2017 SIMB Annual Meeting and Exhibition

Sunday, July 30

7:00 AM - 8:00 AM Workshops 1 and 2 Continental Breakfast

Plaza Court 1 - Concourse Level

7:00 AM - 8:00 AM Workshops 1 and 2 Registration

Plaza Registration - Concourse Level

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

Governors Square 17, Plaza Concourse Level

8:00 AM - 3:00 PM Workshop 1 - Fermentation Basics

Plaza Court 2 - Concourse Level

8:00 AM - 3:30 PM Workshop 2 - Mining Microbial Genomes and Metagenomes for Biological Applications

Plaza Court 3 - Concourse Level

9:00 AM - 6:00 PM SIMB Annual Meeting Registration

Plaza Registration - Concourse Level

4:00 PM - 5:00 PM Opening Remarks and Keynote: George Garrity, Michigan State Univ, SIMB President; Hal Alper, Univ of Texas-Austin, 2017 Program Chair Keynote Speaker: Jack Gilbert, Univ of Chicago

Plaza Ballroom A & B - Concourse Level

5:00 PM - 6:00 PM Science Slam

Plaza Ballroom A & B - Concourse Level

6:00 PM - 8:00 PM Opening Reception/Exhibits Open

Plaza Exhibit - Concourse Level

6:00 PM - 8:00 PM Session: PS1: Poster Session 1: Biocatalysis and Metabolic Engineering

Plaza Exhibit - Concourse Level

P1 Spatial constraints of a chaperone and its substrates for the efficient solubilisation of recombinant proteins

S.C. Jung, L.M. Bui, A. Geraldi, J.H. Lee, S. Lim, M.S. Kim, B.K. Han and S.C. Kim, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South)*

The production of functional recombinant proteins in soluble forms represents a challenging frontier in engineering bacterial hosts. Natural molecular chaperone systems have been widely used to solubilise various recombinant proteins, albeit with limited successes. Here, to facilitate the folding activity of a chaperone via enhancing the interaction with its substrate protein, we either anchored the bacterial chaperone DnaJ to the 3' untranslated region of a target mRNA by fusion with an RNA binding domain in chaperone-recruiting mRNA scaffold (CRAS) system, or coupled the expression of DnaJ and a target recombinant protein using the overlapping stop-start codons 5'-TAATG-3' between the two genes in a chaperone-substrate co-localised expression (CLEX) system. By spatially constraining bacterial molecular chaperones to the location of protein translation, we are able to surpass the in vivo solubilisation efficiency of the native chaperone system and to overexpress aggregation-prone recombinant proteins, producing up to 95% in functionally active soluble forms.

P3 Engineering *Yarrowia lipolytica* for triacetic acid lactone (TAL) production

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Yarrowia lipolytica, an industrially attractive, non-conventional yeast, boasts a high innate capacity to produce acyl-CoA derived molecules such as triacylglycerides. Here we demonstrate the potential of rewiring *Y. lipolytica* to divert this precursor pool away from lipids and into alternative molecules of interest. Specifically, we explored the production of the simple polyketide, triacetic acid lactone (TAL). TAL has been proposed as a biorenewable platform chemical that can be converted into many downstream products including sorbic acid. Previous efforts to produce TAL in hosts such as *E. coli* and *S. cerevisiae* have been limited by the availability of acyl-CoA precursors. We enabled TAL production in the precursor rich host, *Y. lipolytica*, through heterologous expression of 2-pyrone synthase, an enzyme from *Gerbera hybrida* that catalyzes the formation of TAL by condensation of acetyl-CoA and malonyl-CoA. We next performed a series of strain engineering approaches to boost TAL production by metabolically rewiring *Y. lipolytica* for enhanced precursor accumulation. Final strain characterization was conducted in bioreactors to further optimize production titer, rate, and yield. Ultimately, we established a

strain that produced the highest titer of TAL reported to date in any host. Here we present the details of these genetic engineering efforts as well as the production characterization of the resulting strain.

P5 Enzymatic syntheses of (*S*)-ethyl 1-((*S*)-2-(4-cyanophenyl)-2-hydroxyethyl)-piperidine-3-carboxylate and (*S*)-4-(oxiran-2-yl)benzonitrile

A. Singh, Z. Guo and A. Goswami, Bristol-Myers Squibb, New Brunswick, NJ, USA*

BMS-960, (*S*)-1-((*S*)-2-Hydroxy-2-(4-(5-(3-phenyl-4-(trifluoromethyl)isoxazol-5-yl)-1,2,4-oxadiazol-3-yl)phenyl)ethyl)piperidine-3-carboxyl acid) is a S1P1 receptor agonist. At the beginning of the program, KRED-NADPH-114 enzyme catalyzed reduction was identified for the synthesis of the advanced intermediate (*S*)-ethyl 1-((*S*)-2-(4-cyanophenyl)-2-hydroxyethyl)-piperidine-3-carboxylate (**1**). The absolute stereochemistry was assigned based on X-ray crystallography. In order to accommodate a common intermediate approach, an earlier epoxide intermediate (*S*)-4-(oxiran-2-yl)benzonitrile (**2**) was synthesized by KRED-NADH-110 catalyzed reduction of the corresponding α-bromoketone to the chiral α-bromohydrin followed by cyclisation to the epoxide.

P7 Upgrading biomass-derived sugars to HMF/furfural via ketose intermediates

W. Wang, A. Mittal and D.K. Johnson, National Renewable Energy Laboratory, Golden, CO, USA*

As lignocellulosic biomass is considered to be the only sustainable resource with the potential to deliver renewable fuels and biobased chemicals, exploring fermentative or chemical pathways that convert biomass-derived sugars to fuels/chemicals has attracted a lot of interests from many researchers. We are investigating a hydrocarbon pathway from mixed sugars via HMF/furfural. Current processes to produce HMF/furfural generally involve the use of acid catalysts in biphasic systems or solvents such as ionic liquids. However, the yield from transforming glucose to HMF is very low compared to xylose conversion to furfural. In this study, we present an efficient chemical transformation pathway to HMF/furfural via ketose sugars, i.e. fructose and xylulose, which were generated from glucose and xylose via enzymatic isomerization. In enzymatic isomerization, by adding sodium borate to complex with the ketoses, xylose conversion reached equilibrium after 2h with a conversion of 91% and glucose conversion reached 84% after 4h. At 120^{o}C, pH 0.5, and 15 min reaction time, mixed sugars (predominantly ketoses) , were converted to HMF and furfural in yields of 77% and 96%, respectively (based on starting aldose concentrations). These results demonstrate that this combined biological and chemical process could be an effective pathway to simultaneously upgrade glucose and xylose to HMF and furfural intermediates in the production of hydrocarbons.

P9 Characterization of the unique pectin deconstruction system of *Paenibacillus amylolyticus* 27C64

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P. amylolyticus 27C64, a Gram-positive bacterium originally isolated from the hindgut of a *Tipula abdominalis* (aquatic cranefly) larvae, is capable of degrading plant cell wall polysaccharides including pectin. Two previously characterized pectate lyases from this organism have demonstrated broad substrate specificity and one has been used successfully in place of a commercial pectinase cocktail to ferment pectin-rich biomass to ethanol. To identify additional pectinases in this organism, its genome was sequenced and mined using two automated carbohydrate active enzyme annotation tools. Fifteen new putative pectinases were identified including esterases, lyases, and hydrolases predicted to be active on both types of the pectic backbone. In contrast to other systems which typically demethylate pectin extracellularly, this system's sole putative pectin methylesterase appears to be cytoplasmic. Also of note is an enzyme with both a putative RG lyase and RG acetylesterase domain. An enzyme with both of these activities has not yet been described. Two newly identified pectate lyases, *pamy_1763* and *pamy_4669*, have been heterologously overexpressed in *E. coli* and are currently being characterized.

Together these enzymes allow *P. amylolyticus* to use polygalacturonic acid (PGA) as a supplemental carbon source in tryptic soy broth. The system appears to be induced by PGA and susceptible to catabolite repression by a number of sugars. Quantitative real-time PCR is currently being used to confirm transcription of these 17 genes in inducing conditions. To our knowledge, this is the first attempt to characterize a complete pectinolytic system within the *Paenibacillus* genus.

P11 The impact of stress-response related transcription factors on lignocellulosic hydrolysate inhibitor tolerance of *Saccharomyces* strains

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Plant biomass is a desirable feedstock for the production of renewable fuels and chemicals. Unfortunately, pretreatment processes to release sugars locked in plant biomass, or lignocellulosic feedstocks, lead to the production of fermentation inhibitors, such as furfural and hydroxymethyl furfural, resulting in the inefficient fermentation of the lignocellulosic derived sugars by *Saccharomyces cerevisiae*. Numerous transcription factor genes associated with stress response are upregulated in *Saccharomyces* cultures grown in the presence of these inhibitors and overexpression of these transcription factors offers a potential route to improved inhibitor tolerance. Overexpression of one of these transcription factors, YAP1, in *Saccharomyces* strains has led to mixed results with respect to improved lag times in growth and growth rate. In this work, we initially tested overexpression of YAP1 and MSN4, in a *Saccharomyces* lab strain and three environmental strains previously shown to have good tolerance to a number of lignocellulosic inhibitors. While YAP1 and MSN4 overexpression is beneficial to growth of the lab strain at lower inhibitor concentrations, at higher concentrations it was less effective. In contrast, overexpression of YAP1 at different expression levels in the environmental isolates did not improve growth characteristics. Additional stress related transcription factors were also overexpressed in the environmental isolates and failed to improve inhibitor tolerance. It appears overexpression of transcription factors, while potentially leading to improved tolerance of lab strains with inherently weak inhibitor tolerance, is unlikely to be a viable route to increase inhibitor tolerance of environmental strains.

P13 Bioactive peptides from flaxseed protein by alkaline protease of *Bacillus altitudinis*

C.F. Hwang[*], Y.X. Li, Y.A. Chen and Y.C. Fu, Hungkuang University, Taichung City, Taiwan

Flaxseed is rich in ω-3 fatty acid, dietary fiber, protein and lignans, and its residue after oil extraction contains 35-40% of protein not widely used yet. In this study, the alkaline protease of a new isolated strain *Bacillus altitudinis* HK02 from the brine tofu was used to hydrolyze flaxseed protein in order to produce bioactive peptides. The characteristics of extracellular protease from *B. altitudinis* HK02 were further studied and compared with commercial proteases. Besides, a peptide fraction with low molecular weight (<1 kDa) from protein hydrolysates shows the high antioxidant and tyrosinase-inhibiting activity. Five bioactive peptides from the low molecular weight of peptide fraction were purified by semi-preparative HPLC and identified their sequences by MALDI-TOF/MS. The molecular weight patterns of these bioactive peptides are between 384 and 1039 kDa. These peptides according to the above sequence analysis were further synthesized by the Yao-Hong biotechnology Inc. and reconfirmed their bioactivities of antioxidant and tyrosinase-inhibiting ability. Bioactive peptides could be isolated from flaxseed protein directly hydrolyzed by alkaline protease of *B. altitudinis* HK02 and exhibited their potentials to be the functional ingredients for foods and cosmetics.

P15 Towards Understanding Glycosylation in Cellobiohydrolase I

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Fungal cellobiohydrolases from glycoside hydrolase Family 7 (GH7) are critical enzymes used in industrial formulations marketed for second-generation biofuels production. They are often multi-modular, including a large catalytic domain (CD) and a Family 1 carbohydrate-binding module (CBM) connected by a flexible linker. When expressed in fungal hosts, GH7 cellobiohydrolases exhibit *N*-glycosylation of the catalytic domain and *O*-linked glycosylation of the CBM and linker. We have produced a library of mutants lacking single or combined *N*-/*O*-glycosylation sites in a constitutive *Trichoderma reesei* expression system developed in our research group. We have demonstrated that glycosylation plays an important role in the activity and stability of Cel7A from the fungus *Trichoderma reesei* (TrCel7A), with distinct features for the *N*- and *O*-glycans decorating the CD and linker, respectively. To further explore the impact of *O*-glycosylation on the TrCel7A linker and to create a model system to understand the role of glycosylation on this cellulase, we mutated the native TrCel7A linker to either add *O*-glycan sites or replace it with the Cel6A linker. After purification to homogeneity, the mutants were characterized for activity and binding towards different substrates (avicel, cellulose nanocrystals, pretreated corn stover, bacterial cellulose). We employed Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry, Quartz Crystal Microbalance-Dissipation, and Small-Angle Neutron Scattering to characterize the biochemical properties and binding parameters for the various mutants.

P17 Engineered self-assembling protein scaffolds for bioactalytic enzyme cascade

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Spatial organization of metabolic enzymes represents an attractive approach in multi-enzymatic synthesis of valuable chemicals in engineered cells and biocatalysis. Scaffolding of enzymes has the potential to reduce diffusion of reaction intermediates and increase reaction efficiency. Synthetic scaffolds have been shown to significantly increase the function of different designed pathways. Our goal is to create modular and controllable self-assembling cascades of enzymes for the *in vitro* biocatalytic production of commodity chemicals. Here, we build a robust and modular self-assembling protein scaffold using the bacterial microcompartment shell protein EutM from *Salmonella enterica*, and localize cargo proteins to the scaffold using SpyTag-Spycatcher. As proof of concept we show that the SpyTag-Spycatcher system can be used to create isopeptide linkages for covalent localization of fluorescent proteins to EutM forming sheet-like scaffolds, both *in vivo* and *in vitro*. Besides, we will also apply these robust scaffolds for the spatial organization of a multi-enzyme cascade for efficient biosynthesis. Together our results provide an improved scaffold platform for efficient biosynthesis and biocatalysis.

P19 Computational redesign of acyl-ACP thioesterase with improved selectivity towards medium-chain fatty acids at high production levels

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Free fatty acids (FFA) are precursors to important oleochemicals such as alcohols, aldehydes, and wax esters. The current supply of FFA is limited by availability of oil crops such as canola and coconut palm. Demand for oil crops have created tensions between food, fuel, and environmental stewardship. Therefore, microbial hosts are being developed as an alternative production route for FFA. Thioesterases are enzymes that catalyze the last step in microbial FFA production strategies by hydrolyzing the thioester bond in cellular fatty acyl-ACP producing a FFA. Since this step acts as a product sink it is critical to have a thioesterase that is highly active and specific towards the desired chain length to drive its production. Here, we used a computational approach to engineer the *E. coli* thioesterase I (TesA) to improve its specificity towards octanoyl-ACP (C8:0) and lauroyl-ACP (C12:0). TesA native crystal structure was used to guide the algorithm to calculate the binding energy of the substrates upon

configurational changes imposed. Mutants predicted were validated experimentally on *E. coli* and results were used to guide subsequent iterations. After three round of predictions, we found mutants with main specificity towards C8:0 and C12:0. The best mutant exhibited 8-fold increased specificity towards C8:0 compared to wild type without affecting the enzyme activity. This mutant was crystalized to understand structurally the changes made as well as characterized through in-vitro assays to confirm in-vivo results. These successful results highlight the potential of computational approaches combined with experimental validation in protein engineering.

P21 Understanding the stress tolerance of *Saccharomyces cerevisiae* strains evolved for high solids pine fermentations

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Lignocellulosic biomass continues to be investigated as a viable resource for bioethanol production, however the pretreatment process generates inhibitory compounds that impair growth and fermentation performance of microorganisms such as *Saccharomyces cerevisiae*. Naturally evolved strains isolated from industrial settings display high tolerance to inhibitory compounds and improved fermentation performance, but mechanisms underlying improved phenotypes of many strains remain uncharacterized. Therefore, there is precedent for identifying mechanisms necessary for stress tolerance and successful fermentation of pretreated biomass that contains multiple inhibitors. An industrial *S. cerevisiae* strain was subjected to directed evolution and adaptation in high solids pretreated pine and resultant strains, GHP1 and GHP4, exhibited improved growth and fermentative ability. While GHP4 exhibits a constitutive inhibitor tolerant phenotype, GHP1 appears to require continuous selective pressure with a synthetic inhibitor cocktail. Analysis of known inhibitors at the initial and final stages of fermentation identified 13 compounds that exhibited significant changes in concentrations between the performing and nonperforming samples. Differential expression analysis identified 52 genes involved in various cellular processes that may account for improved stress tolerance to multiple inhibitors simultaneously. A number of these were mitochondria-associated and fluorescence microscopy revealed that mitochondria of evolved strains were resistant to the damaging effects of inhibitors in contrast to the parent, supporting improved robustness. The results of this study advance the understanding of stress tolerance of *S. cerevisiae* to biomass derived inhibitory compounds and have direct implications for further development of robust yeast strains for multiple industrial applications.

P23 Alkaliphilic lignocellulolytic enzymes for biomass conversion

B. Li, D. Jabbour and J.D. Walton, Michigan State University, East Lansing, MI, USA*

Lignocellulolytic enzymes such as cellulases and xylanases are the major process cost for conversion of biomass to fermentable sugars. Current commercial sources of biomass conversion enzyme mixtures, mainly from *Trichoderma reesei*, show strong pH dependence, being most active at pH 4-5 and having little activity above pH 7. However, enzymatic degradation of biomass at higher pH (8-10) would have several advantages, including compatibility with alkaline pretreatments, reduced bacterial contamination, and lower inhibition by lignin. By screening environmental samples at high pH, we isolated a novel cellulolytic fungus, *Cladorrhinum bulbillosum,* whose enzymes separately and in mixtures show much increased activity at high pH compared to those from *T. reesei*. The genome of C. *bulbillosum* and its transcriptome under nine growth conditions were sequenced by the DOE Joint Genome Institute. Compared to *T. reesei*, *C. bulbillosum* has more endo- and exo-cellulases in families GH6 and GH7, endo-xylanases in GH10 and GH11, beta-xylosidases in GH43, cellobiose dehydrogenases in AA3, and gluco-oligosaccharide oxidases in AA7. Strikingly, it has 35 lytic polysaccharide monooxygenases (LPMO) of family AA9 compared to 4 in *T. reesei*. A core set of cellulases showing highest expression at pH 9 is being expressed in *T. reesei* Δxyr1 to create synthetic cellulase mixtures active at high pH.

P25 Synergism of hemicellulase and polyethylene glycol with cellulase in the high solids enzymatic hydrolysis of hydrothermally pretreated empty fruit bunches

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High solids loadings hydrolysis of lignocellulose is required to obtain high sugar titers. However, high solids loadings limit enzymatic hydrolysis of lignocellulose possibly due to poor enzyme-substrate interactions. In this study, to overcome this limitation, the synergistic effects of the accessory agents, hemicellulase (i.e., Cellic HTec2) and polyethylene glycol (PEG) 8000, were investigated in the enzymatic hydrolysis of hydrothermally pretreated empty fruit bunches (EFBs). After the optimal addition of Cellic HTec2 and PEG, high enzymatic digestion of the pretreated EFBs was achieved owing to the synergistic effect of Cellic CTec2 and PEG, especially at high solids loadings. For example, at a 21.7% (w/v) solids loading of EFBs, when 2.7 mg of Cellic HTec2 and 62.5 mg of PEG per glucan were used as the accessory agents, the glucose yield obtained from pretreated EFBs using 10 FPU of Cellic CTec2/g glucan was 72.5% of the theoretical max. glucose yield. These results suggested that the accessory agents are effective for the enhanced hydrolysis of lignocellulose using even a commercial cellulase preparation.

P27 Looking from the outside in: substrate-binding proteins are a crucial upstream factor for microbial catalysis

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When considering microbes as miniaturized factories, it is vital that the strategies microbes use to acquire raw materials are given equal importance as to the products they produce. Heterotrophic bacteria rely on protein complexes for the efficient transport of carbon sources to support central cellular metabolism and biosynthesis of useable products. Expansion of ATP binding cassette (ABC) transporters in a microbe's genome can expand its substrate appetite, complementing engineering efforts to include additional families of extracellular enzymes. Heterotrophic, thermophilic microbes are attractive targets for mining of novel ABC transporters, due to the prominence of ABC transporters versus phosphotransferase systems in their genomes. A wide range of ABC transporters were identified in the expanded pan-genome from the plant biomass-degrading, extremely thermophilic genus, *Caldicellulosiruptor*. We have previously explored the use of substrate binding protein gene expression as useful markers for the presence of representative plant carbohydrates. However, functional characterization of these proteins is time consuming, since no detectable product can be easily screened for. As such, this transcriptomic data has been useful in preliminary identification of substrate binding proteins for further characterization of their substrate specificity. Discussed here is the mining of SBP genes from the *Caldicellulosiruptor* pan-genome and thermophilic communities from Yellowstone National Park. Furthermore, we are developing multiple synthetic biology approaches to functionally characterize substrate-binding proteins that will also support protein engineering efforts to improve selectivity.

P29 Synergizing microbial culturing, genome sequencing, asymmetric synthesis and tandem MS for reconstruction of polyketide and alkaloid biosynthesis in *Nocardiopsis* sp. CMB-M0232

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Biosynthetic pathway engineering is rapidly growing by rationally harnessing the enzymatic potential of microbial systems. While a few Gram positive bacteria have offered an extensive array of promising biocatalysts with unique modular functions as polyketide synthases (PKS), or non-ribosomal peptide synthetases (NPRS), the biosynthetic potential of marine *Nocardiopsis* genus largely remain unexplored. Our research is studying a marine actinomycete, *Nocardiopsis* sp. CMB-M0232

isolate as a model organism through an integrated approach involving genome sequencing, metabolic engineering, tandem-MS and asymmetric synthesis to reconstruct multiple biosynthetic pathways leading to PKS, NRPS, alkaloids and their hybrid natural products. These biosynthetic products are potential candidates for clinically relevant drug development. Nocardioazines A and B, from *Nocardiopsis sp.* CMB-M0232, are structurally unique alkaloids featuring a 2,5-diketopiperazine (DKP) core functionalized with indole C3-prenyl as well as indole C3-and N-methyl groups. Bioinformatics analyses of the draft genome afforded the *noz* cluster, split across two regions, and encoding putative open reading frames with roles in nocardioazine biosynthesis, including cyclodipeptide synthase (CDPS), prenyltransferase, methyltransferase, and cytochrome P450 homologs. I will present on recently published work on the biosynthetic pathway to nocardioazines using asymmetric synthesis and mass spectrometry to determine biosynthetic intermediates in the *noz* pathway. The structures of hypothesized biosynthetic intermediates were firmly established through chemical synthesis.

P29A Harnessing the potential of electroactive microorganisms for improved electron transfer charecteristics at reduced graphene oxide functionalized electrode interfaces

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Electroactive microorganisms (EM) mediate oxidation/reduction reactions as well as transfer electrons to the electrode. This bioelectrocatalytic characteristics of the EM make them promising candidates for bioelecochemical applications. The EM can transfer electrons by direct electron transfer with membrane bound conductive proteins/organelles or by electron shuttling compounds. However, for efficient electrocatalytic reaction, the electron transfer at microbe-electrode interphase is the major limitation. Herein, we present the biological reduction of graphene oxide (GO) using *Gluconobacter roseus*. Results shows that the reduced graphene oxide (RGO) presents a biocompatible surface for the prolific growth of microorganisms, and enhanced microbial electrocatalysis when carbon felt electrodes are functionalized with RGO. Biofilms were formed on modified and unmodified carbon felt electrodes using a co-culture of *Acetobacter aceti* and *Gluconobacter roseus* and a microbial consortium associated with food waste. The efficiency of RGO in enhancing the microbial electrocatalysis was analyzed using the carbon substrates glucose and ethanol. The microbial electrocatalysis was investigated in detail using the electrochemical techniques such as cyclic voltammetry and long term chronoamperometry. The RGO modified bioelectrodes produced an enhanced current density of 1 mA/cm^2 and 0.69 mA /cm^2 with ethanol and glucose as substrates, respectively. The developed functionalization strategy will be promising for bioelectrochemical energy/sensor applications.

Keywords: Electroactive microorganisms, Microbial Electrocatalysis, electron transfer kinetics, Reduced Graphene oxide, Bioelectrode

P31 Metabolic engineering of the isopentenyl diphosphate (IPP)-bypass pathway for isoprenol production

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Isoprenol is a valuable compound as a drop-in biofuel and a precursor of commodity chemical such as isoprene. Synthetic microbial system has been developed for the production of isoprenol at ~70% of the theoretical maximum yield using heterologous mevalonate (MVA) pathway. Conventional MVA pathway requires multiple ATPs to produce prenyl diphosphate intermediates such as isopentenyl diphosphate (IPP). To relieve the energy requirement and eventually the aeration cost for industrial application, we developed an IPP-bypass pathway for isoprenol production using the promiscuous decarboxylation activity of mevalonate diphosphate decarboxylase (PMD). The promiscuous activity of PMD, however, is low and limits the flux of mevalonate phosphate (MVAP) to isopentenyl phosphate (IP), and we developed a high-throughput enzyme screening platform for evolution of this enzyme. The screening platform was designed in a way that the catalytic activity of the target enzyme is essential for the growth, and therefore that higher-IP producing *E. coli* strains outcompete strains with slower PMD activity. The screening platform was successfully demonstrated and identified crucial amino acid residues that significantly

improve the PMD activity toward MVAP and increase isopentenol titers up to 2.4-fold. Further pathway optimization led to comparable titer and yield to the level that conventional MVA pathway has achieved, and when more active mutant PMD was used in this optimized pathway, the rate of isoprenol production improved 30% compared to the productivity with wild type PMD. The application of IPP-bypass pathway for isoprenol production will allow the microbial isoprenol production more commercially feasible.

P33 Production of jet fuel precursor monoterpenoids from engineered *Escherichia coli*

T.S. Lee, D. Mendez Perez and J. Alonso Gutierrez, Lawrence Berkeley National Laboratory, Emeryville, CA, USA*

Monoterpenes (C_{10} isoprenoids) are the main components of essential oils and used as precursors for the synthesis of commodity chemicals and high energy density fuels. Plants are major source of these monoterpenes and recently microbial production of monoterpenes has been explored to be a promising alternative. Heterologous expression of the mevalonate (MVA) pathway and several monoterpene synthases in *E. coli* led to the production of various monoterpenes, but in general, the titer of microbial monoterpene production has not been as high as that of various sesquiterpenes probably due to a limited availability of C_{10} intermediates (geranyl diphosphate, GPP) as well as the toxicity of GPP and monoterpene products.

To improve microbial monoterpene production, we heterologously expressed the MVA pathway up to GPP production, and also expressed several monoterpene synthases in *E. coli*. Then we used metabolomics and proteomics tools to optimize the strains. Especially, we engineered the *E. coli*'s native FPP synthase to improve GPP availability for monoterpene production, and from omics data analysis, we discovered that it is crucial to have a basal level of FPP to maintain growth for high titer monoterpene production as well as to achieve a high level of GPP as a precursor for monoterpenes. From this finding, our engineered strains resulted in high production of the jet fuel precursors 1,8-cineole and linalool.

The engineering strategy developed in this work will enable strains that can be used as a general platform for various monoterpenes production.

P35 Engineering *Candida tropicalis* for the production of sebacic acid through the ω-oxidation pathway

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Sebacic acid (SA) is one of the most widely used precursors for the production of nylon, resins, coatings, and adhesives. Currently, SA is only produced from castor oil with a limited supply. To overcome the limited supply of substrate for SA production, it is meaningful to seek alternative ways of producing SA such as through microbial fermentation. In this study, the microbial production of SA from decanoic acid methyl ester (DAME), which is a byproduct from the manufacturing process of biodiesel, was established. First, we identified 5 putative enzymes in the ω-oxidation pathway in *Candida tropicalis*. Secondly, sequential enzymatic steps were validated *in vitro* through the identification of each intermediates using gas chromatography-mass spectrometry. Finally, 58 mg/L of the sebacic acid was produced from DAME by *C. tropicalis*.

P37 A novel aldehyde dehydrogenase in the metabolic pathway of 3,6-anhydro-L-galactose, a major sugar of red macroalgae

S. Yu and K.H. Kim, Korea University, Seoul, Korea, Republic of (South); E.J. Yun, Graduate School, Korea University, Seoul, Korea, Republic of (South)*

Red macroalgae have been emerging as attractive sources for producing biofuels and biochemicals. 3,6-Anhydro-L-galactose (AHG) is one of the main constituents of agar which is the major component of red macroalgae. Recently, the metabolism of AHG and the enzymes related with the AHG metabolism were identified in a marine bacterium by my group. In this study, a novel aldehyde dehydrogenase, AHG

dehydrogenase (AHGD) which catalyzes AHG to 3,6-anhydrogalactonate (AHGA) by oxidation was characterized. Both NAD^+ and $NADP^+$ acted as a cofactor for the oxidation of AHG by AHGD. The optimal pH and temperature of AHGD were investigated and kinetic parameters of AHGD were also calculated. AHGD showed the high substrate specificity toward AHG. The amino acid sequence of AHGD was homologous to aldehyde dehydrogenases in microorganisms which are capable of degrading agar. Since AHGD is responsible for the first step of the AHG metabolism, AHGD might be the keystone enzyme which makes AHG enter its metabolic pathway in microorganisms utilizing red macroalgae.

P39 Development and analysis of novel microbial platforms for syngas to biofuels and high value chemicals

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A major limitation in the conversion of renewable biomass to biofuels and high value chemicals is feedstock recalcitrance. Current methods of conversion primarily involve costly acid or base pretreatment followed by enzymatic hydrolysis to deconstruct biomass, which also requires product separation and fermentation in multiple bioreactors. An alternate method involves gasification of biomass to syngas, producing primarily H_2 and CO. This route to biofuels is attractive because of the feedstock flexibility in biomass gasification, which allows for the complete utilization of biomass (cellulose, hemicellulose, and lignin) in a single step. The biological conversion of gasified biomass into fuels is limited by the lack of syngas-utilizing microbes that are fast-growing, easy to engineer, and tolerant of syngas impurities. Therefore, we analyzed a number of potential syngas-utilizing platform strains. We focused on thermophilic organisms because the use of a thermophilic host takes advantage of the inherently much lower contamination risk in a high-temperature process, which is a significant concern in any industrial-scale mesophilic bioprocess. We selected strains based on their ability to grow rapidly in syngas and utilize CO. Strains were also evaluated for carbon flux through the terpenoid pathway and the availability of genetic tools. Many of the organisms we analyzed do not have established genetic systems, so we are developing the enabling genetic engineering capabilities for transformation via stable plasmid-based expression, and/or targeted gene knock-outs/knock-ins. The ultimate goal of this project is to engineer a syngas-utilizing organism to produce monoterpenes, which are valuable as next-generation biofuels and for industrial applications.

P41 Genome stability in engineered strains of the extremely thermophilic, lignocellulose-degrading bacterium *Caldicellulosiruptor bescii*

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Caldicellulosiruptor bescii is the most thermophilic cellulose-degrader known and is of great interest because of its ability to utilize non-pretreated plant biomass as a sole carbon source. For biotechnological applications, an efficient genetic system is required to engineer it to convert plant biomass into desired products. Genetic technology in *C. bescii* has led to the development of two uracil auxotrophic genetic background strains for metabolic engineering. The first (JWCB005) is based on a random deletion within the pyrimidine biosynthesis genes *pyrFA*, and the second (MACB1018) is based on the targeted deletion of *pyrE* making use of a kanamycin resistance marker. Importantly, an active insertion element, IS*Cbe*4, was discovered in *C. bescii* when it disrupted the gene for lactate dehydrogenase (*ldh*) in strain JWCB018, constructed in the JWCB005 background. Additional instances of IS*Cbe4* movement in other strains of this lineage raised concerns about the genetic stability of such strains and their use as metabolic engineering platforms. In order to investigate genome stability in engineered strains of *C. bescii* from the two lineages, genome sequencing and Southern blot analyses were performed. These analyses show a dramatic increase in the number of single nucleotide polymorphisms, insertions/deletions and

IS*Cbe*4 elements within the genome of JWCB005. At least one daughter strain of this lineage, JWCB018, also contains large-scale genome rearrangements that are flanked by these IS*Cbe4* elements. Such dramatic effects were not evident in the newer MACB1018 lineage, indicating that JWCB005 and its daughter strains are not suitable for metabolic engineering purposes in *C. bescii*.

P43 Cyanobacterial glycogen production and hydrolysis for production of media for industrial bioprocessing

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Cyanobacteria, a branch of photosynthetic microorganisms, as well as terrestrial plants can convert sunlight and carbon dioxide into stored carbohydrates, such as starch and glycogen. Cyanobacterial glycogen production could offer an alternate source of carbohydrates, as compared to terrestrial plants, for biocatalytic processes to produce value-added chemicals. Cyanobacteria offer the potential for higher annual yield than terrestrial plants, and they need neither fresh water nor arable land to grow robustly. This work demonstrates an increased glycogen accumulation in the cyanobacterium *Synechococcus* PCC 7002 through metabolic engineering, and the use of a growth-associated screen for glycogen production. Evaluation of these strains grown in wastewater nutrient sources yielded improved glycogen productivity. Further, optimization of cyanobacterial hydrolysate produced from glycogen rich strains of PCC 7002 supported growth of *Escherichia coli* for production of value-added chemicals. Finally, a techno-economic analysis of proposed industrial processes for producing cyanobacterial hydrolysate has shown that through improvements in biomass titers and separation techniques, such a process could compete with corn-based sugar feedstocks.

P45 A software system for production strain sequence management and analysis

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With the decreased cost of next generation sequencing (NGS) technologies, full genome sequencing of production strains and derivatives has become routine. The management and analysis of strain sequence, variant, and expression data is challenging due to a lack of integrated computational solutions that support workflows from raw next generation sequencing data to biological insight.
As a comprehensive and scalable genome knowledge management suite, Genedata Selector directly addresses the challenge of NGS data analysis and management. The system offers an end-to-end workflow that combines NGS analysis algorithms, interactive visualization, and powerful statistical tools with an integrated database.

Here we demonstrate how complex data sets from NGS derived whole genome and transcriptome sequencing of microbial production strains can be analyzed and integrated in Genedata Selector. Once analyzed and stored in Genedata Selector, the data can be used as a collaborative resource for microbial strain engineering and optimization across an organization.

P47 Improving carbon efficiency in bio-production of citramalate in *Escherichia coli*

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Acrylic polymers are transparent plastics with a global market worth more than £3 billion, and are produced from the monomer, methylmethacrylate (MMA). Currently, MMA manufacturing is energy intensive and uses oil-derived precursors; therefore, sustainable, bio-based alternatives are needed. We have engineered *E. coli* to produce citramalate from glucose, since citramalate can be converted to MMA *via* a patented dehydration-decarboxylation process, followed by methylation. Citramalate is formed by citramalate synthase-catalysed condensation of pyruvate and acetyl-CoA. However, formation of acetyl-CoA results in loss of the greenhouse gas, CO_2, which is undesirable from both economic and

sustainability perspectives. Since carbon neutral ethanol and acetate can be produced using homoacetogenic gas fermentations, we engineered *E. coli* to use ethanol or acetate as co-substrates to produce acetyl-CoA, with pyruvate production from glucose (Fig. 1), to improve carbon efficiency. Ethanol utilisation was achieved by expressing the genes, *alcA* and *aldA* from *Aspergillus nidulans,* whilst the native *ackA-pta* genes of *E. coli* were overexpressed to obtain efficient acetate utilisation. The resulting ethanol- and acetate-utilising strains produced 0.5g/L and 1.82g/L of citramalate in flasks, respectively. Since acetate co-utilisation was the most efficient, we investigated acetate and glucose co-feeding in fed-batch culture, and demonstrated the formation of 15g/L of citramalate. Optimization of the acetate feeding regimes is ongoing to improve carbon efficiency. This study demonstrates the potential of co-feeding systems to develop carbon efficient processes for the biosynthesis of citramalate that could be widened to other commodity chemicals.

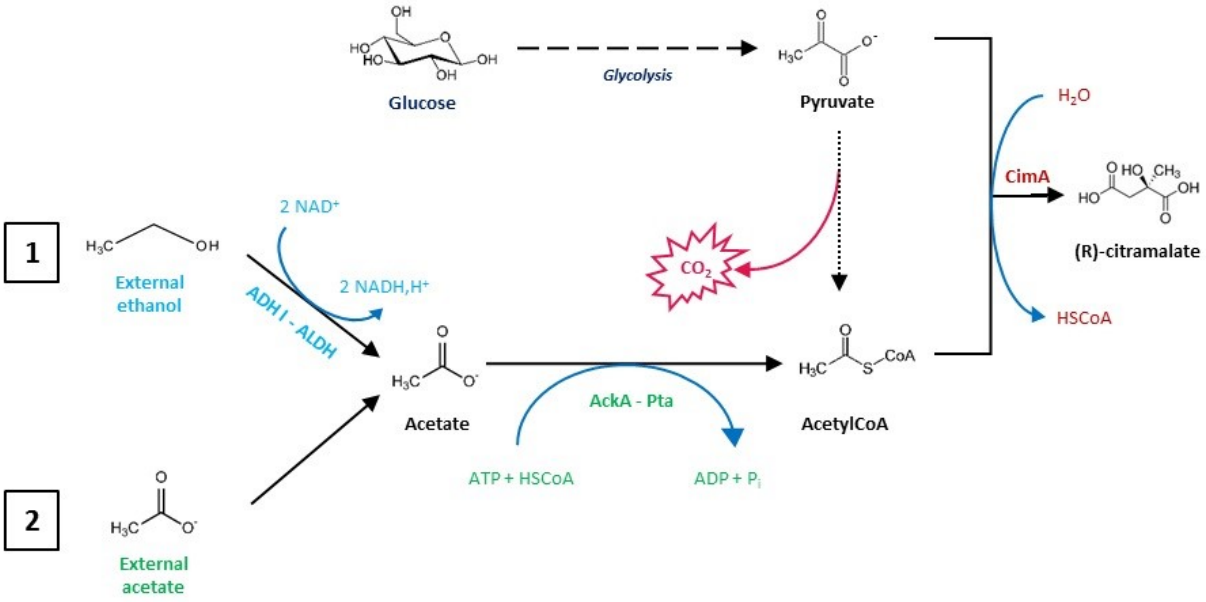

Fig. 1: Summary of the metabolic pathways investigated

1, Ethanol assimilation pathway from *Aspergillus nidulans* consisting of ADH I (alcohol dehydrogenase) encoded by *alcA* and ALDH (acetaldehyde dehydrogenase) encoded by *aldA*;

2, Acetate assimilation pathway composed of AckA (acetate kinase A) and Pta (phosphotransacetylase).

CimA: Citramalate synthase.

P49 Ethyl acetate production by the elusive alcohol acetyltransferase from yeast

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Ethyl acetate is an industrially relevant ester that is currently produced exclusively through unsustainable processes. Many yeasts are able to produce ethyl acetate, but the main responsible enzyme has remained elusive, hampering the engineering of novel production strains. Here we describe the discovery of a new enzyme (Eat1) from the yeast *Wickerhamomyces anomalus* that resulted in high ethyl acetate production when expressed in *Saccharomyces cerevisiae* and *Escherichia coli*. Purified Eat1 showed

alcohol acetyltransferase activity with ethanol and acetyl-CoA. Homologs of *eat1* are responsible for most ethyl acetate synthesis in known ethyl acetate-producing yeasts, including *S. cerevisiae*, and are only distantly related to known alcohol acetyltransferases. Eat1 is therefore proposed to compose a novel alcohol acetyltransferase family within the α/β hydrolase superfamily. The discovery of this novel enzyme family is a crucial step towards the development of biobased ethyl acetate production and will also help in selecting improved *S. cerevisiae* brewing strains.

P51 *In vitro* biosynthesis of 3-hydroxypropionic acid from glucose

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Value-added chemicals and fuels derived from renewable biomass have gained considerable attentions in the past many years due to the environmental problems caused by over exploitation of fossil resources. 3-Hydroxypropionic acid is one of the top twelve value-added planform chemicals from biomass suggested by U.S Department of Energy that has great potential to derive a wide range of commodity chemicals. Many efforts have been devoted in the development of 3-HP production through metabolic engineering. However, most of the fermentation based designs of metabolic pathway used glycerol as feedstock for 3-HP production, because there are inherent difficulties of microbial metabolism for producing 3-HP directly from sugars. *In vitro* biosynthesis, also call *in vitro* metabolic engineering, has emerged as a potential platform for bioproductions without worrying the limits associated with living cells. In this proposal, a novel *in vitro* biosynthetic pathway for 3-HP production was designed and will be constructed by assembling nine enzymes. This innovative pathway is designed to have balance between ATP consumption and generation and vitamin B12 independent which can substantially reduce the cost of production.

P53 Engineering *Escherichia coli* for efficient production of succinate from acetate

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Succinate has been recognized as one of the most important bio-based building block chemicals due to its numerous potential applications. Using the non-food based raw materials to produce commodity chemicals has attracted great attentions. Acetate, a non-food based substrate obtained from multiple biological and chemical ways, is now being paid great attention in bio-manufacturing and have a strong potential to compete with sugar-based carbon source. In this study, an engineered strain *Escherichia coli* MG1655 was constructed for production of succinate from acetate as sole carbon source. The metabolic engineering strategies included the blockage of the TCA cycle, redirection of the gluconeogenesis pathway, enhancement of the glyoxylate shunt, improvement of acetic acid assimilation pathway and increasing aerobic ATP supply through cofactor engineering. Finally, in the experiment of using the resting cells, the engineered strain can accumulated 198.89 mM of succinate with a yield of 0.44 mol/mol in 16 hours, about 88% of the maximum theoretical yield. Our results showed this metabolically engineered *E. coli* strain has a great potential to produce succinic acid using acetic acid as the sole carbon source.

