thought. However, the majority of the genetic loci encoding these compounds are thought to be “silent”. Biosynthetic silence thus represents a significant roadblock to genomics-guided drug discovery, but its biological underpinnings remain poorly understood. Polycyclic tetramate macrolactam (PTM) antibiotic biosynthetic loci enjoy an atypically common distribution within actinomycete genomes. Towards leveraging PTM production as a model to understand biosynthetic silence, our lab has amassed a sizeable collection of PTM-cluster carrying filamentous actinomycetes. This enabled the parallel study of several strains and the use of comparative metabologenomics to reveal specific mechanisms of regulatory control. So far, our results suggest PTM control within the *Streptomyces griseus* clade is complex, implicating both regulatory heterogeneity and metabolic dependency on other biosynthetic loci. Further, our studies reveal differential PTM production profiles from panels of strains harboring identical gene clusters. In sum, our molecular-genetic investigations have now identified candidate mechanisms that determine PTM biosynthetic silence in select families of strains, and inform future rational activation strategies. Further, we find the need to engineer strains for PTM discovery is significantly reduced by a combination of genome mining, tailored metabolomics, and strain library utilization.

8:30 AM S123: Microbial Twitter: using synthetic ecology to study communication and competition in bacteria

J. Chandler, University of Kansas, Lawrence, KS, USA*

Many bacteria exist in polymicrobial communities, where they cooperate and communicate to carry out elaborate social behaviors. Our laboratory is interested in understanding how bacteria use cooperation and communication to control and coordinate activities, such as the production of antibiotics, that maximize competition with other bacteria. Previous studies of competition-related behaviors have been largely limited to single-clone populations, at least in part because direct studies of naturally occurring polymicrobial communities presents many challenges. However, the recent development of laboratory ‘in silico’ models of mixed-strain and mixed-species populations provide an innovative approach to study these behaviors in a controlled, simplified environment. In this talk, I will describe some of the laboratory models we have developed to study bacterial behaviors, such as those used for competition with other strains and species, in the context of more complex communities. Results with these models have shown how the production of bioactive molecules such as antibiotics alters the dynamics of populations in multiple-strain and multiple-species communities, and highlight how these interactions can contribute to the evolution of antibiotic biosynthesis pathways. Our approach has opened new windows into understanding interactions between members of bacterial communities and for understanding the ecology and evolution of antibiotics.

9:00 AM S124: Though Much is Taken, Much Abides: Finding New Antibiotics Using Old Ones

M.R. Seyedsayamdost, Princeton University, Princeton, NJ, USA*

Microbial natural products serve as a dominant source of pharmaceutical compounds and comprise some of our most celebrated cures. Recent studies, however, have been plagued by the frequent rediscovery of old molecules. The underlying reason is that the vast majority of natural product biosynthetic genes in a given bacterium are not significantly expressed, when cultured under standard laboratory conditions. These socalled ‘silent’ or ‘cryptic’ gene clusters represent a large reservoir of bioactive molecules and methods that access them would have a profound impact on natural products research and thereby on drug discovery. In this talk, I will present new strategies that my group has developed for activating silent biosynthetic gene clusters.[1] Application of these approaches to diverse bacteria has unveiled not only the products of silent gene clusters, but also small molecule elicitors, which in most cases are growth-inhibitory or antibiotic in nature.[2-4] These findings have led to the idea that old antibiotics may be used to find new ones. In combination with emerging profiling methodologies, our efforts are beginning to reveal the 'hidden' metabolites encoded in microbial genomes.

References:

[1] Seyedsayamdost, M. R. Proc. Natl. Acad. Sci. USA 2014, 111, 7261.
[2] Xu, F.; et al. J. Am. Chem. Soc. 2017, 139, 9203.
[3] Xu, F.; et al. Nat. Chem. Biol. 2019, 15, 161.
[4] Moon, K. et a. ACS Chem. Biol. 2019, 14, 767.

9:30 AM Break

10:00 AM S125: A bacterium by any other name: symbiont strain variation in toxin production within *Xenorhabdus-Steinernema* interactions

D. Ginete, University of Wisconsin-Madison, Madison, WI, USA, K.E. Murfin, Yale University, New Haven, CT, USA and H. Goodrich-Blair, University of Tennessee-Knoxville, Knoxville, TN, USA*

Bacterial symbionts can have positive or negative outcomes on their hosts. Recent studies show that the sign and degree of an interaction can vary depending on the strain of bacteria; even within a single symbiont species, extensive variation in genes and traits can influence the impact of a symbiont on its host. To establish parameters that dictate symbiotic outcomes, we are identifying factors that govern strain specificity using the association between *Steinernema* nematode hosts and *Xenorhabdus bovienii* bacterial symbionts as a model. These organisms form a nutritional and defensive mutualism that allows them to parasitize insects. Cross-pairing studies indicate that incompatible associations can occur when *Steinernema* nematodes are paired with non-native symbiont strains of the same species as their native symbionts. I will present our genomic, molecular, and cross-pairing analyses to determine causes of this incompatibility. In particular, I will discuss a symbiont-phage-associated ribosome-inactivating protein (RIP) toxin. In general, RIPs depurinate an adenine residue in a target 28S rRNA which causes cellular apoptosis. We found that *S. feltiae* nematode 28S rRNA exhibits substantially more depurination when exposed to a non-native RIP-encoding strain relative to exposure to either RIP deletion mutant or to its native strain. Further, the 28S rRNA of the native host of the RIP-encoding bacterial symbiont does not appear susceptible to depurination. Taken together, these results indicate that a phage region within a bacterial symbiont encodes RIP that is specifically active against non-native nematode hosts. The basis of this specificity, and the biological and ecological consequences of this toxicity are under investigation.

10:30 AM S126: Harnessing the plant microbiome to help farmers sustainably feed our planet

V. Knight-Connoni, Indigo Agriculture, Boston, MA, USA*

Plants rely on a vast array of natural, beneficial microbes to support their health and productivity. The community of microbes living in and around a plant, its microbiome, works in harmony with the plant to provide life-sustaining benefits. The microbiome helps the plant absorb nutrients in the soil and bolsters its resilience to environmental stresses. In many cases, this effect is mediated by the secondary metabolites produced by individual microbes. Microbes have evolved in conjunction with plants over millions of years, in many cases to optimize their combined health and survival. Development of microbial products derived from this unique co-evolution to achieve long-term agricultural sustainability is a growing industry. Indigo Ag, Inc. is focused on identifying microbial products derived from plant-associated microbes. We have developed a broad and sophisticated pipeline to discover microbes that enhance plant performance under laboratory conditions, field trials, and farmer fields. In this session, I will describe the pipeline used to discover the best microbial products and present a case study for secondary metabolites produced by a microbe that allow it to help the plant combat the stresses it encounters in the field.