P55 *Pseudomonas putida* - a versatile cell factory for biotechnology applications

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Pseudomonas putida is a remarkable cell factory for the production of industrially important products. This soil organism has been shown to catabolize aromatics and displays great robustness and high resistance to oxidative stress. This makes it an excellent host to apply molecular and metabolic engineering to

produce advanced biomanufacturing compounds, generate synthetic biology tools, and conduct fundamental research on lignin deconstruction and bioconversion mechanisms.
Based on recent metabolic engineering in a *P. putida* KT2440 strain to produce *cis,cis*-muconic acid from D-glucose, the ß-ketoadipate pathway was additionally modified for the accumulation of ß-ketoadipic acid.[1] ß-ketoadipate can be used as precursor of polymers and has the potential to be further converted into adipic acid, which is of keen interest for coatings, detergents, and nylon synthesis. The engineered strain performance was assessed in 2.5 L bioreactors under continuous feeding of glucose. The combination of metabolic engineering and fermentation process optimization enabled improvement of both ß-ketoadipate titers and metabolic yields.

[1] C. W. Johnson, D. Salvachúa, P. Khanna, H. Smith, D. J. Peterson, G. T. Beckham, *Metab. Eng. Commun.* **2016**, *3*, 111–119.

P57 Engineer *Aspergillus niger* by CRISPR/Cas9 for industrial bioreactor

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The conversion of the biomass into advanced biofuels faces many challenges, one of which is finding the right organism for the job. The filamentous fungus *Aspergillus niger* has been chosen as a biocatalyst for cellulose, hemicellulose, and lignin degradation because it can secrete numerous hydrolytic enzymes, such as lignin modifying enzymes (LMEs) and its genome sequence is available.
However, we currently lack efficient tools for editing and augmenting the *A. niger* genome. While genome editing techniques such as CRISPR/Cas9 editing function in *A. niger*, we are limited by the difficulty of making multiple mutations, restricted selection of markers, and inefficient, expensive and time-consuming methodologies for genome engineering.

Here I present progress towards developing a method for efficiently making multiple genomic mutations via Cas9/gRNAs without the use of selective markers. This technique utilizes several approaches; 1) pyrG positive and negative selection for transient plasmid maintenance, 2) a self-targeting plasmid for selection of Cas9 activity. Once complete, this strategy should remove the need for screening of colonies to identify mutants. Our objective is to first establish this method for genome engineering, and build a library of *Aspergillus niger* strains. Then we will to design two types of bioreactor, a submerged fermentation and solid state fermentation. The objective is to define the best strains and conditions for the productivity of LMEs in bioreactors at a pilot scale for industries.

P59 Interrogating central carbon metabolism via ^{13}C metabolic flux analysis of *Escherichia coli* knockout strains: pathway discovery and model development

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Metabolic and physiological responses to genetic perturbations are of fundamental interest to systems biology and metabolic engineering. They reveal underlying information about network structure including kinetics, regulation, and the presence of otherwise “hidden” reactions. Quantitative prediction of these responses is essential for rational strain design, but has been a persistent challenge in the field. There has been much work done to develop *in silico* predictive models of metabolism, most notably the constraint-based models. Progress in this area has been limited, however, by a lack of high-quality, reproducible experimental metabolic flux (fluxomic) data of genetically perturbed strains.
In this study, state-of-the-art ^{13}C metabolic flux analysis (^{13}C-MFA) approaches were applied to ~50 *Escherichia coli* knockout strains spanning the major pathways of central carbon metabolism. A full physiological characterization of each strain during aerobic exponential growth was performed, including uptake and secretion rates as well as biomass composition. Flux estimates are estimated based on an optimized parallel experimental design, coupled with mass spectrometry measurement of metabolite isotopic labeling and ^{13}C-MFA.

The flux and physiological responses of these strains collectively provide valuable insights into the robustness of central carbon metabolic pathways, as well as areas of likely kinetic limitation. Severely growth-impaired knockout strains identify the most important enzymes in these pathways and the

adaptability of *E. coli* to extreme perturbations. Particularly surprising responses, including the identification of a novel enzymatic activity, will be emphasized. These data are being applied to the assessment of constraint-based metabolic models and the development of kinetic models.

P61 Aromatic metabolism by a non-model, non-conventional oleaginous yeast

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Oleaginous yeasts have long been a target for developing industrial-scale biochemical processes due to their ability to accumulate high amounts of lipids, synthesize complex chemicals and proteins, and robustly metabolize diverse feedstocks. In parallel, interest in lignocellulosic biomass as a feedstock has grown. *Trichosporon oleaginosus,* previously known as *Cryptococcus curvatus*, is a non-model, non-conventional, oleaginous yeast that we have discovered both tolerates and metabolizes lignin-derived phenolics. BLAST analysis suggests a potential putative pathway for metabolism of aromatic compounds, but non-intuitive qPCR results indicates the mechanism could involve a cryptic pathway or an alternative mechanism of aromatic metabolism. RNAseq data was utilized in effort to elucidate relevant metabolic pathways. We have characterized *T. oleaginosus* biomass and lipid accumulation while utilizing glucose, phenol, catechol, 4-hydroxybenzoic acid, and resorcinol as sole carbon sources under nitrogen rich and nitrogen starved conditions. Aromatic metabolism does not hinder lipid accumulation, but toxicity limits how much carbon can be dosed at a single time. As such, we explored different feeding strategies to overcome aromatic toxicity and increase lipid accumulation. Since many recalcitrant waste streams are not homogenous feedstocks, we also characterized growth and lipid accumulation when cells are cultured in dual carbon feedstocks, and found that feeding two carbon sources at once improves lipid accumulation when compared to glucose as a sole carbon source. This work hopes to showcase this yeast as a potential model, oleaginous yeast for aromatic metabolism, with future applications on an industrial level for valorization of recalcitrant feedstocks such as lignin.

P63 Construction of novel strains in *Zymomonas mobilis* uncovers small RNA-driven regulatory networks that lead to improved ethanol tolerance

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Zymomonas mobilis has been identified as a promising cellular factory for biofuels due to its efficient, natural production of and tolerance to ethanol. Recent discovery of ethanol-responsive small regulatory RNAs (sRNAs) in *Z. mobilis* suggested the potential of exploiting these elements for strain engineering. As global controllers of gene expression, sRNAs represent powerful tools for engineering complex phenotypes. In this study, we screen a set of experimentally confirmed sRNAs in *Z. mobilis* for their impact on ethanol tolerance and identify sRNA candidates that have significant impact on ethanol tolerance. We have conducted multi-omics analyses (including proteomics, transcriptomics, and sRNA-affinity tag purification with high-throughput sequencing) of these sRNA-engineered strains to map gene networks under the influence of their regulation. This work has led to the finding that these sRNAs regulate unique combinations of enzymes along pathways known to be relevant to ethanol tolerance such as oxidative stress response, ATP production, redox balance, translation, transport, and DNA repair. Importantly, this work represents the first analysis and application of de novo sRNA-driven strain engineering in non-model *Z. mobilis* that is of relevance to biofuel technologies.

P65 Examination of succinic acid biosynthesis in *Actinobacillus succinogenes* via targeted metabolic engineering

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Succinic acid is a specialty chemical and an important precursor for the synthesis of high-value products that can be applied across many industries, such as for biopolymers, pharmaceutical products, and foods.

Actinobacillus succinogenes, a gram-negative, capnophilic, facultative anaerobic bacterium, produces succinate at high yields from glucose and xylose as a major fermentation product. In addition, succinate production requires the incorporation of CO_2 in the pathway, making this organism an ideal candidate for converting lignocellulosic sugars and CO_2 to a commodity product from a sustainability perspective. We have conducted targeted metabolic engineering in *A. succinogenes* in order to examine flux alterations in the succinate and competitive carbon biosynthetic pathways. Several target genes in the succinate biosynthesis and competitive pathways were identified for up- or down-regulation. Gene knockouts and overexpression and the effect of such manipulations will be discussed in this report.

P67 Accelerating Lignin Valorization Using Cell-Free Pathway Prototyping

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Lignin valorization is important to achieve for biorefinery financial viability. Recently, a biological funneling strategy using *Pseudomonas putida* KT2440 has shown promise for the conversion of a variety of lignin monomers to value-added products, but low final titer and an initial lag phase must be overcome to achieve industrially relevant strains. However, when considering entire heterologous pathways, typical genetic engineering approaches such as directed evolution may not be effective at rapidly converging on optimal enzyme loadings. An alternative strategy, presented here, is cell-free optimization of the pathway of interest. In this study, product pathway enzymes were individually expressed and purified, kinetically characterized, and used to inform a kinetic model of the entire pathway. Following experimental model validation, a genetic algorithm was applied to determine the optimal enzyme loading for high pathway flux. These relative loadings were then genetically encoded through the use of an RBS calculator, and the optimized pathway was integrated into the *Pseudomonas putida* KT2440 genome. Preliminary results will be discussed at this poster presentation.

P69 Enabling efficient ethylene glycol and glycolaldehyde metabolism in *Pseudomonas putida* KT2440

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P. putida KT2440 demonstrates broad substrate specificity for compounds of interest to bioremediation and renewable chemicals production and coupled with its high toxicity tolerance, it is often used for industrial biotechnological applications. Ethylene glycol and glycolaldehyde are of interest as ethylene glycol is a common intermediate in recycling of plastics such as PET and glycolaldehyde (an intermediate in ethylene glycol pathway) is present in biomass-derived streams such as pyrolysis oils, making both of them key targets for catabolism by *P. putida* KT2440. The native strain of *P. putida* KT2440 is not able to efficiently metabolize ethylene glycol or glycolaldehyde. To further expand the ability of *P. putida* for substrates of industrial interest, we enabled enhanced metabolism of ethylene glycol and glycolaldehyde via metabolic engineering.

P71 Discovery of CO_2-fixing one-carbon metabolism in a cellulose-degrading bacterium *Clostridium thermocellum*

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High carbon yield in the bioengineering of heterotrophic bacteria is hindered by carbon loss to CO_2 production. We provide evidence showing *Clostridium thermocellum*, a cellulose-degrading bacterium and a model consolidated bioprocessing (CBP) organism, can fix CO_2 while growing predominantly on cellobiose, a cellulose-derived disaccharide. Combining genomic and experimental data, we demonstrated that the conversion of CO_2 to formate serves as a CO_2 entry point into the reductive one-

carbon (C1) metabolism, and internalizes CO_2 via two biochemical reactions: the reversed pyruvate:ferredoxin oxidoreductase (rPFOR), which incorporates CO_2 using acetyl-CoA as a substrate and generates pyruvate, and pyruvate-formate lyase (PFL) converting pyruvate to formate and acetyl-CoA. We analyzed the labeling patterns of proteinogenic amino acids in putative PFOR and PFL deletion mutants, respectively, and confirmed the dual activities of rPFOR and PFL crucial for CO_2 uptake. These findings demonstrated the metabolic versatility of *C. thermocellum*, which is thought of as primarily a cellulosic heterotroph but is shown here to be endowed with the ability to fix CO_2 as well. Our findings pave the way to future engineering of this bacterium to use cellulose and CO_2 simultaneously as a means to improve microbial carbon efficiency that is constrained by theoretical limitation and to reduce CO_2 in the environment.

P73 Random transposon mutagenesis of the *Saccharopolyspora erythraea* genome reveals additional genes influencing erythromycin biosynthesis

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Genes influencing the production level of the antibiotic erythromycin were uncovered using a random transposon mutagenesis procedure and a micro-screening method. Traditionally, strain improvement mutations are created randomly using chemical or physical mutagens and they are chosen for a particular strain and process based on phenotypic performance alone, with no knowledge of the genotype. The transposon-based strategy used in this study, however, simplifies the characterization of the strain improvement mutation so that favorable mutations can be incorporated into future strain improvement programs directly. In this study, experiments were performed in a *Saccharopolyspora erythraea mutB* strain and over one-thousand mutants were generated. Approximately 3% of the mutants were affected in erythromycin production. Fifteen of these mutants were functionally characterized by DNA sequence analysis. The highest yielding strain in shake flasks was the *cwh1* mutant. The *cwh1* mutation was determined by DNA sequence analysis to be in a gene involved in cell wall biogenesis.

P75 Metabolic engineering of *Escherichia coli* to produce succinate from soybean hydrolysate under anaerobic condition

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Succinate has been designated as one of the top platform chemicals from biomass and many attractive improvements have been reached for bio-based succinate production during last few years. To make it more economic competitive against petrochemical-based succinate, succinate producer CT550/pHL413KF1 was further engineered to efficiently use mixed sugars from soybean hydrolysate to produce succinate under anaerobic conditions. By manipulation of glucose and galactose related sugar transporters, the PTS system was found to be important for utilization of mixed sugars. Galactose uptake was activated by deactivating *ptsG*. In the *ptsG*$^-$ strain, glucose was assimilated mainly through the mannose PTS system while galactose was transferred mainly through GalP. A new succinate producer FZ591 which can produce succinate from the mixed sugars present in soybean hydrolysate efficiently was constructed by integration of the high succinate yield producing module and the galactose utilization module into the chromosome of CT550 delta *ptsG*. The succinate yield reached 1.64 mol/mol hexose consumed (95% of maximum theoretical yield) when mixed sugars was used as a carbon source. Based on the three monitored sugars (glucose, galactose and fructose, the major sugars in soybean hydrolysate), the nominal succinate yield of 1.95 was observed as it can also use some other sugars in the hydrolysate. The bacterial cells can be used for 4 batches in repeated fermentation. In this study, we demonstrate that FZ591 can use a soybean hydrolysate as an inexpensive carbon source for succinate production with high yield under anaerobic conditions, and it has the potential for industrial application.

P79 Unravelling the mechanisms of eucalyptus hydrolysate tolerance in evolved industrial *Saccharomyces cerevisiae* strains

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Bio-based processes for production of fuels and other value-added compounds from raw biomass feedstocks have the potential to be a sustainable alternative to petrochemistry. Second and third-generation biorefineries utilize agricultural and forest residues as the starting biomass. Efficient fermentation of all sugars present in lignocellulosic hydrolysates is crucial for the establishment of economically viable commercial processes. Suitable cell factories must have the robustness to perform such efficient conversion of the substrate into the desired product under sub-optimal and fluctuating environmental conditions, where they have to face the presence of several toxic compounds and other stresses that affect their physiology. The yeast *Saccharomyces cerevisiae* is one of the most promising hosts to function in these environments. In this work, we constructed efficient cell factories based on a diploid industrial *S. cerevisiae* strain. We first engineered it for xylose consumption, using the latest-generation genetic engineering tools developed, and subsequently applied adaptive laboratory evolution to improve growth performance in a pentose-rich hardwood spent sulfite liquor at low pH, derived from eucalyptus. The evolution was performed in sequential liquid batch cultures with increasing hydrolysate concentrations over several months. The obtained strains were characterized and analysed by next generation sequencing, to identify the causative mutations and therefore unravel the mechanisms behind the acquired high tolerance. This project is part of BioREFINE-2G (www.biorefine2g.eu), which is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-613771).

P81 Manipulation of cyanobacterial biomass composition for enhanced HTL biofuel yield and quality

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Cyanobacterial biomass is comprised of essential macromolecular components that are synthesized proportionally (proteins, lipids, DNA, etc.) and carbon/ nitrogen storage compounds (glycogen, PHB, sucrose and cyanophycin etc.) varying in their portions in response to environmental and genetic alterations. For instance, PHB content in cyanobacteria is flexible, and under nutrient starvation (N, P), PHB is accumulated up to 30-50% cell dry weight. Reports from eukaryotic algae suggest that variation in cyanobacterial biomass compositions may affect the outcome of Hydrothermal Liquefaction (HTL) biofuel intermediate (BFI) significantly. The goal of this research is to modify cyanobacterial biomass composition, initially in *Synechocystis* 6803, by engineering biosynthetic pathways of carbon/nitrogen storage compounds and quantitatively investigate the effects of altered biomass composition on HTL BFI yield and quality. The results will in turn guide cyanobacterial engineering to selectively modify relevant pathways toward desired biomass composition. Given the high nitrogen content in cyanobacterial biomass (half of which is protein in normal growth conditions), one of the desirable BFI quality parameters is lower nitrogen content.

P83 Development of synthetic biology tools for the cyanobacterium *Synechocystis* sp. PCC 6803

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Cyanobacteria have served as genetic models in photosynthesis research for decades, and have recently become attractive hosts for the production of fuels and chemicals, due to their ability to utilize sunlight and carbon dioxide. Despite significant advances, there is still an urgent demand for synthetic biology tools for effective genetic manipulation in cyanobacteria. In this study, we have systematically compared a total of 16 natural and chimeric promoters on their capability in expressing the ethylene-forming enzyme (EFE) and the relevant ethylene productivity in the cyanobacterium *Synechocystis* sp. PCC 6803. We

report the finding of a chimeric promoter that is stronger than the previously reported strongest promoters, such as PcpcB and Ptrc, in expressing EFE. A library of ribosome binding sites (RBSs) was rationally designed and systematically characterized for the strength in expressing EFE and thereafter in producing ethylene. Our experimental results demonstrated that the expression of EFE is no longer the rate-limiting step in cyanobacterial ethylene production. These systematically characterized promoters and the methods in the design of RBSs can serve as useful synthetic biology tools to tune the gene expression levels and to pinpoint and mitigate the bottleneck enzymes in cyanobacteria.

P85 Identification and characterization of light-entrained promoters for engineering *Synechocystis* sp. PCC6803 in daily light:dark cycles

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Cyanobacteria are photosynthetic microorganisms which utilize sunlight and carbon dioxide to power cellular metabolism. This property makes them promising chasses for sustainable bio-production of chemicals. A variety of commercially valuable products—such as pigments, fuels, and biopolymers—have been over-produced in the model cyanobacterium *Synechocystis* sp. PCC6803, but further yield increases are necessary to achieve economic viability. Metabolic engineering tools which perform as expected in cyanobacteria are crucial. The goal of this project is to better understand and engineer cyanobacteria cultivated in daily light:dark (LD) cycles, such as those experienced in natural sunlight. In this study, we sought to identify native promoters which are temporally synchronized with light:dark cycles. Using previously published microarray data of transcript accumulation, we identified twenty-one putative light- or dark-entrained promoters and characterized promoter activity in *Synechocystis* sp. PCC6803 using a bioluminescent promoter probe. Four promoters were identified which provided light-entrained bioluminescence. Transcripts from three of these promoters also cycle in LD cycles, while one does not suggesting post-transcriptional regulation. Promoters were applied toward chemical production and titers were compared in continuous light versus light:dark cycles.

P87 New role of pyruvate kinase PykA on the glycolysis in *Escherichia coli*

C. Zhao, H. Dong, Y. Zhang and Y. Li, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China*

Besides the known 3 rate-limiting steps of the glycolysis, that catalyzed by hexokinase, phosphofructokinase and pyruvate kinase, a novel controlling point mediated by pyruvate kinase PykA was found in our study. Two isozymes of pyruvate kinase PykF and PykA are identified in *Escherichia coli*. The PykF is generally considered important, whereas the PykA has less-defined role. But in our study, a *pykA*::Tn5 mutant was screened for increased yield of an end-product derived from pyruvate (*n*-butanol). Under anaerobic conditions, a surprising phenotype was observed that both the cell growth and glucose metabolism of *E. coli* were improved significantly by inactivating the pyruvate kinase PykA. Further, we found that PykA has a very high affinity and catalytic efficiency of ADP, and the *pykA* mutant exhibited elevated intracellular ATP level. It suggested that the role of PykA on the glycolysis might be related to intracellular energy production and need to be reexamined.

P89 The plasticity of cyanobacterial metabolism supports direct CO_2 conversion to ethylene

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The cyanobacterial tricarboxylic acid (TCA) cycle functions in both biosynthesis and energy generation. However, it has until recently been generally considered to be incomplete with limited flux, and few attempts have been made to draw carbon from the cycle for biotechnological purposes. We demonstrated that ethylene can be sustainably and efficiently produced from the TCA cycle of the recombinant cyanobacterium *Synechocystis* 6803 expressing the Pseudomonas ethylene forming enzyme (Efe). A

new strain with a modified ribosome binding site upstream of the efe gene diverts 10% of fixed carbon to ethylene and shows increased photosynthetic activities. The highest specific ethylene production rate reached 718 ± 19 µl l^{-1} h^{-1} per $A_{730\ nm}$. Experimental and computational analyses based on kinetic ^{13}C-isotope tracer and liquid chromatography coupled with mass spectrometry (LC–MS) demonstrated that the TCA metabolism is activated by the ethylene forming reaction, resulting in a predominantly cyclic architecture. The outcome significantly enhanced flux through the remodelled TCA cycle (37% of total fixed carbon) compared with a complete, but bifurcated and low-flux (13% of total fixed carbon) TCA cycle in the wild type. Global carbon flux is redirected towards the engineered ethylene pathway. The remarkable metabolic network plasticity of this cyanobacterium is manifested by the enhancement of photosynthetic activity and redistribution of carbon flux, enabling efficient ethylene production from the TCA cycle.

P91 Repurposing molecular pumps: Tolerance engineering through exploitation of native efflux systems

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Lignocellulosic biomass represents a renewable feedstock that can support large-scale biochemical production processes for fuels and specialty chemicals, although cost-effective conversion still remains a challenge. Toxicity associated with lignocellulose hydrolysate and bio-products is at the center of bottlenecks limiting high production metrics for conversion of renewable resources into value-added chemicals. Tolerance engineering strategies using multidrug resistance (MDR) efflux systems to reduce intracellular toxic chemical exposure and increase bio-product export are promising, but specificity of MDR pumps for industrially relevant compounds lacks comprehensive evaluation. In this work, a library of *Escherichia coli* membrane proteins mainly composed of MDR pumps was screened for their ability to confer tolerance to different lignocellulosic pretreatment inhibitors and aromatic bio-products. Effective pumps were identified using growth-based plasmid enrichment at inhibitory chemical concentrations and overexpression of these pumps was further demonstrated to enhance *E. coli* tolerance to toxic compounds, including furan aldehydes and benzene derivatives. Furthermore, we investigate both chromosomal and vector-based expression strategies to enhance tolerance and demonstrate their application in bioproduction conditions.

P93 Using promoter architecture to guide engineering the strongest known fatty acid inducible hybrid promoter in *Yarrowia lipolytica*

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Recently, there has been significant improvements in the genetic toolbox for the industrial oleaginous microbe, *Y. lipolytica*. These tools range from CRISPR-Cas9 mediated genome editing to libraries of hybrid promoters made from strong upstream activating sequences for tunable strength; however, strong inducible promoters remain undeveloped. In addition to tunable strength, having control of timing of gene expression can improve cellular efficiency by separating the growth and production phase. Inducible regulation is particularly important when certain chemicals produced could be toxic and inhibit growth, or when requiring the ability to switch on production at stationary phase. Here, we demonstrate the development of the only known and strongest fatty acid inducible promoter system in *Y. lipolytica*. The hybrid promoter has nearly 50-fold induction strength relative to glucose with expression ranging from 2 to 10-fold higher than the commonly used native inducible acyl CoA oxidase promoter. We show that this range of tunable induction strengths can be accomplished by different functional elements comprising the hybrid promoter. While repressed in the absence of fatty acids, the hybrid promoter lacks catabolite repression in the presence of either glucose or glycerol in fatty acid containing media. Furthermore, this inducible genetic switch can be can be strongly activated at stationary phase with low concentrations of a wide range of fatty acids ranging from oleic acid to eicosapentaenoic acid. The hybrid promoter activity

correlates well with changes in intracellular fatty acid pools, suggesting its use as a tool for strain engineering.

P95 Engineering a synthetic co-culture system for enhanced co-utilization of lignocellulose-derived sugar mixtures

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Conventional approaches to address the low-efficiency and incomplete conversion of lignocellulose-derived sugars to bioproducts is to engineer single strains, so called microbial 'generalists', to carry the full complement of functions needed to co-utilize sugar mixtures. The potential negative effects of excessive metabolic burden in engineering microbial 'generalists' often leads to reduced fitness and suboptimal production metrics. To address this challenge, we have engineered a synthetic *Escherichia coli* community composed of two unique 'specialists', each capable of metabolizing only glucose or xylose. Xylose uptake was prevented in *E. coli* W by deletion of the xylose transcriptional regulator *xylR*, resulting in the glucose 'specialist'. Glucose consumption was inhibited in the xylose 'specialist' by deletion of multiple glucose transport systems, *ptsIG* and *galP.* Through a series of fermentations investigating the effects of different sugar mixture concentrations and ratios, as well as initial population ratios, the performance of the synthetic 'specialist' community has been compared and contrasted to *E. coli* W. 'Specialist' communities were capable of co-utilizing ~80% of a glucose-xylose sugar mixture (50 g/L for each sugar) within 96 h with identical glucose and xylose consumption rates of 0.39 g $gDCW^{-1}$ h^{-1}, whereas *E. coli* W monoculture consumed only 20% of the supplied xylose. This co-culture strategy was then applied to engineer 'specialist' pairs using the previously engineered *E. coli* strain LY180, leading to a co-culture system capable of co-utilizing glucose-xylose for production of ethanol. This demonstrates the potential of this approach for enhancing the production of valuable bioproducts from biomass sugar mixtures.

P97 Exploring new pathways and strategies for enhancing muconic acid biosynthesis

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The engineering of non-natural pathways is a promising to approach to developing renewable and sustainable alternatives to many conventional petrochemicals. For example, several recent studies have demonstrated how *Escherichia coli* can be engineered for the efficient production of muconic acid which, in turn, can be used as a precursor to adipic acid – a platform chemical used for the synthesis of many different plastics and polymers. To date, no fewer than five different pathways have been reported for the muconic acid biosynthesis from glucose, each involving different precursors of the shikimic acid pathway as well as enzyme chemistries. This unique flexibility arises in part due to the fact that muconic acid is also a naturally-occurring metabolite that arise as a key intermediate in the b-ketoadipate pathway used for degrading an array of aromatics. In this study we demonstrate how three additional routes to muconic acid can be constructed by linking a series of recently-engineering phenol biosynthesis pathways with their subsequent degradation steps. The resultant phenol-dependent pathways support muconic acid production at up to 0.5 g/L in shake flasks. We also report a novel, four-step pathway for muconic acid production from endogenous chorismate via p-hydroxybenzoate which supports muconic acid titers of up to about 1 g/L while circumventing auxotrophic limitations experienced via the original, 3-hydroshikimate-derived route. Moreover, inspired by mechanisms of 'biological funneling' employed by soil microbes for the simultaneous degradation of aromatic mixtures, we investigate a synthetic 'funneling' mechanisms for enhancing muconic acid yields and titers by *E. coli.*

P99 Design and characterization of biological "parts" for thermophilic anaerobic bacteria

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The field of industrial microbiology seeks renewable approaches for the production of high-value chemicals, biofuels, medicines, and food additives utilizing microorganisms as cell factories. Thermophilic (55-60^{o}C) and anaerobic bacteria, including *Thermoanaerobacter saccharolyticum* and *Clostridium thermocellum*, are suitable candidates for the industrial production of valuable compounds. This is due in part to their ability to simultaneously hydrolyze and ferment all major carbohydrates in waste plant biomass, namely cellulose and hemicellulose, into valuable compounds. However, current strain engineering efforts are limited by the lack of characterized genetic "parts" which would allow the development of novel strains with high production rate, titers, and yield. This project aims to develop a series of synthetic promoters and gene regulatory elements that can be used reliably across various thermophilic bacteria. Particularly, we are interested in the development of synthetic promoters that can be regulated upon the introduction of a user-defined chemical signal. Towards that goal, we have identified several thermophilic riboswitches, which are mRNA leader sequences that can regulate gene expression upon binding a small-molecule metabolite, and repurpose them into synthetic biology tools in thermophiles. As a means of characterizing and measuring the strength of these regulatory components in gene expression, we have validated a LacZ reporter assay that can be used at elevated temperatures over 50^{o}C for thermophiles. The characterizations of these genetic elements will expedite the development of these thermophilic bacteria as industrial workhorses, and will also be used as critical tools to deepen our understandings in the genetics and metabolism of thermophilic organisms.

P101 Membrane engineering strategies to improve production of biorenewable fuels and chemicals

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The production of fuels and chemicals from biomass in a manner that is economically competitive with petroleum-based processes is often hindered by microbial inhibition by the product and/or inhibitors in the biomass-derived sugar stream. In many of these cases, this inhibition is due, at least in part, to damage of the microbial cell membrane. This talk will describe rational engineering strategies for engineering the *E. coli* cell membrane on three distinct levels, the fatty acid tails, the phospholipid heads, and the membrane-associated proteins and sugars, and the resulting impact on membrane properties and the tolerance and production of membrane-damaging compounds. Novel engineering strategies identified through the reverse engineering of evolved strains will also be discussed.

P103 Consolidated platform for the bioconversion of single carbon feedstocks to fuels and chemicals

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Single carbon feedstock utilization has been identified as a potential market for which industrial biomanufacturing may prove advantageous over traditional chemical manufacturing (Science, 2017; DOI: 10.1126/science.aag0804). For this to be possible, improvements in biocatalysis of single carbon molecules are necessary. Current approaches to engineer biological C1 conversion involve a metabolic architecture that necessitates the engineering and optimization of pathways associated with carbon assimilation, central metabolism, and product formation. This is a monumental task that has proven to be a challenge owing to the high degree of complexity involved in the design, control, and engineering these systems. Herein, we report on progress towards the development of a novel pathway that consolidates single carbon assimilation with product generation, while eliminating the need for rearrangements associated with central metabolism. The designed pathway makes use of single carbon molecules to directly elongate a growing carbon backbone. This is made possible by our discovery that 2-hydroxyacyl-CoA lyase (HACL1) from *Homo sapiens*, which is natively involved in mammalian alpha-oxidation, is reversible and has high promiscuity for aldehydes of varying chain length. We demonstrate the pathway function *in vitro* and also show that the pathway is functional *in vivo* in *E. coli*, a genetically tractable and industrially relevant host, for the production of 1,2-diols and 2-hydroxyacids. We further demonstrate the

pathway's iterative nature, allowing for consecutive single carbon elongations. We propose that this pathway represents a new metabolic architecture that deviates from the traditional paradigm for engineered biological platforms for fuel and chemical production.

P105 Engineering *Pseudomonas putida* to produce higher yields of polyhydroxyalkanoates from lignin

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Lignin is abundant in plant cell walls, and its complexity makes it difficult to convert into value-added chemicals. When cellulose is converted to biofuels, lignin is typically burned for process heat and electricity. A higher value use would improve the economics of using plant biomass for biofuel. Research has shown the utility of pretreatment to break lignin into monomers, and *Pseudomonas putida* can use those monomers as a carbon and energy source. *P. putida* also synthesizes polyhydroxyalkanoates (PHAs) for storage during carbon excess, which can be used as biodegradable plastics. *P. putida* is promising for converting lignin into value-added chemicals, but its PHA yield is too low. Therefore, we sought to engineer *P. putida* to produce higher PHA yields. Modifications shown to increase PHA production individually were stacked using molecular and microbiology approaches. PhaG (acyl-transferase) and AlkK (acyl-CoA synthase) link fatty acid biosynthesis to PHA production; therefore, the genes encoding those enzymes were overexpressed. PhaC, which polymerizes a growing PHA chain, was overexpressed. Genes encoding enzymes in competing pathways were deleted, including beta-oxidation that competes for 3-hydroxyacyl-CoA intermediates and the PHA depolymerase gene, *phaZ*. The resulting strain was tested by GC-MS for increased PHA production, and it produced twice the amount of PHA produced by the unmodified strain. Additional modifications are being explored to further increase flux to PHA biosynthesis. These strains provide the foundation for further engineering to convert lignin into value-added products.

P107 Anaerobic fungal enzymes are robust to lignin monomers and efficiently hydrolyze lignocellulose for biofuel production

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Inexpensive and reliable methods for lignocellulosic hydrolysis are key to developing abundant and renewable plant biomass as a useable feedstock for the bioeconomy. However, many biocatalysts are expensive and/or limited to only specific feedstocks. An attractive solution for overcoming the selectivity of current biocatalysts is anaerobic fungi. Anaerobic fungi resident in the digestive tract of large herbivores express diverse enzymes that efficiently break down and ferment cellulosic substrates into carbon dioxide, hydrogen gas, and other bioenergy targets including alcohols. Here, we demonstrate that new anaerobic fungal isolates from horse, wildebeest, giraffe, and greater kudu degrade and grow on woody forestry products such as poplar, and an array of agricultural and food wastes. Importantly, several of our fungal isolates are robust against inhibitory lignin monomers. Unlike the industry standard *Trichoderma reesei*, whose enzymes and growth are inhibited up to 50% by increases in select lignin constituents, one of our isolates showed no direct correlation with increasing lignin constituents. We are now isolating and characterizing these enzymes for further study. The enzymatic diversity and this resiliency to lignin composition make anaerobic fungi attractive as microbial platforms for enzymes that may accelerate the development of more economical biofuels from more diverse substrates.

P109 One-step marker-less gene replacement in *Bacillus subtilis*

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Bacillus subtilis, an extensively studied Gram-positive bacterium, has been widely used in industry for the production of pharmaceutical proteins, enzymes, and antibiotics. It is also a model organism for studying sporulation and bacterial chromosome replication. In addition, *B. subtilis* is one of the most advanced model organisms for systems biology study and host for synthetic modules. Despite of its importance, genetic engineering of *B. subtilis* is far from efficient. Almost all of the genome modification methods rely on *E. coli-B. subtilis* shuttle vectors and genetic modification takes much longer time compared with *E. coli*. In addition, a resistance marker is often created for either gene deletion or insertion. The limitation of available resistance markers and long turnover time significantly impede the research related to *B. subtilis*. In this work, we developed an *E. coli* independent method for marker-less gene deletion or insertion. DNA fragments including the homologous fragments, a resistance marker, and an inducibly expressed I-SecI enzyme, are assembled using Gibson assembly and directly transform *B. subtilis*. Double crossover homologous recombination followed by chromosome linearization at a designed I-sceI site will force the removal of the resistance marker and the I-secI, leaving no marker or scar sequence. Using this method the *amyE* gene was deleted and a *gfp* expression operon was inserted into the *amyE* site. This one-step marker-less integration method provides a flexible method for genetic modification in *B. subtilis* with significantly reduced turnover time.

P111 Genetic and cell biological tools in *Yarrowia lipolytica*

E. Bredeweg, K. Pomraning, Z. Dai and S.E. Baker, Pacific Northwest National Laboratory, Richland, WA, USA*

Yarrowia lipolytica is an ascomycete yeast used in biotechnological research for its abilities to secrete high concentrations of proteins and accumulate lipids. In order to expand capabilities of

P113 2,3-butanediol production with metabolically engineered *E. coli*

S. Pflügl, A.M. Erian and C. Herwig, TU Wien, Vienna, Austria*

Due to the prospect of ending crude oil reserves and the need to explore alternative ways to produce building block and fuel chemicals, research efforts for alternative production platforms like biotechnological processes have been increasing. *E. coli*, one of the working horses of biotechnology, has been used extensively in metabolic engineering and bioprocess development approaches to produce a wide variety of different chemicals, including 2,3-butanediol. Here, we present an engineered *E. coli* strain with the ability to produce 2,3-butanediol. Strain construction was done with special emphasis on (i) the use of genes from three different donor organisms and (ii) the use of different promoter strengths for the individual genes to study the effect on the overall flux through the 2,3-butanediol pathway. The obtained construct library was initially screened in shake flasks to identify the most promising construct. Additionally, different host strains of *E. coli* were tested for their suitability to produce 2,3-butanediol. It turned out that genes from *Enterobacter cloacae* subsp. *dissolvens* in combination with *E. coli* W strain showed the highest product titer in small scale batch cultivations. Small scale bioreactor cultivations revealed aeration and pH value as the crucial parameters for 2,3-butanediol in batch cultivations. The use of different carbon sources on defined medium proofed our recombinant strain to be very robust in terms of feed stock utilization and media requirements. These results proof to be a good starting point for further strain and systematic bioprocess development efforts to obtain an economically feasible production process for 2,3-butanediol.

P115 Systems biology of the gas-fermenting acetogen *Clostridium autoethanogenum*

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Gas-fermenting acetogens are promising cell factories for the production of fuels and chemicals from inexpensive waste feedstocks (e.g. syngas, CO). Systems-level description and model-based analysis of

acetogen metabolism is needed to accelerate metabolic engineering. Thus we aimed to characterise and engineer acetogen metabolism using multi-omics fermentation data and a genome-scale metabolic model (GEM).
We started by testing the developed GEM iCLAU786 of *Clostridium autoethanogenum* for finding alternative ATP-generating pathways. Experiments confirmed the amino acid preference predicted by modelling. Moreover, we discovered that arginine catabolism through the arginine-deiminase pathway significantly increases ATP availability, resulting in faster growth and near-abolishment of acetate production. Next, we performed syngas-fermenting chemostat cultures where cells shifted from acetate to ethanol production for maintaining ATP homeostasis at higher biomass concentrations, but reach a limit at a molar acetate/ethanol ratio of ~1. This regulatory mechanism eventually leads to depletion of the intracellular acetyl-CoA pool and collapse of metabolism. We accurately predict growth phenotypes with the aid of transcriptomics data and constraining H_2 utilisation between its use for CO_2 reduction and generating reducing equivalents. Modelling revealed that the methylene-THF reductase reaction was ferredoxin-reducing. Lastly, syngas data was compared to growth on pure CO and a high H_2/low CO gas feed.

Our work provides the first quantitative links between carbon, energy, and redox metabolism in the ancient metabolism of acetogens. The data potentially benefits gas fermentation as a bioprocess by describing an alternative route for supplying cells with ATP and the effects of gas feed composition on autotrophic growth.

P29B Microfluidic droplet-based Sorting of lipase producing bacteria from environments

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Screen processes are routinely performed for obtaining new enzymes from natural microorganisms, as well as for directed evolution of strains from mutant libraries. Recently, droplet microfluidics have quickly emerged as a versatile and powerful tool for single cell screening with ultra-high throughput. In this work, we describe a microfluidic droplet-based pipeline for high throughput screening of lipase-producing bacteria, and validated its performance in the sorting and recovery of lipolytic bacteria from several environmental samples. First, we designed and made an integrated optical system which can be coupled with a standard inverted microscope for laser-activating and recording of fluorescence from picoliter droplets, as well as sorting of droplets using dielectrophoresis in microfluidic channels with a throughput up to 1,000 Hz. The whole fluorescence-activated droplet sorting (FADS) pipeline including picoliter droplet generation, droplet incubation, introduction of fluorogenic substrate using picoinjection, and sorting of droplets based on their fluorescence intensities. We used fluorescein dibutyrate (FDB) as the substrate to quantitatively screen the droplets containing lipase-producing species. The performance was evaluated with a lipase-producing *Pseudoalteromonas lipolytica* strain. The method was validated with three samples collected from highland wetlands, abandoned oil field, and Chinese liquor starter. In total, 36 isolates of 10 genus were obtained with lipolytic activity, validated by lipase plate assays. We believe that this new FADS pipeline can be instrumentalized for high throughput screening of bacterial lipase, as well as other enzymes with industrial importance, providing a high efficient, low consumption, and low cost alternative to current microtiter plate-based screening technologies.

P29C An *in vitro* biocatalytic platform for the production of β-hydroxyacyl chiral building blocks

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Chiral building blocks offer a promising route to semi-synthetically produce natural products and pharmaceuticals with complex stereochemistries. Here we report the development of an *in vitro* biocatalytic platform for the production of chiral β-hydroxyacyl-CoA molecules that function as precursors to diketide and triketide chiral building blocks. By coupling a Coenzyme A generation system (*CoaA, CoaD, & CoaE*) with promiscuous acyl-CoA ligases, we have produced a variety of acyl-CoA species from

pantetheine, ATP, and a carboxylic acid of choice. In the presence of a β-ketoacyl thiolase an acyl-CoA molecule is condensed with acetyl-CoA to form a β-ketoacyl-CoA molecule. The β-ketoacyl-CoA is subsequently reduced by an acetoacetyl-CoA reductase (driven by an NADPH-regeneration system) to produce (3*R*)-3-hydroxyacyl-CoA compounds. Using LC/MS analysis we have determined the production of (3*R*)-3-hydroxybutyryl-CoA and (3*R*)-3-hydroxyvaleryl-CoA from this 7-enzyme one-pot reaction. Ultimately, we seek to produce a wider variety of β-hydroxyacyl-CoA molecules on a preparative scale for use as chiral building blocks.

P129D Metabolic engineering of *Escherichia coli* for glycodiversified flavonoids production

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Sugars are structurally and biologically important units present in most of the currently used natural product based therapeutics. Since, they play significant role in executing biological activities of antibiotic, anticancer, and antifungal compounds; engineering of sugar moieties in natural products is increasingly becoming a tool to design or produce novel biologically potent therapeutics. Flavonoids are considered as bioactive molecules, thus, generation of novel/unnatural derivatives of flavonoid glycosides could have great applications in nutrition, cosmetics, and therapeutics. The novel glycosides could exhibit better stability and bioavailability as many flavonoid *O*-glycosides once ingested, lose their sugar moiety relatively fast and metabolized to other products.

To develop the rapid and sustainable system for the production of different flavonoid glycosides, central metabolic pathway for the production of pool of UDP-D-glucose, UDP-D-xylose, TDP-L-rhamnose, TDP-D-viosamine, TDP 4-amino 4,6-dideoxy-D-galactose, and TDP 3-amino 3,6-dideoxy-D-galactose was engineered in *E. coli* BL21(DE3) cells. Several possible genes were knock-out to divert carbon-flux towards either TDP- or UDP-sugars in *E. coli* cell cytosol. Moreover, biosynthetic pathway genes of respective NDP-sugars were overexpressed from different sources. The genes were assembled in either commercial expression vectors or newly designed synthetic vectors. Glucose facilitator proteins were used to facilitate glucose transport inside the cell. Glycosyltransferases (GTs) from plants and microbes were engaged to transfer sugar moiety from activated NDP-sugars to flavonoid acceptors. As a result, several flavonoids and isoflavonoid glycosides including quercetin 3-*O*-xyloside, kaempferol 3-*O*-glucoside, fisetin 3-*O*-glucoside, fisetin 3-*O*-rhamnoside, novel 4-amino 4,6-dideoxy-D-galactose and 3-amino 3,6-dideoxy-D-galactose sugars conjugated quercetin, kaempferol, and fisetin were produced by fermentation.