8:00 AM - 11:30 AM Session: 23: Fermentation Optimization of Natural Products Sponsored by: BASF

Conveners: Dr. Hartwig Schroeder, BASF SE, Ludwigshafen, Germany

Marshall Ballroom West, Mezzanine

8:00 AM S127: Approach towards productivity improvement of a novel insecticide 'afidopyropen'

Y. Onozaki, S. Hasegawa, K. Kobayashi, K. Ymamamoto, K. Yanai, K. Murashima and K. Oyama, Meiji Seika Pharma Co., Ltd., Tokyo, Japan; H. Anzai, Ibaraki University, Ibaraki, Japan*

The insecticide Afidopyropen was discovered through a collaboration between Meiji and Kitasato Institute, Prof. Satoshi Omura group. Afidopyropen is manufactured by semi-synthesis from pyripyropene A produced by *Penicillium coprobium*. It is an entirely new insecticidal active ingredient which has a unique chemical skeleton and it has a novel mode of action.

Afidopyropen provides highly effective control of insect pests such as aphids, whiteflies and psyllids which already have developed serious resistance to the existing insecticides. Furthermore, afidopyropen is safe to mammalians and the natural environment. Therefore, we believe that afidopyropen will be one of the important tools for pest control in the next generation.

Afidopyropen was commercialized by BASF under the AI trade name of “InscalisTM”. It has already been launched in the U.S., Australia, India and China, and will be globally introduced in the near future.

It is important to cultivate the fungus to produce pyripyropene A (PpA) which is a starting material of Afidopyropen with higher yield for industrialization. Therefore, we tried to obtain a strain to produce PpA with high yield and to optimize the medium culture and growing condition for its production.

First of all, we selected a strain of *Penicillium coprobium* with high productivity by random mutagenesis. However, when scaling up, the productivity was decreased because this fungus is weak against shear stress. For that reason, we selected a shear stress-resistant strain using the same method.

Next, we identified the biosynthesis pathway of pyripyropene in the fungus, cloned the biosynthetiuc gene cluster and generated a recombinant strain to enhance the expression of the biosynthetic genes. In addition, we improved the medium culture and growing conditions in order to scale up to the fermentation volume of 16.5kL. Furthermore, after assessing possible risks we found pressure rise within the fermentation vessel to be the strongest concern regarding productivity.

Through these improvements, we ultimately achieved a 60-fold titer increase on an industrial scale of 165 kL.

8:30 AM S128: Development of novel solid state bioprocess for production of microbial metabolites

F. Alani, McMaster University, Hamilton, ON, Canada*

Solid- state bioprocess or solid-state fermentation is defined as growth of microbial cells in absence or near absence of visible water in the culture. There is recent renewed interest in solid state bioprocess by biotechnology industry. Solid-state bioprocess (SSB) possesses several advantages over traditional submerged bioprocess (SmB). Such advantages include higher fermentation productivity, lower catabolic repression, lower bioreactor size, low water demand, and lower sterility demand. Drawbacks include slow growth, bioreactor design and scale up.

We are proposing novel solid state bioprocess for production of industrially important primary metabolites such as citric acid and lipase enzyme as well as secondary metabolites such as immunosuppressant drug mycophenolic acid. The new developments involve use of novel solid substrates such as DDGS and pearl barley, novel bioreactor design such as packed-bed bioreactor and solid state Rocking BioReactor.

Mycophenolic acid (MPA) was produced from *Penicillium brevicompactum* by both SSB using pearl barley and submerged fermentation SmB using mannitol. It was found that SSB was superior over SmB in terms of MPA concentration (1219 mg/L vs. 60 mg/L after 144 h fermentation), and the product yields were 6.1 mg/g pearl barley for SSB and 1.2 mg/g mannitol for SmB. The volumetric productivities were 8.5 and 0.42 mg/L/ h for SSB and SmB, respectively. The higher volumetric productivity and concentrations makes SSB an attractive alternative to SmB for MPA production.

Detailed study of use SSB for citric acid production from *A.niger* and lipase is also included in this presentation.

9:00 AM S129: Bioactive natural products fermentation at BASF

H. Schroeder, BASF SE, Ludwigshafen, Germany*

The production of natural products by fermentation of microorganisms has a long history for pharmaceutical products widely applied. To leverage natural products for usage as agrochemicals a different level of performance and economics is being needed. Therefore, highly efficient process development is needed, employing technologies such as microbiology, genomics, metabolic engineering, metabolomics as well as process- and media development to only mention a few. We have made significant progress at BASF in the last decade in deciphering biosynthetic pathways, improving strains, transforming lab results into pilot and production leading to new products. Researchers at BASF and collaborators have employed these methods for the generation and production of several different natural compounds as new agrochemicals. Several approaches and experiences will be shared ranging from terpenoid-, alkaloid- and polyketide-class of molecules. The newly introduced insecticide Inscalis is the first example where this work at BASF has entered the plant protection market.

8:00 AM - 11:30 AM Session: 24: Metabolic Engineering for Fuels and Chemicals I

Conveners: Kelsey Yee and **Dr. Peng Xu**, University of Maryland Baltimore County, Baltimore, MD, USA

Marshall Ballroom Southeast, Mezzanine

8:00 AM S130: Post-translational control of engineered metabolic pathways by dynamic assembly of membraneless synthetic organelles

J. Avalos, J. Toettcher and E. Zhao, Princeton University, Princeton, NJ, USA*

Spatial and dynamic control in metabolic engineering has received increasing attention in recent years. We have previously demonstrated that co-localizing enzymes of metabolic pathways in yeast mitochondria enhances chemical production. More recently, we have shown that optogenetic circuits enable dynamic control of engineered metabolic pathways using light inputs to further boost yields, titers, and productivities. In this lecture, I will show how it is possible to combine both, spatial and dynamic control of biosynthetic pathways in the same yeast strain by co-localizing enzymes in synthetic membraneless organelles, whose assembly and disassembly are temporally controlled with light inputs. With this strategy, we are able to the reach the theoretical limits of flux enhancement by enzyme clustering in branched metabolic pathways. These dynamically controlled synthetic organelles offer new opportunities to control engineered metabolic pathways in space and time for enhanced microbial chemical production.

8:30 AM S131: Reinventing chemical manufacturing using biotechnology: Manus Bio approach

C. Santos, Manus Bio, Cambridge, MA, USA*

Chemical manufacturing is undergoing a period of change, from an era focused on petrochemical-derived chemical synthesis or plant extraction to one which leverages engineered chemical biosynthesis in microbes for biomanufacturing. At Manus Bio, we have developed a robust technology platform from discovery to manufacturing which enables the economical and sustainable production of typically rare complex natural products. Our engineered microbes are capable of converting carbon feedstock to

product at high yields and have been adapted to produce a mature pipeline of complex products.

9:00 AM S132: Engineering of an environmental isolate of *Bacillus megaterium* for biochemical production under supercritical CO_2

K.L.J. Prather, J. Boock, A. Freedman and J. Thompson, Massachusetts Institute of Technology, Cambridge, MA, USA; G. Tompsett and M. Timko, Worcester Polytechnic Institute, Worcester, MA, USA*

Continuous processing is a mainstay for chemical production but is far less common for biochemical processes. The increase in productivity and decrease in costs make continuous processing an intriguing option for bulk chemicals where price is a major consideration. Among the various challenges of continuous bioprocessing are the risks of contamination and toxicity of the target products. Supercritical carbon dioxide ($scCO_2$) may provide a means to address both of these issues. $scCO_2$ is an attractive substitute for conventional organic solvents due to its unique transport and thermodynamic properties, its renewability and labile nature, and its high solubility for compounds such as alcohols, ketones and aldehydes. $scCO_2$ is also known for its broad microbial lethality. The isolation and engineering of a microbe that is capable of growth and production in the presence of $scCO_2$ thus represents an opportunity to create a production environment that is both resist to contamination and capable of sequestering toxic products through phase separation. Using a targeted bioprospecting approach by sampling fluid from a natural, deep-subsurface $scCO_2$ well, a strain of *Bacillus megaterium* was isolated that is able to germinate and grow in the presence of $scCO_2$. A modified protoplast-based transformation method permitted the identification of promoters capable of inducible heterologous protein expression in both aerobic and anaerobic conditions. We engineered the *B. megaterium* strain to produce isobutanol from 2-ketoisovalerate by introducing a two-enzyme pathway (2-ketoisovalerate decarboxylase (KivD) and alcohol dehydrogenase (Adh)). Due to the strong partition of the aldehyde to the $scCO_2$ phase, we tested five homologous Adh enzymes and found that YqhD from *E. coli* resulted in greater than 85% conversion when grown aerobically. Isobutanol production was also observed when our recombinant strain was cultured under $scCO_2$. Finally, we have developed a process model for an integrated bioprocess and have found conditions that are comparable if not better than existing *in situ* extraction techniques such as gas stripping.

9:30 AM Break

10:00 AM S133: Metabolic engineering of *Yarrowia lipolytica* for fuels and chemicals production

H. Alper, The University of Texas at Austin, Austin, TX, USA*

The non-conventional yeast *Yarrowia lipolytica* is an excellent platform for the production of biofuels and biochemical, especially those that are derived from acetyl-CoA and malonyl-CoA precursors. Here, we describe recent advances in both the synthetic control of this host as well as applications of metabolic engineering. Initially, we will discuss our novel approaches toward synthetic part design and development including various hybrid promoters, terminators, selection markers, and transposon approaches in the host. Next, we demonstrate the utility of these approaches in the context of large-scale strain engineering efforts that highlight two major products along the way. First, we describe the production of saturated cells containing upwards of 90% lipid content with titers of 40 g/L lipid and demonstrate the ability to divert this product to other oleochemicals of interest. Second, we describe the strain engineering effort for production of a polyketide, triacetic acid lactone, in cells capable of producing upwards of 36 g/L production with over 43% of theoretical conversion yield. We further demonstrate how this product can be converted into a novel material. Finally, this talk concludes with additional examples of biofuels and biochemical of interest, thus showcasing the potential of this host.

10:30 AM S134: Development and commercialization of fermentative isobutanol

C. Smith, Gevo, Inc., Englewood, CO, USA*

Gevo has developed technology based on the best synthetic biology, best classic biology, and best chemical industry production techniques to convert carbohydrates to low carbon chemicals and fuels. Our source of carbon is CO2 in the atmosphere, the GHG itself, captured by plants in the form of carbohydrates. Ideally, the plants are grown with sustainable growing methods wherever possible, building soil quality and carbon levels. We like to capture the protein produced by the plants and supply it to the feed markets and eventually food markets, too. The carbohydrates are separated from the protein and fermented via specially design yeast to make low cost alcohols like ethanol, isobutanol, and or higher alcohols. The alcohols can be sold directly for chemical or fuel use. We especially like to take the alcohols and convert with clean catalytic chemical processes to produce products for renewable jet fuel, gasoline, polyester, rubber products, as well as specialty chemicals, fine chemicals, and ingredients for flavors and fragrances.

11:00 AM S135: Cofactor balance inÂ *Yarrowia lipolytica*Â central carbon metabolism

E. Bredeweg, K. Pomraning, Y.M. Kim, J. Zucker, T. Metz, Y. Gao, W. Qian, W. Cannon and S.E. Baker, Pacific Northwest National Laboratory, Richland, WA, USA*

Production of cell-produced bioproducts is heavily dependent upon monitoring and fostering cellular conditions for their production. Co-factor production, balance and renewal is a key support for oxidation-reduction reactions, such as NAD+/NADH supply during lipid production. We performed multi-omics experiments monitoring central carbon metabolism enzymes, metabolites and global transcripts. We compare mutants in 3 different NAD-production enzymes across 3 different carbon sources. Examination of these conditions and mutants provides insight into regulation and response for metabolic engineering applications.

11:45 AM - 12:30 PM Annual Business Meeting

Madison B, Mezzanine

11:45 AM - 12:45 PM Planning-2020 Annual Meeting

Taylor, Mezzanine

1:00 PM - 4:30 PM Session: 25: Biocatalytic conversion of Lignin

Conveners: Arul Mozhy Varman, Arizona State University, Tempe, AZ, USA and **Joshua Michener**, Oak Ridge National Laboratory, Oak Ridge, TN, USA

Madison B, Mezzanine

1:00 PM S136: Metabolic Engineering of Non-Model Yeast Cutaneotrichosporon Oleaginosus for Valorizing Lignin and Lignin-Derives Aromatics

A. Yaguchi, S. Lee, M. Spagnuolo and M. Blenner, Clemson University, Clemson, SC, USA*

*Cutaneotrichosporon oleaginosus,*previously known as *Cryptococcus curvatus*, is a non-model oleaginous yeast that is known for its ability to metabolize many alternative sugars, including xylose, and toxic lignocellulosic hydrolysate inhibitors such as 5-hydroxymethylfurfural (5-HMF) and furfural. We discovered *C. oleaginosus*also tolerates and metabolizes lignin-derived phenolics, highlighting this organisms' potential to utilize all components of lignocellulosic biomass. *C. oleaginosus*is able to fully metabolize phenol, 4-hydroxybenzoic acid (pHBA), and resorcinol as sole carbon sources, as well as co-utilization with glucose and xylose. We exploited feeding strategies to overcome aromatic toxicity and increased lipid accumulation to over 69% of biomass by weight. Since yeast aromatic metabolism is poorly characterized on a genetic basis, we used transcriptomic analyses to elucidate aromatic metabolic pathways in *C. oleaginosus*and improved the existing genome annotation significantly. Biochemical analysis suggests ortho ring cleavage is used throughout the aromatic metabolic pathways. We also recently demonstrated*C. oleaginosus*metabolizes depolymerized lignin from multiple sources. With such an exceptionally desirable natural phenotype, this yeast could become a preferred host for oleochemical production if novel synthetic biology tools are developed. We identified both strong constitutive and phenolic-regulated promoters to drive expression of. In parallel, we are establishing higher efficiency transformation methods for increasing the pace of engineering efforts. Overall, our work establishes *C. oleaginosus*as a promising platform to robustly convert all components of lignocellulosic biomass into novel high-value oleochemicals.