P116 A Novel Biocontainment Strategy Makes Bacterial Growth and Survival Dependent on Phosphite

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There is a growing demand to develop biocontainment strategies that prevent unintended proliferation of genetically modified organisms in the open environment. We found that the hypophosphite (H_3PO_2, HPt) transporter HtxBCDE from *Pseudomonas stutzeri* WM88 was also capable of transporting phosphite (H_3PO_3, Pt) but not phosphate (H_3PO_4, Pi), suggesting the potential for engineering a Pt/HPt-dependent bacterial strain as a biocontainment strategy. We disrupted all Pi and organic Pi transporters in an *Escherichia coli* strain expressing HtxABCDE and a Pt dehydrogenase, leaving Pt/HPt uptake and oxidation as the only means to obtain Pi. Challenge on non-permissive growth medium revealed that no escape mutants appeared for at least 21 days with a detection limit of 1.94×10^{-13} per colony forming unit. This represents, to the best of our knowledge, the lowest escape frequency among reported strategies. Since Pt/HPt are ecologically rare and not available in amounts sufficient for the growth of the Pt/HPt-dependent bacteria, this strategy offers a reliable and practical method for biocontainment.

P116A Expression of glycosyl hydrolase in *Zymomonas mobilis* enables conversion of cellobiose to biofuel

N. Kurumbang[*] and R. Landick, DOE Great Lakes Bioenergy Research Center, Madison, WI, USA

Biofuels from renewable biomass have the potential to replace petrofuels in future oil shortages while at the same time helping to maintain atmospheric carbon balance. Feedstocks derived from plant biomass contains a significant portion of oligosaccharides that are not utilized by the industrial microorganisms such as Escherichia coli, Zymomonas mobilis or Saccharomyces cerevisiae. For example, about 25% of the total soluble sugar content in AFEX-pretreated corn stover hydrolysate (ACSH) are oligosaccharides that are not consumable by traditional industrial microorganisms resulting in a significant reduction in overall biofuel yield. To address part of this problem, we engineered a Z. mobilis strain capable of heterologous expression of a glycosyl hydrolase enzyme and subjected it to adaptive laboratory evolution. We found that the evolved strain, Z. mobilis ZM4-cb, is capable of converting cellobiose to glucose and using it for cell growth and for production of ethanol. The adaptation proved to be reversible and to be caused by incubation of cells with either cellobiose or sucrose medium. After adaptation, the evolved strain could convert about 50% of cellobiose within 24 h and could grow on a medium containing cellobiose as the only carbon source.

Monday, July 31

7:00 AM - 8:00 AM SIMB News Editorial Board Meeting-Restaurant, Lobby Level near checkin

7:30 AM - 7:40 AM Exhibitor Showcases Autodesk New tools for DNA engineering will transform the world we live in. Genetic Constructor is an extensible CAD software, making powerful genetic engineering accessible in the cloud. Work on genetic designs at higher levels o

Governor's Square 16, Concourse Level

7:30 AM - 5:00 PM Registration

Plaza Registration - Concourse Level

8:00 AM - 11:30 AM Session: 1: Lignocellulosic Conversion Sponsored by NOVOZYMES

Conveners: **Brian Scott**, Novozymes, Davis, CA, USA and **Jennifer Headman**, Novozymes, Davis, CA, USA

Plaza Ballroom F -Concourse Level

8:00 AM S1: Bioprocessing of paddy straw to bioethanol in SSF using thermotolerant *K. marxianus* NIRE-K3

R. Arora, N.K. Sharma and S. Kumar, Sardar Swaran Singh National Institute of Bio-Energy, Kapurthala, India*

The progressive depletion of non-renewable energy sources worldwide, together with the fact that their use has resulted in environmental deterioration and public health problems, has led to development of renewable energy harvesting technologies. Current bioethanol research focusses mainly on waste lignocellulosic feedstocks like agricultural, industrial and municipal solid wastes due to their abundance and renewability. However, the current technologies in use suffer from various drawbacks including low ethanol yield and productivity, co-fermentation of pentose and hexose sugars, low tolerance to product and inhibitors. The present study was carried out using pretreated paddy straw under 0.5% NaOH treatment with 20% solid loading at 15 psi for 10 min. The pretreated straw was treated with Cellic Ctec 2 with enzyme loading of 60 mg enzyme protein g^{-1} dry biomass at 20% biomass loading and thermotolerant isolate *Kluyveromyces marxianus* NIRE-K3 cells at 45 ^{o}C in simultaneous saccharification and fermentation (SSF). SSF configuration was supplemented with medium components with composition (in g l^{-1}) yeast extract, 2.93; K_2HPO_4, 1.99; NaH_2PO_4, 0.24; $MgSO_4$, 0.42; $(NH_4)_2SO_4$, 1.34, pH 5.5. This resulted in maximum ethanol concentration of 25.34 g l^{-1} after 24 h which corresponds to 182.22 g ethanol/kg of raw paddy straw. Thus, it can be concluded that the thermotolerant isolate *K. marxianus* could be exploited for commercial scale bioethanol production from alkali pretreated paddy straw in SSF configuration.

8:25 AM S2: Temperature activation of cellulases

P. Westh, T. Sorensen, R. Tokin, S. Badino and J. Kari, Roskilde University, Roskilde, Denmark; K. Borch, Novozymes, Bagsvaerd, Denmark; B. McBrayer, Novozymes, Inc., Davis, CA, USA*

Recently, there has been much progress within discovery and engineering of thermostable cellulolytic enzymes. This is promising from an industrial perspective as saccharification of lignocellulosic biomass is best conducted at high temperatures. High temperatures are advantageous for different reasons including the repression of bacterial growth and reduction of viscosity, but the most important advantage is an increased reaction rate, which may lead to shorter process time and lower enzyme dosages. However, systematic investigations of temperature–activity relationships for cellulases are rare, and the potential activity gain of an increased process temperature remains uncertain.

We have studied temperature activation of the cellobiohydrolase Cel7A, and some other cellulases. In the range where the enzyme was stable, we found that the activity against a soluble substrate-analog approximately doubled for every 10°C temperature increment (Q_{10}≈2), as it is typical for hydrolases. On insoluble cellulose, however, the degree of temperature activation was much lower. This was shown to rely on a temperature dependent dissociation of enzyme from the substrate surface, which counteracted the inherent acceleration by higher temperature. Interestingly, enzyme variants that were engineered to have higher affinity for the substrate were less prone to dissociation and hence more activated by temperature.

8:50 AM S3: Unraveling the hidden nature of LPMOs: fundamental and applied consequences

B. Bissaro and V.G.H. Eijsink, Norwegian University of Life Sciences, Aas, Norway*

The depolymerization of complex plant biomass represents a major pillar in the Earth´s carbon cycle and relies on a network of enzymatic and chemical reactions that is still full of mysteries. For decades, the degradation of recalcitrant polysaccharides was thought to be mainly achieved by an arsenal of hydrolytic enzymes called glycoside hydrolases (GHs). This paradigm changed in 2010 with the discovery of a new class of enzymes today known as Lytic Polysaccharide Monooxygenases (LPMOs) (Vaaje-Kolstad et al., 2010, *Science*). LPMOs are mono-copper enzymes that have the unique ability to carry out oxidative

cleavage of crystalline polysaccharides, thus making glycosidic chains more accessible for canonical GHs. Inspired by Nature, where LPMOs are notably abundant in wood-rotting fungal secretomes, these enzymes also have become instrumental for the development of economically sustainable lignocellulose biorefineries (Johansen et al., 2016*, Biochem.Soc.Trans.*). However, the mode of action of LPMOs remains largely enigmatic, which potentially hampers their optimal industrial harnessing.
We have recently been considering the possibility that the classification of LPMOs as "classical" monooxygenases may need to be revised. Here, we provide compelling evidence that H_2O_2, and not O_2, is the co-substrate of LPMOs during the reaction of polysaccharide oxidation (Bissaro et al., 2016, *bioRxiv* 097022). These findings are shattering established dogmas and offer new perspectives regarding the mode of action of copper enzymes and the enzymatic conversion of biomass in Nature. We will also illustrate how the design of industrial lignocellulose biorefining processes may be improved based on our recent findings.

9:15 AM Break

9:45 AM S4: Determinants of lytic polysaccharide monooxygenase reactivity on polysaccharide substrates

K. Salomon Johansen, Chalmers University of Technology, Gothenburg, Sweden*

Lytic polysaccharide monooxygenases (LPMOs) are industrially important enzymes that are found widespread in the microbial world. LPMOs carry out oxidative cleavage of glycosidic bonds in recalcitrant polysaccharides such as cellulose and thereby boost the activity of glycosyl hydrolases. LPMOs have evolved to become substrate controlled but in the absence of substrate and in the presence of oxygen and a reducing agent, LPMOs release reactive oxygen species as a product of each redox cycle. Liberation of reactive oxygen species may cause chemical chain reactions that are detrimental to enzymes and to the microorganism. Oxidative inactivation of commercial cellulase mixtures has been shown to be a significant factor influencing the overall saccharification efficiency and the addition of catalase can successfully be used to protect cellulases mixtures from inactivation. In contrast, addition of hydrogen peroxide to the saccharification slurry after liquefaction had a clear negative effect on glucose yield. It is thus important to be able to understand the details of oxygen activation at the mononuclear copper active site of LPMOs. We have studied the interaction between two closely related members of the AA9 enzyme family and several polysaccharides. Although both enzymes acts on a range of polysaccharides, there are distinct differences which will be discussed.

10:10 AM S5: Cellulosic ethanol from corn kernel fiber

M. Tse, DuPont Industrial Biosciences, Palo Alto, CA, USA*

Gen 1.5 describes the process of converting corn kernel fiber into ethanol. This process can give up to 10% more ethanol gallons, improve corn oil recovery by up to 70% and yields DDGs with improved digestibility. We will provide a review of current Gen 1.5 technologies in the market, and the prerequisites for this technology. We will discuss the science behind mechanical, chemical and enzymatic corn fiber treatment. We will present results on corn fiber saccharification and fermentation using enzymes and describe how different cellulolytic enzymes act on corn fiber. We will also provide an overview of the value of Gen 1.5 technology.

10:35 AM S6: Expression of fungal cellulases in the oleaginous yeasts for biofuels production

M. Zhang, Q. Xu, H. Wei, E. Knoshaug, M. Alahuhta, W. Wang, J.O. Baker and M.E. Himmel, National Renewable Energy Laboratory, Golden, CO, USA; H. Alper, The University of Texas at Austin, Austin, TX, USA*

One of the technical barriers for biofuels production from biomass is the costly cellulases required to break down lignocellulosic feedstocks to sugars due to the general recalcitrance of plant cell walls. To overcome this hurdle, a “one pot” or “consolidated bioprocessing (CBP)” biomass processing scheme has been proposed, which includes cellulase production, cell wall polymer hydrolysis, and sugar fermentation in a single step. Simultaneous production of cellulases in fermentation host organisms for ethanol production especially in *Saccharomyces cerevisiae* has been explored. We are investigating the potential of the oleaginous yeasts as CBP host for production of long chain hydrocarbon biofuels. Cellobiohydrolase I (CBH I) is a key cellulase required for effectively break down cellulose and is found difficult to be functionally expressed in fermentation organisms at high level. While the most active *Trichoderma reesei* CBH I failed to be expressed successfully, recently researchers discovered the *Talaromyces emersonii-Trichoderma reesei* chimeric cellobiohydrolases I (Te-Tr chimeric CBH I) can be functionally expressed in *S. cerevisiae*. We showed here that the chimeric CBH I as well as its newly constructed fusion proteins can be expressed in oleaginous yeasts, *Lipomyces starkeyi* and *Yarrowia lipolytica.* All parent chimeric CBH I and fusion proteins are highly active and converted pretreated corn stover substrates. In addition, we will also discuss the co-expression of fungal cellulases including CBH I, CBHII and EGII in the oleaginous yeast and their impact on lipid accumulation.

11:00 AM S7: Isolation of levoglucosan-utilizing bacteria and crystallographic characterization of levoglucosan dehydrogenase

A. Arya and M.A. Eiteman, University of Georgia, Athens, GA, USA*

Pyrolysis is one means of creating bio-oils, bio-chars, and syngas useful as combustible biofuels from lignocellulosic materials. The pyrolysis process also generates high yields of the anhydrosugar levoglucosan. Because this sugar is not readily metabolized by typical production organisms, there has been an interest in expressing enzymes which catabolize levoglucosan in production organisms. Levoglucosan dehydrogenase (LGDH) is the only known bacterial enzyme to act on levoglucosan, and previously only one species of *Arthrobacter* has been observed to express LGDH. It was proposed that intracellularly this enzyme oxidizes levoglucosan to 3-keto-levoglucosan, which then chemically hydrates to 3-keto-glucose before reducing this substrate to glucose. Is this enzyme and its associated metabolic pathway specific to a particular taxonomic group of bacteria? What residues or structural moieties make LGDH specific to levoglucosan conversion?

Four bacterial strains were isolated showing growth on levoglucosan and LGDH activity. Through Illumina sequencing these isolates were determined to be *Microbacterium* sp., *Paenibacillus* sp., *Shinella* sp., and a strain of *Klebsiella pneumoniae*. The gene *lgdh* has been identified in all isolates, and we are presently looking at neighboring genes for other enzymes critical to levoglucosan conversion.

To understand how LGDH interacts with levoglucosan, the LGDH from *Arthrobacter phenanthrenivorans* Sphe3 was crystallized. X-ray diffraction data were used to assemble enzyme structures, revealing that while major structural features including the Rossman-fold for NADH binding and the carbohydrate active site are conserved relative to known dehydrogenases, LGDH has several different residues in the carbohydrate-binding site likely responsible for its preferential activity on levoglucosan.

8:00 AM - 11:30 AM Session: 2: Electric Microbiomes

Conveners: **Gemma Reguera**, Michigan State University, East Lansing, MI, USA and **Dr. Orianna Bretschger**, J. Craig Venter Institute, La Jolla, CA, USA

Governors Square 15, Concourse Level

8:00 AM S8: Exoelectrogens in the gut microbiota and their influence on development of the host immune system

A. Ericsson, University of Missouri, Columbia, MO, USA*

Exoelectrogenic microbes capable of transferring electrons to an extracellular acceptor are attractive means of converting organic waste into usable end products, and many exoelectrogens are found in host-associated communities such as the gut microbiota (GM). As certain eukaryotic cell types including lymphocytes migrate within the body in response to both chemical and electrical gradients, we hypothesized that differences in the exoelectrogenic capacity of gut microbiota contribute to differences in gut lymphocyte recruitment during development. Initial testing of mice from different commercial sources revealed vendor-dependent differences in the exoelectrogenic capacity of GM, regardless of host genetic background, and this difference correlated with lymphocyte numbers in the gut. This exoelectrogenic capacity and greater abundance of gut lymphocytes could both be transferred via gavage of fecal material. Migration of green fluorescent protein (GFP)-expressing lymphocytes following intravenous transfer to two groups of mice lacking an endogenous adaptive immune system differed depending on the exoelectrogenic capacity of the GM. Gene expression assays found no difference in canonical chemokine expression in the host. However, microbial communities demonstrating current production in microbial electrolysis cells (MECs) demonstrated significantly greater expression of genes relevant to electron transfer including OmcA and MtrC, and consistently showed fine pilus-like structures connecting bacterial cells within MECs. Current studies performed during maturation of both host and microbiota reveal stark differences in the *Proteobacteria* content in the different GM cohorts which correlate with electron transfer and lymphocyte numbers found in adults, suggesting that these mechanisms are active very early in life.

8:30 AM S9: Optimizing electromethanogenesis using stimulus-induced metatranscriptomic information

O. Bretschger, Aquam, LLC, San Diego, CA, USA; K. Carpenter, Columbia University, New York, NY, USA; S. Ishii and S. Suzuki, JAMSTEC, Kochi, Japan; S. Phadke, J. Craig Venter Institute, La Jolla, CA, USA; M. Flynn and J. Hogan, NASA Ames Research Center, Mountain View, CA, USA*

Electromethanogenesis is the process whereby microbes utilize electricity as an energy source, and reduce carbon dioxide to methane in a bioelectrochemical system (BES). This process may have several benefits toward the sustainable production of biofuels; however, a fundamental understanding about the mechanisms underlying how microbial communities utilize electricity to reduce CO_2 may have broader implications towards the synthesis of several useful products including high value chemical precursors. Here we utilized a novel stimulus-induced metatranscriptomic approach, in combination with metagenomics, to identify the dominant microbial taxa in an enriched electromethanogenic biofilm; and quantify the dynamic responses of key genes associated with electron uptake, hydrogen production/utilization and carbon dioxide reduction in duplicate BES reactors.

Results from the metagenomic analyses show that the same strains of methanogens, sulfate reducers and fermenters were present in the replicate reactors; however, they were present in different relative abundances, which may explain the overall differences in electricity consumption and methane production. Further, removing the availablity of electricity as an energy resource induced different functional responses from each microbial community, which also indicated that the relative abundance of species impacts how they functionally cooperate in BESs. Future work will address the specific microbial interactions that correlate with these performance differences.

9:00 AM S10: Industrial microbiology wires up

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

In Nature, highly efficient and diverse consortia of microbes cycle carbon and other elements in electrochemical reactions that discharge metabolic electrons onto soluble and insoluble metals. Driving these reactions are electric microbes with the ability to electronically couple their metabolism to other consortia members and minerals. Studies in model electric microbes such as the iron-reducing *Geobacter* bacteria have identified several strategies by which microorganisms can potentially exchange electrons. Strategies include the production of conductive peptides that assemble to form protein nanowires, interactions with conductive minerals, and synthesis of electroactive exopolysaccharide matrices that allow for the formation of current-harvesting biofilms on electrodes. Molecular understanding of these

mechanisms of electron transfer opens opportunities to custom-design and mass-produce conductive peptides for integration in electronic devices. Furthermore, the recombinant peptides can be assembled *in vitro* to make synthetic protein nanowires. Of special significance is the ability of the recombinant peptides and protein nanowires to retain the ability to bind and reductively precipitate metals, a process that could be harnessed to develop sensors and deployable devices for bioremediation. The ability of these bacteria to form electroactive biofilms on electrodes can be harnessed to engineer bioelectrodes and power electro-fermentations that mimic the electronic coupling of fermentative and electric microbes in Nature. The bioelectrodes harvest current from unwanted fermentation products, simultaneously increasing product titers and/or productivity and reducing the cost of downstream purification schemes. By 'wiring up' a synthetic consortium, new fermentation-based technologies become economically feasible.

9:30 AM Break

10:00 AM S11: Redox regulation of community behavior in *Pseudomonas aeruginosa*

L. Dietrich, Columbia University, New York, NY, USA*

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

10:30 AM S13: "*Candidatus* Tenderia electrophaga" is responsible for cathodic current in an aerobic biocathode community

S. Glaven, A. Malanoski, B. Eddie, Z. Wang, M. Yates, L. Tender and N. Lebedev, Naval Research Lab, Washington, DC, USA; N. Kotloski, George Mason University, Fairfax, VA, USA*

Bacteria perform extracellular electron transfer (EET) enabling electrical interactions between biofilms and surfaces. Our understanding of the microbial electrical wiring enabling this connection is remarkably limited. Understanding electrode EET could result in leap-ahead advancements in microbial electrosynthesis and microbial bioelectronics. Using a meta-omics approach we have expanded our understanding of uncultivable electroautotrophs, bacteria presumed to "eat" electricity and use the energy gained to fix CO_2. Metagenomic, metaproteomic and metatranscriptomic data supports the discovery of an electroautotroph from a marine biocathode microbial community referred to as Biocathode-MCL, "*Candidatus* Tenderia electrophaga". Initial metagenomic analysis of one MCL bioelectrochemical system (BES) resulted in 16 bin genomes. To further resolve bin genomes and compare community composition across replicate MCL BES, we performed shotgun metagenomic and 16S rRNA gene (16S) sequencing at steady state current. Clustering pooled reads from replicate BES increased the number of bin genomes to 20, over half of which were >90% complete. Direct comparison of unassembled metagenomic reads and 16S OTUs predicted higher community diversity than the assembled/clustered metagenome and the

predicted relative abundances did not match. When 16S OTUs were mapped to bin genomes and genome abundance scaled by 16S gene copy number estimated relative abundance was similar to metagenomic analysis. The relative abundance of "*Ca.* Tenderia electrophaga" was correlated with increasing current, further supporting the hypothesis that it is an electroautotroph. Results presented here provide guidance for metagenomic and 16S sequencing of low complexity microbial communities that may contain previously uncharacterized microorganisms, such as those associated with biocathodes.

8:00 AM - 11:30 AM Session: 3: Metabolic Engineering for Fuels and Chemicals I Sponsored by REG

Conveners: **Mark Blenner**, Clemson University, Clemson, SC, USA and **Priti Pharkya**, Genomatica, Inc., San Diego, CA, USA

Plaza Ballroom A & B - Concourse Level

8:00 AM S14: Metabolic engineering of bacteria for production of oleochemicals

B.F. Pfleger, University of Wisconsin - Madison, Madison, WI, USA*

Advances in metabolic engineering, synthetic biology, and other bioengineering disciplines have expanded the scope of what can be produced in a living organism. As in other engineering disciplines, synthetic biologists want to apply a general understanding of science (e.g. microbiology and biochemistry) to construct complex systems from well-characterized parts (e.g. DNA and protein). Once novel synthetic biological systems (e.g. enzymes for biofuel synthesis) are constructed, they must be engineered to function inside evolving cells without negatively impacting the host's physiology. In this talk, I will describe pathways for producing high-value commodity chemicals derived from fatty-acids and how my group and others have combined synthetic biology and systems biology to improve oleochemical production in bacteria using sustainable feedstocks. The talk will describe the critical regulatory points in native fatty acid metabolism, strategies for deregulating the pathway, and alternatives that by-pass it altogether. I will highlight the use of heterologous plant and bacterial enzymes to alter the chain length distribution of products from common long-chain molecules to higher-value medium-chain analogs. I will also describe our collaborative efforts with the Maranas lab at Penn State to use computational tools to redesign the substrate specificity of enzymes used to produce medium chain oleochemicals. Here, we have used the IPRO software to identify a small (~60) library of thioesterase mutants that resulted in a new variant that produces 2-fold more fatty acid with over 50% C_8 chain lengths, molecules not produced by the native enzyme.

8:30 AM S15: The emerging organism engineering industry

J. Cui, Ginkgo Bioworks, Boston, MA, USA*

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

high resolution-accurate analytical methods to develop engineered microbes. Finally, I'll touch on how our improvements in manufacturing organisms lend to opportunities outside of cultured ingredients.

9:00 AM S16: Design rules for engineering a nutrient assimilation system in *Saccharomyces cerevisiae*

V. Endalur Gopinarayanan and N. Nair[*]*, Tufts University, Medford, MA, USA*

Nutrient assimilation is a key first step in designing synthetic biological systems to produce value-added products. However, design rules to implement synthetic regulatory systems that control nutrient assimilation are relatively underdeveloped. Nature generally prefers nutrient-responsive regulation of metabolic genes for growth and metabolite production through genome-scale regulatory systems called regulons. Yet, metabolic engineers have largely eschewed such systems for simplistic constitutive gene overexpression to achieve the same objective of rapid nutrient assimilation. In this study, we used a model eukaryote, Saccharomyces cerevisiae and compared the two strategies for their growth benefits in a native nutrient, galactose. We observed significant growth benefits with the regulon over constitutive expression. We then tested if the observation holds true even in the case of a synthetic regulon that senses and responds to a non-native nutrient, xylose. To that end, we re-engineered the galactose regulon to function as a xylose sensing regulon through directed evolution of galactose sensor to sense xylose, incorporation of a dual positive feedback loop to emulate the feedback in galactose regulon and placed a minimal set of xylose metabolic genes under the xylose regulon. When compared with constitutive expression strategy, the xylose regulon exhibited significantly higher growth rates in xylose, better nutrient consumption and better growth fitness. While strategies and rules have been mapped out for production of downstream metabolites and proteins, this is the first work that provides a general design guideline for incorporating a non-native nutrient assimilation system by comparting several regulatory strategies to direct efficient non-native nutrient assimilation.

9:30 AM Break

10:00 AM S17: Strategies for metabolic engineering and optimization of *S. cerevisiae* for the production of sustainable, next-generation functional and specialty ingredients

P. Prochasson[*]*, Evolva, Lexington, KY, USA*

Humankind has long relied upon yeast for making wine, beer and bread. Today, *S. cerevisiae* has become the go-to yeast for producing sustainable, next-generation specialty and functional ingredients by fermentation. Evolva works almost exclusively with *S. cerevisiae*, leveraging it for the production of ingredients like stevia sweeteners, nootkatone, and valencene, which all use the same metabolic pathway to produce the isoprene precursor farnesyl pyrophosphate (FPP). With baker's yeast, brewing and biotechnology, Evolva can sustainably produce in a single fermentation batch the same volume of Valencene that would otherwise require 1 million metric tons of Valencia oranges. We present an approach of combinatorial genome integrations of the mevalonate pathway genes into yeast to maximize carbon flux towards terpene production. Flux imbalance during the engineering process can negatively affect terpene production, especially when scaled-up. Therefore, we apply a stepwise engineering method, taking advantage of the "push and pull strategy" to minimize the accumulation of inhibitory concentrations of metabolites and maximize carbon flux to product formation. The end result is an efficient, cost-effective and sustainable approach to producing high-value, next-generation ingredients.

10:30 AM S18: Metabolic engineering of *Pseudomonas putida* KT2440 for production of muconic acid from sugar

C.W. Johnson, P. St John, D. Salvachúa, H. Smith, D.J. Peterson, P. Khanna, G. Dominick and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Recent interest in muconic acid as a biological product stems from its efficient and environmentally-friendly chemocatalytic conversion to adipic acid, a precursor to polymers such as nylon 6,6 and terephthalic acid. The saprotrophic soil bacterium *Pseudomonas putida* KT2440 generates muconic acid as an intermediate in the catabolism of lignin-derived aromatic molecules. Here we describe a *P. putida* KT2440-based strain engineered to accumulate muconic acid produced from sugars by an exogenous pathway bridging the shikimate pathway for aromatic amino acid synthesis to aromatic catabolism and model-driven approaches to increase the flux of carbon to this product. The resulting strain achieves a yield of >30% (mol/mol) from glucose, on par with the highest performing strains reported, without the use of plasmids or auxotrophic mutations that would preclude utility in a commercial process.

11:00 AM S19: The development of a biofabricated leather material production process through the synergy of high yielding collagen production process and a bottom-up recapitulation of leather.

D. Williamson, L. Dai, J. Borden, B. Purcell and H. Varadaraju, Modern Meadow, Ny, NY, USA*

Modern Meadow is a New York based company pioneering a new age of biofabricated materials. By harnessing the power of design, biology, and engineering, Modern Meadow is creating new materials produced through novel processes enabling new design and performance possibilities beyond those in either traditional leathers or synthetics. Engineering at the subcellular and macroscopic level offers the opportunity to take a bottoms-up approach enabling the creation of exciting new materials with truly tunable properties based on the best that both biology and material science has to offer.

This presentation will provide an overview of the company, technical approaches to create the worlds first biofabricated leather materials and the properties for this new family of materials. Here at Modern Meadow we have successfully developed a series interconnected processes enabling the creation of leather materials using the basic building block of leather - collagen. The process was started with the production of an engineered type 3 collagen produced via a highly efficient protein production process. The purified collagen protein was subsequently transformed into a complex hierarchy of fibrils and fibers through a trade secret protected process. This collagen biocomposite was subsequently converted into a biofabricated leather material through the application of conventional tanning and retanning chemistries. These materials have been shown to exhibit a property set similar to traditional leather.

8:00 AM - 11:30 AM Session: 4: Screening of Natural Products for Drug Discovery

Conveners: **Sung Ryeol Park**, Baruch S. Blumberg Institute/Natural Products Discovery Institute, Doylestown, PA, USA and **Dr. Ashootosh Tripathi**, University of Michigan, Ann Arbor, MI, USA

Plaza Ballroom E - Concourse Level

8:00 AM S20: Discovery of agricultural fungicides from natural products

D. Hahn, Q. Xiong, S. Fotso, P. Graupner, C.A. Adame, G. Davis, C. Yao, K. Meyer and C. Klittich, Dow AgroSciences, Indianapolis, IN, USA*

A key driver in the development of new antifungal agents is the desire for new modes of action (MoA) to address expanding resistance to existing fungicides, open up options for new treatment regimens and/or reduce side effects. Unfortunately, the 12 drugs approved for antifungal treatment in humans represent only four antifungal MoA. Control of fungal diseases is also important in the agricultural setting, however,

unlike clinical drugs, at least 100 compounds representing over 30 MoA are used for fungicidal control in agriculture. New fungicidal MoA are essential to maintain robust tools to combat fungal diseases in the field setting. Natural products from microbes have played an important role in agriculture through the discovery of novel fungicides and new fungicidal MoA. The natural products strobilurin A and pyrrolnitrin resulted in the development of new fungicides with new MoA. Other natural products like the atpenins target existing fungicidal MoA. Through the screening of natural products and microbial extracts from a wide range of natural sources from around the globe, Dow AgroSciences has discovered a number of novel lead fungicidal chemistries. Among these lead chemistries is the novel natural fungicide the alveolides, from the fungus *Microascus alveolaris*. Natural fungicides, although potent *in vitro*, often lack the robust physical properties necessary for efficacy in the field setting (e.g. UV-stability, rainfastness). Using chemistry we are working to develop robust agricultural fungicides with new MoA to enhance the farmer's toolbox for maximizing agricultural yields.

8:30 AM S21: Pathway-targeted approaches to decode orphan NRPS and PKS pathways

J. Crawford, Yale University, West Haven, CT, USA*

Modular nonribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) enzymes represent multidomain systems responsible for the synthesis of a broad range of natural products with pharmaceutical value. The majority of these types of enzymes in expanding sequence databases are orphan, and their small molecule products remain unknown. Additionally, these enzymes can engage in "hybrid" systems to expand the structural and functional repertoire of their products. Here we discuss "pathway-targeted" and "domain-targeted" approaches to decode these orphan pathways with surgical genetic and metabolic precision. "Pathway-targeted" networking interfaces with an existing molecular networking pipeline (GNPS) and provides a visually appealing output to map relative fold-change levels, isotopic labeling information, and genetic parameters onto a targeted secondary metabolic pathway. "Domain-targeted" metabolomics combines genome editing with pathway analysis to define the general timing and functions of individual catalytic domains within these fascinating enzyme systems at the cellular metabolic level. Specifically, domain-targeted metabolomics provides a functional readout of the catalytic domain contributions to the pathway-dependent metabolome – "multidomain signatures" – which can be assigned to every node in a molecular network. These general approaches will be highlighted primarily in the context of two selected pathways, the "pepteridines" and the "colibactins." The pepteridines are new signaling metabolites from an unprecedented type of hybrid system that links NRPS machinery and pteridine synthase machinery. The colibactins are colorectal cancer-associated NRPS-PKS hybrid metabolites, and domain-targeted metabolomics defined their heterocycle assembly steps and revealed new metabolites with potent genotoxic scaffolds.

9:00 AM S22: Microbes to medicine: Development of a *Millennial* drug discovery platform

A. Tripathi, P. Schultz, J. Wu, C. Xi and D.H. Sherman, University of Michigan, Ann Arbor, MI, USA; S.R. Park, Baruch S. Blumberg Institute/Natural Products Discovery Institute, Doylestown, PA, USA*

If discovery of new antibiotics continues to wane while the ability of drug resistant pathogens continues to surge, society's medicine chest will soon lack effective treatments against a multitude of serious infections. To put the situation into context, over the last 30 years no new class of antibiotics has been introduced to mankind.[1] Moreover, the majority of pharmaceutical efforts during the past six decades have focused on the synthetic enhancement of a limited set of unique core scaffolds.[2] From these perspectives, it was envisioned that a more sustainable route to combat antibiotic resistance is the discovery of novel classes of antimicrobials, which would require a greatly improved antibiotic discovery process, and greater access to unique microbial targets. Here, I will describe a robust high throughput antibacterial discovery platform involving key resistance mechanism as target against gram-negative (*A. baumannii*) pathogenic microbes. The success of the approach provided solutions for two major bottlenecks that impede the drug discovery pipeline i.e., identification of novel drug leads and overcoming

resistance due to indirect microbial targeting.[3] Moreover, biosynthetic characterization of the discovered antibiotic unraveled a convergent nature of the gene cluster machinery.[4] This genetic bifurcation was then further explored to biochemically analyze and structurally characterize the substrate scope of a key gatekeeper enzyme. The study led us to isolate novel congeners through targeted precursor incorporation, resulting in production of a potent *Acinetobacter baumannii* biofilm inhibitor. Further information on the analytical approach that is being applied for identification of new classes of antibiotics will be discussed.

9:30 AM Break

10:00 AM S23: Mining uncultured bacteria with comparative metagenomics and metatranscriptomics

I. Miller, S. Carlson, J. Lopera, I. Miller, W. Rose and J. Kwan, University of Wisconsin-Madison, Madison, WI, USA; M. Puglisi, Chicago State University, Chicago, IL, USA; R. Kerby and F. Rey, University of Wisconsin - Madison, Madison, WI, USA*

Bacteria are incredibly talented at making intricate small molecule structures, that are frequently bioactive. However, the vast majority of bacteria have never been cultured in the laboratory, representing a potentially rich vein of unexploited biosynthetic potential. Amongst bacteria that are least likely to be cultured are a group that often produce ecologically important natural products - symbionts of higher eukaryotes. Ecologically important natural products are likely to have exquisite activity against an evolved target, and if there are related therapeutic targets, such compounds could be potential drug leads. We have devised an automated method of "binning" metagenomic contigs into individual microbial genome bins, allowing the study of complex microbial communities at the resolution of single species. We will present the application of this method to determine the response of individual bacteria in a complex marine sponge microbiome to the influx of non-symbiotic species, including the upregulation of specific biosynthetic pathways. Through the correlation of upregulated biosynthetic pathways with upregulated compounds and activities, such as bacterial biofilm inhibition, we are able to discover both compounds and their biosynthetic pathways in parallel, in a microbial ecology-guided manner.

10:30 AM S24: Bacterial communication in situ

A. Condren, K. Zink, C. Clark, S. Costa, B. Murphy and L. Sanchez, University of Illinois at Chicago, Chicago, IL, USA*

Microbiome studies have laid the groundwork demonstrating that bacteria are major players in host health, however the exact mechanisms by which these bacteria influence or protect hosts represents a major gap in knowledge. There are strong correlative observations from microbiome surveys that suggest microbes play essential roles in host function, but very little work has been done to uncover the chemical mechanisms underlying host-microbe interactions. We have developed orthogonal approaches to study natural products in situ. First, we have developed a matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) pipeline to rapidly profile metabolic fingerprints directly from Petri dishes. We simultaneously applied this technique to probe two model host-microbe interactions; one being a symbiotic relationship leading to colonization of epithelial cells by one specific bacterium (*Vibrio fischeri* and the Hawaiian bobtail squid), and the other exploring how enteric pathogens can colonize and infect a host vertebrate (*V. cholerae* and zebrafish). Both of these systems are native to the macroorganism and microbe, allowing us to fully characterize the underlying chemistry involved in colonization, infection, and maintenance of the relationship. We use a combination of MALDI-TOF imaging MS techniques and orthogonal molecular networking approaches to probe these two distinct systems.

11:00 AM S25: Screening a natural products library for HBsAg secretion inhibitors

J. Clement, S. Rawat, D. Solaiman and T. Zhou, Baruch S. Blumberg Institute, Doylestown, PA, USA; M. Goetz, M. Todd and S.R. Park, Baruch S. Blumberg Institute, DOYLESTOWN, PA, USA*

The inhibition of hepatitis B virus S-antigen (HBsAg) secretion is a potentially useful target for the development of therapeutic agents for the treatment of chronic HBV infection. We have recently developed an assay for screening for HBsAg secretion inhibitors with several improved features over previous assays. Using this assay and selected follow-up assays, we have identified several extracts derived from actinomycete fermentations with HBsAg secretion inhibitory activity. We will present our results in the isolation and identification of the compounds responsible for this activity.

8:00 AM - 11:30 AM Session: 5: Recent Advances in Microbial Control

Conveners: **Robert Donofrio**, Neogen Corporation, Lansing, MI, USA and **Christopher Cornelison**, Georgia State Univeristy, Atlanta, GA, USA

Governor's Square 14 - Concourse Level

8:00 AM S26: Modifications to a biofilm growth reactor method for multikingdom biofilm studies

D. Walker, Montana State University, Bozeman, MT, USA*

The Standardized Biofilm Methods Laboratory (SBML) at the Center for Biofilm Engineering was designed to meet research and industry needs for standard analytical methods in order to evaluate innovative biofilm control technologies. SBML staff and students develop, refine, and publish quantitative methods for growing, treating, sampling, and analyzing biofilm bacteria. The SBML works with international standard setting organizations such as AOAC International, ASTM International, IBRG, and OECD on the approval of biofilm methods. To date, five biofilm methods have been standardized through ASTM International. These methods grow *Pseudomonas aeruginosa* biofilms, a representative and ubiquitous bacterium found in many environments. The study and testing of other bacterial biofilms is also of interest to many industry and academia. Modifications to the standard method are therefore required with respect to growth temperature, media type and/or concentrations, or incubation times. The SBML has developed a protocol with modifications to standard method ASTM E2562-12 for growing a multi-kingdom biofilm, which includes *Legionella pneumophila* and *Vermamoeba (Hartmannella) vermiformis*. This protocol is the first stage in the development of a cooling tower model (or other environments where *Legionella* is of concern) designed for *in vitro* efficacy testing.

8:30 AM S27: Assessment of hydrophobic and hydrophilic copper-treated fabric for susceptibility to fungal growth

D. Price, Interface Inc., Lagrange, GA, USA; D.G. Ahearn and R. Simmons, Georgia State University, Marietta, GA, USA*

The responses of common environmental fungi of the genera *Aspergillus, Curvularia, Fusarium* and *Trichoderma* to exposures to textiles treated with polymer composites based on copper nanoparticles (CuNP) are ill-defined. Recently various natural fiber textiles (cotton-cellulose) and synthetic polymers both with superhydrophobic capacities have been marketed. Herein, we comparatively challenge proprietary hydrophobic synthetic textiles containing CuNP with *A. brasiliensis* and a mixed species challenge (ASTM G21) under essentially non-nutritive conditions. Additional challenges were conducted by atmospheric exposures in an environmental chamber (ASTM D3273). Samples for light microscopy were photographed with a Leica S6 stereo microscope and a Canon T3i camera in RAW format. Scanning electron microscopy was performed with a Tescan Vega 3 SEM; microanalysis with an EDAX Element EDS detector on the SEM. Compared to cellulose controls with confluent growth (ASTM G21)), hydrophobic-copper-treated fabric supported only latent sparse splotches of growth, but with well-defined

conidiophores and conidia of apparently select species, particularly *A. brasiliensis*. Similar growth of *A. brasiliensis* was recovered in the AATCC Method 30 test. Select fungi attached to fibers from the atmosphere. Hyphal development was sparse, but again with defined conidia and conidiophores. Hydrophobicity and microcycle conidiogenesis were implicated as factors for the survival-recovery patterns of the fungi. The data are reviewed vs that from prior studies with silver–treated materials.

9:00 AM S28: Interactions between bacterial isolates from different soils

E.D. Elder, Auburn University, Auburn University, AL, USA*

In spite of the urban location, the southwestern portion of the Auburn University campus includes a forested area that has largely been left untouched. The trees occur in groupings of southern yellow pines interspersed with a variety of hardwoods including pecans, oaks, and dogwoods. The areas selected for this research were within a grouping of the southern yellow pines and within a grouping of the native pecans. Soil samples, collected from each area, were used to make soil extracts which were incorporated into soil extract agars. Additional soil samples were collected, diluted, spread plated on soil extract agar, and incubated at 36^0 C. Morphologically distinct colonies were isolated and described. The distinctive colonies were then applied to streak plates in a manner that allowed each distinctive colony to be grown adjacent to every other distinctive colony – isolates from soil under pine tree were exposed to isolates from soil under pine trees and isolates from soil under pecan trees were exposed to isolates from soil under pecan trees. Following incubation at 36^0 C, zones where the isolates interacted were measured to determine whether the isolates showed synergistic, antagonistic, or neutral interactions. There were consistently fewer distinctive isolates from the soil under the pines (5 applicable colony types) than from the soil under the pecans (7 applicable colony types). The pine isolates all showed synergistic interactions while the pecan isolates showed 83.33 % synergistic interactions and 16.66% antagonistic interactions. No neutral interactions were measured for pine or pecan organisms.

9:30 AM Break

10:00 AM S29: Contact-independent microbial control using biologically-derived volatile organic compounds

K.T. Gabriel and S.A. Crow Jr., Georgia State University, Atlanta, GA, USA; C.T. Cornelison, Kennesaw State University, Kennesaw, GA, USA*

Volatile organic compounds (VOCs) are organic chemicals typically characterized as having low molecular weight, low solubility in water, and high vapor pressure. Consequently, they readily evaporate from liquid to the gaseous phase at standard temperature and pressure. VOCs are produced by many microorganisms as a result of both uninduced and induced metabolic pathways. Volatile-based microbial inhibition in environments such as soil is well-founded, with numerous antimicrobial VOCs and formulations having been identified. Inhibitory VOCs are of particular interest as microbial control agents, as low concentrations of gaseous VOCs have been observed to elicit significant antimicrobial effects. It is believed that this contact-independent antagonism may present unique advantages over traditional microbial control methods, particularly where contact-dependent treatment methods are either impractical or inconvenient. This method may be of particular benefit for managing infections where disease may become pervasive in the population, such as with white-nose syndrome (WNS) of bats.
VOCs were screened for their in vitro ability to inhibit growth of select pathogenic fungi. A dispersal system was developed that entailed circuit and software engineering as well as quantitative analysis to validate consistent and accurate dispersal of potential treatment compounds and formulations. Ex and in situ treatment trials were conducted to determine efficacy of promoting the reduction of disease severity and increasing survivorship of infected bat populations. The identification of volatile-based inhibitory compounds, in conjunction with a novel method for accurate and automated delivery, could prove a promising treatment and prophylactic in combatting microbial pathogenesis and contamination.