1:30 PM S137: Expanding the molecular tookit for lignin valorization by rapid pathway identification in non-model microbes and prospecting for novel aromatic compound-degrading bacteria

G.N. Presley, D. Garcia, R.J. Giannone, O.N. Cannon, J.G. Elkins and J. Michener, Oak Ridge National Laboratory, Oak Ridge, TN, USA; D.M. Klingeman, Biosciences Division and BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Lignin-rich waste streams from plant biomass-based fuel can be microbially upgraded to lucrative chemical precursors. However, known biological decomposition pathways are limited to a few well-studied model organisms. This work describes efforts to isolate novel lignin-based aromatic compound-degrading bacteria from environmental samples and to identify the requisite decomposition pathways in non-model bacteria. To date we have isolated over 200 bacterial strains with the capacity to degrade lignin monomers and/or dimers and have screened for their capacity to degrade a variety of lignin-based aromatic compounds. Twenty-nine of these strains have been submitted for whole genome sequencing to date with a goal of sequencing at least seventy-five. Comparative genomics will be used to determine the genetic basis of these ligninolytic phenotypes. Additionally, the genetics of lignolysis in the aromatic compound-degrading bacterium, *Novosphingobium aromaticivorans*, was investigated using Tnseq/barseq. These efforts identified novel enzymes useful in biological lignin valorization including a β-1 dimer-cleaving deformylase (LsdB) formerly classified as a hypothetical protein. This protein was shown to work in concert with lignostilbene dioxygenase to produce vanillin from a model β-1 dimer. This work has also identified a novel two-component 2Fe-2S guaiacol demethylase (GdmAB) with no homology to other known enzymes with this functionality. Efforts to biochemically characterized these enzymes are ongoing. The same techniques are being used to identify lignin monomer decomposition pathways in a syringate-degrading strain of *Pseudomonas fluorescens*. Several candidate genes important for syringate metabolism have been

identified using Tnseq/Barseq and proteomics. These genes are being examined for their biological role. This work aims to expand the available molecular toolkit for biological lignin valorization.

2:00 PM S138: Metabolism of S-lignin by *Pseudomonas putidaÂ* KT2440

C.W. Johnson, S. Notonier, A. Werner, A. Amore and G.T. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; L. Dumalo and L.D. Eltis, University of British Columbia, Vancouver, BC, Canada; P.E. Abraham, E.A. Hatmaker, R.J. Giannone, A.M. Guss and R.L. Hettich, Oak Ridge National Laboratory, Oak Ridge, TN, USA; L. Wang and C.D. Maranas, The Pennsylvania State University, University Park, PA, USA*

Lignin is a heterogeneous polymer produced in the plant cell wall by radical coupling of *p*-coumaryl (H-lignin), coniferyl (G-lignin), and sinapyl (S-lignin) alcohols. Deconstruction of S-lignin yields molecules such as syringic acid and syringaldehyde that can be readily metabolized by soil bacteria such as *Sphingobium* sp. SYK-6 and *Novosphingobium aromaticivorans* DSM12444. *Pseudomonas putida* KT2440 is capable of metabolizing the products generated by metabolism of G- and H-lignin using pathways that are well described. In this work, we have demonstrated that *P. putida* KT2440 is also capable of metabolizing syringic acid and syringaldehyde and describe these pathways using *in vitro* and *in vivo* approaches. Culturing conditions and strain engineering strategies are identified that enable efficient assimilation of these substrates. Finally, we extend this work to describe the conversion of S-lignin monomers to the biopolymer precursor 2-pyrone-4,6-dicarboxylic acid (PDC), again using a pathway that is native to, but previously undescribed in, *P. putida* KT2440.

2:30 PM Break

3:00 PM S139: Natural and engineered utilization of methoxylated aromatics by *Methylobacterium* species

C. Marx, T. Ticak, A. Baugh and S. Stolyar, University of Idaho, Moscow, ID, USA; J. Lee, Global Viral, San Francisco, CA, USA*

Microbial lignin degradation faces many barriers, one of which is an abundance of methoxy substituents. Demethoxylation of lignin-derived aromatic monomers in aerobic environments releases formaldehyde, a potent cellular toxin that organisms must eliminate in order to further degrade the aromatic ring. We have discovered that several species of the genus *Methylobacterium*, a plant-associated genus of methylotrophs in which aromatic catabolism has not previously been described, are able not only to catabolize lignin-derived aromatic compounds but also to use the potentially toxic methoxy groups for growth. Critically, whereas non-methylotrophs excrete formaldehyde during growth on vanillate, *Methylobacterium* do not. Comparative genomics has indicated that aromatic catabolism is ancestral to the *M. nodulans* and *M. aquaticum* clades, but has also been acquired horizontally by other *Methylobacterium* species. Genome content largely predicted the ability of *Methylobacterium* strains to grow on various aromatic compounds, and metagenomic data indicate widespread presence of these organisms in plant-associated niches. Pathways for methoxylated aromatic use can be introduced into *M. extorquens* PA1 and enable it to grow on aromatics. Over a short period of experimental evolution on protochatechuic acid, strains of *M. extorquens* PA1 grow quite well, and further introduction of genes encoding vanillic acid use permit growth on this methoxlated aromatic compound. Furthermore, we have identified a novel beta-cleavage enzyme that directs carbon from beta-ketoadipate into the TCA cycle in a novel manner. These advances pave the road for development of strains that produce valuable bioproducts like butanol from lignin-derived aromatics.

3:30 PM S140: RNA-Seq reveals regulatory mechanisms in *Novosphingobium aromaticivorans*

A. Linz, W. Kontur, J. Perez, K. Myers, T. Donohue and D. Noguera, University of Wisconsin-Madison, Madison, WI, USA*

Some microbes are suited for lignin valorization because of their ability to funnel many different compounds into a limited number of metabolic precursors. We are studying the aromatic degrading bacterium, *Novosphingobium aromaticivorans*, since it can efficiently consume lignin monomers and dimers derived from chemical depolymerization processes and can be genetically modified to accumulate potentially valuable bioproducts. We propose that understanding how these metabolic pathways are regulated can help to engineer a strain suitable for industrial scale production, which would need to always produce a large amount of the desired compound. To better understand transcriptional regulation in *N. aromaticivorans*, we performed RNA-Seq on cultures grown on varying lignin monomers and used patterns of gene expression to infer regulatory relationships. We found evidence for multi-level regulation of aromatic degradation pathways, with a combination of global and specific transcriptional regulators controlling levels of expression. We identified key regulators and used homology to predict their regulation mechanisms. Finally, we used co-regulation to propose new genes involved in aromatic degradation, including degradation of diketones produced by current depolymerization methods. The results generated by this research can be used to identify new gene targets for metabolic engineering and to manipulate expression levels for maximum production of desired compounds from depolymerized lignin.

4:00 PM S141: Defining Process-Structure-Property Relationship for Lignin-based Products to Promote Biorefinery Sustainability and Cost-Effectiveness

J.S. Yuan, Synthetic and Systems Biology Innovation Hub, Texas A&M university, College Station, TX, USA*

Biocatalytic conversion of lignin into value-added products represent a major challenge for the sustainability and cost-effectiveness of modern biorefinery. Nevertheless, the quality and yield of lignin-derived products heavily depends on the fundamental understanding of the process-structure-property relationship of lignin and its derived products. In particular, there is an imperative need to define the relationship between lignin chemical characteristics and performance of products like carbon fiber, asphalt binder, nanoparticles, and bioconversion processes. Furthermore, lignin chemistry can be defined by either feedstock design or biocatalytic process development. In the past several years, our multidisciplinary team has significantly advanced the understanding of how to tailor lignin chemistry to improve the performance of lignin-based products. For carbon fiber, we have revealed that carbon fiber mechanical and conductive performance heavily depends on the molecular weight, uniformity, chemical linkages, and functional group profile of lignin. The chemical characteristics determines the miscibility of lignin with guest polymer and thus impacts the crystallite content and size, resulting in carbon fibers with various performance. For nanoparticles, we have shown that the functional groups, chemical linkages, molecular weight, S/G ratio, and lignin condensation all could impact the hydrogen bond networks and electron double layers, which could in turn define the size, uniformity, and stability of lignin nanoparticles. For asphalt binder modifiers, the functional groups and molecular weight also impact the interaction with asphaltene, thus defining the high temperature and low temperature performance of asphalt binder. For bioconversion, we have found that low molecular weight lignin are more ready to be converted by microorganisms. Together with the fundamental understanding, we have developed a series of pretreatment and fractionation processes to promote biocatalytic conversion of lignin into value-added products. Overall, lignin chemistry is crucial for the performance of various high value products. The fundamental understanding transformed the biorefinery design, where new pretreatment, fractionation, and feedstock development strategies are being developed to tailor lignin chemistry toward best-performing lignin-based products.

1:00 PM - 4:30 PM Session: 26: Bioconversion of waste to products

Conveners: Kang Wu, University of New Hampshire, Durham, NH, USA and **Dr. Navanietha Krishnaraj Rathinam**, South Dakota School of Mines and Technology, Rapid City, South Dakota. USA., SD, USA

Marshall Ballroom North, Mezzanine

1:00 PM S146: Microbiome Exploration and Discovery

N. Mouncey[*]*, E. Eloe-Fadrosh, N. Kyrpides, S. Roux and N. Ivanova, DOE Joint Genome Institute, Walnut Creek, CA, USA*

The cross-cutting nature of microbiome research in environmental sciences, health, agriculture, energy, and natural and built environments requires the development of new solutions and community coordination to tackle grand challenges that will accelerate basic discovery and lead to transformative advances. The exponential growth of microbiome data over the past few decades has ushered in a new era of biology, shifting the focus from descriptive observations and small-scale experimental paradigms to data-driven exploration and hypothesis generation, enabled by and relying on a rapidly growing suite of transformative data science strategies. The DOE JGI is a leader in microbiome data science through its user-centric scientific programs that have expanded the microbial tree-of-life, discovered numerous new viruses and host associations, explored microbe-plant interactions, and discovered new biochemical pathways that play important roles in nutrient cycling and microbial survival. I will discuss some of our recent scientific studies, as well as provide an overview of the new National Microbiome Data Collaborative.

1:30 PM S143: Commercial Scale Production of Low Carbon Fuels and Chemicals from Waste Gases

M. Köpke[*]*, LanzaTech Inc., Skokie, IL, USA*

Rapid population growth and climate change are posing some of the most urgent challenges to mankind and have intensified the need for low-cost manufacturing of fuels, chemical-building blocks, materials and food from sustainable resources.

Gas fermentation using autotrophic microorganisms offers a sustainable path to these products from a range of local, highly abundant, waste and low-cost resources. LanzaTech has pioneered a gas fermentation process using anaerobic acetogenic microbes capable of fixing carbon oxides.

While 10 years ago, acetogens were considered genetically inaccessible and mass-transfer of gases was considered a major scale up hurdle, LanzaTech has since developed a suite of synthetic biology tools successfully scaled up the process from the laboratory bench to full commercial scale. In May 2018, LanzaTech successfully started up a world-first commercial scale (48k MTA) gas fermentation plant using emissions from the steel making process as feedstock.

The technology has been demonstrated with a diverse range of additional low-cost feedstocks including waste gases from other industries (e.g., processing plants or refineries) or syngas generated from any biomass resource (e.g., unsorted and non-recyclable municipal solid waste, agricultural waste, or organic industrial waste).

In order to maximize the value that can be added to the array of gas resources that the process can use as an input, LanzaTech has established a unique biofoundry that enables automated strain engineering of anaerobic organisms and strain screening in context of flammable and toxic CO and H2 gases. Through this platform, LanzaTech has demonstrated direct production over 50 different products from gas.

2:00 PM S144: Project LIBERTY Update: Commercial Scale Conversion of Corn Stover to Ethanol

C. Sarks, POET Research, Sioux Falls, SD, USA*
Cellulosic biofuels have long been an attractive renewable energy option. Project LIBERTY is currently the world's largest operating cellulosic biofuel plant with a nameplate capacity of 20-25 million gallons per year. At LIBERTY, corn stover is collected before undergoing acid pretreatment followed by enzymatic saccharification and fermentation to produce ethanol. This presentation will cover the past, present, and future of Project LIBERTY highlighting struggles, changes, successes, and potential.

2:30 PM Break

3:00 PM S145: Screening for biotechnological potentials of yeasts isolated from sugar-rich fruits in the fermentation of spoilt fruits

T. Adeleye, S. Kareem, B. Chima, O. Oladunni and Z. Ayodele, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria*
Yeast strains associated with fruit surfaces have been reported to be are capable of bio-conversion of a wide range of sugars to industrial products. However, strain selection for biotechnological applications is determined by the physiological properties of the yeasts isolates. The aim of this study was to isolate high-fermenting yeasts with potentials for biotechnological applications. Yeasts were isolated from selected fruits samples bought from local markets and plucked from trees in Abeokuta, South-Western Nigeria. Primary screening for rate of sugar fermentation was done. Selected isolates were further screened for ethanol tolerance, thermotolerance and osmotolerance. Juice was extracted from the spoilt fruits picked from market wastes, by simple methods, sterilized and diluted to a sugar concentration of 40%. Laboratory scale fermentation of the extracted juice (1L) by 2 selected isolates was studied for a period of 10 days. The selected isolates identified as *Rhodotorula species* (osmotolerant) and *Saccharomyces species* (thermotolerant) were found in association with the green apple and hog plum respectively. After the 10day fermentation, a significant reduction in total dissolved solids (fermentable sugars) was observed in both samples inoculated with *Rhodotorula species* (70- 40°Brix) and *Saccharomyces species* (70- 36°Brix). An increase in specific gravity (1.084- 2.00) was observed after fermentation by *Rhodotorula species*. While a decrease in (1.92- 1.50) with alcohol yield of 54.18% was obtained from sample fermented by *Saccharomyces species*. This study reveals the potentials of indigenous yeasts for industrial applications.