10:30 AM S30: Recent trends in microbial control for energy production.

J.S. Rajan, Dow Microbial Control, Collegeville, PA, USA*

Advanced stimulation techniques like hydraulic fracturing and enhanced oil recovery are water-intensive processes that are susceptible to bacterial contamination. Left unchecked, the microbes will contribute to reservoir souring, corrosion of pipelines and equipment, and formation plugging. Studies using modern molecular approaches are able to track organisms found in surface water, and have shown that many of these microorganisms survive and remain metabolically active in deep well environments. Our understanding of microbial populations—both inherent and developing—in the deep well environment as well as in flow-back waters has grown, especially with increased reuse of produced water. Repurposing of such produced waters, which was previously disposed off by deep-well injection, have now acquired additional significance since the implication of such disposal practices in seismic activities in some regions of the US. Moreover, recycling of produced water for hydraulic fracturing is a common practice in circumstances where water availability has become scarce, and the additives used, including biocides, are expected to provide performance under conditions that are no longer suitable for their effectiveness. More recently, superior testing techniques have been employed to simulate the effect of heat aging on produced waters and successfully predicting the ability of biocides to survive and be active under deep well conditions. Such techniques are gaining acceptance for not only preparing waters, but to decontaminate these wells and protect their reservoirs, contributing to the sustainability of energy production and preserving a very valuable natural resource.

11:00 AM : Discussion Session - 5th RAMC

R. Donofrio, Neogen Corporation, Lansing, MI, USA*

Discussion session with organizing committee for the 5th RAMC conference 2018

11:30 AM - 1:00 PM Lunch - All registered attendees

Plaza Exhibit - Concourse Level

11:30 AM - 1:00 PM Quarter Century Club Luncheon (Members Only)

Plaza Court 2 - Concourse Level

1:00 PM - 4:00 PM Session: 10: Genome Mining of Fungal Natural Products

Conveners: **Jonathan D. Walton**, Michigan State University, East Lansing, MI, USA and **Scott E. Baker**, Pacific Northwest National Laboratory, Richland, WA, USA

Plaza Ballroom D - Concourse Level

1:00 PM S55: Nanoscale clustering of enzymes in a fungal sesquiterpene biosynthetic pathway

H.C. Kistler, University of Minnesota, Saint Paul, MN, USA*

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

1:30 PM S56: Exploring fungal polyketide *C*-methylation through combinatorial domain swaps

P. Storm and C. Townsend, Johns Hopkins University, Baltimore, MD, USA*

Fungal polyketide natural products are frequently *a*-methylated by *S*-adenosyl methionine (SAM)-dependent *C*-methyltransferases (CMeT) embedded in the polyketide synthase (PKS) architecture. A survey of non-reduced (NR) fungal polyketides demonstrates programmed *C*-methylation of the growing polyketide intermediate, but the source of this control is not clear. We combined recent NR-PKS CMeT structural data with combinatorial domain swaps of several CMeT-containing NR-PKSs to explore this programmed modification and gauge the compatibility of non-cognate domains. Product profiles are dependent on the concentration and identity of the CMeT and reflect the native methylation pattern of the CMeT. Our results are consistent with kinetic control of *C*-methylation during polyketide biosynthesis, such that only programmed positions on the PKS-bound intermediate are methylated before the next round of extension occurs. The ability of this class of fungal PKS CMeTs to methylate various chain-length intermediates suggests that these methyltransferases may be a useful tool for unnatural biosynthesis of fungal polyketides.

2:00 PM S57: Reading fungal genomes to discover antifungal natural products

G. Bills, University of Texas Health Science Center at Houston, Houston, TX, USA*

Natural products from fungi have been critical to the development of antifungal agents, including griseofulvin, echinocandins, and strobilurins. Other outstanding preclinical and clinical antifungal leads (sordarins, enfumafungin, aureobasidin) have also originated from fungi. The argument that fungi offer superior sources of agents that regulate fungal growth, interact with essential fungal proteins, or modulate the activities of key fungal pathways has been validated by chemo-genetic profiling of microbial products across a compendium of essential proteins encoded by the *Candida albicans* genome. Genome sequencing enables retrospective searches for newly characterized biosynthetic gene clusters. But can this approach guide discovery of new antifungals belonging to unrecognized structural classes? We will relate our experiences in mapping the distribution of gene clusters of two classes of antifungal agents. Although genome mining approaches can uncover the potential chemical diversity encoded by a set of related gene clusters, the small number of available fungal genomes limits the approach. Therefore, tracking down historical records and the strains responsible for producing reported antifungals, e.g.,

echinocandins, was needed to guide genome mining. Although most antifungal screening has historically centered on *C. albicans* and *Aspergillus* spp., other major pathogens, like *Cryptococcus* species, were typically only tested for antifungal susceptibility when the spectrum of *C. albicans* lead compounds were evaluated. We contend that *Cryptococcus*-centric screening of fungi for natural products presents outstanding prospects for the discovery of new antifungal therapies. Targeting fungi with complex secondary metabolism, but have rarely been included in screening programs to date, can enhance these prospects.

2:30 PM Break

3:00 PM S58: Scalable expression of cryptic fungal biosynthetic gene clusters for the discovery of novel natural products

C. Harvey, Stanford University, Palo Alto, CA, USA*

For decades, fungi have been an important source of medically relevant natural products (NPs). Recent advances in DNA sequencing have revealed that the biosynthetic potential of fungal genomes is much deeper than previously realized. Difficulties in culturing and genetically engineering many fungi, combined with the fact that many NP biosynthetic gene clusters (BGCs) are not expressed under standard laboratory conditions has lead to much of this biosynthetic potential remaining untapped. Here we describe the realization of a pipeline based in *S. cerevisiae* encompassing bioinformatic tools for BGC curation, genetic parts for BGC refactoring, and improved DNA assembly for BGC building.
With this pipeline, we have successfully detected novel NPs from several previously unstudied fungal BGCs, and have structurally characterized a subset of the BGC-associated compounds. Our pipeline demonstrates how high-throughput synthetic biology tools can facilitate the rapid discovery of complex chemical scaffolds of potential pharmaceutical relevance and their production in model fungal hosts.

3:30 PM S59: A cytotoxic protein from the mushroom *Coprinus comatus* possesses a unique glycan binding fold and specificity

P. Zhang, K. Li, G. Yang, S. Bruner and Y. Ding, University of Florida, Gainesville, FL, USA*

Glycans possess significant chemical diversity and glycan binding proteins (GBPs) recognize specific glycans to translate their structures to functions in various physiological and pathological processes. The discovery and characterization of novel GBPs and characterization of glycan-GBP interactions is therefore significant to provide potential targets for therapeutic intervention of a wide range of diseases. Here, we report the biochemical, functional and structural characterization of a 130-amino-acid protein, Y3, from the mushroom *Coprinus comatus*. Biochemical studies of recombinant Y3 from a yeast expression system demonstrated the protein is a unique GBP. Additionally, we showed that Y3 exhibits selective and potent cytotoxicity toward human T-cell leukemia Jurkat cells as compared with a panel of cancer cell lines via inducing caspase-dependent apoptosis.. Screening of a glycan array demonstrated GalNAcb1-4(Fucα1-3)GlcNAc (LDNF) as a specific Y3-binding ligand. To provide a structural basis for function, the crystal structure was solved to a resolution of 1.2 Å, revealing a compact single-domain αβα-sandwich motif. Two monomers were dimerized to form a large ten-stranded, antiparallel β-sheet flanked by α-helices on each side, representing a novel GBP fold. A large glycan binding pocket extends into the dimeric interface and docking of LDNF identified key residues for glycan interactions. Disruption of residues predicted to be involved in LDNF/Y3 interactions resulted in the significant loss of binding to Jurkat T-cells and severely impaired their cytotoxicity. Collectively, these results demonstrate Y3 to be a novel GBP with selective cytotoxicity toward human T-cell leukemia cells and indicate its potential use in cancer diagnosis and treatment.

1:00 PM - 4:30 PM Session: 11: Student Oral Presentation Session

Conveners: **Stephanie Gleason**, DuPont Industrial Biosciences, Cedar Rapids, IA, USA;**Sonya Clarkson**, Conagen, Inc., Bedford, MA, USA and **Katherine J. Chou**, National Renewable Energy Laboratory, Golden, CO, USA

Governor's Square 14 - Concourse Level

1:00 PM S60: Effects of agricultural wastes and energy crops anaerobic co-digestion on anaerobic microbes and corresponding digestion performance

Y. Zhong[*] and W. Liao, Michigan State University, East Lansing, MI, USA

In the past decades, microbial communities of anaerobic digestion (AD) have been intensively investigated, with majority of these studies focusing on the correlation between microbial diversity and biogas production. However, currently there is a lack of comprehensive research on the relationship between microbial communities and compositional changes of the solid digestate (AD fiber). Therefore, a distinct understanding on the relationship between mixed feedstock, microbial communities, biogas production, and solid digestate quality should be concluded to promote AD technology for the next-generation biorefining. The objective of this study was to understand the responses of microbial communities to different feedstock combinations and ratios of anaerobic co-digestion and their influences on biogas production and solid digestate quality. Three feedstock combinations (dairy manure with corn stover, dairy manure with switchgrass and dairy manure with miscanthus) and two feedstock ratios (4 to1 and 3 to 2) were investigated with a completely randomized design. The 16S rRNA gene-based 454 pyrosequencing, Terminal Restriction Fragment Length Polymorphism (T-RFLP) and clone library were used to investigate the communities. Microbial communities were also statistically correlated with performance parameters such as total solids reduction, biogas production, and AD fiber quality (cellulose, xylan, and lignin).

1:25 PM S61: Engineering *Yarrowia lipolytica* for triacetic acid lactone production

C. Palmer[*], K. Markham and H. Alper, The University of Texas at Austin, Austin, TX, USA

Yarrowia lipolytica, an industrially attractive, non-conventional yeast, boasts a high innate capacity to produce acyl-CoA derived molecules such as triacylglycerides. Here we demonstrate the potential of rewiring *Y. lipolytica* to divert this precursor pool away from lipids and into alternative molecules of interest. Specifically, we explored the production of the simple polyketide, triacetic acid lactone (TAL). TAL has been proposed as a biorenewable platform chemical that can be converted into many downstream products including sorbic acid. Previous efforts to produce TAL in hosts such as *E. coli* and *S. cerevisiae* have been limited by the availability of acyl-CoA precursors. We enabled TAL production in the precursor rich host, *Y. lipolytica*, through heterologous expression of 2-pyrone synthase, an enzyme from *Gerbera hybrida* that catalyzes the formation of TAL by condensation of acetyl-CoA and malonyl-CoA. We next performed a series of strain engineering approaches to boost TAL production by metabolically rewiring *Y. lipolytica* for enhanced precursor accumulation. Final strain characterization was conducted in bioreactors to further optimize production titer, rate, and yield. Ultimately, we established a strain that produced the highest titer of TAL reported to date in any host. Here we present the details of these genetic engineering efforts as well as the production characterization of the resulting strain.

1:50 PM S62: Chemical and bioactivity screening of secondary metabolites from Oregonian bacterial strains

C. Zhu[*], C. Lew and S. Loesgen, Oregon State University, Corvallis, OR, USA

Soil-dwelling bacteria have given us a remarkable chemical diversity and an unprecedented amount of bioactive small molecules, such as the clinical used approved anticancer drugs doxorubicin, mitomycin, and many antibiotics, such as erythromycin and kanamycin. Here we present the chemical and bioactivity

screening of seventeen bacterial strains isolated from soils collected near Bend Oregon from an arid, high desert area. Species diversity was assessed using 16S rRNA gene sequencing, while chemical diversity was explored via untargeted comparative metabolomic analysis based on liquid-chromatography coupled mass spectrometry (LCMS) derived data. Organic extracts were prepared from each bacterium cultivated in malt-based liquid media and tested for their respective anti-bacterial and cytotoxic activities. Antibacterial screening was carried out against four human pathogens, *Enterococcus faecium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, by broth dilution method. Cell viability was evaluated against a panel of human cell lines to assess their potential for the treatment of cancer. Five model systems were used to determine the ability of extracts to inhibit the growth of human colon cancer adenocarcinoma (HCT-116), breast adenocarcinoma (MCF-7), lung carcinoma (A549), malignant melanoma (SK-Mel-5), and prostate adenocarcinoma (PC-3) via MTT cell proliferation procedures. Noteworthy, six extracts (35%) showed strong inhibition with less than 50% cell proliferation at 10 µg/ml in all cell lines. Active compounds are purified and characterized by HPLC and NMR. Principle component analysis of organic extract LCMS data revealed drastic differences in the metabolic profiles found in these panel of high desert derived bacteria

2:15 PM S63: Developing the thermotolerant yeast *Kluyveromyces marxianus* as a microbial host for volatile ester biosynthesis

A.K. Loebs, R. Engel, C. Schwartz and I. Wheeldon, University of California Riverside, Riverside, CA, USA*

The yeast *Kluyveromyces marxianus* is a promising candidate for chemicals biosynthesis. It has rapid growth kinetics at temperatures upwards of 45 °C and can produce short and medium chain volatile esters and ethanol at high rates. Of particular interest is its capacity to produce ethyl acetate at rates upwards of 2 g/L/hr in aerated cultures. Both ethanol and ethyl acetate production rely on the activity of alcohol dehydrogenases to oxidize acetaldehyde to ethanol, which serves a substrate for ethyl acetate production. Little is known about the ester production pathways in *K. marxianus*. Prior findings suggest the presence of alcohol acetyltransferase (Atf) activity and the absence of significant reverse esterase activity. Our studies suggest the presence of one ATF and seven different alcohol dehydrogenase (ADH) genes in *K. marxianus*. In this work, we design a hybrid-synthetic RNA polymerase III promoter to create a CRISPR-Cas9 genome editing systems for *K. marxianus*. This system was used to construct a disruption library of ADH and ATF genes to study their function in volatile metabolite production. ATF disruption and overexpression analyses suggest a limited role of Atf in overall ethyl acetate biosynthesis in *K. marxianus*. ADH2 disruption resulted in reduced ethanol production along with accumulation of acetaldehyde suggesting an importance of Adh2 in ethanol production. The terminal step of ethyl acetate synthesis remains puzzling but overexpression of ADH7 shows promiscuous activity towards ethyl acetate production from hemiacetal. These findings serve as starting point for metabolic engineering approaches towards ethyl acetate and longer chain esters production.

2:40 PM Break

3:10 PM S64: Measuring dynamic interactions and metabolic fluxes in microbial communities

N. Gebreselassie and M. Antoniewicz, University of Delaware, Newark, DE, USA*

In nature, microorganisms don't exist in isolation, rather they are part of complex interacting systems. Recent efforts in metabolic engineering have also taken advantage of multi-microorganism systems to enhance product yields and productivities. Despite these advances, quantitative characterization tools to elucidate microbial metabolism and drive engineering efforts are still lacking for multi-microorganism systems. For example, metabolic flux analysis, a widely used approach for metabolic engineering of pure cultures, has not been developed and applied towards multi-microorganism systems.
In this work, we present a novel approach for performing ^{13}C-metabolic flux analysis (^{13}C-MFA) in co-cultures. We demonstrate for the first time that it is possible to determine population dynamics, metabolic

interactions (e.g. cross-feeding of molecules between cells), and metabolic flux distributions in multiple species simultaneously without the need for physical separation of cells or proteins, or overexpression of species-specific products. We have applied this methodology to study an interacting synthetic co-culture consisting of a glucose consuming wild-type *E. coli* strain and a glucose non-consuming *ΔptsI Δglk E. coli* strain. Here, the *ΔptsI Δglk* strain relies on acetate produced by wild-type *E. coli* for cell growth. We have performed a complete characterization of this interacting system using our dynamic co-culture ^{13}C-MFA methodology. In addition to measuring intracellular metabolic fluxes for individual strains in the co-culture, for the first time, population dynamics and acetate cross-feeding was fully characterized. This work lays the foundation for more detailed studies of complex interacting microbial communities including systems consisting of more than two species.

3:35 PM S65: Uncovering the role of branched-chain amino acid transaminases in *Saccharomyces cerevisiae* isobutanol biosynthesis

S. Hammer and J. Avalos, Princeton University, Princeton, NJ, USA*

Engineering metabolic pathways in yeast is a promising strategy for producing commodity and high-value chemicals from renewable sources. Yeast are particularly attractive hosts for the production of branched-chain higher alcohols (BCHAs), which they naturally synthesize in trace amounts during ethanol fermentation. BCHA production is complicated by separation of the upstream and downstream biosynthetic pathways between the mitochondria and the cytosol, as well as the presence of branched-chain amino acid transaminases (BCATs) in both compartments. In the past, researchers have deleted or overexpressed the yeast mitochondrial (*BAT1*) or cytosolic (*BAT2*) BCAT genes in order to reduce BCHA production and improve the flavor and aroma profiles of fermented alcoholic beverages. However, these studies have yielded inconsistent results regarding the effects of BCATs on isobutanol production. Recently, there has been a renewed interest in understanding the role of these transaminases in the production of BCHAs – specifically isobutanol – as advanced biofuels. This work aimed to resolve previous conflicting results and conclusively determine the role of BCATs in the biosynthetic route from glucose to isobutanol. Manipulation of transamination activity alone enabled us to increase isobutanol production by more than 15-fold over wild type strains. Addressing the intracellular transport bottleneck implicated in our transamination activity experiments led to an additional 92% increase in isobutanol production. This work answers the longstanding question of the role of transamination activity in BCHA biosynthesis and develops valuable strains for future optimization of isobutanol production.

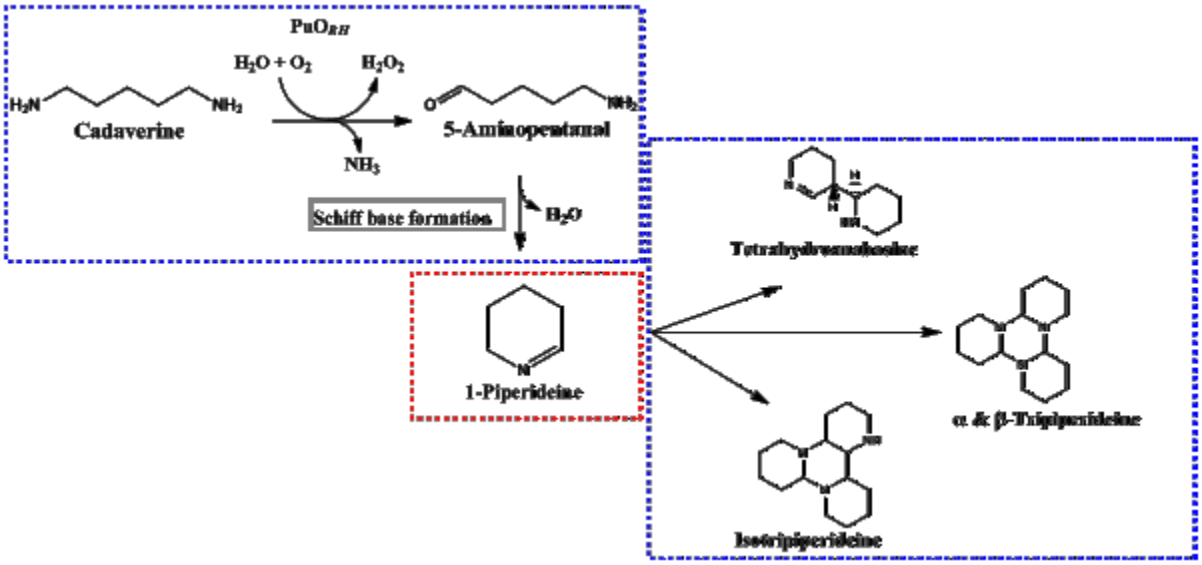
4:00 PM S66: Bioproduction of 1-piperideine using engineered *Escherichia coli* strains

V. Anyanwu, J. Lebeau, A. Grimsley, S. Hall, A. Pordea and G. Stephens, University of Nottingham, Nottingham, United Kingdom*

Abstract

Although the bioproduction of complex, functionalised *N*-heterocycles has been reported, bioproduction of unsubstituted platform *N*-heterocycles has not yet been achieved. Therefore, the suitability of putrescine oxidase from *Rhodococcus erythropolis* (**PuO*RH***) for bioproduction of Δ^1-piperideine was studied. **PuO*RH*** catalyses the oxidation of cadaverine to 5-aminopentanal. Although this product is known to cyclise spontaneously into Δ^1-piperideine, direct formation of this product catalysed by **PuO*RH*** has not been demonstrated, except by using *o*-aminobenzaldehyde as a reagent to trap Δ^1-piperideine and shift the equilibrium for cyclisation. The **PuO*RH*** gene was cloned and expressed in *E. coli* BL21 (DE3) using the pET20b vector, and the His-tagged enzyme was purified. Steady state kinetics of **PuO*RH*** were determined by monitoring oxygen consumption; the K_M and k_{cat} values were 0.24 ± 0.05 mM and 26.6 ± 0.08 s^{-1} for cadaverine, and 0.17 ± 0.03 mM and 147.4 ± 0.4 s^{-1} for putrescine, respectively. Whereas the k_{cat}/K_M values are lower than those reported using peroxidase-coupled assays, this result should represent the true kinetics of **PuO*RH***. Using the purified enzyme, the conversion of cadaverine to Δ^1-piperideine was

demonstrated qualitatively using LC-ESI-MS and ^{1}H NMR; 5-aminopentanal could not be detected. Δ^1-Piperideine also formed the corresponding dimer and trimers, a known spontaneous reaction, and the product ratio could be adjusted by varying the pH. This preliminary study indicates that **PuO*RH*** is suitable for bioproduction of Δ^1-piperideine. The next steps are to optimise the reaction conditions, quantify, extract and purify the products, and develop whole cell bioproduction of *N*-heterocycles from renewable feedstocks.



1:00 PM - 4:30 PM Session: 6: Biocatalytic Conversion of Lignin into Valuable Products

Conveners: **Aditya Bhalla**, DuPont Industrial Biosciences, Palo Alto, CA, USA and **Navanietha Krishnaraj Rathinam**, South Dakota School of Mines and Technology, Rapid City, SD, USA

Plaza Ballroom F -Concourse Level

1:00 PM S31: Engineering *Pseudomonas putida* to convert aromatic compounds into fuels and chemicals

A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Pseudomonas putida KT2440 is a highly robust bacterium capable of efficiently utilizing a variety of carbon sources, including aromatic compounds derived from lignin. Recently, *P. putida* has been engineered to valorize the lignin stream of a lignocellulosic biomass pretreatment process. While genetic tools exist that allow rational manipulation of the *P. putida* chromosome, the toolkit for engineering *P. putida* is underdeveloped when compared to platform organisms such as *Escherichia coli*. To help accelerate engineering of *P. putida* for the conversion of lignin into fuels and chemicals, we developed a rapid genome integration system using the site-specific recombinase from bacteriophage Bxb1 to enable rapid, high efficiency integration of DNA into the *P. putida* chromosome. We also developed a library of synthetic promoters with various UP elements, -35 sequences, -10 sequences, and ribosomal binding sites fused to the fluorescent reporter mNeonGreen, resulting in a 150-fold range of expression levels. We are using this and other tools to engineer *P. putida* to produce value-added products from lignocellulosic biomass and the aromatic compounds therein. Through a combination of gene deletion and heterologous expression, we have doubled the yield of medium chain length polyhydroxyalkanoates

from aromatic substrates, including depolymerized lignin. We are leveraging these results to expand the range of products that can be made by *P. putida* for lignin valorization.

1:30 PM S32: Bacterial enzymes for lignin degradation and production of renewable aromatic chemicals

T.D.H. Bugg, University of Warwick, Coventry, United Kingdom*

The lignin content of lignocellulose and lignin-containing wastes represents a possible resource for production of aromatic chemicals, using biocatalysis or chemocatalysis. My research group has been studying the enzymology of bacterial lignin degradation, and the metabolic engineering of bacterial lignin degradation for production of renewable chemicals.

We have previously identified peroxidase DypB in *R. jostii* RHA1 as a lignin-oxidising peroxidase [1]. We have identified a further Dyp1B peroxidase from *Pseudomonas fluorescens* [2] and a manganese superoxide dismutase from *Sphingobacterium* T2 [3] that have activity for lignin oxidation, whose mechanism for lignin oxidation is currently being studied. Through gene deletion of the vanillic acid breakdown pathway of *Rhodococcus jostii*, vanillin has been generated as a bioproduct from lignocellulose breakdown [4]. By re-routing the pathways for lignin degradation, we have also been able to generate two pyridine dicarboxylic acids which can be used as monomers for synthesis of renewable bioplastics.

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2. R. Rahmanpour & T.D.H. Bugg, *Arch. Biochem. Biophys*., **574**, 93-98 (2015).
3. G.M.M. Rashid, C.R. Taylor, Y. Liu, X. Zhang, D. Rea, V. Fülöp & T.D.H. Bugg, *ACS Chem Biol*, 10, 2286-2294 (2015)
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2:00 PM S33: Developing laccase-catalyzed conversions of lignin

J.O. Rich, United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Peoria, IL, USA*

Identifying suitable reaction conditions remains an important task in the development of practical enzyme catalysts. Laccases play an important role in the biological break down of lignin and have great potential in the deconstruction of lignocellulosic feedstocks. We have examined 16 laccases, both commercially prepared and crude extracts, for their ability to oxidize veratryl alcohol in the presence of various solvents and mediators.

While many literature reports have described single enzyme-mediator pairs, we will describe a combinatorial approach to the laccase-mediated system (LMS) where 16 enzymes were each paired with 30 different previously reported mediators. We determined the pH optimum (4.4–6.4) and operational temperature range (25–80 °C) for each enzyme. The LMS reaction was optimized to achieve near quantitative yields in the oxidation of veratryl alcohol to veratrylaldehyde.

Although each of the mediators had been previously reported to serve as a laccase-mediator, it is clear that for a given substrate, it is necessary to examine a wide variety of enzyme-mediator combinations, and this combinatorial approach will enable the rapid identification of a suitable enzyme-mediator combination and optimization of reaction conditions. The application of the LMS for the modification of lignin model compounds, including novel biobased products from these reactions, will also be discussed.

2:30 PM Break

3:00 PM S34: Microbial valorization of lignin: Using bacteria to depolymerize short lignin oligomers and convert aromatic compounds into valuable commodities

W. Kontur and T. Donohue, University of Wisconsin-Madison, Madison, WI, USA; D. Noguera, University of Wisconsin - Madison, Madison, WI, USA*

As part of the Great Lakes Bioenergy Research Center's goal to generate biofuels and other valuable commodities from lignocellulosic biomass, we are investigating the use of bacteria to valorize lignin. Lignin is a hetero-polymer of aromatic units that has historically been considered a waste product to the biofuel industry, since there are few economically feasible uses for it. In addition, lignin's recalcitrance to degradation contributes to the difficulty of deconstructing plant biomass, and methods that can partially degrade lignin generally release water-soluble aromatic compounds that are toxic to many microbes. Our goal is to develop bacterial systems to both depolymerize lignin-derived materials and convert the constituent aromatic compounds into valuable commodities. We found that *Novosphingobium aromaticivorans* can cleave the β-aryl ether bond commonly found between aromatic units in lignin; we are pursuing this organism as a chassis for converting lignin oligomers into aromatic compounds that can be used as commodities (e.g vanillin) or as feedstocks for bacteria that only metabolize monomeric aromatics. Additionally, we found that enzymes from this and similar bacteria could be employed within *in vitro* depolymerization systems.

3:30 PM S35: Platform chemicals and materials from microbial aromatic catabolism

C.W. Johnson, D. Salvachúa, N. Rorrer, B.A. Black, D.R. Vardon, N.S. Cleveland, W. Michener, T. Vander Wall and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Soil microbes commonly catabolize aromatic compounds via ring-opening dioxygenase enzymes from central aromatic intermediates such as catechol and protocatechuate. The ring-opened reaction products are then further metabolized, eventually being converted to central carbon metabolism. However, there are multiple compounds between the central aromatic intermediates and the tricarboxylic acid cycle that exhibit significant and potentially interesting chemical functionality for biopolymer applications, only some of which have been appreciably produced to date. We have engineered multiple strains of the robust soil microbe, *Pseudomonas putida* KT2440, to produce several of the metabolic intermediate compounds between protocatechuate and catechol via both *ortho*- and *meta*-cleavage pathways. Bioreactor cultivations demonstrate significant differences in product yield, titer, product stability, and toxicity. For the target molecules, we develop analytical techniques to quantify the compound and facile bench-scale separations methods for isolation and purification. Lastly, we demonstrate the potential for new materials via multiple examples, many of which exhibit performance-differentiated material properties from their current petroleum-derived counterparts. Overall, this study demonstrates the potential for diverse aromatic catabolic pathways to produce new platform chemicals for biopolymer and other novel materials and chemicals applications.

4:00 PM S36: Assimilation of lignin-breakdown products by lignolytic and non-lignolytic bacteria and fungi

M. Dailey, D.C. Hayes, D. Ye, T. Simoes, L. Appelhans, M.S. Kent and J.A. Timlin, Sandia National Laboratories, Albuquerque, NM, USA*

Lignin is typically 15-28% of the total dry weight of biomass, making it a significant potential source of revenue, yet it is currently a waste stream that is usually burned to generate power. Efficient, controllable conversion of lignin to useful molecular building blocks has been elusive. While much lignin breakdown occurs extracellularly, the breakdown products, a substantial fraction of which are mono- and di-aromatics, are known to be taken up by lignolytic fungi and bacteria and metabolized intracellularly. Assimilation of these breakdown products is likely an important part of the lignin conversion, yet very little

is currently known about the substrate range and kinetics of transport into microbes. Here, we have studied the uptake of five lignin breakdown products in the lignolytic fungus, *Phanerochaete chrysosporium* and the lignolytic bacterium, *Enterobacter lignolyticus* SCF-1. These results are compared to *Saccharomyces cerevisiae* and *Escherichia coli*, which have no known lignolytic activity. Cultures were grown in media supplemented with individual lignin breakdown products. Molecular uptake was determined via LC-MS of the cell lysate following stringent washing. Additionally, click-chemistry enabled fluorescence microscopy was performed for select lignin breakdown products to determine the percentage of cells that were positive for the compound and the distribution in relative abundance throughout the cell population. The experiments show differential uptake and kinetics across the lignin breakdown products and between the lignolytic vs non-lignolytic organisms. These results open up the possibility of engineering these organisms to optimize transport in increase efficiency in the biofuel production pathway.

1:00 PM - 4:30 PM Session: 7: Metabolic and Spatial Interactions in Microbial Communities

Conveners: **Aaron T. Wright**, Pacific Northwest National Laboratory, Richland, WA, USA and **Natalie Sadler**, Pacific Northwest National Laboratory, Richland, WA, USA

Governors Square 15, Concourse Level

1:00 PM S37: Linking intracellular metabolism to microbial ecosystem dynamics in structured environments.

W. Harcombe, University of Minnesota, St Paul, MN, USA*

The inter-species exchange of metabolites plays a key role in the spatio-temporal dynamics of microbial communities. This raises the question whether ecosystem-level behavior of communities can be predicted using genome-scale models of metabolism for multiple organisms. We developed a modeling framework that integrates dynamic flux balance analysis with diffusion in a structured environment. Our computational approach provides quantitatively accurate predictions of how species ratios fluctuate in synthetic 2 and 3-species communities. We further used the modeling approach to elucidate how location mediates interactions between colonies of *Escherichia coli* or *Salmonella enterica* grown on different carbon sources. Interestingly, we find that geometry, not distance, is the best predictor of microbial interactions. Our work highlights that location is an important determinant of microbial interactions. Further we demonstrate that models of intracellular metabolism can quantitatively predict ecological interactions, and microbial community dynamics in complex structured environments.

1:30 PM S38: The microbial metabolic response to drought and implications for carbon cycling in tropical forest soils.

N. Bouskill, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Climate model projections for tropical regions show clear perturbation of precipitation patterns leading to increased frequency and severity of drought in some regions. Previous work has shown declining soil moisture to be a strong driver of microbial trait distribution, however, the feedback between changes in metabolic functional potential and ecosystem properties related to carbon cycling are poorly understood. Herein I leverage a year long drought experiment in a tropical forest to demonstrate that drought-induced shifts in microbial metabolic traits and activity shape, and are shaped by, the composition of dissolved and soil-associated carbon. Shifts in metabolic traits toward osmolyte and hygroscopic compounds subsequently suppress the efflux of carbon dioxide following soil rewetting. Furthermore, this metabolic response to drought appears to condition (biologically and physically) the soil matrix, notably through the production of polysaccharides, particularly in experimental soils pre-exposed to drought. In this talk I will expand on the findings of this study with further discussion as to the importance of microbial metabolic

traits in the formation and evolution of soil organic matter. I will conclude by discussing how these traits can, in part, determine the vulnerability of soil carbon to future climate change.

2:00 PM S39: Linking phylogenetic identity and biogeochemical function of uncultivated marine microbes with novel mass spectrometry techniques

R. Mueller[*] and S. Bryson, Oregon State University, Corvallis, OR, USA; Z. Li, R.L. Hettich and C. Pan, Oak Ridge National Lab, Oak Ridge, TN, USA; J. Pett-Ridge and X. Mayali, Lawrence Livermore National Lab, Livermore, CA, USA

Resource availability influences marine microbial community structure, suggesting that population-specific resource partitioning defines discrete niches. Identifying partitioning patterns among diverse populations in natural environments, thereby characterizing functional guilds within the complex communities, remains a challenge for microbial ecologists. We used proteomic stable isotope probing (proteomic SIP) and NanoSIMS analysis of phylogenetic microarrays (Chip-SIP) along with 16S rRNA gene amplicon and metagenomic sequencing to concurrently characterize the assimilation a variety of stable isotope labeled substrates, and to define overall changes in communities collected from coastal marine environments. Interestingly, observed increases and decreases in relative abundance for individual populations did not correlate well with directly measured substrate assimilation. Use of these complementary SIP approaches revealed differential assimilation of substrates into protein and ribonucleotide biomass among taxa, indicating distinct metabolic routing of substrates that is not apparent by genome sequence data alone. Substrate assimilation trends indicated significantly conserved resource preferences among populations, suggesting that functional guilds within marine microbial communities are phylogenetically cohesive. Additionally, clear differences were observed in the functions expressed and in the types of enzymes synthesized *de novo* by distinct taxa in these communities, indicating divergent environmental growth strategies of these populations. By integrating 'omics methods with stable isotope probing, we have developed a high-throughput and sensitive approach to define the niches of organisms *in situ* and to characterize the functional responses of individual populations to competitive interactions, allowing deeper insight into how communities as a whole adapt to environmental change.

2:30 PM Break

3:00 PM S40: Comparative metagenomics clarifies the gut microbiome's functional association with health and evolution

T. Sharpton[*], Oregon State University, Corvallis, OR, USA

The gut microbiome contributes to animal health and homeostasis. Unfortunately, the precise mechanisms through which the gut microbiome operates to influence animal physiology remain elusive, which consequently challenges the development of disease diagnostics and therapeutics. We recently conducted a series of comparative metagenomic investigations to uncover potential mechanisms through which the gut microbiome interacts with its animal host to influence physiology. First, we analyzed over 2,000 gut metagenomes from a variety of human clinical populations to statistically model how the metabolic pathways encoded in the microbiome associate with health. This analysis revealed aspects of microbiome metabolism that are perturbed in diseased humans, many of which are common to multiple disease types. Second, we compared microbiome samples spanning many vertebrate species to quantify how the microbiome may have diversified over evolutionary timescales. Our analysis reveals properties of the microbiome that are relatively conserved across host species, which indicates that they may have been anciently integrated into the gut microbiome and subsequently retained, potentially because they promote host fitness. Collectively, these investigations clarify the aspects of the gut microbiome that may yield disease when perturbed and implicate their potential interaction with host physiology.

3:30 PM S41: The role of gut microbiota in obesity among pregnant women and children

M. Stanislawski, D. Dabelea, B. Wagner, M. Sontag, N. Iszatt and C. Lozupone, University of Colorado, Aurora, CO, USA; C. Dahl and M. Eggesbø, Norwegian Institute of Public Health, Oslo, Norway*

Recent research suggests that gut microbiota may play a key role in obesity and metabolic diseases; less is known about what role it plays in the development of these conditions in children. Using a large Norwegian observational cohort, we have investigated the relationships between obesity and the gut microbiota of pregnant women and their infants. We explored gut microbial characteristics as a potential mechanism to explain the association between maternal overweight and the development of obesity in offspring. We found differences in the maternal gut microbiota at the time of delivery with both pre-pregnancy overweight/obesity (OW/OB) and with excessive GWG. The infant gut microbiota was predictive of BMI at age 12; strikingly, the microbiota at 2 years of age predicted 53% of the variation in BMI at age 12 even though the infants who became OW/OB at age 12 did not significantly differ in BMI z-scores at this age. Maternal OW/OB also showed consistent associations with the infant gut microbiota taxa predictive of childhood BMI. The findings of this research could have implications for public health efforts to prevent obesity because the gut microbiota of pregnant women and children are alterable through non-invasive means, such as diet, prebiotics, and probiotics. Furthermore, our results suggest that the gut microbiota may also offer potential as an early biomarker for obesity, which could help to target prevention efforts more effectively.

4:00 PM S42: Functional and spatial characterization of diverse microbiomes by activity-based protein profiling

A.T. Wright, N. Sadler, C. Whidbey, J. Rosnow, L. Anderson, P. Bohutskyi and B. Killinger, Pacific Northwest National Laboratory, Richland, WA, USA*

Within microbes and microbial communities, from host-associated to those occurring within terrestrial, aquatic, or other environments, nutrient allocation and utilization is a key factor driving microbiome interactions and functions. We have developed chemical activity-based probes based upon B vitamins, or as mechanism-based inhibitors of metabolic enzyme families such as cellulases, glucuronidases, and proteases. By employing these chemical probes, we capture extracellular enzymes involved in polymeric carbon metabolism, nutrient transporters and intracellular protein interactions. In mammalian gut microbiomes we use probes to isolate distinct functional subpopulations of microbes, that are subsequently sequenced. Our complement of probes is enabling a functional analysis in live microbiomes of nutrient scavenging and utilization. Our results are identifying new enzymes, proteins, and regulators playing pivotal roles in the control of cell growth, response to stress, and other activities that potentially are leading to coordination of cell behavior in complex multicellular systems.

1:00 PM - 4:30 PM Session: 8: Fermentation of Non-Conventional Organisms

Conveners: **Kathrine Allikian**, Callaghan Innovation, Lower Hutt, New Zealand and **Tim Cooper**, Novozymes, Raleigh, NC, USA

Plaza Ballroom E - Concourse Level

1:00 PM S43: Production of methylobacteria for applications in the agro-ecosystem

S. Chauhan, N. Sweeney, S. Camp, K. Nannapaneni, K. Allen and D. Jimenez, NewLeaf Symbiotics, St Louis, MO, USA*

NewLeaf Symbiotics (NLS) is an agricultural biologicals company built upon a content-rich bioinformatics platform and a vast collection of methylotrophic bacteria isolated from the phytobiome. NLS focuses on *Alphaproteobacteria*, particularly pink-pigmented facultative methylotrophs (PPFMs) of the genus *Methylobacterium*. Methylobacteria are ubiquitous colonizers of surfaces and tissue of plant leaves, roots

and seeds. Genomes of methylobacteria in the NLS collection are deep sequenced and annotated. The genomes of lead strains are closed. Correlations between genotypes and phenotypic responses are organized within a proprietary database, the Prescriptive Biologics™ Knowledgebase (PBK). Functional genomics reveal critical roles for PPFMs in plant health and immunity and automated host imaging and phenomic analyses support *in-silico* predictions. Select PPFM strains are assigned to the growing NLS pipeline for product development, manufacturing and commercialization efforts. Leads are selected using commercial crop varieties in field plots configured to assess microbial trait contributions. Fermentations conducted at 2-10L facilitate bioprocess development and optimizations for 100-10,000L scale production, downstream concentration and stable formulations. Procedures are implemented for product quality assurance throughout the production steps. Production materials are tested *in-vitro* and *in-vivo* in growth chamber and greenhouse tests. Formulations and application methodologies are developed for compatibility with conventional inputs and crop management. Methylobacteria best fit in agricultural biologics space as supplements to traditional active ingredients via seed treatment, in-furrow or foliar applications. Crop inoculations are host genetics and methylobacteria dependent. Four years of global field testing confirmed the effects of methylobacteria on pests, diseases, and nutrient stress.

1:30 PM S44: The Bioexpression and Fermentation Facility: Fifty years of strange growth

D. Blum[*]*, University of Georgia, Athens, GA, USA*

Production of microbes at high temperature and/or under anaerobic conditions presents challenges compared to traditional fermentation of platform strains e.g. *E. coli* and *Pichia*. The Bioexpression and Fermentation Facility (established 1967) provides biotechnology services in Fermentation, Protein purification, cell culture and monoclonal antibodies. Using both sterilize in place (SIP) and autoclavable systems, microbes are produced to meet customer specifications including a variety of unique conditions. Exploration of best growth conditions as well as integration of downstream processing is critical to project success. Modern process development approaches using design of experiments or other methodologies have traditionally not been used to optimize conditions of these non-conventional fermentations. A historical perspective will be presented as well as how modern approaches could improve yield and product quality.

2:00 PM S45: Cullture optimization of *Epicoccum purpurascens* for fermentative production of antifungal compounds

S. Villas-Boas[*] *and M. Fokin, University of Auckland, Auckland, New Zealand; A. Lee and K.W. Bang, The University of Auckland, Auckland, New Zealand*

The control of fungal phytopathogens such as *Botrytis cinerea* and *Sclerotinia sclerotiorum* has a significant impact on agriculture and the food industry. Current control methods employ synthetic fungicides to reduce the occurrence of fungal pathogens and subsequently improve crop yields of acceptable quality. However, recent backlash against the use of synthetic chemicals and associations with adverse health effects and environment impact has led to legislative restrictions to the use of these compounds. A strain of *E. purpurascens* isolated in New Zealand has been found to secrete a range of water-soluble polyketide pigments with broad activity against many common plant pathogenic fungi. These antifungal compounds can be naturally produced via fermentation and are readily secreted by the fungal biomass. The bioactive compounds have shown little toxicity to animals and plants in addition to be quickly biodegraded by bacteria in the environment. We have characterised the primary metabolic pathways involved in the biosynthesis of these antifungal compounds using metabolomics combined with ^{13}C-labelled substrates of *E. purpurascens* grown under solid-state and submerged fermentation. Their biosynthesis seems to be primarily controlled by nitrogen catabolite repression and the medium pH. Optimal growth conditions for production of these bioactive molecules were defined and titter as high as 60 $g.L^{-1}$ has been obtained in laboratory conditions.

2:30 PM Break

3:00 PM S46: The use of non-conventional organisms in fermentation

F. Agbogbo[*]*, Cytovance Biologics, Oklahoma City, OK, USA*

In the production of therapeutic proteins, different hosts are chosen depending on the type of products to be made. Some of the conventional microbial hosts that are typically used are *E. coli* and *P. pastoris*. In some cases, no-conventional organisms have been used in the production of therapeutic proteins. In this presentation, examples will be provided on some of the non-conventional microorganisms that were evaluated.

3:30 PM S47: Dark fermentation, physiology and regulation in the hyperthermophilic archaeon *Thermococcus kodakarensis*

J. Walker[*] *and T. Santangelo, Colorado State University, Fort Collins, CO, USA*

Currently employed model microbes, typically yeasts and bacteria, enjoy success due in large part to genetic systems that compensate for their limited metabolisms. However, these platforms remain restricted to similar feedstocks, necessitate the introduction, regulated and balanced expression of heterologous pathways for substrate degradation, suffer from significant and expensive contamination issues, have large fresh water requirements, are limited by product inhibition, and typically require expensive substrate pretreatments that both introduce toxic byproducts and increase total costs. Alternative systems must be developed in parallel with modifications and improvements to existing platforms. The advantages of the hyperthermophilic Archaea have long been recognized, but only within the last decade have genetic techniques allowed the full potential of these organisms to be more completely exploited. We developed a near complete genetic system for the marine archaeon *Thermococcus kodakarensis*, providing unlimited and unprecedented access to archaeal chemistries and rational and iterative strain construction. We, together with many members of the archaeal community, have simultaneously identified and characterized a host of enzyme activities promoting the complete saccharification of cellulose as well as the degradation of chitin, peptides, and starch. The combination of established genetic and biochemical techniques are now poised for a grand merger producing a whole-cell hyperthermophilic biocatalyst for biofuel production, and our long-term goal is to establish *T. kodakarensis* as a biofuel production platform. We will discuss recent advances in strain construction, regulated transcription systems and new replicative vectors.

4:00 PM S48: Symbiotic growth of photoautotrophic and heterotrophic microorganisms

B. McConnell[*] *and M. Antoniewicz, University of Delaware, Newark, DE, USA*

Climate change due to rising atmospheric greenhouse gas concentrations is one of the most threatening problems facing humanity. Methods for capturing greenhouse gasses and producing biofuels have gained much attention. Microalgae and cyanobacteria can be used to capture carbon dioxide from point-source emitters and produce biofuels at high levels. Open bioreactors are needed to economically grow these photoautotrophs; however, heterotrophs often contaminate open bioreactors. The presence of heterotrophs can be advantageous in some cases, but frequently they are detrimental to the growth of photoautotrophs.

In this work, we show that *Chlorella vulgaris* UTEX #395 (a unicellular, non-motile, photosynthetic microalga) releases significant amounts of polysaccharides and proteins during photoautotrophic growth, termed “photosynthate”. Using *C. vulgaris* spent media, we isolated heterotrophic microorganisms from soil samples that can grow on the released photosynthate and used ^{13}C-labeled photosynthate to confirm that soil microbes efficiently consumed it. In a co-culture with *C. vulgaris* the soil microbes significantly improved overall biomass production. Using pulse-chase tracer experiments in a co-culture of *C. vulgaris* and soil microbes, we demonstrated that soil microbes efficiently consumed photosynthate while *C. vulgaris* received carbon dioxide in return. The symbiotic exchange between the organisms where each partner benefited from the other was quantified using ^{13}C-tracers. We also demonstrate that soil microbes

increase biomass production in co-culture with *Synechocystis* sp. ATCC 27184 (a unicellular, freshwater cyanobacterium). The increased understanding of photoautotroph-heterotroph interactions obtained in this study may help to improve the possibility of economically viable large scale biofuel production from waste carbon dioxide sources.

1:00 PM - 4:30 PM Session: 9: Metabolic Engineering for Fuels and Chemicals II - Sponsored by ZYMERGEN

Conveners: **Zengyi Shao**, Iowa State University, Ames, IA, USA and **Rachit Jain**, GreenLight Biociences, Inc, Medford, MA, USA

Plaza Ballroom A & B - Concourse Level

1:00 PM S49: Systematic and comprehensive genome engineering for improved microbial performance

S. de Kok, Zymergen Inc., Emeryville, CA, USA*

Identifying, understanding and ultimately engineering the complex web of genes responsible for any cellular phenotype is extremely difficult and traditionally requires years of exhaustive study. For example, optimizing metabolic flux to a native or non-native small molecule, or increasing the diversity of carbon sources that a microbe can use are well-studied topics and have been the topic of hundreds of papers over decades of research. The burgeoning bioeconomy requires that we find a general purpose and rapid solution to phenotypic optimization, otherwise new developments will follow the traditional slow, expensive and high-risk path to commercialization. To address this problem, Zymergen has built a powerful platform for automated and high-throughput microbial strain improvement. To both improve the economics of current large-scale fermentation processes and accelerate the industrialization of novel bio-products, the platform has been designed to be flexible, highly reliable, and host-agnostic. Using this platform, Zymergen takes a data-driven approach to biology, capturing as much data as possible and using heuristically-guided search processes that embrace the complexity of biology.

1:30 PM S50: Evolutionary robust many-regulator CRISPRi arrays for dynamic regulation of metabolic networks

A. Reis, S. Halper and H. Salis, Penn State University, University Park, PA, USA*

We present a new approach to dynamically knocking down the expression of many enzymes within an endogenous metabolic network, utilizing a newly developed toolbox of CRISPRi regulators designed for maximum evolutionary robustness. CRISPRi employs the deactivated version of Cas9 and single-guide RNA (sgRNA) regulators to bind to targeted DNA sites and repress transcription. Here, we show how to design, build, and utilize Very Long sgRNA Arrays (VLSA) with as many as 20 sgRNA regulators, targeting up to 20 enzymes for dynamic repression. Notably, VLSAs can be synthesized as a single DNA fragment with high assembly efficiency and success rate, greatly accelerating the metabolic engineering design-build-test cycle. We show how to use VLSAs to redirect carbon flux in metabolic networks towards the production of desired fuels and chemicals.

2:00 PM S51: Raman spectroscopy and peptide-guided surface enhanced Raman scattering (pgSERS) in metabolic and enzyme engineering

R. Senger, Virginia Tech, Blacksburg, VA, USA*

Raman spectroscopy can return molecular composition information of growing cell cultures in a non-destructive manner. The analysis can be performed inexpensively, through a glass barrier, and in a

matter of seconds, which has led to the term “Near Real-Time Phenotyping.” This technique has been used to study the transition of *E. coli* cell membrane fatty acids (and measure membrane fluidity) upon exposure to toxic levels of n-butanol. It was then used to screen strains for improved fatty acids production when a genomic library was enriched in *E. coli* in the presence of butanol toxicity. In addition, with a computational pipeline involving principal component analysis and linear discriminant analysis, Raman data can be used to characterize whole cell phenotype changes. With this technique, the mechanism of action of antibiotics (including uncharacterized antimicrobials) was probed by tracking *E. coli* phenotype changes with exposure. In a further application, the presence of gold or silver nanoparticles can amplify the Raman signal of their immediate vicinity by several orders of magnitude. Thus, locating gold or silver nanoparticles to specific sub-cellular locations can provide localized molecular composition information. This has been accomplished by a technique called “Peptide-Guided Surface Enhanced Raman Scattering (pgSERS),” where silver nanoparticles are bound to short peptides that selectively locate in a cell based on hydrophobicity or other charge interactions. Here, it is demonstrated how pgSERS probes can be assembled *in vitro* or *in vivo* and can target intracellular locations in a bacterial cell selectively.

2:30 PM Break

3:00 PM S52: MixoFerm as a platform for biochemical and biofuel production with reduced CO_2 emissions

B. Tracy, White Dog labs, Inc., New Castle, DE, USA*

Traditional fermentation processes for the production of the majority of biochemicals and biofuels produce CO_2 because of decarboxylation reactions, which limits the final mass yields of products. To overcome this limitation, we have developed a fermentation technology called MixoFerm™ (also known as anaerobic, non-photosynthetic mixotrophy), which uses microorganisms capable of simultaneously consuming both organic (e.g., sugars) and inorganic (e.g., CO_2, CO, or H_2) substrates. With this technology, product mass yields for many biochemical or biofuel products can be increased by at least 50%, and processes can be designed that result in no CO_2 production. As a first demonstration of this technology, acetone, an important commodity chemical and a feedstock for poly(methyl methacrylate) (PMMA) production, was chosen. Through traditional fermentation, the theoretical maximum conversion of sugar into acetone is 32wt%, but with MixoFerm™, the mass yield can be increased to at least 45wt%, an improvement of 50% over the previous theoretical maximum. Accordingly, we metabolically engineered a strain of *Clostridium ljungdahlii* to produce acetone by expressing a synthetic acetone operon, and then deleted metabolic pathways that produced undesired side products, like isopropanol. Finally, the strain was engineered to utilize glucose by introducing a heterologous glucose transporter. With the ability to improve product yields, MixoFerm™ is a robust and flexible platform technology to improve process economics and product life-cycle analysis.

3:30 PM S53: Metabolic engineering of oleaginous yeast *Yarrowia lipolytica* for omega-3 long chain fatty acid production

D. Gao and M. Blenner, Clemson University, Clemson, SC, USA*

Omega-3 long chain fatty acids are essential for human health and development. The stagnant production of omega-3 fatty acids from fish oils no longer meet the demands of the fast growing aquaculture and nutraceutical markets. A sustainable and economic source of omega-3 fatty acids is urgently needed. Here we report a systematic approach to engineer non-conventional yeast *Y. lipolytica* for eicosapentaenoic acid (EPA) production from non-sugar substrates such as cheap plant oils and rendered animal fats. We first established an alternative EPA biosynthesis pathway into *Y. lipolytica* by heterologous expression of a set of elongases and acyl-CoA dependent desaturases, which use acyl-CoA instead of phosphatidylcholine as substrate, resulting in a high level of EPA production, while avoiding accumulation of undesired intermediate fatty acids. We also enhanced the assimilation and synthesis pathways of triacylglyceride, and disrupted its degradation pathway to improve the efficiency of utilizing

exogenous lipids as building block for EPA production. By redirecting acyl-CoA flux towards fatty acid elongation and desaturation, we further increased the amount of EPA that was incorporated into triacylglycerol. Our results illustrate the potential for using lipid-based substrates to produce omega-3 fatty acids in a non-conventional industrial yeast.

4:00 PM S54: Engineering the oleaginous yeast *Rhodosporidium toruloides* for the production of lipids and lipid-based chemicals

C. Rao[*]*, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Natural lipids can be used to produce a wide variety of compounds, including fuels (biodiesel), lubricants, surfactants, solvents, waxes, and creams. These lipid-derived compounds, or so-called oleochemicals, potentially offer a renewable alternative to traditional petroleum-based manufacturing. Oleaginous yeast naturally produce lipids from simple sugars when some other essential nutrient such as nitrogen is limiting. In these regards, they potentially offer an economical and renewable route for natural lipid production from low-cost, plant-based sugars. In this talk, I will discuss the metabolic engineering of *Rhodosporidium toruloides,* a red basidiomycetous yeast, for the production of natural lipids. This oleaginous yeast naturally produces lipids at high titers from a wide variety of common sugars, including glucose, xylose, cellobiose, arabinose, and sucrose. However, far less is known about this oleaginous yeast than the model *Yarrowia lipolytica*. In addition, the genetics are still quite primitive. Despite these limitations, we have made significant progress increasing lipid production in *R. toruloides*. In particular, we have been able to engineer a strain that doubles lipid production during batch growth and quintuples it during fed-batch growth using glucose as a feedstock. However, these strains have significantly lower productivities when xylose is the feedstock. I will also discuss some of the challenges and opportunities associated with xylose along with our efforts to engineer better xylose utilizing strains.

5:00 PM - 7:00 PM Reception/Exhibits Open

Plaza Exhibit - Concourse Level

5:30 PM - 7:30 PM Session: PS2: Poster Session 2: Environmental, Fermentation/Cell Culture, Natural Products, Special Topics

Plaza Exhibit - Concourse Level

P2 Microbial fuel cell design to increase power production by microbial electrogens in wastewater

J. Romero[*]*, T. Roane and J.D. Park, University of Colorado Denver, Denver, CO, USA*

Microbial fuel cells (MFCs) rely on ‘electrogenic’ bacteria to produce electricity, offering an innovative form of sustainable energy with further investigation. With potentially dozens of electrogenic bacterial species inhabiting wastewater, growth conditions satisfying the metabolic needs of these organisms, thus enhancing electricity production, are still being determined. The objective of this study was to enhance the metabolic activity of electrogenic bacteria in MFC reactors to encourage extracellular electron release, resulting in the production of current and voltage to produce power. MFC designs tested included a ‘box’ reactor design with different anodes (titanium and iron oxide) and different chemical compositions of growth medium. Among the MFC designs tested, an iron oxide anode in conjunction with a vitamin and mineral-enriched medium was found to increase power production. Within 30 days of incubation under 1000 Ω resistance, this MFC design produced an average of 334 mV, a 7-fold increase over a design

consisting of a titanium anode and a less enriched medium under the same timeframe and resistance. With the former design, analysis of the iron oxide anode fibers under fluorescent light microscopy showed evidence of a diverse anode biofilm. Bacterial 16S rDNA sequencing of the electrogenic community in free solutions and in anode biofilms collected at different time points during MFC maturation showed diverse bacterial communities. Continuing studies will further optimize MFC conditions to meet the metabolic needs of the mixed electrogenic bacterial communities.

P4 Multiple electron transfer components participate in the first step of dioxin metabolism

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Sphingomonaas wittichii RW1 is of great interest for its diverse metabolic activities. *S. wittichii* RW1 uses a unique angular dioxygenase system to break down the recalcitrant carbon structure of dioxin. Previous studies showed that the RW1 dioxygenase possibly functions with multiple ferredoxins and reductases. However, how many genes are actually involved in the electron transfer remains to be elucidated. Here we report the characterization of the essential electron carrier proteins, a ferredoxin and a reductase implicated in dioxin metabolism by generating single and/or double knockout mutants of each electron transfer component. The single and double knockout mutants were examined on minimal medium supplemented with dioxin or dibenzofuran for growth ability. Screening tests revealed that either RedA1 or RedA2 and Fdx1 or Fdx3 are able to serve as an electron donor and to oxidize NADH in the first step of the dioxin degradation pathway. The single knockout mutant of each of the electron transfer components showed no effect when *S. wittichii* RW1 was grown on dioxin or dibenzofuran while the double knockout mutant of the same component abolished growth on both of the substrates, confirming that two electron carrier proteins, Fdx1 and Fdx3, RedA1 and RedA2 participate in transferring electrons to the initial dioxygenase. This proves that the two ferredoxins and the two reductases are reciprocal in their function. Our work provides in vivo physiological proof of the role of the two reductases and two ferredoxins in dioxin metabolism thus complementing the previous in vitro protein purification experiments.

P6 ^{13}C metabolic flux analysis in microbial communities: An integrated multi-scale modeling approach

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Syntrophy, or cross-feeding, is the co-existence of two or more microbes whereby one feeds off the products of the other. Recently, we have developed an integrated multi-scale modeling approach that allows, for the first time, to dissect interactions in microbial communities using ^{13}C tracers. Specifically, to quantify metabolism and identify cross-feeding interactions we have developed a compartmental multi-scale ^{13}C metabolic flux analysis approach that quantifies metabolic fluxes for multiple cell populations in microbial communities without physical separation of cells or proteins. As a specific example, in this presentation, I will illustrate our investigations of metabolic interactions between several *E. coli* mutants that are unable to grow on glucose in minimal medium by themselves, but can grow on glucose when cultured together. Using our novel tools, we have quantified metabolic interactions (i.e. metabolite cross-feeding) in four distinct synthetic *E. coli* co-cultures. We also applied adaptive laboratory evolution and next-generation whole-genome sequencing to elucidate how syntrophic interactions evolve and are strengthened through adaptive co-evolution.

Overall, the methods we have developed for studying microbial communities enable a new area of investigations, allowing us and others to dissect complex microbial systems that are of significant importance in biology but cannot be investigated with current tools. More broadly, by better understanding syntrophic relationships at the genetic, molecular, cellular and systems levels we are generating new knowledge on microbial syntrophy that enables us to ensemble synergistic interactions in engineered microbial communities for novel industrial biotechnology applications.

P8 The genome of *Varunaivibrio sulfuroxidans* TC8T, an alphaproteobacterium from a shallow-water gas vent in the Tyrrhenian Sea shows potential for heavy metal detoxification and metabolic adaptations to its native habitat

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Varunaivibrio sulfuroxidans, type strain TC8^{T}is a mesophilic, facultatively anaerobic, facultatively chemolithoautotrophic bacterium,isolated from shallow-water marine gas vent located at Tor Caldara ,Tyrrhenian Sea. The draft genome of *V.sulfuroxidans*comprises 3045253 bp with 59.42% G+C content and has 2837 predicted protein coding genes. There are 46 predicted tRNA and 3 rRNA genes in the genome. In addition to the CO2 emissions, terrigenous organic carbon is also available at Tor Caldara; an adaptation to this is reflected in *V. sulfuroxidans'*genome, which encodes for both the Calvin cycle as well as the Kreb's cycle and glycolysis pathway. The genome encodes for a complete SOX system, complete dissimilatory sulfate reduction pathway and an incomplete assimilatory sulfate reduction pathway. This shows another metabolic adaptation to an environment where there is an abundance of hydrogen sulfide. The genome has a complete set of genes required for nitrogen fixation as well as denitrification. *V. sulfuroxidans*is exposed to both reducing and oxidizing conditions on a temporal and spatial scale at Tor Caldara. As a result, along with nitrate respiration it can also respire oxygen, which is confirmed by the presence of cytochrome c oxidase in the genome. The genome of *V.sulfuroxidans*also encodes for several heavy metal resistance genes including the mer operon, genes for arsenate resistance and genes for selenate detoxification. One intact prophage and six CRISPRs are found in the genome. Overall, the genome of *V. sulfuroxidans*reflects its versatile adaptations to the dynamic nature of its native habitat.

P10 Analysis of CO_2/H_2 and cold-responsive transcriptomes of *Acetobacterium bakii* DSM 8239

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About 80% of terrestrial habitats is found to be permanently cold, and cold-active acetogenesis contributes significantly to the global carbon cycle. However, understanding of the cold adaptation mechanism of psychrotolerant acetogens is quite limited. Here, we elucidated the genome and transcriptomes of *Acetobacterium bakii* DSM 8239 along with the regulatory elements for transcription under CO_2/H_2 and cold (10°C) condition. We constructed a near-complete genome sequence (4,319,181 bp) encoded reliable annotations, and then we determined CO_2/H_2 and cold responsive transcriptomes consisting of 2,052 genes using strand-specific RNA-seq. Remarkably, most of the acetogenesis-associated genes were highly up-regulated under the cold condition, despite growth in the heterotrophic condition. Hinted by the perturbations of secondary RNA structures play a major role in the transcription regulation under cold condition, we determined the genome-wide transcription start sites (TSSs) by using the differential RNA-sequencing. Overall, we identified 1,379 purine-rich TSSs along with well conserved TATA box in promoter regions, and Shine-Dalgarno (SD) motifs in 1,100 5'-untranslated regions (5'-UTR). Interestingly, a group of 252 genes including acetogenesis-associated genes with coherent expression patterns contains longer 5'-UTR length (a median of 89 nt) with lower RNA folding free energy than others (a median of 46 nt). Furthermore, the cold-responsive transcriptional signatures indicating attenuation were found in their 5'-UTR regions. This phenomenon suggests that post-transcriptional regulation is necessary for cold-adaptation of psychrotoloerant acetogens. Thus, the results provide the underlying

mechanism for cold-adaptive acetogenesis as well as the useful genetic information of *A. bakii* for potential future application in metabolic engineering.

P12 Bioprocess optimization in leading cellulosic feedstocks miscanthus and corn stover for bioethanol production.

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With the mandate of 36 billion gallons of ethanol production by 2030 set by Renewable Fuel Standard Association (RFA) and Energy Independence Security Act (EISA), US it is important to investigate ethanol production from cellulosic energy crops. While corn ethanol production has achieved commercial limit of 15 billion gallons, the remaining deficit of 21 billion gallons of ethanol from cellulosic biomass is still in its infancy. Cellulosic Ethanol production is influenced by several factors, which include feedstock loading and enzymes loading. Increasing enzyme loading for saccharification can be quite expensive. Saccharificaion is critical to the success of cellulosic biofuel production, which determines primarily the amount of sugar released. Miscanthus giganteus and corn stover are used for biofuel production. The objectives of this study were to 1) determine the optimal substrate loading and 2) enzyme loading for maximum ethanol production. Study utilized commercial Miscanthus var. Freedom and corn stover; fermentation and saccharification optimization using response surface methodology (RSM) were investigated. The preliminary results indicated that the best enzyme and substrate loading were 20 and 10 percent respectively for both feedstocks.

P14 Characterization of a silent pathway for biphenyl degradation in *Sphingomonas wittichii* RW1

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Sphingomonas wittichii RW1 is known for its unique ability to degrade dibenzofuran and dibenzo-*p*-dioxin. Even though this stain was unable to use biphenyl as a sole carbon source, our previous experiments showed that its angular dioxygenase can attack biphenyl at a lateral position producing 2,3-dihydro-2,3-dihydroxybiphenyl. Furthermore, the 2,2,3-trihydroxybiphenyl dioxygenase involved in the DBF and DD pathway, *dbfB1*, showed activity towards 2,3 dihydroxybiphenyl and has strong homology with extradiol dioxygenases of biphenyl degraders. All these facts collectively led us to hypothesize that the same genes for DBF and DD degradation can show activity towards intermediates from the biphenyl pathway and that the only missing step would be the *cis*-dihydrodiol dehydrogenase. To prove our hypothesis, the gene for *cis*-dihydrodiol dehydrogenase, *bphB*, from the biphenyl degrader *Sphingobium yanaikuyae* B1 was placed downstream the *fdx3* gene under the control of the constitutive promoter of the *dxn* locus in *S. wittichii* RW1. Interestingly, this engineered strain grew on biphenyl with a doubling time of 3.2 hours revealing a hidden pathway for biphenyl degradation in RW1. To determine the ring cleavage dioxygenase and the hydrolase involved in this pathway, multiple mutants for these enzymes were tested for their ability to grow on biphenyl after introducing a pRK415 vector carrying *bphB.* Results showed the involvement of two different ring-cleavage dioxygenases and two different hydrolases in the biphenyl degradation pathway. This work demonstrates that the enzymes in the upper pathway for DBF and DD degradation have a wide substrate range with activity towards other aromatic hydrocarbons.

P16 Lipopeptide production and petroleum hydrocarbon utilization by *Bacillus amyloliquefaciens*

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Several *Bacillus amyloliquefaciens* isolates from wheat residue have been studied as biological agents to control wheat diseases. Notable traits of isolates 1BA and 1D3 include growth with high salt (10% NaCl), at temperatures up to 50˚ C and over a wide pH range. Isolates 1BA and 1D3 also produce lipopeptides

such as surfactin and iturin. Their production of lipopeptides and tolerance to some environmental stresses led to the current study to better understand their metabolic capabilities; and whether they could grow and metabolize petroleum hydrocarbons such as kerosene.
BIOLOG Gen III plates were used to study and further characterize *Bacillus amyloliquefaciens* 1BA and 1D3. Both showed tolerance to high salt concentration supporting previous studies. They also grew in the presence of lithium chloride, potassium tellurite, and sodium bromate. Both isolates grew at pH 5, with almost identical carbon source utilization fingerprints. However, D-serine and quinic acid and N-acetylglucosamine were utilized by 1BA but not by 1D3.

In broth studies, 1BA and 1D3 were grown in tryptic soy broth (TSB) with 1.0 % kerosene. Gas chromatography (GC) was done to monitor petroleum hydrocarbon utilization. The $C_{13}H_{28}$ and $C_{12}H_{26}$ initially observed in sterile control TSB and at day 5 in bacterial cultures were consumed by the bacteria after 15-day incubation period at 25° C. Ongoing studies will further examine petroleum hydrocarbon utilization of the kerosene, and test for long chain and aromatic hydrocarbon utilization and lipopeptide production by these two isolates.

P18 Isolation and characterization of rhizobacteria PGPR and its effect on growth promotion in potato (*Solanum tuberosum*) under greenhouse conditions

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The agriculture has been characterized by the intensive use of chemical fertilizers to maintain high yields, without taking into account its negative effects. This generates the need to develop technologies which favor the nutrition of plants. Due of this the objective of this work was isolate native rhizobacteria from soils from central Mexico and evaluate their plant growth promoting ability in potato cultivation (*Solanum tuberosum*). 120 bacteria were isolated from maize (56) and potato (64) rhizosphere, and purified, characterized and preserved at -80 ° C. Their effect on germination and root elongation in tomato seeds was evaluated. A group of 4 bacteria was selected according to their capacity to produce AIA, ACC desaminase and/or solubilize phosphates, tolerance to agrochemicals and growth promoting effect. The isolated selected was identified according to the sequence analysis of the 16S rRNA gene. The results showed that the treatments inoculated with the strains PT41PS and MT53PS showed a significant difference in the Germination Index and significantly increasing in height (67%), dry weight of stem, root and tuber (three more times) of the plants with respect to the commercial product and to the uninoculated control. The strains identified as *Enterobacter cloacae* and *E. asburiae* produces AIA (7.97 and 6.53 mg L^{-1}), ACC deaminase (0.137 and 0.015 mM α-ketobutyrate) and solubilizes phosphate (SI=4.95 and 2.38 respectively). Both strains tolerate high concentrations to different agrochemicals. This suggests that they can be used as a biofertilizer for potato cultivation under greenhouse conditions in the central region of Mexico.

P20 A small pH control system for six fermentation reactors

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Several companies are offering systems that allow for multiple simultaneous fermentation vessels with independent controls (temperature, pH, oxygen, agitation, antifoam, etc.). However, many of these systems are overly complex and expensive if only pH control is necessary. For this reason, we developed a simple and inexpensive system that can control pH in up to six small fermenters.
The fermentation pH was measured by standard pH probes that were interfaced with a Data Acquisition (DAQ) module (LabJack, Lakewood, CO) but the standard DAQ software lacked control functions. Therefore, we constructed a Visual Basic program in Excel to record and control the pH by communicating with relays connected to pinch valves for dispensing base (NaOH). The control system was tested with ANKOM Technology RF Gas Production System (Macedon, NY) vessels used for ethanol fermentation with continuous gas production monitoring.

P22 Inhibitor resistant non-conventional yeast strains for lignocellulosic biomass hydrolysate fermentation

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To efficiently use lignocellulosic biomass hydrolysates as fermentation media for bioethanol production, besides being capable of producing significant amount of ethanol, the fermenting host should also meet the following two requirements: (1) resistant to the inhibitory compounds formed during biomass pretreatment process, (2) capable of utilizing C5 sugars, such as xylose, as carbon source. In our laboratory, a screening was conducted on microorganisms collected from environmental sources for their tolerance to hydrolysate inhibitors. Several unique resistant yeast strains were selected and identified, among which completely unexplored species. A unique *Wickerhamomyces anomalus* strain was discovered that was able to produce ethanol in various biomass hydrolysates, both with and without oxygen. Besides, the strain could assimilate xylose and use nitrate as N source. These physiological characteristics make this an interesting strain for bioethanol production from lignocellulosic biomass hydrolysates.

P24 Dark fermentation, physiology and regulation in the hyperthermophilic archaeon *Thermococcus kodakarensis*

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Currently employed model microbes, typically yeasts and bacteria, enjoy success due in large part to genetic systems that compensate for their limited metabolisms. However, these platforms remain restricted to similar feedstocks, necessitate the introduction, regulated and balanced expression of heterologous pathways for substrate degradation, suffer from significant and expensive contamination issues, have large fresh water requirements, are limited by product inhibition, and typically require expensive substrate pretreatments that both introduce toxic byproducts and increase total costs. Alternative systems must be developed in parallel with modifications and improvements to existing platforms. The advantages of the hyperthermophilic Archaea have long been recognized, but only within the last decade have genetic techniques allowed the full potential of these organisms to be more completely exploited. We developed a near complete genetic system for the marine archaeon *Thermococcus kodakarensis*, providing unlimited and unprecedented access to archaeal chemistries and rational and iterative strain construction. We, together with many members of the archaeal community, have simultaneously identified and characterized a host of enzyme activities promoting the complete saccharification of cellulose as well as the degradation of chitin, peptides, and starch. The combination of established genetic and biochemical techniques are now poised for a grand merger producing a whole-cell hyperthermophilic biocatalyst for biofuel production, and our long-term goal is to establish *T. kodakarensis* as a biofuel production platform. We will discuss recent advances in strain construction, regulated transcription systems and new replicative vectors.

P26 Production and antimicrobial activity of glycolipid biosurfactant produced by *Pseudomonas aeruginosa* NSU-1 isolate

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Biosurfactants, which are amphiphilic compounds produced by microorganisms, have excellent surface tension-lowering activity with biotechnological and therapeutic potentials. Besides their surfactant and environment-friendly properties, additional value-added property such as antimicrobial activity makes them good candidates for applications in the control of infectious agents to further heighten their commercial interests. In this study, the *in vitro* antimicrobial activity of glycolipid biosurfactant produced by *Pseudomonas aeruginosa* NSU-1 from cassava wastewater was evaluated against multidrug resistant clinical isolates of *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* using the microdilution method. The biosurfactant showed potent antimicrobial activity with minimum inhibitory concentration and

minimum bactericidal concentration of between 25 and 50 mg/ml concentrations against the pathogens tested. The results of this study indicate that the *Pseudomonas aeruginosa*NSU-1 biosurfactant has antimicrobial property against the microbial strains tested. This capability suggests the potential of the biosurfactant for application in the treatment and/or prevention of certain infections.

P28 Scaling-up the production of a second-generation biofertilizer

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The green revolution technology have provoked several adverse effects to the environment due to indiscriminate use of pesticides, herbicides and nitrogen fertilizers. The usage of beneficial bacteria to enhance yield of crops represent an alternative to replace the consumption of chemical fertilizer and toxic pesticides. Even though inoculation of plants with beneficial bacteria is centuries old and several research groups around the world have been working in isolation and description of new bacterial species, just few microbial inoculants appear on the commercial market, probably due to little scientific efforts focused on the development of production technology. Second generation inoculants include bacterial consortia able to promote plant growth more effectively than a single bacterium which represent desirable inputs for organic farming schemes. However, the process development for the production of a multi-species microbial inoculant in bioreactors is not an easy task. In this work, we describe the challenges faced when the production process of a multi-species inoculant was scaled-up from Petri dishes to bioreactor. This second-generation microbial inoculant contains in its formulation six plant-growth promoting bacteria with different functions: nitrogen fixers, biocontrol agents, growth-hormone producers and phosphate solubilizing bacteria. In order to decrease production cost, a co-cultures strategy was proposed demonstrating that the number of fermentation runs can be reduced making this process more feasible at commercial scale.

P30 Production of vicatx1 toxin in the cytoplasm of *Escherichia coli* using a novel promoter

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Heterologous protein expression in E. coli is easy to handle at large-scale, cheap and well characterized. However, this bacterial system presents few limitations especially when expressing proteins with post-translational modifications, such as disulfide bond formation. Disulfide bonds are required for proper protein folding, stability and activity. They are formed into the covalent bond by the oxidation of thiol groups between two cysteine residues in the protein taking place into the endoplasmic reticulum of eukaryotes and the periplasm of prokaryotes. Although *E. coli* cytoplasm can produce a large amount of recombinant protein, it is not favorable for the formation of disulfide bonds due to its highly reducing environment. Extensive efforts have been made to overcome these limitations to improve soluble expression of different disulfide-bonded proteins in the cytoplasm of *E. coli.* Disruption of pathways involving thioredoxin reductase and glutaredoxin can effectively promote disulfide bond formation but with low protein yields due to impaired growth of the host strains. In this work we evaluated the ability of E. coli to produce a disulfide-bonded protein, VICaTx1 toxin, in the cytoplasm using the oxygen-induced expression system. The elevated oxygen concentration used in this system has dual function: change the redox environment of cytoplasm and induce the expression. This proposal represents an economical and functional alternative of recombinant protein production, since it does not require costly chemical inducers or expensive purification processes.

P32 Fermentation in a basic research setting: a case study

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Fermentation and cell culture are a driving force for the biotechnology and pharmaceutical industry, because an increasing number of valuable therapeutic products are now being produced through biological processes rather than small molecule chemical synthesis. However, fermentation's role in a basic research environment, such as government institutes or academic universities, has not been greatly appreciated. In this case study, *Paracoccus denitrificans (Pd),* a bacterial ancestor of mitochondria, was used as a model to study the electron transport chain (ETC) of oxidative phosphorylation. A well-controlled fermentation played a key role in protein expression of the full set of intact ETC components for the study. An online off-gas analyzer was coupled with the fermenter, which provided insightful information on *Pd*'s oxygen uptake rate and CO_2 exhaust rate during exponential growth. The close collaboration between the fermentation core facility and the physiology research group provided a unique opportunity to develop fermentation process aimed at decoding the mysteries of energy homeostasis

P34 Evaluation of the antibacterial potential of a probioticated beverage from a mixture of Orange, Sugarcane and Moringa extracts

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In developed and developing countries, functional foods and diet are now embraced to reach and maintain optimal health, wellbeing or performance beyond regular nutrition. Consumption of probiotics is a natural way of promoting consumer health. This study was carried out to evaluate the viability of probiotic lactobacilli in a mixture of orange, sugarcane and moringa juice extracts at two different temperatures with a view to producing acceptable and health beneficial probiotic beverage. *Lactobacillus* species isolated from fermented maize grains and sorghum samples were characterized and inoculated into the sterile beverage samples. Samples were then stored at ambient (26 ± 2^{o}C) and refrigeration (4 ± 1^{o}C) temperatures over a period of three weeks and the viability of the probiotic isolates investigated using standard methods. Using broth culture method, antibacterial effect of the probiotic isolates on food-borne pathogens (*Staphylococcus aureus* and *Escherichia coli*) was also determined. Findings showed that *Lactobacillus plantarum* strains remained viable in the beverage for 21 days. The *L. plantarum* gotten from both sorghum and maize increased from an initial count of 1.0 x 10^6 to 5.00 x 10^6 and 8.50 x 10^6 CFU/ml respectively in samples kept at ambient temperature. The results further showed that the probiotic isolates exhibited varying degree of inhibition against the food-borne pathogens with greater inhibition shown against *E. coli*.

P36 A case study for bio-pesticide: Roquette proteins combination significantly reduce the lag-phase

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Roquette has developed multiple nitrogen sources for fermentation industry applications, cost-effective Solulys™, Tubermine™ potato protein and Nutralys™ pea protein, as well as the protein hydrolysates. Solulys™ is not a "by-product", rather a corn steep type product developed specifically by Roquette. The tightly controlled manufacturing process is aimed at producing a characteristic product profile that differentiates Solulys™ from typical corn steep liquor (CSL).

In this study, *Bacillus thuringiensis* (ATCC 33679) was selected as a model to verify the performance of Roquette Solulys™ compare to typical corn steep liquor (CSL), and combinations of Solulys™ with either Tubermine™ or Nutralys™ were evaluated. All the benchmarks were applied at the equal nitrogen level and the DASGIP parallel bioreactor system was employed for the studies.

As results, comparing to typical corn steep liquor (CSL), Roquette Solulys™ improved the productivity by reducing almost 50% lag-phase time; furthermore, the combination of Solulys™ with Tubermine™ or Nutralys™ resulted in the reduction of lag-phase time up to 80%.

P38 Coupling xylitol dehydrogenase with NADH oxidase improves L-xylulose production in *Escherichia coli* culture

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L-xylulose is a rare sugar generated from xylitol by an NAD^+-dependent xylitol-4-dehydrogenase (XDH) from *Pantoea ananatis*. Since the specific conversion of xylitol to L-xylulose involves the formation of NADH, the continued presence of NAD^+ is necessary to drive the process. In this study, we co-expressed water-forming NADH oxidase (NOX) from *Streptococcus pneumoniae* with XDH in wild-type *E. coli*. Controlled batch processes at the 1 liter scale demonstrated that final equilibrium L-xylulose/xylitol ratio was correlated to the ratio of intracellular NAD^+/NADH, with 69% conversion of xylitol to L-xylulose and a yield of 0.88 g L-xylulose/g xylitol consumed attained for MG1655/pZE12-*xdh*/pCS27-*nox* growing on glycerol. Intermittently feeding carbon source was ineffective at increasing the final L-xylulose concentration because introduction of carbon source was accompanied by a reduction in NAD^+/NADH ratio, confirming the importance of maintaining a high NAD^+/NADH ratio. A batch process using 12 g/L glycerol and 22 g/L xylitol generated over 14 g/L L-xylulose after 80 h, corresponding to 65% conversion and a yield of 0.89 g L-xylulose/g xylitol consumed.

P40 Kinetic Model Development for Ethanol Production by *Parageobacillus thermoglucosidasius* Using Food Waste

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Use of corn, and sugarcane etc. for bioethanol production surfaced food vs fuel debate, and initiated drive for the search of alternative sources for bioethanol production. Food waste which forms a major portion of municipal solid waste, can be used as an alternative source providing a sustainable solution for both bioethanol production and municipal solid waste. The objective of the current study was to investigate kinetic parameters for batch ethanol fermentation by *Parageobacillus thermoglucosidasius* using simple sugars, and extend the studies for kinetic model development using food waste. *Parageobacillus thermoglucosidasius* was grown on several different carbon sources: glucose, xylose, galactose, and lactose, maltose, mannose, cellobiose, fructose, and starch. The microbial growth pattern, substrate utilization, and product formation were recorded for data analysis and kinetic model development. Under facultative anaerobic conditions, *Parageobacillus thermoglucosidasius* produced ~0.19 g ethanol/g glucose, and 0.043g ethanol/ g xylose along with lactate, acetate, and formate as other fermentative products. Similar product profile was obtained with other carbon sources. The data collected was used to calculate several kinetic parameters, to develop a kinetic model. The kinetic model thus developed will be used to predict the theoretical amount of ethanol obtainable after fermentation with the food waste, by *Parageobacillus thermoglucosidasius*. Successful development of a kinetic model for the production of ethanol by *Parageobacillus thermoglucosidasius* can provide insight for further improving and optimizing the bio-process for ethanol production from food waste. Also, the kinetic model can provide useful information for the techno-economical analysis of the process.

P42 Fermentation process development for *Streptomyces albus* as a novel platform for biofuel production from cellulosic sugars

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Due to the difficulty of placing internal methyl groups on a hydrocarbon chain using fatty acid synthases (FASs), we have employed polyketide synthases (PKSs) capable of producing hydrocarbon chains with branches near the center of the chain. Engineering of PKS pathways favors a native host; *Streptomyces* strains have the ability to functionally express hybrid PKSs and metabolize a wide variety of carbon

sources including both 5 and 6-carbon sugars. While *Streptomyces* are widely employed for production of antibiotics, their suitability for industrial production of biofuels and chemicals is currently unknown. Two engineered *Streptomyces albus* strains producing a mixture of short chain ketones were tested for fermentation development. One strain produces methyl isobutyl- and methyl isopropyl ketones, while the second strain produces a mixture of ethyl isobutyl- and ethyl isopropyl ketones. *Streptomyces* species generally require complex media for production of secondary metabolites - we were able to double product titers and eliminate several complex media components by substituting cellulosic hydrolysates for glucose, insoluble starch, and glycerol. Use of alkali-pretreated corn stover hydrolysate as the primary carbon source led to highest ketone production (280 mg/L in shake flasks); this condition was chosen for scale-up to 2L fed-batch fermentation. The impact of varying process parameters, including temperature, pH, dissolved oxygen concentration was established at the 2L scale. Optimal temperature for ketone production was found to be 30 ºC. Performing fed-batch fermentation with corn stover hydrolysate improved ketone titers.

P44 Carotenoid value addition of Corn Syrup using red yeast fermentation

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Corn ethanol industry is critical in realization of renewable energy mandate. Corn ethanol produces several co-products/byproducts such as CO_2, Distillers Dried Grain with Solubles (DDGS) and corn syrup. Some of these co-products are known to have very low value. Attempts have been made to develop value added products from co-products. Some of the examples are extraction of Zein protein from DDGS and Carotenoid enriched DDGS. The value addition to co-products enhance sustainability to ethanol companies by generating extra revenue when ethanol's price comes under fluctuation. Astaxanthin and β-barotene are two commercially important natural carotenoids in food and feed industry. These natural carotenoids are produced by microbes such as algae and red yeasts. The overall objective of this study was to produce corn syrup as feedstock for production of astaxanthin and β-carotene using red yeast *Phaffia rhodozyma*. Process optimization and scale-up studies in the bench-top bioreactor will be discussed.