3:30 PM S142: Enhancing yields and efficiency of corn wet milling through enzymes

B.J. Vidal, Novozymes North America, Franklinton, NC, USA*

For decades, the corn wet milling process has been the leading industrial process for the manufacture of corn starch and other byproducts. Advances in mechanical separation efficiencies and milling practices have contributed to yield improvements over time; yet despite these advances, current wet mills continue to see significant losses in their recovery of starch and protein. Because enzymes are capable of hydrolyzing substrates with high specificity, they can be used to target components in the matrices that bound these valuable products within "lower-value" streams. As an example, Novozymes has launched the first full-scale proven enzymatic solution called Frontia Fiberwash® for enhancing the release of starch and protein that are bound up with the corn wet-mill fiber. This is achieved through a combination of enzyme activities that work synergistically to solubilize non-starch polysaccharides in the endosperm matrix. The industrial application of this enzyme solution has resulted in cost-saving benefits to wet mills through increased recovery and yield of starch and protein, and through energy savings due to reduced drying requirement.

4:00 PM S159: Genome annotation and engineering of a non-model fungus for high level production of cellulolytic enzymes

S.S. Yazdani, International Center for Genetic Engineering and Biotechnology, New Delhi, India*
Abundantly available lignocellulosic biomass in the form of agricultural residues is considered a viable feedstock for production of second-generation biofuels. However, highly crystalline nature of this residue makes it recalcitrant towards enzymatic digestion. The fungal kingdom is majorly responsible for recycling the decaying biomass on earth. We therefore built a mathematical model to screen for highly efficient cellulase producer and selected *Penicillium funiculosum* for its ability to digest the plant residues most effectively. Upon genome sequencing, annotation and proteomic study, it was found that cellobiohydroloase I (CBH1) was secreted in largest quantity in addition to several other cellulases and hemicellulases, and large number of proteins with auxiliary activities were also detected. When PfCBH1 was purified and compared with *Trichoderma reesei* CBH1 for its activity and structural features, PfCBH1 demonstrated 6-fold higher specific activity and much higher tolerance towards its product. Genome engineering tools for *P. funiculosum* were developed and homolog of a catabolite repressor, Mig1, was deleted. This led to >2-fold increase in enzyme expression. Overexpression of transcriptional enhancer and some of the key cellulolytic enzymes further increased the enzyme activity. The enzyme composition from this engineered non-model fungus could be an excellent resource for production of lignocellulosic ethanol.

1:00 PM - 4:30 PM Session: 27: Metabolic Engineering for Fuels and

Chemicals II

Conveners: Sijin Li and **Sudeep Agarwala**

Marshall Ballroom Southeast, Mezzanine

1:00 PM S147: Rapid Engineering of Natural Product Biosynthetic Pathways

H. Zhao, Departments of Chemistry, Biochemistry, and Bioengineering, Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Natural products produced by plants and microorganisms are a prolific source of therapeutic drugs. However, the biosynthetic pathways for most natural products consist of many enzymes and are quite complex in their gene architecture and regulation, which poses a major challenge in metabolic engineering. In this presentation, I will introduce a few new tools and strategies that my laboratory has developed for engineering of natural product biosynthetic pathways. One of them is a two-tiered pathway refactoring strategy which has been successfully used to discover novel ribosomally synthesized and post-translationally modified peptides. Another tool is to assemble any target natural product biosynthetic pathway in a highly efficient and standardized manner using PfAgo-based artificial restriction enzymes. A third strategy is to combine an integrated robotic system with machine learning to optimize the flux through a biosynthetic pathway. As proof of concept, this strategy was used to rapidly optimize the lycopene biosynthetic pathway by combinatorically varying the promoter strength in the pathway and evaluating less than 1% of all the possible combinations. These new tools and strategies should be generally applicable to any biosynthetic pathway for production of fuels, chemicals, materials, and drugs. Finally, a variety of CRISPR-based genetic tools have been developed for metabolic engineering of Streptomyces species, a talented group of natural product producing bacteria.

1:30 PM S148: Achieving high-efficiency production of pharmaceutical opiates in engineered yeast

P. Facchini, University of Calgary, Calgary, AB, Canada*

Plants remain the commercial source for several high-value metabolites including the pharmaceutical opiates morphine, codeine and thebaine in opium poppy. Thebaine is used to produce semi-synthetic derivatives such as oxycodone, hydrocodone and naloxone. De novo biosynthesis of pharmaceutical opiates in engineered yeast has been demonstrated, but product yields have been far below the threshold required for commercial feasibility. Major factors contributing to low product titers in the first engineered strains include (i) an incomplete understanding of opiate biosynthetic pathways and (ii) the misconception that all required plant genetic components were known. Though joint academic-industry partnerships, we have established an effective pipeline to identify and resolve the metabolic bottlenecks responsible for the low yield of opiates in engineered yeast, which includes the integrated discovery and deployment of new plant genes essential for efficient metabolic performance in heterologous production systems. Two key categories of novel opiate biosynthetic genes include those encoding (i) enzymes catalyzing previously characterized spontaneous metabolic conversions and (ii) transporters responsible for cellular translocation of opiates in the plant. Our recent discovery and deployment of the opium poppy genes encoding thebaine synthase (THS) and neopinone isomerase (NISO) dramatically improve opiate product yields by precluding the formation of undesirable by-products, which are favored in the corresponding spontaneous reactions, both in the plant and in engineered yeast strains. Similarly, our detection of a benzylisoquinoline alkaloid uptake permease (BUP), which is a purine permease-type importer involved in the uptake of opiates and upstream pathway intermediates to laticifers (the cellular site of opiate accumulation in the plant) further increases heterologous pathway performance. The isolation of recalcitrant opiate metabolism genes, and the resulting refinement in our understanding of opiate biosynthesis in the plant, have advanced the prospect of opiate production in engineered yeast substantially closer to commercial feasibility. The discovery of these genes and the consequences of their deployment in engineered yeast strains will be discussed.

2:00 PM S149: Development of A Yeast-Based System To Elucidate Phytosteroid Biosynthesis

S. Xu, UC Riverside, Riverside, CA, USA and Y. Li, University of California, Riverside, Riverside, CA, USA*

Plant natural products are important sources of pharmaceuticals, with more than 10% of the WHO listed essential medicines of flowering plant origin. However, the biosynthetic pathways of most phytochemicals are not fully elucidated - enzymes catalyzing certain steps in the proposed pathway are unknown. Without the comprehensive understanding of the biosynthesis of plant NPs, it is challenging to produce these expensive molecules and difficult to advance the discovery of novel plant NPs from the rapidly growing transcriptome data. Baker's yeast *Saccharomyces cerevisiae* has been demonstrated to be a promising biotechnological production platform for plant natural products. Thus, *S. cerevisiae* has been considered to be a promising heterologous host to simultaneously reconstitute and elucidate plant natural product biosynthesis. Here, through a combination of metabolic engineering, strain evolution, and pathway reconstruction, a yeast-based system was developed to reconstitute and thereby elucidate the biosynthesis of phytosteroid and derivatives.