P46 Effects of seed culture conditions on *Clostridium acetobutylicum* performance in batch fermentation

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Clostridium acetobutylicum has been studied for decades as a means of producing acetone, butanol and ethanol (ABE) from various carbon sources. Butanol is of particular interest: unlike ethanol, it can replace up to 85% gasoline in cars designed to use standard petrol without engine modification. With technological improvements reducing the price of petroleum products, batch fermentations of *C. acetobutylicum* have not been commercially viable since the mid-20th century. Because of relatively low productivity (up to 1.2% v/v butanol in batch fermentation over 36-48 h) and solvent toxicity to *C. acetobutylicum*, a continuous fermentation approach is required for commercial viability. A robust batch *C. acetobutylicum*fermentation process is necessary as a prerequisite to developing a continuous fermentation process for butanol production.

After observing inconsistent and unreliable growth and ABE productivity in batch fermentations inoculated with confluent seed cultures, we examined the effects of other characteristics of the seed culture on production fermentation performance: seed culture optical density (OD_{600}), age, pH and residual glucose concentration. Butanol production levels were used to benchmark those runs where successful growth in production fermentation was achieved. We found that seed culture pH (5.0 ± 0.3, from initial 6.2) and residual glucose concentration (10 g/L ± 2 g/L, from initial 20 g/L) were critical parameters contributing to successful production fermentation performance, independent of seed culture age or OD_{600}. While production fermentations inoculated with seeds that did not meet these criteria also grew occasionally, only those meeting these criteria reliably grew and produced expected levels of butanol.

P48 Biopolymers produced by a thermophile: production, characterization and applications

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Exopolysaccharides (EPSs) are high molecular weight carbohydrate biopolymers composed of sugar residues linking together through glycosidic bonds, and secreted by microorganisms into surrounding environment with certain properties and functions. The biosynthesis of EPSs is one of the adaptations developed by various kinds of extremophiles to assist the microbial communities to endure extremes of temperature, salinity, and pH. Recently, extremophiles are considered as potential producers of EPSs with a wide range of physicochemical, structural, and rheological properties which may hardly be found in more traditional polymers. However the accumulative knowledge for the structural and functional properties of extremophilic EPSs is still limited. In this research, a thermophilic strain, *Geobacillus* sp. WSUCF1 which produced unique EPSs has been studied. The strain WSUCF1 could utilize glucose as optimum carbon source to produce significant amount of EPSs. Two purified EPSs were obtained through ion-exchange and gel filtration columns, and both of them demonstrated relatively high degradation temperature. Monosaccharide composition analysis showed these EPSs were composed by mannose and glucose with various molar ratios. The EPS-based films were developed through solvent casting using glycerol as plasticizer, and their characterizations, such as surface morphology, thermal stability, mechanical properties and biocompatibility are currently under investigation. EPSs from WSUCF1 strain will likely provide a valuable resource for exploitation in novel biotechnological processes and opportunities in various industrial fields including bio-based polymeric materials and antitumor-drug carrier. Meanwhile, the research of extremophilic EPSs will also expand the scope and provide new research direction for the microorganisms isolated from harsh environments.

P52 Testing of on-line optical cell biomass probe linearity and accuracy across changing process conditions

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A long-standing challenge for on-line optical biomass probes has been to maintain biomass prediction accuracy across changing process conditions, particularly for organisms requiring highly variable rates of agitation and aeration in order to avoid oxygen-limited growth. For microbial organisms grown to high cell biomass densities, being able to accurately track the biomass across the entire growth cycle from inoculation to harvest has been difficult to achieve due to the limited linearity range of traditional on-line methods and variation in sensor readings with changing process conditions. A comparison study of the sensitivity and linearity of different commercially available on-line optical probes to changing process conditions over a wide range of biomass densities will be described. In particular, the effects of changing agitation and aeration conditions on biomass prediction error for a methanotrophic organism will be compared for two commercially available on-line optical reflectance probes.

P54 Bacteriocin production from Lactic acid bacterial using spent coffee ground as feedstock

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Soluble coffee, being one of the world's most popular consuming drink, produces considerable amount of spent coffee ground (SCG) along with its production. The SCG could function as a potential lignocellulosic feedstock for production of bioproducts. The objective of this study was to evaluate the production of bacteriocin by *Lactobacillus plantarum* and *Lactobacillus paracasei* using spent coffee ground as feedstock. Bacteriocins are ribosomally-synthesized antibacterial peptides. These compounds have attracted significant attention because of their possible applications as non-toxic additives for food preservation and prevention of food spoilage by food-borne pathogenic bacteria. The bacteriocin produced by *Lactobacillus plantarum* and *Lactobacillus paracasei* showed a broad inhibitory activity

against Gram-positive and Gram-negative bacteria including *Lactobacillus casei, Escherichia coli* and *Bacillus cereus.* Based on Tricine–SDS–PAGE, the BLIS is approximately 3.0 kDa in size. This study is useful for the further optimizing of fermentation and developing of biorefinery using SCG as feedstock at large scale.

P56 Production of methylobacteria for applications in agro-ecosystems

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NewLeaf Symbiotics (NLS) is an agricultural biologicals company built upon a robust bioinformatics platform that generates intellectual property and a rich pipeline consisting of bacteria isolated from the phytobiome. Most microbial biocontrol products consist of *Firmicutes*, *Actinomycetes* or *Fungi.* NLS focuses on *Alphaproteobacteria,* particularly pink-pigmented facultative methylotrophs, abbreviated as PPFM(s). Functional genomics analyses reveal critical roles for these bacteria in plant growth and immunity. Methylobacteria are found as plant epiphytes or endophytes in association with plant seeds, leaves and roots.

All methylobacteria in the NLS collection are deep sequenced. Select strains are advanced to close individual genomes to a single contig and annotated within a proprietary database known as the Prescriptive Biologics™ Knowledgebase (PBK). The PBK combines high-content screening analytics and computational biology. It informs our pipeline, enabling correlations between genotypes and phenotypic responses. Automated host imaging and phenome analyses confirm *in-silico* predictions. NewLeaf Symbiotics has produced its lead strains at scales ranging from 2-L to >60,000-L through media improvements, fermentation process optimizations, downstream process development and end-product formulations. Production materials are iteratively tested for performance *in-vitro* and *in-vivo* in growth chamber and greenhouse screens. Product leads are selected using commercial crop varieties in field plots configured to assess microbial trait contributions. Global field tests confirm the effects of NewLeaf's proprietary *Methylobacterium* strains on pests, diseases, and nutrient stress via seed treatment, in-furrow or foliar application. Crop benefits are host genetics and PPFM strain dependent.

P58 Microencapsulation of *Lactobacillus paracasei* with a polysaccharide extraced from *Ficus awkeotsang Makino*

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Many researches have recommended that the probiotics must be presented at a minimum concentration in order to provide health benefits. However, the vulnerability of probiotics at low pH has limited their optimal use as nutraceuticals. Microencapsulation is suggested to be a promising approach for introducing viable probiotics through oral administration because the encapsulation matrix can provide a physical barrier against adverse environmental conditions. The aim of this study is to increase the viability of Lactobacillus plantarum during exposure to simulated conditions of the gastro-intestinal tract by microencapsulation with the polysaccharide extracted from the seed of Ficus awkeotsang Makino. Ficus awkeotsang Makino is a native Taiwanese plant and its seed polysaccharide can be crosslinked with divalent or trivalent cation to form a gel with a good physical stability. Three different cation solutions, including CaCl2, ZnCl2, FeCl3, were used as crosslinking agents to prepare microencapsulation of L. plantarum in the Ficus polysaccharide beads using extrusion method. The result shows that the Ficus polysaccharide has high loading capability of L. plantarum (98.51%) in all cases. The survivability and release rate will also be tested and reported as well as other physical properties of the microbeads.

P60 The *Streptomyces spectabilis* NRRL-2792 genome: de novo sequencing for identification of secondary metabolites and genetic engineering studies

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This is the first report of a fully annotated genomic sequence of *Streptomyces spectabilis*, NRRL 2792, isolated and identified by The Upjohn Co. in 1961. Sequencing was done using the Illumina HiSeq 2000 sequencing platform, followed by PCR based gap-closure to yield a single scaffold. The chromosome is linear, 9.5 Mb long, with a very high GC content of 72.4% and contains approximately 8000 genes. No evidence was found for any large linear or circular plasmid. Bioinformatics analysis identified 45 putative secondary metabolite biosynthetic gene clusters including metacycloprodigiosin and albaflavenone. This information can also be utilized for the creation of novel synthetic antibiotics or enhancing expression of existing ones. Additionally, the sequence provides information about various regulatory, resistance and biosynthetic genes, and allows other NGS based applications like RNA-Seq to aid candidate selection for genetic engineering. Using the sequence information, we were able to identify that the BT1, R4, TG1, VWB and φC31 phage integration systems can be used in *S. spectabilis* for genetic engineering and were also able to determine the approximate number of copies it might deliver.

P62 A study of synthetic promoters for maximizing the activity of a superoxide dismutase in *Streptomyces venezuelae*

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We present studies using a ΦC31-based integrative plasmid to study the effect of various promoters on the translational activity of a two-gene cassette of a native superoxide dismutase (SOD) and a reporter gene GusA in *S. venezuelae*. Five constitutive promoters (ermEP1*, KASO*, GAPDH, p21M, and SF-14), previously studied in various other streptomyces were selected for this study. *S. venezuelae* was specifically selected for this study owing to its outstanding potential as a host for heterologous gene expression of novel secondary metabolites.

Through analysis of genomic sequence, available at the NCBI database, two SOD genes and a single copy of ΦC31-*attB* site were identified. A single gene annotated as Ni-SOD was selected for the construction of the variable promoter driven two gene cassettes used for our study. Promoter strength was quantified by measuring total SOD activity using an inhibition-based microtiter-assay, specific for SOD activity. Total SOD specific activity was used essentially as a pseudo-reporter for identifying the strongest promoter for use with *S. venezuelae*. GusA activity was used to further rank the promoter activity. In essence, this study implements a unique, dual protein based activity assay to evaluate individual promoter strength.

From these studies, we found that promoters p21M and KASO* gave highest SOD and GusA activity in *S. venezuelae*. GAPDH appeared to be the weakest promoter. Enhanced SOD activity could provide benefits to sustained viability. These results likely have utility in augmentation of heterologous gene expression and production of various secondary metabolites, particularly in obligate aerobes, such as *S. venezuelae*.

P64 Exploring fungal polyketide *C*-methylation through combinatorial domain swaps

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Fungal polyketide natural products are frequently *a*-methylated by *S*-adenosyl methionine (SAM)-dependent *C*-methyltransferases (CMeT) embedded in the polyketide synthase (PKS) architecture. A survey of non-reduced (NR) fungal polyketides demonstrates programmed *C*-methylation of the growing polyketide intermediate, but the source of this control is not clear. We combined recent NR-PKS CMeT structural data with combinatorial domain swaps of several CMeT-containing NR-PKSs to explore this programmed modification and gauge the compatibility of non-cognate domains. Product profiles are dependent on the concentration and identity of the CMeT and reflect the native methylation pattern of the CMeT. Our results are consistent with kinetic control of *C*-methylation during polyketide biosynthesis, such that only programmed positions on the PKS-bound intermediate are methylated before the next round of extension occurs. The ability of this class of fungal PKS CMeTs to methylate various chain-length intermediates suggests that these methyltransferases may be a useful tool for unnatural biosynthesis of fungal polyketides.

P66 Enhanced electrotransformation of *Leuconostoc citreum*

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An enhanced electroporation protocol was developed for *Leuconostoc*. Improved electroporation is important for understanding natural product formation and to enhance biotechnology applications of this bacterial genus. Previously reported low transformation efficiencies for this microbe were probably due to an impermeable cell wall and poor cellular repair following electrical pulse. The goal of this study was to improve the electrotransformation efficiency in *L. citreum* by using treatments that permeablize the cell wall or aid cell repair / resuscitation following electrical pulse. First, *L. citreum* was electro-transformed using varying concentrations of pGK12 and transformants were selected on chloramphenicol agar. Each electro-transformation was performed in triplicate. In general, transformation efficiency improved as less plasmid DNA was used from 1.0 µg to 0.1 µg. To enhance permeability, *L. citreum* cells were treated with concentrations of glycine, penicillin, or lysozyme and then electro-transformed. Statistically significant increases in electro-transformation efficiency were obtained when cells were treated with optimal concentrations of glycine, penicillin, or lysozyme compared to the control (no treatment). To enhance cell repair, competent cells were treated with either 2-mercaptoethanol or Oxyrase or mixing during the resuscitation period following the electrical discharge. All post-electroporation treatments increased transformation efficiency compared to the control. In summary, electro-transformation efficiencies of *L. citreum* were improved by testing plasmid DNA concentration, and by using treatments that either helped permeablize the cell wall or improve cell repair during resuscitation. The enhanced electro-transformation protocol can be used to help understand the genetics of *Leuconostoc* and improve biotechnology applications.

P68 Interconversion of fungal epithiodiketopiperazines with α,β-polysulfide bridges

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Marine microbes are a prolific source of structurally diverse and functionally significant natural products. We isolated and identified one fungal strain *Penicillium steckii* YE from the blank band layer of infected coral samples collected in FL. Five known and three new (Penigainamide A-C) epithiodiketopiperazines (ETP) alkaloids featured with one rare α,β-polysulfide bridge and 1,2-oxazadecaline moiety were identified from the crude extract of fungal fermentation. The structures of Penigainamides A and B were assigned on the basis of comprehensive spectroscopic data, while HRMS and tandem MS/MS analysis led to the structural determination of Penigainamde C as well as all other identified ETPs. Some isolated ETPs underwent substantial degradation into other analogs including one new ETP (Penigainamide D). The observed degradation paths led to a plausible interconversion relationship among nine identified ETPs, which can unify chemical diversity of this subtype of fungal ETPs. Adametizine A, the most abundant alkaloid in the fungal fermentation, showed a potent anti-aging activity toward *Caenorhabditis elegans* and 100 *µ*M of adametizine A substantially expanded the lifespan of the wild type animals by 9.1%.

P70 Functuional analysis of an aminoacyl-tRNA-dependent peptide synthase involved in the biosynthesis of a streptothricin-related compound

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Glycylthricin, a biosynthetic intermediate of BD-12, possesses a glycine side chain, which were covalently attached to an amino sugar moiety (streptothrisamine) *via* a peptide bond. We have reported that, in BD-12 biosynthesis, the peptide bond formation between streptothrisamine and the glycine residue is

catalyzed by a Fem AB-like enzyme (Orf11) in a Gly-tRNA^{Gly}-dependent manner. We have therefore hypothesized that Orf11 homologues with different substrate specificities allow us to generate new ST-related compounds, and we searched *orf11* homologue genes in the draft genome database of streptomycetes.
Based on bioinformatic analysis, we found a gene cluster (*sba* gene cluster) homologous to the BD-12 biosynthetic gene cluster in which the *orf11* homologue gene (*sba18* gene) was identified. To clarify the substrate of Sba18, we investigated the substrate specificity using a recombinant Sba18 (rSba18). In the enzyme reactions with aminoacyl-tRNAs (aa-tRNAs) from *Escherichia coli*, rSba18 was found to accept Ala-tRNA^{Ala} and Ser-tRNA^{Ser} as well as Gly-tRNA^{Gly} as the donor substrates and to catalyze the peptide-bond formation between streptothrisamine and the amino-acid side chain. The resulting new compounds designated as alanylthricin and serylthricin were further demonstrated to exhibit antibacterial activity. To produce these compounds *in vivo*, we introduced the *sba18* gene into a *Streptomyces* strain producing streptothrisamine. We expected that a transformant would produce three compounds, glycylthricin, alanylthricin and serylthricin. However, the transformant provided only glycylthricin. In this presentation, we will discuss about the results and the mechanism of substrate recognition in the aa-tRNA-dependent peptide forming enzyme.

P72 Indigenous *Pseudomonas* from maize rhizosphere (*Zea mays* L.) with strong biocontrol potential for fusariosis management

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Indigenous *Pseudomonas* from maize rhizosphere (*Zea mays* L.) with strong biocontrol potential for fusariosis management

Phytopathogens causing fusariosis in maize are a potential threat to grain quality and availability in affected regions of the world. Native biological agents are becoming ideal options over chemical agents for plant disease management of such in South Africa. Out of 200 native *Pseudomonas* isolates identified from the maize rhizosphere of ten farms in the North West Province of South Africa, 20 were further evaluated for their antifungal and antibacterial activity. Seven isolates ($PS2_2$, $PS9_1$, $PS1_{22}$, $PS6_4$, $PS1_1$, $PS7_2$, $PS6_8$) showed consistent *in vitro* biosuppressive effects against 7 microbial pathogens (*Fusarium graminearum* and *Fusarium culmorum*, *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Moxarella cartarrhalis* 25240, *Pseudomonas aeruginosa* ATCC 27853). Sensitivity to 9 antibiotics by the seven isolates and antimicrobial potential of the filtered crude methanolic extract of $PS2_2$, $PS9_1$, $PS6_4$, were determined. Three housekeeping genes *rpoD*, *gyrB* and 16S rRNA, were used to obtain partial sequences from the DNA extract of the *Pseudomonas* isolates. We also used specific primers to detect antibiotic genes (hydrogen cyanide, phenazine, 2, 4-DAPG, diacetlyphloroglucinol and pyrrolnitrin) and antibiotic resistant genes responsible for the biosuppressive and resistant ability of the isolates. The detection of these nonribosomal peptides synthetases genes in these isolates makes them a probable biofungicide candidate. This work emphasizes the significance of genomic based approach for the identification of biocontrol agents useful for plant disease management.

P74 Internalization of ParE toxin peptide analogue by rhamnolipids liposomes

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ParE- ParD is a toxin-antitoxin (TA) system where ParE is the toxin and ParD the antitoxin. ParE is able to inhibit the DNA gyrase activity, blocking bacterial DNA replication, regulating cell growth. ParE3, a ParE peptide analogue has DNA gyrase and topoisomerase IV inhibition activities already described, but its antimicrobial activity is impaired by its difficult bacterial cell membrane permeability. Considering the biotechnological potential of this peptide, the internalization of ParE3 into rhamnolipids liposomes was

proposed as an alternative to improve its plasmatic membrane permeability. ParE3 was chemically synthesized by solid phase methodology, purified, analyzed by HPLC and characterized by MS-ESI. Gel electrophoresis assays were employed to evaluate the ability of the peptide to inhibit the supercoiling reaction of DNA gyrase and DNA relaxation by topoisomerase IV. Liposomes were produced by film lipid hydration method and characterized by Dynamic Light Scattering (DLS) to determine size and zeta potencial. Microbiological assays were performed by broth microdilution method. ParE3 showed inhibition activity of gyrase and topoisomerase IV with IC_{100} = 25 μmol L^{-1}. Incorporated liposomes showed a 50% increase on average size when compared to empty liposomes. Zeta potential average for incorporated liposomes was -28 mV whereas for empty liposomes was -15 mV. The peptide Incorporation Efficiency (I.E.) was approximately 40%. The microbiological assay suggests an increase in bacterial growth inhibition by peptide incorporated into liposomes. ParE3 peptide internalization into rhamnolipid liposomes was successful and increased cell permeability and bioavailability, consequently better results for microbial inhibition were obtained.

P76 The role of simulation and scheduling tools in bioprocess development and manufacturing

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The successful scale up and commercialization of bio-fuels and biomaterials is a challenging task that requires collaboration of professionals from many disciplines. Process simulators and other computer aids can facilitate this task by assisting scientists and engineers to analyze the integrated production process and answer questions such as: How should the facility be designed in order to achieve a desired annual throughput? What would the capital and operating costs be for the facility? What changes are required in an existing multi-product facility to accommodate production of a new product? What process development efforts could have the highest return on investment? What is the impact of raw material prices on process costs and overall profitability? What is the impact of scale on process economics? Our experience in addressing the above and other related questions will be presented using an industrial example related to the production of lysine for animal feed.

P78 Large scale algal oil production for bio-fuel use: techno-economic analysis and evaluation

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Microalgae are promising organisms for producing bio-fuels and bio-materials in a sustainable way. These microorganisms are photosynthetic, have minimal nutrient requirements, and are capable of fixing carbon dioxide and converting it into lipids and other bio-materials. However, substantial R&D work is still required for making algae bio-fuels competitive with fossil fuels. Computer simulation provides an effective way to understand the requirements of a process in terms of equipment, raw materials, support utility systems, etc. With this information, the capital and operating costs associated with the process can be estimated. In addition, the key parameters that have a major impact on cost and product yield can be readily identified. The findings from such analyses can be used to focus further R&D studies in order to optimize the critical steps of a process. The ability to experiment with alternative process setups and operating conditions using a computer model reduces the costly and time-consuming laboratory and pilot plant effort. This poster presents the results from such an analysis for the production and purification of algal oil which can be readily converted into biodiesel.

P80 Evaluation of ethanol production from renewable cellulosic resources using process simulation tools

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Over the past three decades there has been intense investigation on the development of fuel producing processes that are based on the use of renewable agricultural materials as feedstock. This activity is driven primarily by the quest for fuel self-reliance and carbon emission reductions. The main effort has been concentrated on bio-ethanol and bio-diesel which have been shown to give motor engine performance similar to that of conventional petroleum-based fuels. In addition to product characteristics, however, process economics play an equally important role in any successful product commercialization. In this work, realistic process simulation models have been developed in order to analyze the economics of corn-stover to ethanol conversion. This poster will illustrate how such models can guide R&D work and facilitate process optimization.

P82 Molecular and functional characterization of secondary metabolic gene clusters in *Aspergillus flavus* Strains 70 (Af70) and 3357

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Aspergillus flavus is a saprophytic fungus that can invade and contaminate agronomically important crops. The fungus produces a number of toxic secondary metabolites, such as aflatoxin, which are synthesized from genes located in close proximity with each other on the chromosome. *A. flavus* has approximately 55 such gene clusters. Two strains of *A. flavus* commonly used in molecular, developmental, and pathogenicity studies have genomic sequences available, 70 and 3357. A comparison of the strains reveal significant differences in growth, toxin production, and the presence of secondary metabolic gene clusters. One putative secondary metabolic gene cluster that is present in strain 70 but not found in other *A. flavus* isolates, including strain 3357, contains genes predicted to encode a polyketide synthase, two p450s, a Zn(2)–Cys(6) transcription factor and other decorating enzymes. Analysis of the molecular profiles of the secondary metabolic gene clusters in 3357 and 70, with special focus on this unique secondary metabolic gene cluster, will be discussed.

P84 Producing analogues of the natural product antibiotic teixobactin via directed biosynthesis

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Teixobactin is a novel depsipeptide antibiotic active against pathogens without the development of detectable resistance. The producing microbe, *Elftheria terrae*, is a Gram negative bacteria isolated using a novel technique for growing previously uncultivatable microorganisms. DNA sequencing predicts teixobactin is produced by a non-ribosomal peptide synthetase mechanism that incorporates the 11 amino acids including enduracidinine, 4 D-amino acids, and a methylated phenylalanine. Our work focused on using known methyltransferase inhibitors to generate des-methyl phenylalanine analogues of teixobactin. Varying concentrations of sinefungin, aminopterin, D-methionine, or DL-ethionine were added to deep-well plate cultures of *E. terrae* at the on-set of teixobactin production. LC-MS analysis of the culture extracts treated with the methyltransferase inhibitors revealed the presence of a peak eluting slightly earlier (polar) on reverse-phase and with a 14 mass unit reduction in molecular weight compared to teixobactin. Both attributes are consistent with the generation of a des-methyl analogue. Current work consists of scaling up the production of this analogue, purifying it by chromatography, and determining its structure.

P86 Advancing biofuel production and drug discovery through the development of molecular tools: Unlocking the full potential of anaerobic fungi

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Anaerobic fungi are an attractive platform for biotech with novel chemistries for biofuels production and efficient biomass degrading properties that remain unexploited due to a lack of tools. These anaerobic

fungi are an early-branching class of fungi that thrive in the strict anaerobic environments of large herbivore guts. Their role in these GI tracts is primarily the breakdown of lignocellulosic material where they are responsible for nearly 50% of cellulose degradation. Such performance in cellulose-rich environments makes them promising candidates for industrial applications where their robust enzymes may reduce the cost of biomass deconstruction. Additionally, anaerobic fungi contain many unexamined polyketide synthases (PKS) that are versatile production platforms for drugs and fuels. Recently, we have isolated and characterized five newly classified anaerobic fungi from the fecal matter of large herbivores. Due to the limited genomic information that is currently available, we have begun to isolate and clone important enzymes such as glycoside hydrolases and PKS using degenerate primers to explore the biosynthetic landscape of these organisms. Pairing these primers with a newly-developed colony PCR method allows us to quickly screen the fungal systems for valuable enzymes. In parallel, we are evaluating the capability of a naturally competent stage of the fungal life cycle to introduce DNA that directly modifies cellular function. This work ultimately allows us to access the molecular realm of the anaerobic fungi, and will create new avenues for biotechnology by harnessing their biosynthetic genes.

P88 The NPDI collection and natural product research

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The Natural Products Discovery Institute (NPDI) houses the former Merck and Schering-Plough natural products libraries, which is available to researchers in the scientific community, to screen for pharmaceutical, agricultural, cosmeceutical, and nutritional agents. The combined collection comprises over 100K extracts from plants and microbial fermentation samples. The NPDI also houses a collection of >4,000 fungi and actinomycete microorganisms. Recent screening of the NPDI collection for pharmaceutical uses has yielded hits from extracts, which, in 80% of the cases, have not been previously identified as biologically active. Recently, we have begun accumulating purified DNA preparations from our diverse microbial collection as an additional resource for drug discovery in the genomics era. Exploration of the actinomycete genomic DNA sequences has demonstrated some interesting metabolic profiles. In addition, improvement of microbial strains and fermentation process has been achieved, aiming at overproducing industrial natural products. Furthermore, many of the plants in the collection have a history of medicinal usage. Herein, we will describe the NPDI productive resources which researchers can access through collaborations or fee-for service arrangements, as well as recent natural product researches performed at the NPDI.

P90 Biosynthesis of platensimycin and platencin: why use one enzyme when you can use two, or four?

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Platensimycin (PTM) and platencin (PTN) are highly functionalized bacterial diterpenoid natural products that target bacterial and mammalian fatty acid synthases. In the early steps of PTM and PTN biosynthesis, there are two seemingly innocuous transformations that are performed: a stereoselective hydroxylation of C7 and CoA activation of the C19 carboxylic acid. Although two enzymes were initially predicted to catalyze these transformations, an oxygenase and an acyl-CoA synthetase, respectively, we found through a series of in vivo, in vitro, and structural biology experiments, that six enzymes were needed to yield the hydroxylated and activated intermediate. PtmO3 and PtmO6, α-ketoglutarate dioxygenases, both catalyze hydroxylation of the diterpenoid skeleton yielding an *S*-configuration at C7. PtmO8 and PtmO1, short chain dehydrogenases with 37% protein sequence identity, catalyze oxidation and stereoselective reduction, respectively, to epimerize the hydroxyl group into an *R*-configuration at C7. Similarly, another proposed one (bifunctional) enzyme process, CoA activation, occurs through two distinct monofunctional enzymes. PtmA1 adenylates the C19 carboxylic acid and PtmA2 completes the reaction by thioesterification with CoA. PtmA2 represents the first example of an acyl-CoA ligase that performs only the second half of the usual two-step CoA activation process. This counterintuitive approach to biosynthesis, using more enzymes when one would be more efficient, reveals an unexpected and interesting aspect of the evolution and enzymology of PTM and PTN biosynthesis.

P92 Investigating plant and fungus derived antimicrobials for aerosol application

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For thousands of years, plants and microorganisms have served as a rich source of bioactive compounds and hold enormous potential for use in many industries. While natural product discovery efforts have significantly dwindled due to advancements in high-throughput synthetic chemistry, bioactives from nature remain as an attractive source of novel compounds with a wide range of application. My research aims to investigate antimicrobial compounds derived from natural sources with the prospective application as aerosols for air sanitization. Candidate plant and fungal sources chosen for my research have previously demonstrated antimicrobial activity including two plant leaf extracts, and six rare fungal isolates from an in-house culture collection at The University of Auckland. Crude extracts from the fungal and plant candidates were obtained to first characterize their spectrum of activity against common environmental microorganisms through agar diffusion assays. Next, efficacy of the active extracts was assessed by obtaining minimum inhibitory concentrations to identify candidates with the greatest potential for future application as aerosols. Further work will involve evaluation of the bioactives physico-chemical properties, purification of the active compound using high-performance liquid chromatography, and chemical characterization by liquid or gas chromatography – mass spectrometry. Once a promising candidate(s) is identified, a stable formulation will be developed for integration into a solenoid valve dispenser, and its safety assessed in compliance with international standards.

P94 Identification of plant phyllosphere associated bacteria that inhibit the growth of pathogens on fresh produce

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Aims: To identify phyllosphere associated bacteria (PAB) that prevent the growth of pathogenic bacteria such as *Listeria monocytogenes*. Methods and Results: We isolated PAB from various types of fresh produce by washing the produce in PBS + 0.01% Tween80 with glass beads for 1 hr shaking. The produce was removed and the liquid was plated onto 5 types of bacterial culture agar plates: de Man, Rogosa, and Sharp (MRS), Tryptic Soy Agar (TSA), Reasoner's 2A (R2A), Pseudomonas Agar Base (PAB), and Pseudomonas Isolation Agar (PIA), and incubated at 30^{o}C for 24 hrs. Isolates were screened for the ability to inhibit the growth of *Listeria monocytogenes* pGFP in a high throughput in vitro fluorescent assay. Isolates that inhibited the growth of *L. monocytogenes* were assayed for cross-reaction to inhibit the growth of *Salmonella enterica* and *Escherichia coli*. The 16S genes of selected isolates were amplified by PCR, sequenced, and aligned to obtain consensus sequences, and identified by BLAST. We isolated eleven PAB (Lmono01 – Lmono11) that inhibited the fluorescence of *L. monocytogenes* pGFP in vitro by 10 to 50-fold. Moreover, Lmono01 could also inhibit the growth of *S. enterica* pGFP and *E. coli* pGFP in vitro.

P96 Epicoccaene: a novel antifungal compound derived from *Epicoccum purpurascens*

A. Lee, S. Swift and S. Villas-Boas, The University of Auckland, Auckland, New Zealand*

Secondary metabolites produced by fungi have great potential as sources of new antimicrobial drugs, including natural antifungals. Fungi are known to produce a diverse range of bioactive compounds, and there are a wide range of fungi that are yet to be studied. Thus, the possibility of discovering novel antifungal compounds from fungi is very high. In the Metabolomics Lab at the University of Auckland, we discovered epicoccaene; an antifungal secondary metabolite with a potential novel mode of action. It is a water-soluble yellow pigment produced by the fungus *Epicoccum purpurascens.* Unlike any other antifungal compounds that are available in the market, it shows specific activity against moulds, but not yeasts. The aim of this research is to elucidate the antifungal mode of action of epicoccaene. Agar diffusion assays were used to assess antifungal activity in crude extract of *E. purpurascens*. Then, solid-

phase extraction followed by preparative high-performance liquid chromatography further purified epicoccaene. Ultra-performance liquid chromatography-quadrupole-orbitrap, and gas chromatography–mass spectrometry following trimethylsilyl derivatization were used to confirm the presence and purity of the compound. The structure of epicoccaene was determined by nuclear magnetic resonance spectroscopy. The molecular mass of epicoccaene is 612.2934 with the molecular formula of $C_{34}H_{44}O_{10}$. This indicates it is an isomer of orevactaene and epipyrone; the HIV-1 Rev protein inhibitor and a commercial antifungal compound respectively, which are produced by *E. purpurascens*. The structures proposed for each isomer showed that they share the same polyene chain with different polar sides, exhibiting different biological activities.

P98 Identification of cyanobacterial PPTases with broad- substrate scope

G. Yang, Y. Zhang, N. Lee, M. Cozad and Y. Ding, University of Florida, Gainesville, FL, USA*

Cyanobacteria are promising but poorly tapped source of structurally and functionally diverse secondary metabolites, particularly polyketides (PKs), nonribosomal peptides (NRPs) and their hybrids. Recent genomic studies revealed astonishing biosynthetic potential of cyanobacterial species. However, the translation of cyanobacterial genetic information into chemicals is severely lagging behind, primarily because of the lack of capable synthetic biology tools. Sfp-like phosphopantetheinyl transferases (PPTases) are essential to functionalize carrier proteins (CPs) for the synthesis of PKs, NRPs and their hybrids. Cyanobacterial Sfp-like PPTases are thus critical to the research of cyanobacterial natural products but remain largely unstudied. Herein, we disclosed detailed characterization of six Sfp-like PPTases selected from six cyanobacterial strains by phylogenetic analysis. Biochemical characterization of these recombinant cyanobacterial PPTases was carried out using 11 recombinant CPs of various known and silent biosynthetic pathways from both cyanobacterial and *Streptomyces* strains. The PPTases from *Anabaena* sp. PCC7120 (APPT), *Microcystis aeruginosa* NIES843 (MPPT) and *Synechocystis* sp. PCC6803 (SPPT) possessed both broad substrate promiscuity and high catalytic efficiency. Furthermore, transient APPT, MPPT and Sfp all supported the growth of the *Synechocystis* sp. PCC6803 mutant whose native *PPTase* gene was inactivated. Moreover, these PPTases in the cell lysates of *Synechocystis* sp. PCC6803 functionalized the selected CPs in biochemical assays. These cyanobacterial PPTases with broad substrate scopes can become useful tools to synthesize cyanobacterial natural products through *in vitro* and *in vivo* synthetic biology approaches.

P100 3,6-Anhydro-L-galactose, a rare sugar found in red macroalgae, as a novel anticariogenic sugar to replace xylitol

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Due to increasing demands for dietary sugars and rising concerns of dental caries, the significance for alternative anticariogenic sugar substitutes is growing. *Streptococcus mutans* is one of the main microorganisms that cause dental caries. Xylitol is widely used as an anticariogenic sugar substitutes because it inhibits *S. mutans*. However, the inhibitory effect of xylitol is exhibited only at high concentrations. Therefore, alternative anticariogenic sugar substitutes, which may replace xylitol, is highly desired. In this study, the inhibitory effects of 3,6-anhydro-l-galactose (AHG), obtained from agar which is the main carbohydrate of red macroalgae (*Rhodophyta*), on *S. mutans* were evaluated for the first time. With 5 g/L of AHG, the cell growth of *S. mutans* was retarded, and in particular, the lag phase was prolonged by 10 h, compared to those of the control without AHG. With 10 g/L of AHG, the cell growth and lactic acid production of *S. mutans* were not measured. Whereas, even with a much higher concentration of xylitol (i.e., 40 g/L), the cell growth of *S. mutans* was still observed. Therefore, the inhibitory activity of AHG was much higher than that of xylitol. These results suggest that AHG can be used as a novel anticariogenic sugar to prevent dental caries. To our knowledge, this is the first study on the anticariogenic activity of AHG.

P102 Genome mining to predict β-lactone and olefin biosynthesis

S.L. Robinson, J.K. Christenson and L.P. Wackett, University of Minnesota, St. Paul, MN, USA*

Natural products containing β-lactone rings have demonstrated anti-tumor, anti-obesity, and anti-microbial properties but no enzyme was identified for β-lactone biosynthesis. We recently reported the discovery of the first β-lactone synthetase enzyme in a *Xanthomonas campestris* olefinic hydrocarbon biosynthesis pathway. We now performed biochemical analyses that concretely link β-lactone natural product biosynthetic gene clusters (BGCs) with olefinic hydrocarbon BGCs. The β-lactone intermediate in this pathway is transformed to the final olefin product by an unprecedented β-lactone decarboxylase, OleB. We hypothesized that BGCs lacking an OleB homolog would secrete β-lactone natural products. Indeed, two *Streptomyces* strains in which OleB homologs were absent were shown to produce the clinically-relevant β-lactone compounds lipstatin (Orlistat, Xenical) and ebelactone A. Standard sequence alignments tools used to identify OleB and OleC homologs returned thousands of nonspecific hits within the diverse α/β-hydrolase and AMP-dependent ligase/synthase superfamilies. To improve specificity, we developed a bioinformatics pipeline using Hidden Markov Models to detect *ole*-like BGCs. We trained a support vector machine classifier to predict the natural products of the *ole* pathways as β-lactone compounds or olefins. Experimental screening of diverse organisms with homologs in the *ole* pathway was used to validate our approach. We present this predictive tool to aid in the discovery of new β-lactone natural products and advance drug development.

P104 Synergy via target promiscuity drives structural diversity in bacterial akylquinolone biosynthesis

Y. WU, Princeton University, PRINCETON, NJ, USA and M. Seyedsayamdost, Princeton University, Princeton, NJ, USA*

Bacteria are a dominant source of secondary metabolites with exquisite, sometimes clinically-useful, biological activities. In contrast to the specificity of primary metabolism, secondary metabolite biosynthetic pathways are diversity-oriented and usually give rise to a number of variants, rather than a single product. It remains unclear why bacteria have evolved to encode structurally diverse molecules within a given biosynthetic pathway. Herein, we have addressed this question by investigating the detailed functions of two analogues of 4-hydroxyl-2-akylquinolines (HAQs) from *Burkholderia thailandensis*. The HAQs are a family of secondary metabolites with a well-established promiscuous biosynthetic pathway that operates in a number of Proteobacteria. Using bioactivity assays and bacterial cytological profiling, we found that two HAQ analogues, 4-hydroxyl-3-methyl-2-nonenylquinolone (HMNQ) and 2-n-heptyl-4-hydroxyquinoline N-oxide (HQNO), inhibit bacterial growth by acting in a synergistic fashion. HQNO inhibits the cytochrome bc1 complex, while HMNQ dissipates the proton motive force. Together, they disrupt energy production with two distinct mechanisms. At the same time, our studies revealed that HMNQ and HQNO also target a second pathway: both inhibit pyrimidine synthesis, and thereby, DNA biosynthesis. On the basis of these results, we pose synergy and target promiscuity as the functional rationale for the diversity-oriented biosynthesis of HAQs.

P106 Butyrate, a gut bacterial metabolite, induces human endogenous retrovirus expression in cultured human neural stem cells

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The bidirectional communication between the brain and the gastrointestinal tract is commonly referred to as the "**Brain-Gut Axis**". Of interest to us is the study of the effect(s) of gut microbial metabolites on the central nervous system (CNS), specifically the brain.

Metabolites produced in the gut are generated by many commensal microbes, especially strict anaerobic bacteria. Well known metabolites produced from dietary fiber are short chain fatty acids (SCFAs) such as butyrate, acetate, propionate and succinate. They are believed to play an important role in human health and disease (Tan et al., 2014). We are proposing that SCFAs may lead to the induction of transcription of human endogenous retroviruses (HERVs) that make up about 8% of human DNA in all cells, including brain cells. Transcripts of HERVs have been found in the blood of individuals with CNS diseases such as:

amyotrophic lateral sclerosis, schizophrenia, bipolar disorder, multiple sclerosis and Creutzfeldt-Jakob Disease (prions) **(**Mortelmans et al., 2015).

The cultured human neural stem cells that were used were derived from NIH approved H9 (WA09) human embryonic cells. Our study revealed induction of HERV-K expression in these cultured cells that were exposed to sodium butyrate. The expression of the HERV-K family mRNA and protein was determined by RT-PCR and immunoblot analysis, respectively. Reproducibility of these results will be evaluated with another type of brain cell, HBEC-5i, a cell line that is used mostly in studies that model the blood-brain barrier and thus would represent a potential barrier to microbial products entering the brain.

P108 Epitope modifications on computationally broadly reactive influenza vaccines to investigate differential antibody response

A. Sicam and T. Ross, Center for Vaccines and Immunology, University of Georgia, Athens, GA, USA*

Designing a universal influenza vaccine remains a challenge due to the diversity and high mutation rate of circulating strains. To develop a broadly reactive influenza vaccine, a methodology termed computationally optimized broadly reactive antigens (COBRA) was used to design novel hemagglutinin (HA) vaccines. COBRA H3 antigens expressed as viral-like particles (VLP) in cell culture have been shown to elicit broader breadth of antibody responses, detected by hemagglutination inhibition assay (HAI), against H3N2 vaccine and co-circulating strains compared to wild-type HA antigens that were represented in commercial influenza vaccines. Previous mouse study revealed that H3 COBRA vaccines T-10 and T-11, which differ only by 4 aa, elicit different HAI responses against H3N2 vaccine and 2004-2007 co-circulating strains. T-11 exhibited broader response breadth than T-10; T-11 elicited HAI-reactive antibodies against A/Mississippi/1/1985 (Miss/85), A/Brisbane/10/2007 (Bris/07) and other co-circulating strains from 2004-2007.

This study investigates which epitope/s differentiate/s T-10 and T-11 antisera HAI reactivity. For this, distinct T-11 epitopes were introduced in the T-10 HA sequence by site-directed mutagenesis. Generated mutants were expressed as VLPs and screened in mice for the elicitation of antibodies with HAI activity. We show here that glycine introduction at site 158 in T-10 results in antibody response elicitation against A/Brisbane/10/2007 and some of the 2004-2007 co-circulating strains. Other T-11 epitopes, specifically at residue 328, will be explored in the future to determine how these contribute to the differential HAI response observed between sT-10 and T-11. Determining critical regions would inform future COBRA designs and structure-based prediction of protection.

P112 A framework to identify micro-organisms subject to the *Canadian Environmental Protection Act*

Z. Saikali, Environment and Climate Change Canada, Gatineau, QC, Canada; S. Bernatchez, M. Breton and V. Anoop, Health Canada, Ottawa, ON, Canada*

In Canada, new organisms, including certain industrial micro-organisms, are subject to the *Canadian Environmental Protection Act* (1999) and must undergo a risk assessment for environmental and human health effects before being imported or manufactured. This assessment is carried out jointly by Environment and Climate Change Canada and Health Canada when a proponent presents an application under the New Substances Notification Regulations (Organisms) (NSNR(O)).