2:30 PM Break

3:00 PM S150: Enabling Metabolic Engineering Through High Throughput DNA Solutions

N. Raynard, E. Lee, S. Chen and R. Nugent, Twist Bioscience, San Francisco, CA, USA*

As our understanding of biological pathways continues to evolve, scientists today have an increased power to optimize pathways through combinatorial testing. Testing enzymes from different sources or modulating expression of a protein through different promoters can greatly alter the yield of the final product when designing a pathway. A limiting area of this workflow is the creation of these different pathway combinations. Here we show advancements in metabolic engineering methods using Twist Bioscience's high-throughput DNA assembly method. We will highlight metabolic engineering through combinatorial DNA assembly and gene pools. Gene pools are a collection of diverse non-clonal DNA sequences that can be used in a variety of downstream applications including pathway engineering, peptide screening and high throughput screens. Using these technologies, we constructed a pathway with multiple domains to build a diverse and uniform clonal pool with greater than 100,000 unique combinations and a gene pool of 250,000 sequences. Through Next Generation DNA Sequencing we will show sequences are incorporated in equivalent ratios and with very low error rates. Utilizing these technologies, Twist provides researchers access to hundreds of thousands of variants in their pathway of interest, enabling high-throughput screening of complex genetic constructs.

3:30 PM S151: An Engineered *E. coli* Nissle for the treatment of Phenylketonuria (PKU)

V. Isabella, D. Lubkowicz, B. Ha, M. Castillo, S. Rowe, Y. Millet, C. Anderson, A. Fisher, K. West, P. Reeder, M. Momin, C. Bergeron, S. Guilmain, P. Miller, C. Kurtz and D. Falb, Synlogic, Cambridge, MA, USA*

Phenylketonuria (PKU) is a human metabolic disease characterized by the inability to metabolize phenylalanine (Phe), resulting in significant neurotoxicity. As a novel therapeutic treatment, we engineered Escherichia coli Nissle (EcN) to express the Phe-metabolizing enzyme phenylalanine ammonia lyase (PAL) in response to anoxic conditions within the mammalian gut. Co-expression of a high affinity Phe uptake system, pheP, resulted in a 7-fold increase in the rate of Phe degradation by PAL in vitro. In a mouse model of PKU, administration of our synthetic strain, SYNB1618, reduced Phe concentration in the blood by 38% compared with the unengineered EcN control, independent of dietary protein intake. Additionally, we established that the breakdown product of Phe by PAL, trans-cinnamate (TCA), was quantitatively converted to hippurate and excreted in urine in vivo, and could act as a non-invasive biomarker of SYNB1618 activity. In healthy Cynomolgus monkeys, we found that SYNB1618 significantly blunted an increase in serum Phe after an oral dietary challenge. Additionally, SYNB1618 was detectable in murine and primate feces after a single oral dose, permitting the evaluation of pharmacodynamic properties. Finally, in a phase I dose escalation trial in healthy human volunteers, SYNB1618 administration resulted in a dose-dependent recovery of urinary hippurate excretion. Our results define a strategy for the translation of live synthetic bacterial therapeutics for the treatment of metabolic disease.

4:00 PM S152: Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

X. Luo and A. Lechner, UC Berkeley, Emeryville, CA, USA; M. Reiter, C. Denby, Y. Zhang, A. Grzybowski and J. Keasling, UC Berkeley, Berkeley, CA, USA; L. d'Espaux, J. Wong, S. Harth, W. Lin, H. Lee, C. Yu, J. Shin, K. Deng, V.T. Benites, G. Wang, E. Baidoo, Y. Chen, I. Dev and C.J. Petzold, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Cannabis sativa L. has been cultivated and used around the globe for its medicinal properties for millennia. Some cannabinoids, the hallmark constituents of *Cannabis*, and their analogues have been investigated extensively for their potential medical applications. However, the study and medicinal use of cannabinoids has been hampered by the legal scheduling of *Cannabis*, the low in planta abundances of nearly all of the dozens of known cannabinoids, and their structural complexity, which limits bulk chemical synthesis. Here we report the complete biosynthesis of the major cannabinoids cannabigerolic acid, Δ^9-tetrahydrocannabinolic acid, cannabidiolic acid, Δ^9-tetrahydrocannabivarinic acid and cannabidivarinic acid in *Saccharomyces cerevisiae*, from the sugar galactose. To accomplish this, we engineered the native mevalonate pathway to provide high flux of geranyl pyrophosphate and introduced a heterologous, multi-organism-derived hexanoyl-CoA biosynthetic pathway. We also introduced the *Cannabis* genes that encode the enzymes involved in the biosynthesis of olivetolic acid, as well as the gene for a previously undiscovered enzyme with geranylpyrophosphate:olivetolate geranyltransferase activity and the genes for corresponding cannabinoid synthases. Furthermore, we established a biosynthetic approach that harnessed the promiscuity of several pathway genes to produce cannabinoid analogues. Feeding different fatty acids to our engineered strains yielded cannabinoid analogues with modifications in the part of the molecule that is known to alter receptor binding affinity and potency. Our work presents a platform for the production of natural and unnatural cannabinoids that will allow for more rigorous study of these compounds and could be used in the development of treatments for a variety of ailments.

1:00 PM - 4:30 PM Session: 28: Emerging Technologies in Natural Products

Conveners: Jie Hu, Dow AgroSciences LLC, Indianapolis, IN, USA;**Michael Freeman**, University of Minnesota-Twin Cities, St. Paul, MN, USA and **Don Hahn**, corteva

Marshall Ballroom West, Mezzanine

1:00 PM S153: Biosynthetic Design of Nonribosomal Peptides

H. Kries, Leibniz-HKI, Jena, Germany*

Nonribosomal peptide synthetases (NRPSs) protect microorganisms against environmental threats by producing siderophores or antibiotics, for instance, and are predisposed for biosynthetic engineering because of their modular molecular structure. We have explored several strategies for the redesign of NRPS specificity. Notable examples are the incorporation of a clickable amino acid through targeted binding pocket mutagenesis [1] or specificity transfer through swapping of small protein fragments [2]. Incorporation of clickable amino acids has further enabled a strategy for high-throughput sorting of mutagenized NRPSs displayed on yeast [3]. Here, we demonstrate the addition of DNA templates to nonribosomal peptide synthetases to facilitate NRPS reprogramming. We have deconstructed the NRPS for the cyclic decapeptide gramicidin S into modules that were later reassembled on a DNA template using specific DNA binding domains. The amounts of various peptide products reacted strongly to the module sequence encoded on the DNA. Several unnatural peptide sequences could be synthesized with the same set of engineered modules, although turnover rates were compromised. In the future, DNA programmable NRPSs perfected by laboratory evolution might provide facile access to natural product-like peptides.

[1] Kries, H. et al., Angew. Chem. Int. Ed. Engl. 2014, 53 (38), 10105.
[2] Kries, H. et al., Chem. Biol. 2015, 22 (5), 640.
[3] Niquille, D. L. et al., Nat. Chem. 2018, 10 (3), 282.