The accurate identification of a micro-organism constitutes the cornerstone of the risk assessment conducted under these regulations. However, accurate identification can represent a challenge, and past experience has shown that there exists a need for detailed technical guidance for proponents and regulators on the use of current methods of microbial identification. This guidance, embodied in a newly-developed 'Microbial Identification Framework for Risk Assessment' (MIFRA), outlines a standardized, logical and tiered approach for the collection and analysis of data leading to microbial identification that is appropriate for risk assessment under the NSNR(O). Additionally, the MIFRA helps to deal with issues of relatedness or similarity of the notified micro-organism to other micro-organisms, and takes into consideration how well different taxonomic groups are described in the literature. This tool will help proponents and regulators to achieve consistency in the analysis of information pertaining to micro-

organism identification. Methods used in microbial identification will be discussed and case studies will be presented to illustrate the approach for a variety of micro-organisms (bacteria, yeasts, fungi, one virus and one micro-alga).

P114 Metabolic engineering and pH control strategy for high production of 2,3-butanediol from glycerol by *Raoultella ornithinolytica* B6

T. Kim and Y. Um, Korea Institute of Science and Technology, Seoul, Korea, Republic of (South)*

Microbial production of 2,3-butanediol (2,3-BD) from renewable resources is an attractive alternative to chemical processes. 2,3-BD is a platform chemical with a wide range of industrial applications. Sugar-based 2,3-BD fermentation has been extensively studied due to the high 2,3-BD production capabilities of various microorganisms. Alternatively, biological 2,3-BD production from crude glycerol, which is a major by-product from biodiesel facility, has been studied. However, in glycerol-based 2,3-BD fermentation, 1,3-propanediol (1,3-PD) is a problematic by-product because its similar physicochemical characteristics to 2,3-BD cause difficulty in downstream processes. Herein, 2,3-BD production without 1,3-PD accumulation was investigated using *Raoultella ornithinolytica* B6, which is deficient in the 1,3-PD synthesis pathway. The optimal fermentation conditions for 2,3-BD production were found to be 25°C, 400 rpm, and pH control with a lower limit of 5.5, respectively. Notably, a significant pH fluctuation which positively affected 2,3-BD production was generated by simply controlling the lower pH limit at 5.5. In fed-batch fermentation, *R. ornithinolytica* B6 produced 79.3 g/L 2,3-BD, and a further enhancement of 2,3-BD production (89.5 g/L) was achieved by overexpressing the homologous *budABC* genes, which is directly involved in conversion of pyruvate to 2,3-BD. Moreover, *R. ornithinolytica* B6 harboring pUC18CM-*budABC* produced 78.1 g/L 2,3-BD with the yield of 0.42 g/g and the productivity of 0.62 g/L/h using pretreated crude glycerol, which were the highest values for 2,3-BD production from glycerol among 2,3-BD producers without the 1,3-PD formation. The results presented here demonstrates *R. ornithinolytica* B6 as a promising 2,3-BD producer from glycerol.

P114A Enabling microbial utilization of lignin-derived monomers

K. Davis, M. Rover, L.R. Jarboe, Z. Wen, R. Smith and R.C. Brown, Iowa State University, Ames, IA, USA; D. Salvachúa and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Biomass contains lignin, a polymer of phenolic molecules, which provides stability and protection to the plant. Many economic models indicate that the addition of value to lignin is essential for the economic viability of the conversion of biomass to renewable fuels and chemicals. Some microorganisms, such as *Pseudomonas putida*, can convert or metabolize some aromatic and phenolic molecules. Previous reports have shown that *P. putida* can be engineered to funnel multiple phenolics through its central metabolic pathways [1]. A funneling approach can be advantageous because thermally decomposed lignin can be composed of hundreds of different molecules many of which can be aromatic or phenolic in nature. Phenolic-rich fractions of pyrolyzed biomass have low solubility and therefore cannot be easily accessed by microorganisms in aqueous cultures. In addition, microorganisms can be negatively affected by inhibitors present in processed biomass streams. Here, we have developed an emulsion of phenolic monomer-rich product derived from fast-pyrolyzed red oak using a combination of Tween® 20 and Span® 80. *Pseudomonas putida* KT2440 grew in emulsions of the phenolic monomer-rich product. Studies suggest that the emulsion allowed *P. putida* to access molecules with low solubility in aqueous culture. In addition, the emulsion seemed to have an additional benefit of decreasing the toxic effects of the phenolic molecule *p*-coumarate.

Refs: 1. Johnson, C. W.; Beckham, G. T. *Metabolic Engineering* 2015, 28, 240-247.

Tuesday, August 1

6:15 AM - 7:15 AM Annual 5K Fun Run/Walk Meet in hotel lobby. Run begins at 6:15 am-Signed Waiver Form needed for participation. Forms available at registration desk or in the lobby prior to the run

7:30 AM - 5:00 PM Registration

Plaza Registration - Concourse Level

8:00 AM - 11:00 AM Session: 13: Synthetic Biology Tools and Applications

Conveners: **Nanette Boyle**, Colorado School of Mines, Golden, CO, USA and **Carrie A. Eckert**, Renewable and Sustainable Energy Institute (NREL/University of Colorado, Boulder), Golden, CO, USA

Plaza Ballroom A & B - Concourse Level

8:00 AM S73: How to efficiently access the mutational landscape

G. Pines, J. Winkler, A.D. Garst, M. Bassalo and R.T. Gill, University of Colorado Boulder, Boulder, CO, USA*

The mutational landscape of a gene or a genome is an abstractive multidimensional space in which mutations are measured according to their contribution to fitness. Such landscapes are case-specific and are commonly portrayed as three dimensional for convenience purposes. The aim of bioengineers is to find the global maximum, i.e., the highest peak within the landscape while avoiding local maxima “traps”. Since it is impossible to evaluate complete landscapes experimentally, methods are developed to increase the chances of finding the best performing mutants. Three approaches will be discussed: reducing the screening load of semi-rationally designed libraries, systematically scanning through single point mutations, and increasing mutational accessibility by refactoring the genetic code.

8:30 AM S74: Continuous *in situ* recombination system to speed up strain development in *Escherichia coli*

K.C. Kao, Texas A&M, College Station, TX, USA*

Adaptive laboratory evolution is a powerful tool for strain development. In an evolving population, individuals with beneficial mutations are selected for, and become enriched, in the environment. However, the rate of adaptation can be limited by the frequency of beneficial mutations; and competition amongst co-occurring beneficial mutations can lead to a loss of information. Here we describe the use of horizontal gene transfer (HGT) in conjunction with tunable mutation rate to more rapidly develop complex phenotypes in *E. coli*. We have previously developed a “genderless” strain of *E. coli* proficient in continuous HGT during normal culturing conditions. In this work, we introduced an inducible mutator system to the genderless strain to allow modulation of mutation rate to enhance the supply of mutations during ALE. The system was characterized to determine the influence of HGT and mutation rate. Our results indicate HGT and increasing mutation rate can act together to speed the rate of adaptive laboratory evolution. The system was further leveraged to more rapidly combine different complex phenotypes, to help expedite strain development of more industrially relevant phenotypes.

9:00 AM S75: Engineering modular tunable biosensors responsive to cellular health for gene regulation and pathway optimization

R. Chatterjee, Y.H. Wang, K.Z. Lee, E. Hillman, L. Readnour and K. Solomon, Purdue University, West Lafayette, IN, USA*

Microbial chemical factories increasingly rely on biosynthetic pathways incorporating toxic intermediates that inhibit cellular growth and product formation. A promising strategy to overcome this challenge is dynamic regulation of production pathways to limit toxic intermediate accumulation, and maintain cells at optimal health. While dynamic control may be implemented with natural transcription factors as sensors that recognize and respond to a given metabolite, sensors for many intermediates are largely unknown. Here, we develop modular transcriptional regulators that consist of a tunable elastin-like polypeptide (ELP) biosensor and transcription factor to directly respond to general indicators of cellular stress for dynamic control of any biosynthetic pathway. ELPs are engineered proteins that reversibly self-assemble at a critical temperature, pH, or ionic strength programmed by their tunable primary sequence. When fused to transcription factors such as orthogonal sigma factors and *tetR,* ELP self-assembly suppresses gene transcription at targeted promoters in a reversible switch-like fashion. We show that ELP-transcription factor fusions operate as precise temperature repressible switches over a range of programmed physiological temperatures. Similarly, we demonstrate that these regulators respond to small variations in intracellular pH that precede significant cellular damage. The tunable responsive nature of these synthetic regulators make them ideal regulators for pathway optimization, with potentially broad utility across organisms with appropriate transcription factor design.

9:30 AM Break

10:00 AM S76: Building a biological foundry for next-generation synthetic biology

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

Inspired by the exponential growth of the microelectronic industry, synthetic biologists have been attempting to build biological foundries for rapid genetic design and cellular prototyping. In this talk, I will briefly discuss the challenges and opportunities in synthetic biology and highlight our recent work on the development and application of novel foundational synthetic biology tools. Specifically, I will introduce the Illinois Biological Foundry for Advanced Biomanufacturing (iBioFAB) that we have been establishing to automate the design-build-test-analyze cycle and discuss its three potential biotechnological applications. The first is the rapid and high throughput synthesis of transcription activator-like effector nucleases (TALENs) for genome editing applications. The second is the discovery, characterization, and engineering of novel natural product biosynthetic pathways for drug discovery and development. The third is the design, construction and optimization of biochemical pathways and microbial factories for economic production of chemicals and fuels.

10:30 AM S77: Opportunities for facility-enabled science at the DOE Joint Genome Institute (JGI)

Y. Yoshikuni, Lawrence Berkeley National Lab, Walnut Creek, CA, USA*

Fundamental unsolved problem in genomics is the need for high-throughput approaches to bridge the gap between the availability of DNA sequence data and our ability to assign biological function to it. The DOE JGI's niche is the development of a diversity of large-scale experimental and computational capabilities to link sequence to biological insights relevant to energy and environmental research. This will range from preparing material and applying functional capabilities prior to genomic analysis to post-sequence processing and manipulations to enable the Institute's users to carry out sequence-to-function studies that are beyond the capabilities of individual laboratories. Here, I will discuss new sequence-to-

function capabilities that have been established at the DOE JGI and some scientific examples enabled with these capabilities (Schwander et. al. Science 2016 and Tsementzi et al. Nature 2016).

8:00 AM - 11:30 AM Session: 12: Industrial Biocatalysts for Carving A Better Tomorrow

Conveners: **Bhupinder Singh Chadha**, Guru Nanak Dev University, Amritsar, India and **Rajesh Sani**, South Dakota School of Mines & Technology, Rapid City, SD, USA

Plaza Ballroom F -Concourse Level

8:00 AM S67: Engineering *Clostridium thermocellum* for cellulosic hydrogen and biofuels platform

W. Xiong, L. Magnusson, J. Lo, L. Warner, K.J. Chou and P.C. Maness, National Renewable Energy Laboratory, Golden, CO, USA; P. Lin and J.C. Liao, University of California, Los Angeles, Los Angeles, CA, USA*

Clostridium thermocellum is a thermophilic anaerobe that exhibits one of the fastest growth rates in cellulose while yielding ethanol, formate, lactate, acetate as well as copious amounts of hydrogen. This microbe therefore has immense potential for the production of hydrogen or biofuels in a consolidated bioprocessing (CBP) configuration using waste lignocellulosic biomass as the feedstock. Hydrogen production is likely catalyzed by two putative bifurcating hydrogenase enzymes using both reduced ferredoxin and pyridine nucleotides as the electron donors. Manipulating the interconversion of these redox cofactors therefore could play an important role in modulating hydrogen and biofuels production. Via feeding ^{13}C-bicarbonate to the culture medium, we recently discovered that *C. thermocellum* can fix CO_2 via the reductive C1 metabolic pathway while actively hydrolyzing cellobiose, the latter a biomass-derived sugar. This finding demonstrates the metabolic flexibility of this microbe leading to higher carbon-conversion yield. Work is ongoing to optimize parameters in bioreactor performance to convert pretreated corn stover biomass to hydrogen and expand the carbon substrate utilization profile to realize *C. thermocellum*as a CBP microbe for the production of the next-generation biofuels and bioproducts.

8:30 AM S68: Metabolic engineering for enhanced *trans*-4-Hydroxy-L-proline production in *Escherichia coli*

B. Lin, H. Zhou and Y. Tao, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China*

Central metabolic pathways provide precursors for chemicals biosynthesis. However, precursor competition between synthetic pathway and the central metabolic pathways restrict the yield of target product. To resolve this problem, we develop a strategy called "center metabolic pathways coupling" in which the reaction of the biosynthesis pathway is "embedded" in center metabolic pathways. By the pathways coupling, the target product synthetic pathway is necessary for the cell growth. Metabolic strategy of "central metabolic pathways coupling" take advantage of the flux of central metabolic pathways to solve the problem of precursor competition and resulted in improved production of target product. By this strategy, the reaction catalyzed by proline-4-hydroxylase for biosynthesis of *trans*-4-Hydroxy-L-proline was coupled with the central metabolic pathways in *E.coli* to enhance the production of *trans*-4-Hydroxy-L-proline. This work also demonstrated the feasibility of strategy "central metabolic pathways coupling" in the 2-oxoglutarate dependent dioxygenase catalyzed reactions

9:00 AM S69: Integrated process for extraction of wax as a value-added co-product and improved ethanol production by converting both starch and cellulosic components in sorghum grains

N.P. Nghiem, Eastern Regional Research Center, Agricultural Research Service, USDA, Wyndmoor, PA, USA and J. O'Connor, Clemson University, Clemson, SC, USA*

Grain sorghum is a potential feedstock for fuel ethanol production due to its high starch content, which is equivalent to that of corn, and has been successfully used in several commercial corn ethanol plants in the United States. Some sorghum grain varieties contain significant levels of surface wax, which may interact with enzymes and make them less efficient toward starch hydrolysis. On the other hand, wax can be recovered as a valuable co-product and as such may help improve the overall process economics. Sorghum grains also contain lignocellulosic materials in the hulls, which can also be converted to additional ethanol. An integrated process was developed, which consisted of the following steps: 1. Extraction of wax with boiling ethanol, which also is the final product of the integrated process; 2. Pretreatment of the de-waxed grains with dilute sulfuric acid; 3. Mashing and fermenting of the pretreated grains to produce ethanol. During the fermentation, commercial cellulase was also added to release fermentable sugars from the hulls, which then were converted to additional ethanol. The advantages of the developed process are illustrated with the following results:

1. Wax extracted (determined by weight loss): ~0.3 wt% of total mass.
2. Final ethanol concentration at 25 wt% solid using raw grains: 86.1 g/L.
3. Final ethanol concentration at 25 wt% solid using de-waxed grains: 106.2 g/L (23.3 % improvement).
4. Final ethanol concentration at 25 wt% solid using de-waxed and acid-treated grains (1 wt% H_2SO_4) plus cellulase (CTec 2): 117.8 g/L (36.8 % improvement).

9:30 AM Break

10:00 AM S70: Production and extraction of triacylglycerols from lignocellulose-grown bacteria

B. Wang, J. Gill, R. Young and K.H. Chu, Texas A& M University, College Station, TX, USA*

Lignocellulosic biomass is abundant renewable resource for production of different biofuels and bioproducts. However, no study has explored the use of lignocelluosic biomass for the production of bacterial lipid, triacylglycerol (TAG), which can be used for lipid-based liquid biofuel production.
In this study, we examined four TAG-accumulating *Rhodococcus* stains (*R. opacus* PD630, DSM43205, M213 and *R. jostii* RHA1) for their ability to grow in the presence of known inhibitory compounds, like furfural, 5-hydroxylmethyl furfural (5HMF), vanillin, vanillic acids and trans-p-coumaric acid (TPCA). Our results show that all the tested *Rhodococcus* strains can tolerate individual inhibitory compounds at low concentrations. Furthermore, strain PD630 can use TPCA, vanillic acid and vanillin as growth substrates. Vanillic acid-grown cells produced different lengths of fatty acids. These strains were also able to grow on hydrolysates of corn stover, sorghum, and grass, while producing TAGs. Bacteriophages were explored as a means for extracting TAG-bearing *Rhodococcus* strains. We have demonstrated TAG released from the TAG-bearing *Rhogodoccus* after phage infection.

This is the first study reporting the growth of TAG-accumulating *Rhodococcus* strains on known inhibitory compounds in hydrolysates and three different sources of lignocellulosic biomass. It is also the first report using phages as bioextractors to release TAGs from TAG-accumulating bacteria. The results of this study suggest that these *Rhodococcus* strains are ideal for bacterial lipid production from lignocellulosic biomass and phage-based extraction technology is a promising means for TAGs extraction.

10:30 AM S71: Approaches for developing thermophilic fungal strains as source of novel cellulolytic and auxiliary enzymes

B. Singh Chadha, Guru Nanak Dev University, Amritsar, India*

This study reports thermophilic fungi as important source of lignocellulolytic enzymes. Zymographic and IEF based analysis revealed that these strains produce catalytically and functionally distinct xylanases.

The detailed secretome analysis using LC–MS/MS orbitrap also indicated to distinct spectrum of glycosyl hydrolases (cellulase/hemicellulase), polysaccharide lyases (PL) and carbohydrate esterases (CE) in addition to cellobiose dehydrogenase (CDH) indicating to the presence of functional classical and oxidative cellulolytic mechanisms. The protein fractions in the secretome resolved by ion exchange chromatography were analyzed for ability to hydrolyze differently treated substrates. This strategy in tandem with peptide mass fingerprinting led to identification of metal dependent protein hydrolases with no apparent hydrolytic activity, however, showed 5.7 folds higher saccharification in presence of Mn2+. Adding different protein fractions to Cellic CTec2 resulted in enhanced hydrolysis ranging between 1.57and 3.43 folds indicating the enzymes from these fungi as catalytically efficient. Genes coding for xylanases (GH10 and GH11) from *Malbranchea cinnamomea* were cloned (McXGH11 and McXGH10) and expressed in *Pichia pastoris.* The observed xylanase activity of McXGH11 (571.98 U/ml) was 7.73 folds higher when compared to parent strain (73.91 U/ml). McXGH11 was purified and found to be thermostable at 70˚C and catalytically efficient against variety of substituted xylans. Improved saccharification of alkali and acid treated rice straw and bagasse (~1.37 folds) was observed using enzyme cocktail comprising of McXGH11 and CellicCTec2 (2:8 ratio) when compared to cellicCTec2 alone at same protein loading rate of 5.7 (mg/g biomass).

11:00 AM S72: Genomic analyses and potential applications of Malaysian rare extremophiles

K.M. Goh[*]*, University Teknologi Malaysia, Johor, Malaysia*

Extremophilic microorganisms have attracted much interest in the realm of biotechnology, especially in relation to industrial processes (i.e. ‘white biotechnology’). The advancements in whole genome sequencing have prompted a paradigm shift in the discovery of biocatalysts. With genome data, amplification and cloning of genes have become very feasible. Most enzymologists and structural biologists are more attracted to studying proteins with low sequence similarity to other known sequences owing to their novelty. One potential method to mine underexplored biocatalysts genes is to isolate a rare genus and explore its proteins. In this communication, two interesting bacteria isolated from a saline hot spring and seawater is described. Using complete genome and transcriptome analyses, data indicated that halophilic *Jeotgalibacillus malaysiensis* adopted a combination of approaches, including the uptake or synthesis of osmoprotectants, for surviving salt stress. We then cloned and biochemical characterized the recombinant beta-glucosidase from this bacterium. In a separate work, a bacterium designated as strain RA was isolated from a saline hot spring sample. Analyses of the 16S rRNA gene and housekeeping gene sequences suggested that the strain could represent a new genus of *Rhodothermaceae*. Whole-genome shotgun sequencing of strain RA was performed using a PacBio RS II sequencing platform. Bacteria strain RA genome consists of 106 glycoside hydrolases and 9 AA auxiliary activities. A xylanase from this bacterium was chosen, purified and characterized. Both enzymes from this rare extremophiles are active on various substrates and hence suit the requirements for wide industrial applications that include biofuel production.

8:00 AM - 11:30 AM Session: 14: Development of Microbiome Standards: Improving the Interpretation and Comparability of Microbiome Data Sponsored by ATCC

Conveners: **George Garrity**, Michigan State University, East Lansing, MI, USA and **Tasha Santiago-Rodriguez**, ATCC-Center for Translational Microbiology, Union, NJ, USA

Governors Square 15, Concourse Level

8:00 AM S77A: Metagenomics for plant disease resistance and ocean acidification

J.F. Kim, Yonsei University, Seoul, Korea, Republic of (South)*

With the advent of the genomics era powered by high-speed high-throughput next-generation sequencing technologies, life science is being transformed and biological research and development have been significantly accelerated. We applied metagenomic approaches to study model experimental ecosystems. Host-microbiota relationships in a plant disease will be presented as the first example: the role of microbiome in disease resistance or susceptibility of tomato varieties against bacterial wilt for the plant rhizosphere. In the talk, microbes and the marine environment will also be presented: marine mesocosm experiments that investigate the influences of ocean acidification and global warming to the dynamics of marine microbial communities.

8:30 AM S81: Validation of uniquely identifiable molecular barcodes as spike-in reference standards for microbiome applications

T.M. Santiago-Rodriguez, J. Schuyler, R. McMillan, G. Kaur, J. Pace and R. Cano, ATCC-Center for Translational Microbiology, Union, NJ, USA; D. Mittar, ATCC, Manassas, VA, USA*

A shortcoming in microbiome studies is the lack of reference standards needed to standardize DNA extraction, library preparation, sequencing, and analysis. To address these issues, we have developed uniquely identifiable molecular barcodes in the form of synthetic DNA and recombinant whole cells that can be used as spike-in reference standards. To analyze their efficacy, five differentiable synthetic DNA molecular barcodes were spiked in staggered copy numbers (10^5 and 10^6) into porcine DNA samples and quantitated using bioinformatics tools. Additionally, *Escherichia coli* and *Lactobacillus gasseri* recombinants, each with a unique barcode, were also spiked into porcine fecal samples at copy numbers of 10^6 and 10^5, respectively. DNA was then extracted using three commercially available kits, followed by 16S rRNA gene (V3-V5) sequencing using the Illumina MiSeq platform. Mapping the 16S rRNA gene reads against each of the molecular barcodes as references provided a high level of detectability and quantification. Spiking synthetic DNA with the differentiable molecular barcodes resulted in lower recovery (10^2-10^3 copies) as compared to the recombinant *E. coli* (10^6). *Lactobacillus gasseri* recombinant recovery was the lowest (10^1). The differential recovery of the molecular barcodes may be due to their loss during DNA extraction, library preparation, and/or sequencing depth. Further, the Operational Taxonomic Unit picking strategy may have an effect on the diversity and taxonomy analyses. Overall, these results support that spike-in controls can be used as reference standards in 16S rRNA gene analyses as a means to strengthen the validity of findings in microbiome research.

9:00 AM S80: GSC metadata standards in action: MetaSUB and ISD

L. Schriml, University of Maryland School of Medicine, Baltimore, MD, USA*

The Genomic Standards Consortium (GSC) has developed and implemented a suite of metadata standards to capture contextual metadata for genomic sampling and sequencing projects. The GSC family of minimal information standards are most commonly used in the form of checklists information about the Minimal Information (MI) about and (x) Sequence(S) to adequately describe all phases of a sequencing project. This presentation will describe how the GSC MIxS standards were recently applied to data derived from the study of microbiome communities; the first from Crete soil samples and the second from subway surface samples as part of the larger MetaSUB project, which was designed to improve city utilization and urban planning by through detection, measurement and design of metagenomic studies in urban environments.

9:30 AM Break

10:00 AM S79: What can Genome Quality Scores for a hundred thousand bacterial genomes tell us about standards for metagenomics?

D. Ussery, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

Several different methods for calculating quality scores of genomes have been applied to more than a hundred thousand bacterial genomes. Only a tiny fraction (about 5%) of them are complete, and about two-thirds of the draft genomes are of good enough quality for reliable and robust comparisons. Similarly, there thousands of metagenome projects available, but the quality of the sequencing is variable. Various possible methods will be discussed to evaluate metagenomic sequence quality.

10:30 AM S78: Some thoughts and observations on “taxon calling”

G. Garrity, Michigan State University, East Lansing, MI, USA*

For over two decades, we have followed the changes in the taxonomy and nomenclature of prokaryotes with validly published names. When properly applied, these names symbolize what is known about a particular taxon and serve as a means of accessing and indexing information in the literature and in databases. However, these names may change in an unpredictable manner; leading to a loss of information, possible misinterpretation or misidentification of unknowns. In our effort to understand how well-regulated subject language terminologies evolve (e.g., prokaryotic nomenclature), we tracked the changes occurring in the underlying gene and genome sequences data on which the current nomenclature is largely based. A benefit of this tracking is that we can pose questions about methodological variation and hidden biases that can impact classification, identification and naming including: the effect of different alignments, different marker genes, sequence quality and length, algorithms and cut-off points to define taxonomic boundaries. The focus of this lecture will be to explore the benefit of a well-curated and carefully annotated reference database that can be used to evaluate methods for identifying and naming prokaryotes and used to re-annotation and updating of analyses of metagenomes and microbiomes at a finer grain of resolution that is currently used.

11:00 AM S82: Open session for public comments

G. Garrity, Michigan State University, East Lansing, MI, USA*

Open discussion

8:00 AM - 11:30 AM Session: 15: Mechanistic Insight to Natural Product Biosynthesis and Engineering

Conveners: **Yi Tang**, University of California Los Angeles, Los Angeles, CA, USA and **Yit-Heng Chooi**, University of Western Australia, Perth WA, Australia

Plaza Ballroom E - Concourse Level

8:00 AM S83: Engineering biosynthesis of the anticancer alkaloid noscapine in yeast

Y. Li, University of California, Riverside, Riverside, CA, USA and C. Smolke, Stanford University, Stanford, CA, USA*

Noscapine is a potential anticancer drug isolated from the opium poppy *Papaver somniferum*, and genes encoding enzymes responsible for the synthesis of noscapine have been recently discovered to be clustered on the genome of *P. somniferum*. Here, we reconstitute the noscapine gene cluster in *Saccharomyces cerevisiae* to achieve the microbial production of noscapine and related pathway

intermediates, complementing and extending previous *in planta* and *in vitro* investigations. Our work provides structural validation of the secoberberine intermediates and the description of the narcotoline-4′-O-methyltransferase, suggesting this activity is catalysed by a unique heterodimer. We also reconstitute a 14-step biosynthetic pathway of noscapine from the simple alkaloid norlaudanosoline by engineering a yeast strain expressing 16 heterologous plant enzymes, achieving reconstitution of a complex plant pathway in a microbial host. Other engineered yeasts produce previously inaccessible pathway intermediates and a novel derivative, thereby advancing protoberberine and noscapine related drug discovery.

8:30 AM S84: Revealing the chemical logic of diverse non-heme iron enzyme catalysis in natural products assembly

W.C. Chang, J.L. Huang and J. Dicks, North Carolina State University, Raleigh, NC, USA; Y. Guo, J. Li, M. Kurnikova and I. Kurnikov, Carnegie Mellon University, Pittsburgh, PA, USA*

Non-heme iron, 2-oxo-glutarate and molecular oxygen dependent enzymes catalyze a broad array of synthetically challenging transformations, ranging from hydroxylation, demethylation, halogenation, epimerization, endoperoxidation, ring expansion and etc. In the last decade, mechanistic investigations on this enzyme family have revealed an emerging paradigm where diverse reaction outcome can be attributed from: (1) the electronic structures of the Fe(IV)-oxo species; (2) participation of polar or redox-active residues in the secondary coordination sphere; and (3) the intrinsic properties of the substrates. AsqJ, a dual-functional enzyme, catalyzes sequential desaturation and epoxidation reactions in biosynthesizing 4'-methoxy-viridicatin. To explore the chemical logic governing the AsqJ reaction outcomes, combining mechanistic probe design, transient kinetics dissection and spectroscopic characterization, we provide experimental support for AsqJ catalyzed desaturation where the cationation or hydroxylation pathway operates. Furthermore, for the first time, the involvement of the Fe(IV)-oxo and the hydroxyl (or water) ligand tautomerism in the non-heme iron enzyme catalyzed epoxidation has been discovered.

9:00 AM S85: Post-PKS enzyme complexes

J. Rohr, University of Kentucky, Lexington, KY, USA*

Type I (more) and type II (less) PKSs are well known and thoroughly studied. However, the post-PKS tailoring steps are still viewed a linear sequence of events catalyzed by single, independent enzymes. This picture is rapidly changing, since more and more multiple-function enzymes and/or co-dependent enzymes were discovered in context with post-PKS tailoring steps. For example, multiple function enzymes as well as enzyme complexes of were observed in the post-PKS tailoring steps of mithramycin and gilvocarcin biosyntheses. The gilvocarcin group of anticancer antibiotics is characterized by the unique benzo [*d*]naphtho[1,2-*b*] pyran-6-one chromophore with D-fucofuranose at the 4-position. To achieve this chromophore, polyketide biosynthesis is carried out by a type II polyketide synthase (PKS), and a tailoring enzyme complex is proposed to convert an angucyclinone intermediate (dehydrorabelomycin) into the defuco-gilvocarcin chromophore. Its components, consisting of GilOII, GilM and GilR, established a complicated and intriguing collaboration to share an FAD co-factor necessary for all three enzymes. While the basic functions of these enzymes have been determined, a more complete biochemical characterization is still needed to understand their interplay and substrate channeling. Likewise, the two crucial saccharide chains as well as the highly functionalized pentyl side chain attached at 3-position, all hallmarks of the pharmacophore of the aureolic acids anticancer drugs, are assembled using at least two post-PKS tailroing enzyme complexes, in which some of its components also play multiple roles.

9:30 AM Break

10:00 AM S86: Disfavored *exo*-adduct generation by a modified octalin-forming Diels–Alderase

K. Watanabe, University of Shizuoka, Shizuoka, Japan*

The octahydronaphthalene (octalin) structure formed as a result of an intramolecular [4+2] cycloaddition is widely present among bioactive natural products isolated from fungi. During the biosynthesis of Sch 210972, the dirigent protein CghA is proposed to perform a Diels–Alder cycloaddition on a straight-chain substrate to forge the core octalin structure of the compound. To examine the mechanism of this reaction in details, the 2.1Å-resolution crystal structure of CghA was determined, providing us with the first X-ray structure of a eukaryotic Diels–Alderase of fungal origin. Based on the structural information, further biochemical and computational studies were conducted to determine the mechanism of how CghA imposes a stereoselectivity on a complex cyclization reaction. This work expands our knowledge and understanding of the emerging and potentially widespread class of natural enzymes capable of catalyzing stereoselective Diels–Alder cycloaddition reactions.

10:30 AM S87: Identification of novel Diels Alderases from fungal biosynthetic pathways

Y. Tang, University of California Los Angeles, Los Angeles, CA, USA*

Diels Alder cycloaddition is an important reaction for construction of multicyclic compounds with efficiency and stereocontrol. In recent years, several enzymes that can catalyze the Diels Alder reaction in bacterial natural product biosynthetic pathways have been identified. However, no biochemical confirmation of Diels Alderase activities has been demonstrated for fungal pathways, despite the wide occurrence of natural products that are proposed to derive from such cyclizations. In this talk, we will discuss the discovery and characterization of two new Diels Alderases from fungal polyketide biosynthetic pathways. Both genetic and biochemical reconstitution were performed on both enzymes to demonstrate the reactions that are catalyzed. The implication of the discoveries on genome mining of other polycyclic natural products will also be discovered.

11:00 AM S88: Biosynthesis of beta-lactone natural products

L.P. Wackett, J.K. Christenson and J.E. Richman, University of Minnesota, St. Paul, MN, USA; S.L. Robinson, University of Minnesota, Minneapolis, MN, USA*

Beta-lactones are biosynthesized by bacteria as intermediates in the production of membrane hydrocarbons and as end products of natural product biosynthetic pathways. We have identified a class of bacterial enzymes that synthesize beta-lactones from beta-hydroxy acid precursors. These beta-lactone synthetases were first identified as OleC proteins that function in the biosynthesis of long-chain *cis*-olefinic hydrocarbons and have been identified in the genomes of hundreds of bacteria dispersed throughout the eubacterial phylogenetic tree. The beta-lactone synthetases require ATP and surprisingly show a lack of stereochemical preference for different beta-hydroxy acid diastereomers. beta-Hydroxy acids that react with OleC-type proteins are generated from fatty acyl-CoA precursors via a Claisen-condensation reaction catalyzed by OleA that forms a beta-keto acid, followed by a ketone reduction reaction catalyzed by OleD. Some bacteria have an additional enzyme, OleB, that processes beta-lactones via a decarboxylation reaction that generates *cis*-olefins. In at least one bacterium, OleB, OleC and OleD proteins self-assemble into large (~2 MDa) multi-enzyme assemblies. These assemblies may have evolved to prevent the release of free beta-lactone intermediates into the cytoplasm of the bacterial host. There are commonalities between the synthesis of beta-lactones for membrane hydrocarbons and for natural product biogenesis and this will be discussed.

8:30 AM - 11:30 AM Session: 16: Systems Biology: A Guide to Optimize Fermentation and Cell Culture Sponsored by DOW AGROSCIENCES

Conveners: **Stephen Van Dien**, Genomatica, Inc., San Diego, CA, USA and **Deepti Tyagi**, Moderna Therapeutics, Cambridge, MA, USA

Plaza Ballroom D - Concourse Level

8:30 AM S89: Collaboration between strain engineering and fermentation scientists is key to developing large scale industrial fermentation processes

N. Dowe, National Renewable Energy Laboratory, Golden, CO, USA*

Our laboratory is tasked with developing and demonstrating pilot scale biological processes capable of producing low-cost sustainable fuels and fuel intermediates from biomass. The development process for biological conversion of biomass to fuels is highly dependent on strain engineering; often a combination of gene insertions and deletions within a microbial pathway based on a systems biology approach. Evaluation of new strains' performance often occurs at small scale on plates or in shake flasks. Once strains are performing sufficiently at small scale, the strain engineering and fermentation scientists map out strategies for integration at the bench scale. A close working relationship is necessary, particularly when the developed strains are targeted for large scale fermentation processes. The iterative and collaborative process between fermentation development and strain engineering informs areas in which strain improvement and scale-up should focus. For example, the production of 2,3-butanediol from *Zymomonas mobilis* will be presented to illustrate the challenges our strain engineering and fermentation process development scientists face within their own disciplines and how critical it is for the two groups to collaborate to solve the technical challenges in developing industrial strains for the production of fuels from biomass.

9:00 AM S90: Leveraging Genomatica's integrated technology platform for the successful application of diagnostic fermentation in industrial biotechnology

G. Scalcinati, Genomatica Inc., San Diego, CA, USA*

Genomatica has established an integrated, systems-biology based technology platform that employs data-driven strategies to rapidly design, characterize and improve industrial microorganisms and bioprocesses. This platform combines predictive computational modeling with robotic high-throughput strain screening and enzyme engineering, precision fermentation, and a suite of systems biology approaches including whole genome sequencing, transcriptomics, proteomics, metabolomics, and ^{13}C-flux analysis. Achieving commercial targets requires a "whole process" approach, with parallel optimization of the organism, the fermentation process, and downstream product recovery. As the design of the integrated process evolves, the bottlenecks in the organism can shift, and vice versa. Addressing this challenge requires an iterative and integrative approach that combines generation and analysis of heterogeneous data types across multiple scales (well-plates to lab-scale & industrial-scale bioreactors). This presentation will showcase how the synergy between systems and fermentations are important to identify process bottlenecks, guide the design of a variety of organisms and processes, and ultimately enable commercialization. We'll share our hard-won experience, including how commercial scale conditions have to be taken into consideration in the earliest phases of strain design; how to choose fermentation and downstream processing approaches based on techno-economic analyses; and how to apply our systems bioengineering and diagnostic fermentation platform to design strains that optimize metabolism and product yield under conditions compatible with "at-scale" constraints.

9:30 AM Break

10:00 AM S91: Mapping plasmid and genome evolutionary failure modes by deep sequencing

D. Deatherage and J. Barrick, University of Texas at Austin, Austin, TX, USA*

Mutations are an inevitable outcome of DNA replication, yet they must be avoided in engineered cells to maintain reliable and predictable function. Different choices in editing genomes or constructing plasmids can make a design more or less prone to mutations. Undesirable 'failure' mutants can rapidly take over cell populations when non-producing cells have a growth advantage. If the mutations that were most likely to compromise a particular strain could be diagnosed, its DNA sequences could potentially be redesigned to delay the evolution of reduced product yield. We have combined sequence-based enrichment, error-correction via molecular barcodes, evolutionary time series data, and the *breseq* computational pipeline into a high-throughput method for recovering the diversity and trajectories of failure mutations. We have demonstrated this approach in two model systems. First, we profiled mutations leading to the rapid loss of costly plasmid-based expression of yellow fluorescent protein in three different *E. coli* hosts. Second, we performed 'evolvome' sequencing of eight chromosomal genes known to mutate rapidly in *E. coli* evolved under the conditions of the Lenski long-term evolution experiment (LTEE). These data enabled us to calculate the fitness benefits of more than 100 unique mutations across these genes and to characterize the nature of adaptive mutations in each gene. We analyzed the same samples from the perspective of the 'mobilome' by profiling how new copies of IS elements were inserting genomewide. We anticipate that characterizing and then solving these types of evolutionary failure modes will be useful for optimizing the functions of engineered microbes.

10:30 AM S92: Dynamic metabolic control: A new paradigm for robust and scalable fermentation processes

M. Lipscomb, DMC Limited, Boulder, CO, USA*

Major barriers currently impede the successful commercialization of integrated bio-refineries and large-scale industrial bioprocesses to produce high value chemicals. Despite substantial recent advances in metabolic engineering and synthetic biology, it has proven much more difficult than expected to integrate a well-characterized production pathway into a living host and balance the complex requirements of both growth and production. Another challenge in the field is the routine predictable scale-up from small-scale screening due to the complex responses of microbial systems to environmental variables. Finally, the demonstration of multi-product bio-refineries (analogous to petroleum refineries) has not been achieved due to the custom design specifications for each bio-process that is developed. To overcome many of these challenges, we developed Dynamic Metabolic Control (DMC) technology to utilize a universal, standardized two-stage bioprocess for any desired product molecule. This approach decouples growth from production and enables the dynamic minimization of metabolism to only those components necessary for product formation. Importantly, the resultant biocatalysts are robust to the production environment and we have demonstrated predictable performance across scale from high-throughput microfermentation to multi-liter scale for numerous products.

11:00 AM S93: Physiological and systems level characterization of *Caldicellulosiruptor bescii* responses to growth at acidic pH

P. Manga and K. Sander, University of Tennessee/ ORNL, knoxville, TN, USA; M. Rodriguez Jr., ORNL, Oak Ridge, TN, USA; D.M. Klingeman and R.L. Hettich, Biosciences Division and BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN, USA; R.J. Giannone and S. Poudel, BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN, USA; N.L. Engle, T.J. Tschaplinski and S.D. Brown, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Caldicellulosiruptor bescii is an anaerobic hyper thermophile (70-80^{o}C) that can utilize a wide range of substrates. However, inhibitors released from biomass can result in unfavorable growth conditions and limit bioconversion to products. Medium as well as intracellular pH are conditions critical for growth and prone to change in effect of inhibitory compounds. Growth pH for *C. bescii* as currently reported is a narrow range of 6.8-7.3. In this study, we examined the physiological and systems level responses of *C. bescii* to grown at acidic pH. Samples collected from bottles, controlled batch and chemostat systems were subjected to growth, product and integrated omics profiling. *C. bescii* displayed the ability to maintain growth at pH 5.5 – 7.2 at 0.1 hr^{-1} dilution rate in chemostat on avicel. In batch reactors, lowering pH from 7.2 to 6.0 at mid-log phase led to significant increase in growth and product yield. We hypothesize, the observed improvement in growth and productivity is due to increased efficiency of ATP generation at higher membrane potentials driven by proton motive force. Time course transcriptomics data from these reactors revealed a sugar ABC transporter operon and PQQ redox cofactor biosynthesis like operon to be consistently highly over-expressed, in treated cells. Proteomics detected >1650 total proteins in both control and treated samples with 36 proteins significantly differentially expressed between conditions. Overall integrated omics analysis indicates that active proton transport, metabolic flux re-routing, potential pH sensing or signaling elements and passive homeostasis related strategies are employed by *C. bescii* for pH/pmf maintenance.

11:30 AM - 1:00 PM Lunch - All registered attendees

Plaza Exhibit - Concourse Level

11:30 AM - 4:00 PM JIMB Senior Editors' Luncheon Meeting

Governors Square 17, Plaza Concourse Level

1:00 PM - 3:30 PM Session: 18: Advances in Downstream Procession for Fermentation and Cell Culture

Conveners: **Dr. Frank Agbogbo**, Cytovance Biologics, Oklahoma City, OK, USA and **Jeroen den Hollander**, DSM Biotechnology Center, Delft, Netherlands

Plaza Ballroom D - Concourse Level

1:00 PM S101: Making scents of waste: Fermentative production of high-value flavour and aroma compounds from food industry by-products

M. Lindsay, D. Greenwood, N. Granucci and S. Villas-Boas, The University of Auckland, Auckland, New Zealand*

Perfume and flavouring agents have been valued by civilisations for thousands of years. Today, modern fragrance and flavour ingredients can comprise up to 5% of the total cost of many consumer goods/products. This USD 25 billion industry continues to expand in particular, due to consumer demand for natural alternatives to artificial additives. We know that fermentation can be used to naturally enrich the volatilome of many organic substrates for example, grape juice vs wine. Therefore, microbial bioconversion of readily available primary industry waste may be an attractive solution to naturally produce fragrance and flavour chemicals compared with expensive traditional methods. We have completed over 50 different fermentations using substrate derived from primary industry waste including:

juice and wine by-products, olive pomace and waste onions. A range of fungi were used to conduct laboratory-scale liquid-, solid- and/or submerged-state fermentations. For each, sterile substrate was inoculated with a single fungal strain. The volatilome of both fermented and unfermented substrate was then analysed using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). Fermented profiles were compared to unfermented substrates to identify *de novo* production of flavour and aroma compounds. Volatile profiling showed that hundreds of compounds were produced, of which many were identified using the NIST2014 library as well as an in-house library. Several valuable compounds were produced including those with mint, rose, cherry and honey characters. These are now the focus of optimisation and scale-up trials.