1:30 PM S154: Biocatalytic C–H Oxidation as an Enabling Tool for Natural Product Synthesis

H. Renata, The Scripps Research Institute, Jupiter, FL, USA*

Direct oxidation and functionalization of C–H bonds, though ubiquitous in Nature's biosynthetic machinery, have only recently gained traction as a viable strategy in chemical synthesis. Despite rapid advances in the past decade, contemporary chemical methods for C–H oxidation still face significant challenges in achieving useful levels of selectivity on complex scaffolds. In contrast, the oxidative enzymes that have evolved to perform these transformations are capable of achieving unprecedented levels of selectivity. However, the applications of these catalysts in organic synthesis have remained largely overlooked. This talk will describe our recent efforts in developing a new design language in chemical synthesis that centers on the application of biocatalytic C–H functionalization logic. Case studies will focus on the use of this platform in the chemoenzymatic syntheses of complex natural products.

2:00 PM S155: Convergent evolution and novel chemistry in natural products related to fosmidomycin

E. Parkinson, Purdue University, West Lafayette, IN, USA, K.S. Ju, The Ohio State University, Columbus, OH, USA and W.W. Metcalf, University of Illinois, Urbana, IL, USA*

The fosmidomycin class of natural products, which share a hydroxyaminopropylphosphonate scaffold, are 1-deoxy-D-xylulose 5-phosphate reductoisomerase inhibitors with potent antibacterial, antimalarial and herbicidal activities. Although the biosynthetic pathway for one of these natural products (FR-900098), produced by *Streptomyces rubellomurinus,* has been extensively characterized, the genetic and biochemical traits required for biosynthesis of fosmidomycin have yet to be elucidated. We performed a series of physiological, biochemical and genetic experiments using the native fosmidomycin producer, *S. lavendulae*. Surprisingly, we were unable to elicit production of fosmidomycin by *Streptomyces lavendulae* under any growth condition. Instead, we observed the major product to be dehydrofosmidomycin, an unsaturated derivative of fosmidomycin that is an even more potent inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase. Due to the high structural similarity of dehydrofosmidomycin and FR-900098, one would expect their biosynthesis to be highly homologous. However, this is not the case. Analysis of the *S. lavendulae* genome revealed a potential biosynthetic gene cluster, which differed significantly from that required for synthesis of FR-900098. Heterologous expression of the *S. lavendulae* gene cluster confirmed its role in production of dehydrofosmidomycin. Characterization of biosynthetic intermediates produced, with and without supplementation of labeled precursors, allowed further insight into the biosynthetic pathway. In vitro biochemical experiments revealed that the biosynthetic pathway involves conversion a two-carbon phosphonate precursor into the unsaturated three-carbon product via a highly unusual rearrangement reaction, catalyzed by the 2-oxoglutarate dependent dioxygenase DfmD. Overall, these studies suggest that the ability to produce FR-900098 and dehydrofosmidomycin arose via convergent evolution.

2:30 PM Break

3:00 PM S156: Canvass: A Crowd-Sourced, Natural-Product Screening Library for Exploring Biological

Space

K. Wan, NCATS, NIH, Rockville, MD, USA*

A pilot effort by the National Center for Advancing Translational Sciences (NCATS) to broadly survey the biological potential of natural products using automation and high-throughput screening will be presented. Natural products and their derivatives continue to be wellsprings of nascent therapeutic potential. However, many laboratories have limited resources for biological evaluation, leaving their previously isolated or synthesized compounds largely or completely untested. To address this issue, the Canvass library of natural products was assembled, in collaboration with academic and industry researchers, for quantitative high-throughput screening (qHTS) across a diverse set of cell-based and biochemical assays. Our efforts to characterize the library and analyze the resultant assay data, as well as noteworthy activities discovered, will be described.

3:30 PM S157: Harnessing genomic information to engineer the biosynthesis of novel antibiotics

L. Foulston, Ginkgo Bioworks Inc, Boston, MA, USA*

The increasing occurrence of antibiotic resistance, and frequently multi-drug resistance, in a wide range of bacterial pathogens has led to an urgent need for new antibiotics, particularly for the Gram-negative organisms. Ginkgo Bioworks' Genome Mining platform offers a unique opportunity to access novel chemical matter, inspired by nature, to address this critical problem. Utilizing a database of over 135,000 actinomycete genome sequences we have bioinformatically screened these organisms for the capacity to produce novel natural products. The identified novel clusters are cloned and introduced to a heterologous host system. Manipulation of the transcriptional context of the biosynthetic gene cluster is used to ensure efficient gene expression. Our high-throughput fermentation-extraction system is then used to assess production of novel molecules through activity assays and by mass identification. By integrating the power of our Genome Mining Platform with the scale of the Ginkgo Bioworks Foundry, we aim to identify Gram-negative active antibiotics with novel chemical structures and unique mechanisms of action.

4:00 PM S158: Natural Products Discovery and Optimization Coupled With High Throughput Strain Engineering

C. Coates, Zymergen Inc., Emeryville, CA, USA*

Natural products have been used as therapeutics for thousands of years. However, applications of natural products have advanced far beyond medicine and span all areas of our lives, including agriculture, food, personal care products, and the manufacture of everyday items. At Zymergen our mission is to create a vibrant, sustainable future through biology. Our molecular technology platform enables every step from discovery to advantaged production economics. Our metagenomics platform opens up the 99% of untapped microbial potential by enabling culture-free access to unparalleled pathway diversity. Far larger than any other known private or public sequence database, this collection provides us with unprecedented access to microbial genetic diversity as a source of novel building blocks and tailoring enzymes and allows us to expand the breadth and performance characteristics of the molecules we can engineer. Prototyping and rapid strain improvement capabilities have enabled rapid production of a broad range of natural product classes in diverse hosts.

1:00 PM - 4:30 PM Session: ST-2: Federal Perspective on Biomanufacturing R&D

Conveners: Philip Laible and **Laurel Harmon**

Madison A, Mezzanine

1:00 PM S160: Biomanufacturing growth in the industrial microbiology.

B. Bextine, Darpa, J. Fitzgerald, BETO and T. Anderson*

Biomanufacturing is a rapidly growing area of industrial microbiology. It is being driven by revolutions in synthetic biology and accelerated by innovations in automation, bio/chem hybrid engineering, and machine learning. These advancements contribute greatly to support the missions of multiple government agencies and Federal research and development programs. This panel will bring together representatives from the Department of Energy (Offices of Biological and Environmental Research and Energy Efficiency and Renewable Energy), the National Institutes of Health, and the Defense Advanced Research Programs Agency. Each will describe their organization's mission and research programs, the goals of their R&D activities, and present their views of research needs and gaps. The panelists will discuss (i) the role of each agency in supporting the rapid progression of biomanufacturing technologies and (ii) opportunities for working with each agency. The location of the annual meeting provides a unique opportunity to gather this diverse set of representatives to describe, compare, and contrast the roles of biomanufacturing/synthetic-biology research within the Federal R&D ecosystem.

Thursday, July 25

8:00 AM - 12:00 PM SIMB New Board of Directors meeting

Madison B, Mezzanine