1:30 PM S102: Pushing productivities for lactate fermentation on lignocellulosic hydrolysates in membrane bioreactor mode

W. Van Hecke and H. De Wever, VITO, Mol, Belgium; S. Verhoef and W. Groot, Corbion, Gorinchem, Netherlands; M. Saric, ECN, Petten, Netherlands; A. de Haan, TU Delft, Delft, Netherlands*

Polylactic acid (PLA) became the first bio-based polyester manufactured on an industrial scale. Its production price is mainly affected by feedstock costs and by the efficiency of the production process. Lignocellullosic biomass offers potential for cost reductions on the longer term. For high volume, low margin products, continuous fermentation technology offers advantages in comparison to (fed-)batch technology.

The aim of our work was to investigate continuous lactate fermentations at increased cell densities on simulated lignocellulosic hydrolyzate and wheat straw hydrolyzates using Ca(OH)2 suspensions as base.

To this end, a set-up with external tubular membranes was designed and constructed and long-term continuous and axenic fermentations were run in membrane bioreactor mode.

Fouling of the membranes proved to be a major bottleneck in the process. Automatization circumvented this problem, allowing constant operating conditions throughout extended periods of time.

Maximal productivities of 30.5 g.kg-1.h-1 could be reached in the prototype combined with an average lactate concentration of 85.4 g.kg-1 and complete consumption of C5 and C6 carbohydrates.

Hence, significant performance improvements in comparison to batch and (regular) continuous conditions were demonstrated.

2:00 PM S103: Advanced alcohol recovery methods

S. Kohl, White Energy, Plano, TX, USA*

The fermentative production of alcohol has made significant advancements over the past decade. These improvements have been able to raise the terminal concentration of alcohol in the end product as well as produce non-ethanol alcohols at sufficient yields and speed to be industrially relevant. One of the newer advents in commercial alcohol production is novel recovery techniques. A vacuum recovery method has been developed for both ethanol and butanol production. This process has advantages in that the alcohol product is removed from the fermentation broth during ethanol production. This process substantially removes end product inhibition allowing the fermentation process to proceed faster and to higher “net” titre that previously available. Discussion of this technology at both laboratory and full-scale implementation will be given along with comparison to the standard finished beer distillation process used in most commercial applications.

2:30 PM Break

3:00 PM S104: Advances in downstream processing of biotherapeutic proteins from microbial sources

S. Fisher, Cytovance Biologics, Oklahoma City, OK, USA*

Downstream Process of Biotherapeutic proteins involves multiple unit operations including cell homogenization and clarification, typically several chromatography steps, and Tangential Flow Filtration for final formulation. In addition, some microbial products need to be refolded. This presentation will highlight advances in each area as well as challenges in incorporating new technology.

1:00 PM - 4:00 PM Session: 19: Industrialization of Synthetic Biology and Metabolic Engineering Sponsored by INTREXON

Conveners: **Patrick J. Westfall**, Zymergen, Inc, Emeryville, CA, USA and **James Kealey**, Intrexon Corporation, South San Francisco, CA, USA

Plaza Ballroom A & B - Concourse Level

1:00 PM S105: Collaborative chemical and bioprocess development and scaling to accelerate commercialization of synthetic biology- and industrial biotech-based products

T. Pray, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Robust and flexible process development, piloting and demonstration is key to scaling up and commercializing new products important for both emerging and established companies in the bioeconomy using fermentation, chemistry, separations, and / or purification processes. The US Dept. of Energy's (DOE) Advanced Biofuels - *and Bioproducts* - Process Demonstration Unit (ABPDU), located at Lawrence Berkeley National Laboratory (LBNL) in the San Francisco Bay Area is pioneering new collaborations to accelerate this development and de-risk technologies with our industry, academic and National Lab partners. Over the past 3-4 years, our experienced and well-trained team has worked with over 30 start-up and larger companies from around the country and overseas to develop and scale processes in our facility for products as diverse as chemical intermediates, biomaterials, biofuels and proteins to enable process debottlenecking and commercialization using our world-class bench-to-pilot scale process and analytical equipment and software. The ABPDU serves as a collaboration facility where researchers and developers can incubate and improve their processes, demonstrate their technology's feasibility, produce kg-scale product volumes for application testing, and create technology transfer packages for scale-up / scale-down prototyping with other facilities. This presentation will highlight several key outcomes from our partnered projects and describe our technical and project management approaches, including our focus on industry-friendly IP and cost terms, and client access both to our seasoned technical team and to our facility and expanding capability and equipment.

1:30 PM S106: A platform for rapidly engineering methanotroph biocatalysts to produce industrial products from natural gas

K. Dietzel, Intrexon, South San Francisco, CA, USA*

Natural gas is one of the most economical and abundant sources of carbon currently available. Methanotrophic bacteria use the methane in natural gas as a source of carbon and energy and have been successfully grown at industrial scale for biomass production. Compared to other industrial microorganisms such as *E. coli* and *S. cerevisiae*, a limited set of techniques for genomic manipulation and metabolic engineering are available for methanotrophic bacteria and significantly less is known about their metabolism and biochemistry. 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 industrial products. This platform consists of a complete set of genetic tools that enable facile host modification, a detailed metabolic model, a high throughput screening 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 how these tools are enabling rapid strain improvement for a variety of industrial products.

2:00 PM S107: From models to microbes and back: How automating strain design produced hundreds of hits

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

Engineering microbial metabolic networks to cost-effectively convert feedstocks into high-value products requires navigating a vast and difficult-to-measure phenotype-genotype landscape. Quantitatively characterizing this landscape is key to shrinking the development cycle to achieve kilogram-scale quantities of new target molecules from years to months. To do this we first automated pathway discovery, using metabolic models to identify biochemical routes that convert plant sugars to over five hundred diverse target metabolites. Using these routes as a foundation, we then devised a Design-of-Experiment (DOE)-inspired approach to strain design. In a nutshell, this method combinatorically adds proteins and other regulatory elements from Amyris' genetic parts library and results in strain designs that broadly explore native and non-native metabolism, but are crossed enough to draw robust conclusions. This method of strain design differs significantly from classic DOEs such as definitive screening, Box-Behnken, or central composite designs, which are unable to handle the unusual shape of the combinatorial space at hand. We subsequently constructed thousands of strains using this approach and measured end-point product titers using high-throughput chromatography and mass spectrometry. Significantly, this approach successfully led to the microbial production of over a hundred target molecules, some with no precedent in the literature.

2:30 PM Break

3:00 PM S108: Applying high-throughput genome engineering to optimize microbes for industrial bioprocesses

S. Hoover[*]*, Zymergen, Inc., 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, efficiency, and reliability. This platform integrates several core technologies including custom software, high-throughput laboratory automation, and machine learning algorithms. We have developed custom scientific computing tools for specifying and tracking the creation of designer microbes. 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. Additionally, throughput allows us to test thousands of design ideas across many library types to identify the ideas that are beneficial. 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.

3:30 PM S109: Developing green cell factories: The journey to cyanobacterial fuels and chemicals

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

Cyanobacteria are attractive systems for metabolic engineering due to the relative ease of genetic manipulation and their potential of producing a variety of valuable chemicals and fuels directly from carbon dioxide. This relatively young field of research has yet to engineer a strain of cyanobacteria that reaches the productivity and titers needed for industrial relevance. While there are promising examples of g/L titers being achieved from engineered strains, rapid progress in the field is limited by an overall lack of understanding of how to best engineering cyanobacteria grown in periods of alternating light:dark

conditions, by a lack of understanding of the systems level changes in these conditions and how they are regulated, and by a lack of molecular tools developed specifically for photosynthetic microorganisms. This talk will focus on our efforts to address these limitations. More specifically, I will discuss our work on developing a counter-selection marker and a promoter library for use in cyanobacteria and on our progress to engineer cyanobacteria for the production of fuels and chemicals.

1:00 PM - 4:30 PM Session: 20: Novel Insights from Metagenomics and Microbiomes and Their Biotechnological Applications

Conveners: **Raul J. Cano**, California Polytechnic State University, San Luis Obispo, CA, USA and **Andreas Schirmer**, REG Life Sciences, LLC, South San Francisco, CA, USA

Governors Square 15, Concourse Level

1:00 PM S110: Application of next generation sequencing and bioinformatics for rapid and accurate pathogen detection and characterization of the microbiome

R. Colwell, University of Maryland, College Park, MD, USA*

Next generation sequencing (NGS) combined with high-resolution bioinformatics, offers powerful method for detection, identification, and characterization of pathogenic microorganisms (bacteria, viruses, fungi, and parasites). This approach to diagnosis of infectious disease agents and infectious diseases offers accuracy, speed, and actionable information, the sequencing within one or two days and bioinformatics analysis within minutes. We have applied this method in clinical studies, including retrospective case control studies comprising samples known and unknown etiology, as well samples from healthy individuals. The results are exciting and demonstrate detection and identification of pathogens can be accomplished well within the time frame of a single day or so. Furthermore, microbiome analysis can be used to differentiate healthy, diseased, and asymptomatic carriers, including individuals in early stages of infection and disease. Results of studies accomplished to date show that disease state of patients reveals multiple pathogens, the microbial communities of healthy humans of diverse geographic locations tolerate different levels of pathogenic microorganisms and antibiotic resistance in their microbiomes. Different rates of antibiotic resistance were detected in geographically diverse populations. Analysis of the human microbiome and the microbial ecology of aquatic reservoirs and wastewater reuse treatment plants has provided insight into the complex interactions of the microbial populations of these ecosystems. Precision offered by next generation sequencing coupled with powerful bioinformatics makes possible a much more complete understanding of the microbiology of human populations and their environment. Thus, it is clear that a Genomics Center at North South University is both timely and valuable resource for Bangladesh.

1:30 PM S111: From data to discovery: unveiling microbiome-based product candidates from your biospecimens

T. DeSantis, Second Genome, South San Francisco, CA, USA*

The opportunities for distilling microbiome data into commercial products are surfacing rapidly. However, data distilleries are faced with challenges in broad variations in biospecimen preparation, assay selection, bioinformatics pipelines and statistical approaches, as evidenced by discordant microbiome observations found in the literature. Awareness of these sources of variation enables well-structured machine learning at cloud compute scale to find diagnostic biomarkers of disease, beneficial microbes and therapeutic microbial secretions. Furthermore, the benefits of adding PhyloChip analysis on top of NGS workflows will be discussed, with special attention to the benefits of zero barcodes, zero multiplexing, internal controls within each sample, automation, low technical variation and production of non-sparse

matrices. Integration of NGS and PhyloChip data into a unified bioinformatics analysis allows confident product candidate nominations.

2:00 PM S112: Connecting the dots in complex disease: The microbiome as a potential map

M. Carlin[*] and T. Yates, The Biocollective, Centennial, CO, USA

This session will discuss the microbiome as a potential lens through which to view the trajectory of a complex disease. Using Parkinson's disease as an example, we will discuss the environmental factors that can potentially impact the microbiome and the course of disease. This session will discuss The BioCollective's unique approach to collecting broad-based population samples from healthy and disease specific groups with the goal of collecting, connecting and correcting the microbiome to change the course of chronic disease. Dr. Yates will present data on collection and preservation methods to maximize culturing.

2:30 PM S113: Describing the root-associated microbiomes of maize and soybean using comparative metagenomics

R. Williams[*], Monsanto Company, Chesterfield, MO, USA

Soil microorganisms interact with root surfaces (rhizosphere) and within root tissues (endosphere), affecting host plant productivity and driving soil biogeochemistry. *Zea mays* L. (corn) and *Glycine max* L. (soybean) together are the most dominant agricultural products globally. The root-associated microbiomes of these crops can therefore influence microbial biodiversity and functional capacity of soil on a global scale. However, in-depth characterization of these microbiomes currently does not exist. In this study, we sampled corn and soybean root associated microbiomes (rhizosphere and endosphere) across central USA through amplicon sequencing (16S rRNA) and shotgun metagenomics. Using a novel iterative assembly approach based on the bacterial diversity of each sample, we generated high quality reference metagenomes for corn and soy root-associated microbiomes that are the largest to date. Soy microbiomes were enriched in *Rhizobiales* and nitrogen fixation genes following classical models of legume-rhizobia mutualisms. The root-associated microbiomes of corn were more diverse and enriched in a variety of nutrient acquisition strategies, suggesting that multiple resource limitation creates more niche space for microorganisms to coexist. Our reference metagenomes represent a publicly available resource that will assist in determining microbial-based targets for improving agricultural productivity and sustainability.

3:00 PM S114 [Withdrawn]

3:30 PM S115: Autometa: automated extraction of microbial genomes from shotgun metagenomes

I. Miller[*], E. Rees, I. Miller, J. Baxa, J. Ross, J. Lopera, R. Kerby, F. Rey and J. Kwan, University of Wisconsin - Madison, Madison, WI, USA

Culture-independent sequencing (metagenomics) is a powerful, high resolution technique enabling the study of microbial communities *in situ*. With modern sequencing technology and bioinformatics, individual genomes can be assembled and extracted directly from environmental samples containing complex microbial communities by a process known as metagenomic "binning." However, available binning programs suffer from methodological and practical shortcomings, such as the requirement of human pattern recognition, which is inherently unscalable, low-throughput, and poorly reproducible. Some methods also require the assembly of pooled samples, which can lead to poor assemblies in the case of inter-sample strain variability. We therefore devised a fully-automated pipeline, termed "Autometa," which incorporates machine learning principles to separate pure microbial genomes from single shotgun

metagenomes. Autometa uses Barnes-Hut Stochastic Neighbor Embedding to analyze 5-mer frequency in the contiguous sequences (i.e., "contigs") produced by *de novo* metagenomic assembly. The DBSCAN algorithm is then used to identify groups of contigs (i.e., genome "bins") with congruent 5-mer frequency patterns. Unsupervised machine learning is then employed to optimize clustering for purity of genome bins, measured by the presence of gene markers known to occur as single copies in isolated strains. In preliminary tests, Autometa recovered more pure and complete genomes from simulated, synthetic, and environmental metagenomic samples as compared to available programs such as MaxBin and MetaBAT. We are actively integrating supervised machine learning to further refine the binning process and using our current implementation of Autometa to study natural product biosynthesis in marine invertebrate microbiomes.

1:00 PM - 4:30 PM Session: 21: Diversity Session

Conveners: **Raul J. Cano**, California Polytechnic State University, San Luis Obispo, CA, USA and **Laura R. Jarboe**, Iowa State University, Ames, IA, USA

Governor's Square 14 - Concourse Level

1:00 PM S116: Diversity at Ginkgo

J. Cui, Ginkgo Bioworks, Boston, MA, USA*

Like many other technology companies, Ginkgo has seen a problem with gender diversity. Since the company was founded in 2008, the team averaged ~27% women, but by the beginning of 2016—a time of rapid growth—that number went to 23%. With strong belief in fostering a diverse and inclusive environment, Ginkgo made the decision to focus on gender diversity in recruiting and hiring after noticing this disturbing trend. By the end of 2016 the company had tripled in size and the effort to improve diversity had paid off: Ginkgo reached 37.5% women and was committed to continuing to improve and to expanding efforts in 2017, to maintain that upward trend and to consider other aspects of diversity including age, sexual orientation, race, ethnicity, national origin, and educational background, among others. It has been shown for groups from academic labs to large business that diverse teams are more effective and more productive; focusing on diversity earlier in a company's life ensures that the team will be built on these values. Building an inclusive culture and recruiting talent from groups that have been systemically undervalued can also provide young companies with a sustained advantage, by attracting amazing talent that is being missed by larger companies. Together we hope to succeed in our mission to make biology easier to engineer.

1:30 PM S117: Microaggressions: an obstacle in the workplace, or an opportunity for growth?

A. Prescott, AB Mauri, St. Louis, MO, USA*

Microaggressions—subtle, often unintentional forms of discrimination— are an obstacle under-represented minorities face in the STEM community. To those affected, microaggressions can make historically marginalized social groups feel undervalued or unwelcome in a laboratory or degree program, and can be a contributing factor in their decision to continue pursuing a STEM career. These inadvertent offenses can also be indicative of the unconscious biases held by those perpetrating microaggressions, which can lead to more serious forms of discrimination. With the proper mindset, these unintended offenses can become an opportunity for dialogue and growth between the recipient and perpetrator, leading to a more inclusive laboratory or classroom environment.

2:00 PM S118: Building community for deaf and hard-of-hearing scientists

S. Smith, G. Buckley and J. DeCare, Rochester Institute of Technology, Rochester, NY, USA; S. Barnett and S. Dewhurst, University of Rochester Medical Center, Rochester, NY, USA*

For many complex reasons, deaf and hard-of-hearing individuals have historically been significantly underrepresented compared to their hearing counterparts in research-based science professions that require the highest levels of education for career entry and advancement. Recently, the NIH included people with disabilities as underrepresented groups of individuals who might qualify for training programs aimed to increase science workforce diversity. As a result, the University of Rochester and the Rochester Institute of Technology have collaborated to implement three new NIH-funded pipeline training programs for aspiring deaf and hard-of-hearing scientists, including the Rochester Institute of Technology Research Initiative for Scientific Enrichment (RIT-RISE) Scientists-In-Training Program for undergraduates, the Rochester Bridges to Doctorate (RBD) Program for graduate students, and the Rochester Postdoctoral Partnership Program (RPPP) for postdoctoral fellows. The Rochester training pipeline will generate and disseminate best practices for training aspiring deaf and hard-of-hearing scientists so that we can build a thriving community for them and eliminate this unwanted disparity in the science workforce.
This 30-minute presentation will focus on the following topics pertinent to developing best practices for training deaf and hard-of-hearing scientists:

- Unique characteristics of deaf/hard-of-hearing individuals that contribute both to their historical obstacles accessing science careers and to the diversity benefits that they can bring to the science workforce
    - Variable communication strategies and variable English writing skills
    - Incomplete access to incidental information
    - Social and internalized stigma
    - Diminished career awareness and expectations
    - Unique elements important to successfully train aspiring deaf/hard-of-hearing scientists
- Address English writing skills
- Include deaf/hard-of-hearing mentors as role models

2:30 PM Break

3:00 PM Panel Discussion

1:00 PM - 5:00 PM Session: 17: Extremophilic Enzymes

Conveners: **Noha M. Mesbah**, Suez Canal University, Ismailia, Egypt and **James Coker**, University of Maryland University College, Baltimore, MD, USA

Plaza Ballroom F -Concourse Level

1:00 PM S94: Extremophiles: Source of a highly efficient biocatalysts

J.M. Blamey, Swissaustral LLC, Athens, GA, USA*

The discovery of extremophilic microorganisms and their novel enzymes has developed an increasing interest for using them in industrial applications.
Currently, the repertoire of enzymes which are used in industrial processes is limited by properties such as substrate concentration, sheering forces, temperature, pH and organic solvents, all common features in industrial settings. In general, extremozymes have an overall inherent stability and they are able to overcome most of these conditions.

The unique features of each group of extremophiles can be exploited to provide enzymes with specific applications.

Swissaustral has focused in the discovery and development of industrial enzymes exclusively derived from extremophilic microorganisms. It has built one of the largest and more diverse collections of novel and unique microorganisms from different extreme environments. This extreme bio-bank is permanently growing in order to provide the industry with the most complete source of extremozymes as novel biocatalytic solutions for non-standard technological challenges.

Here we present some of the enzymes we have been discover and developed as biocatalysts from extremophiles.

1:30 PM S95: High versatility of extremozymes in continuous flow biocatalysis

M. Planchestainer, M. Contente and F. Paradisi, University of Nottingham, Nottingham, United Kingdom; J. Cassidy, University College Dublin, Dublin, Ireland; F. Molinari and L. Tamborini, University of Milan, Milan, Italy*

We have investigated a variety of halophilic enzymes from halophilic organisms to date (the archaeon *Haloferax volcanii* and the halo-tolerant bacterium *Halomonas elongata*) with the specific aim of identifying new and robust biocatalysts able to withstand the presence of a variety of organic solvents and with a versatile active site to accommodate a broad range of substrates. Halophilic alcohol dehydrogenases (ADHs) and amine aminotransferases (AAT) from halophilic organisms alike show an unusually broad substrate scope, excellent tolerance to organic solvent and ease of manipulation. The enzymes have been successfully immobilized on an epoxy-resin which allows for reusability of the biocatalysts over 10 times. The immobilized biocatalysts can also easily be adapted to reactions in flow, under controlled pressure. Flow-biocatalysis is in its infancy with respect to more traditional chemical reaction and the potential for industrial applications is significant. Our enzymes are particularly tolerant to mechanical stress (flow and pressure) and we have achieved conversion rates above 99% with the amino transferase from *Halomonas elongata*. We will be presenting here our latest progress in the field of flow biocatalysis.

2:00 PM S96: That which doesn't kill you, makes you stronger: how halophile proteins adapted to salt

C. Evilia, L. Cobani, J. Kuhlmeier and J. Rosentreter, Idaho State University, Pocatello, ID, USA*

All extremophiles have protein adaptations that allow them to survive in their extreme environment. While the thermophiles and psychrophiles have adaptations to combat the entropic forces that are prevalent in their extreme heat and cold environments, the halophiles have a different problem. Halophiles not only grow in extreme concentrations of metal salts, but they also retain high concentrations in their cytoplasm. For their proteins to remain stable and functional, their structures have had to adapt to challenges to intra- and intermolecular interactions that come with the highly ionic environment. One protein feature that halophiles have acquired is a strong negative surface charge. Because it is not well understood if this charged surface plays a passive role to keep ions away or has a more active, specific binding role, we set out to determine if cations were binding and, if so, to measure their binding affinity. Using the *Halobacterium salinarum* and *E. coli* cysteinyl-tRNA synthetases and a modified equilibrium dialysis apparatus with atomic adsorption spectroscopy, we could measure relatively tight binding of group 1 cations to the halophilic protein whereas no binding to the *E. coli* protein was detected. Research is underway to determine if the halophilic protein's negative surface charge provides specific binding sites for the cations. By characterizing these interactions, we are getting closer to determining what the optimal design might be for proteins to remain functional and stable in extreme salts. These understandings are crucial to design more robust enzymes for potential biotechnological applications.

2:30 PM Break

3:00 PM S97: Deconstructing CelA: Functional studies of CelA a multimodular cellulase from *Caldicellulosiruptor bescii*

R. Brunecky, D. Chung, N. Hengge, N. Sarai, M.E. Himmel and Y. Bomble, National Renewable Energy Laboratory, Golden, CO, USA; J. Russel and J. Westpheling, University of Georgia, Athens, GA, USA*

The thermal tolerant CelA from *Caldicellulosiruptor bescii* is one of the most active cellulose degrading enzyme we have tested to date. In the saccharification of a common cellulose standard, Avicel, CelA outperforms mixtures of other commercially relevant exo- and endoglucanases. The modular structure of CelA is defined as: GH9-CBM3c-CBM3b-CBM3b-GH48 and the enzyme is extremely thermostable and highly active at elevated temperatures. From earlier transmission electron microscopy studies of cellulose following incubation with CelA, we discovered morphological features that suggest CelA is capable of not only the common surface ablation/fibrillation strategies driven by processivity, but also of forming extensive cavities of roughly the size of the enzyme. Surprisingly, unlike fungal enzymes CelA is not affected by high cellulose crystallinity, a major problem for traditional fungal cellulases. It has been proposed that CelA, and possibly other multi-functional glycoside hydrolases, act in novel manner when deconstructing cellulose. To further our understanding of these processes, we have recently created and tested fragments of CelA in order to specifically examine its subdomain activities on insoluble substrates as well as the role glycosylation may play in CelA activity.

3:30 PM S98: Lanthanide biochemistry:beyond methylotrophy

N.C. Martinez-Gomez, N. Good, O. Walser and P. Roszczenko, Michigan State University, East Lansing, MI, USA; E. Skovran, San Jose State University, San Jose, CA, USA*

Lanthanides are strong Lewis acids but their low aqueous solubility in natural environments led enzymologists to reason that their bioavailability was limited. A direct link between lanthanides and methylotrophy has recently been established, via pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenases (MeDH) during methane and methanol oxidation. Our work focuses on the impact of lanthanides on methylotrophic metabolism of *Methylobacterium extorquens* AM1. Using RNA-sequencing analysis, we identified genes encoding two PQQ-dependent alcohol dehydrogenase systems that were upregulated in the presence of lanthanides, XoxF-MeDH and the ethanol dehydrogenase ExaF-EtDH. We have purified and biochemically characterized these enzymes, showing that they incorporate lanthanide metals in the active site, and have gained insights into protein structural features to differentiate metal binding. Further, both ExaF-EtDH and XoxF-MeDH catalytic properties are altered by different lanthanide ligands. XoxF-MeDH exhibits methanol oxidation capacity similar to reported non-lanthanide-dependent enzymes. ExaF, however, is the most efficient PQQ-dependent EtDH reported to date, extending lanthanide-dependent enzymes to multicarbon metabolism. Further, our biochemical studies have shown that *M. extorquens* produce a siderophore-like molecule that resolves mechanistically the puzzling question about solubilization of lanthanides at neutral pH. Overall, our data shows that lanthanide-dependent biochemistry has superior oxidation capacity and/or increased catalytic efficiency for the oxidation of alcohols in a microorganism that is efficient for the solubilization of lanthanides, opening new avenues for the use of methylotrophs as a platform for green technology.

4:00 PM S99: Production and application of soluble hydrogenase I from *Pyrococcus furiosus*

C.H.P. Wu and M.W.W. Adams, University of Georgia, Athens, GA, USA*

Hydrogen gas is a potential renewable alternative energy carrier that could supplement the increasing demand for energy in the future. However, the current methods of commercial hydrogen production are based on fossil fuels and/or are cost-prohibitive. Thus, alternative renewable methods are needed for industrial hydrogen production. Biological-based systems have a great deal of potential and the enzyme hydrogenase, which catalyzes the reduction of proton to generate hydrogen, is an essential component of such systems. The soluble hydrogenase I (SHI) from hyperthermophile *Pyrococcus furiosus* has been

studied extensively and has already been used in various biotechnological applications. In particular, it is used in an *in vitro* synthetic pathway for hydrogen production that converts sugars and water to hydrogen and carbon dioxide. This *in vitro* system has a theoretical yield (12 H_2/glucose) that is three-times higher than that of *in vivo* biological fermentations (4 H_2/glucose). In order to scale up the *in vitro* pathway, a large scale method for preparing SHI is required. In addition, SHI is being used as a model hydrogenase to better understand the mechanism of hydrogen catalysis. Such information could assist in designing and engineering more efficient hydrogen production catalysts. In this presentation, the properties of SHI, the efforts to scale up its purification and its potential applications will be described.

4:30 PM S100: Extremophilic organisms for the production of biocatalysts and value-added products

S. Payne, P. Blum, N. Buan and J. Aldridge, University of Nebraska, Lincoln, NE, USA*

While the production of biocatalysts by mesophilic organisms is the industrial standard, technical hurdles are encountered when conditions become unfavorable for growth or enzymatic activity. Similarly, biosynthesis of certain products can be constrained by the native limitations of mesophilic pathways. Extremophilic organisms are a reservoir of proteins and enzymes capable of functioning in unfavorable conditions and often have non-standard metabolic pathways that make them favorable for producing certain products. *Sulfolobus solfataricus* is a hyperthermoacidophile archaean isolated from terrestrial hot springs. It is the source of a hot-acid resistant cellulase (Extremase) that has proved highly effective in the pre-processing of lignocellulose biomass for bioethanol production while reducing treatment costs. Extremase production employs an *S. cerevisiae* secretion system and has been combined with related enzymes to achieve a more potent hydrolytic cocktail for lignocellulose saccharification. *S. solfataricus* also demonstrates value in the production of the natural compound class terpenes, which include common flavor and scent compounds, up to highly valuable antitumor, antibiotic, and anti-inflammatory agents. Unlike standard industrial organisms, its highly active mevalonate pathway provides abundant substrate for engineered terpene cyclase enzymes. In addition, efficiency of harvest for volatile compounds improves at high growth temperatures. The isolation of extremase and the production of value-added terpenes demonstrate the industrial potential of extremophilic systems for biocatalysis and product synthesis.

6:00 PM - 7:00 PM Banquet Reception - Exhibits Open

Plaza Exhibit - Concourse Level

7:00 PM - 10:00 PM Annual Banquet, Award Presentations and Banquet Speech: "Stayin' Alive: A Musical Look at Contemporary Food Safety Issues" Carl Winter, University of California-Davis

Plaza Ballroom E - Concourse Level

Wednesday, August 2

7:30 AM - 1:00 PM Registration

Plaza Registration - Concourse Level

8:00 AM - 11:00 AM Session: 23: Fermentation Methods in the Food and Beverage Industry

Conveners: **Mark Berge**, MedImmune, Gaithersburg, MD, USA and **Dr. Helene Ver Eecke**, Metropolitan State University of Denver, Denver, CO, USA

Plaza Ballroom F -Concourse Level

8:00 AM S125: Who's really in control? Recent trends in starter, non-starter lactic acid bacteria research in cheese.

T. Oberg, Leprino Foods Company, Denver, CO, USA*

Modern cheese making utilizes defined starter cultures during production for consistent results in finished product, though it is known that the non-starter lactic acid bacteria (NSLAB) community drives the characteristics of the cheese throughout shelf life or aging. These characteristics can be detrimental to the finished product causing flavor defects, soft body, and gassing. Changes in manufacturing practices, and intentional manipulation of the NSLAB, has provided some benefit, but unanticipated issues to have arisen. Recent microbiological studies of cheese indicate *Lactobacillus curvatus* has become a predominant species of the NSLAB population. Whole genome sequencing and annotation performed on cheese isolates has shown genes coding for pathways that would give *Lb. curvatus* a competitive advantage in cheese, and recent studies have shown the addition of *Lb. curvatus* during cheese production causes gas defects in cheddar cheese during the first month of ripening. Recent research has also identified a novel obligately heterofermentative NSLAB, which appears to cause late gas defects in commercially aged cheese. Named *Lactobacillus wasatchensis*, it can survive HTST pasteurization, can grow at the salt concentration and ripening temperature of aging cheese, and can grow on starter lysate as it's only nutrition source. When *Lb. wasatchensis* was added to milk prior to experimental cheesemaking trials, it produced gas during ripening at elevated ripening temperatures. Cheese is a dynamic microbial ecosystem and given their metabolic capabilities, there will always be a need to understand the interaction of starter and NSLAB in cheese.

8:25 AM S126: Advances and challenges in Baker's yeast industrial production

S. Trupia and J. Evans, AB Mauri, St. Louis, MO, USA*

Bread-making is a type of solid-state fermentation that has been practiced for millennia. Historically, and as late as the 1920s, bread was made from a starter culture and was not industrially produced. Later, utilization of dried, liquid or compressed *Saccharomyces cerevisiae* became the *de facto* way of baking bread, especially for large commercial bakers because it is faster and has a limited role in the sensory profile of bread, as compared to starter, sourdough-type cultures. For the purposes of industrial production of baker's and distiller's yeast, strains of *S. cerevisiae* have been typically selected for robustness under non-ideal physiological conditions, resulting in higher specialization and lower genetic diversity. As bread baking technology advances, for example with the demand for yeast tolerance to frozen dough conditions or higher sugar doughs, some of the traits that we find in a 'regular' industrial strain of *S. cerevisiae* could become a hindrance. New baking challenges require further understanding of the response to these stresses. We will discuss recent advances made in the study of yeast physiology during bread fermentation, how gene expression analysis has been used to understand the factors that influence the behavior of yeast and its response to external stress conditions, and what place does genetic editing or engineering of yeast has or should find in a fundamental human artifact such as bread.

8:50 AM S127: Fermentation technology for yeast extract manufacture

C. Ribeiro, DSM Biotechnology Center, Delft, Netherlands*

The DSM mission is to create brighter lives for people today and generations to come. Yeast extract is one of the many Biotechnological products produced by DSM. But what is yeast extract and what it is used for? How is it produced?

The yeast cell factory can be used to produce several marketable products: either based on its biomass or metabolites. As yeast extract is a biomass based product, optimal biomass production during fermentation is crucial, with biomass yield on sugar and productivity as important parameters. The fermentation yield has an impact on sugar costs and the fermentation productivity has impact on fixed costs. These parameters can be maximized by choosing appropriate fermentation conditions.

Furthermore, an optimal biomass composition is also of major importance since it determines the taste enhancing properties of the yeast extract.

As increasing productivity impacts biomass composition, the optimal yeast production process for yeast extract preparation requires a balance between fermentation productivity and biomass composition at the end of fermentation.

9:15 AM Break

9:45 AM S128: Purposeful *Pichia* pitching: Analyzing beer deliberately fermented with *Pichia*, a yeast genus that may be unknowingly prevalent in barrel-aged beer

H. Ver Eecke, J. Lambert, L. Fetter, G. Stout and M. Spindler, Metropolitan State University of Denver, Denver, CO, USA*

Modern craft brewing is trending towards increased barrel-aged beer. These barrels are of unknown microbial make-up, which can lead to unpredictable products. Avery Brewing contributed samples from their barrel-aged projects. Previously, the VerEecke lab analyzed these samples using culture-dependent (growth on 6 different media) and culture-independent (next generation amplicon sequence analysis) techniques. Bioinformatic analysis of all bacterial/archaeal 16SrDNA genes and all fungal ITS regions revealed a community with low richness dominated by *Lactobacillus* and *Pichia,* each comprising >98% of their respective domains. *Lactobacillus* is conventional in sour beer production, but *Pichia* has not been. *Pichia membranifaciens* is used often in smear-surface-ripened-cheese fermentation for flavor development and unwanted mold inhibition. Use of *Pichia spp.*is prevalent in the biofuel industry due to its ability to tolerate relatively high alcohol concentrations, break down plant components (xylose and cellulose), and produce lipids. This xylose/cellulose degradation may elucidate why *Pichia* may be prevalent in wooden barrels and/or fruit based sours. As a wine contaminate investigated ~50 years ago *P.membranifaciens* was found to produce alcohol and certain fermentation byproducts that have traditionally been thought of as off-flavors, but with the current renaissance of craft beer that promotes eclectic beer styles, these flavors may be desirable. We brewed a hop-less golden ale and compared various fermentations: *Saccharomyces cerevisiae*(WLP001) exclusively, *P.membranifaciens*(YB4326) exclusively, and combined pitches of WLP001 and YB4326. Beer samples were analyzed via sensory analysis and gas-chromatography-mass-spectroscopy. Future analyses will investigate hop-tolerance, *Brettanomyces* comparisons, and aging qualities as well as molecular assessment of *Pichia* ubiquity in barrels.

10:10 AM S129: From grape must to wine: towards a better knowledge of yeasts achievements

J. Noble, Lallemand SAS, Blagnac, France*

Fermentation of grape must, a crucial step of winemaking, consists in the conversion of sugars into ethanol, CO_2 and numerous other metabolites by yeasts. Most of the fermentations are performed by selected yeasts, essentially from *Saccharomyces cerevisiae* species, inoculated under dried active forms. Such strains have been selected for their specific properties and are well characterized. They contribute greatly to the aromatic balance of wines.
Since many years, knowledge about wine yeasts steadily increased thanks to numerous scientific studies and the gain in the understanding of their metabolism is huge. Consumption and requirements in nutrients (sugars, lipids, nitrogen, sulfur, vitamins, minerals), synthesis and production of biomass and metabolites (ethanol, glycerol, acids, alcohols, esters, sulfur compounds) have been characterized. Current work combining different levels of study, from physiologic and metabolomic to transcriptomic and genetic, provides us a global overview and allows us to deepen our understanding.

Furthermore, following the market trends and consumers preferences, demand for new wine yeast strains combining different properties of interest or adapted to winemaking conditions and global climate change, is continuously increasing. To meet those requirements, new methods based on the generated knowledge can be implemented such as clonal selection, metabolic engineering or hybridization. Some of those new wine yeasts and the science behind them will be presented.

10:35 AM S130: Primary souring: a novel bacteria-free method for sour beer production

K. Osburn, S. Metcalf, D. Nickens, C. Rogers, C. Sausen, J. Tennessen and M. Bochman, Indiana University, Bloomington, IN, USA; J. Amaral, Mainiacal brewing Company, Bangor, ME, USA; R. Caputo and J. Miller, Wild Pitch Yeast, LLC, Bloomington, IN, USA*

In the beverage fermentation industry, especially at the craft or micro level, there is a movement to incorporate as many local ingredients as possible to both capture terroir and stimulate local economies. In the case of craft beer, this has traditionally only encompassed locally sourced barley, hops, and other agricultural adjuncts. The identification and use of novel yeasts in brewing lags behind. We sought to bridge this gap by bio-prospecting for wild yeasts, with a focus on the American Midwest. We isolated 284 different strains from 54 species of yeast and have begun to determine their fermentation characteristics. During this work, we found several isolates of five species that produce lactic acid and ethanol during wort fermentation: *Hanseniaspora vineae*, *Lachancea fermentati*, *Lachancea thermotolerans*, *Schizosaccharomyces japonicus*, and *Wickerhamomyces anomalus*. Tested representatives of these species yielded excellent attenuation, lactic acid production, and sensory characteristics, positioning them as viable alternatives to lactic acid bacteria (LAB) for the production of sour beers. Indeed, we suggest a new LAB-free paradigm for sour beer production that we term “primary souring” because the lactic acid production and resultant pH decrease occurs during primary fermentation, as opposed to kettle souring or souring via mixed culture fermentation.

11:00 AM S131: Exploring the bioconversion of fruit and vegetable by-products using a food grade non-*Saccharomyces* yeast

N. Granucci, P. Harris and S. Villas-Boas, The University of Auckland, Auckland, New Zealand*

Fermented foods and beverages became a new trend in the natural and health food space. An attractive and innovative option is to combine non-conventional microorganisms with alternative substrates to produce novel fermented products. Therefore, in this work we compared solid-state (SSF) and liquid-state fermentation (LSF) of three abundant fruit and vegetable by-products produced by the juice and beverage industry using a non-*Saccharomyces* yeast species. Apple, orange and carrot pomaces were chemically characterized before and after the fermentations to assess the yeast ability in converting these substrates into a nutritionally enriched food product. Extended bromatological analysis of the fermented substrates were carried out including total protein, soluble sugars, fat, dietary fiber, nucleic acid, and mineral composition. In addition, we used metabolomics tools to determine the fine changes in biochemical composition of the substrates after fermentation. Results demonstrate that both fermentation approaches increased the content of protein, fat and minerals in parallel to increased antioxidant activity and marked

reduction in soluble sugars content. SSF showed the highest increase in protein and decrease in soluble sugars levels. SSF also resulted in the highest increase in unsaturated fatty acids, in particular oleic and linoleic acids. Therefore, the fermentation process increased significantly the nutritional value of the fruit and vegetable by-products that are abundant food grade substrates usually wasted in landfills.

8:00 AM - 11:00 AM Session: 24: A3 (China-Japan-Korea) Foresight Network on Chemical and Synthetic Biology of Natural Products

Conveners: **Eung-Soo Kim**, Inha University, Incheon, Korea, Republic of (South) and **Yasuo Ohnishi**, The University of Tokyo, Tokyo, Japan

Plaza Ballroom E - Concourse Level

8:00 AM S132: Improved chain release of engineered polyene derivatives

Y. Zhou, Z. Qi, Q. Kang, J. Zheng, Y. Zhao and L. Bai, Shanghai Jiao Tong University, Shanghai, China*

Polyene polyketide antibiotics are widely used for antifungal therapy and food preservation in food industry. Recently, a few chemical and genetic studies have shown that modifications on exocyclic carboxylic group of amphotericin resulted in a significant decrease of the cytotoxicity, which encouraged us to genetically modify natamycin. However, the lower yields of its derivatives are hampered their full application.

Through inactivation of the P450 monooxygenase gene *scnG*, two natamycin derivatives, compounds **1** and **2**, with improved pharmacological activities were detected in *scnG*-inactivation mutant. In order to obtain high yields, several site-directed mutations were firstly introduced into the thioesterase (TE) domain, under the guidance of TE homology modeling, and a by-product **3** was and converted into a full-length linear compound **6**. Alternatively, when DH-KR12 didomain was replaced by DH-KR11 didomain, the yield of **1** and **2** was improved by 3-fold and without any accumulation of **3** and **6**. Subsequent specific accumulation of **1** or **2** was achieved via further overexpression or disruption of *scnD*.

The specificity of TE domain towards natamycin or decarboxylic derivatives has been further investigated through crystallization of pimTE, molecular docking, site-directed mutagenesis, in vitro biochemical assay, and in vivo site-specific modification of the TE domain. The crystal structure of pimTE with a resolution of 2.07 A was obtained, which shows interesting features on dimerization, substrate binding, and interaction with the hydrophobic tetraene region of natamycin. Mutagenesis based on the molecular docking of **6** with pimTE shed new lights on the yield improvement of decarboxylic polyene antibiotics.

8:30 AM S133: Unprecedented cyclization mechanisms in the biosynthesis of heterocyclic secondary metabolites.

Y. Katsuyama and Y. Ohnishi, The University of Tokyo, Tokyo, Japan*

Natural products show diverse structures, which are enhanced by a wide variety of chemical reactions. Cyclization is one of the important reactions that contribute to the structural diversity of natural products. Cyclization reactions have been extensively studied by many researchers and diverse reaction mechanisms have been proposed. However, there are still a number of heterocycles whose biosynthetic mechanisms remained unclear. Benzastatin derivatives are natural products isolated from several *Streptomyces* species. They have unique heterocyclic structures, such as indoline and tetrahydroquinoline, which are probably derived from geranylated *p*-aminobenzoic acid derivatives. Although they have some interesting bioactivities including an antiviral activity, their biosynthetic pathways remained unclear. We focused on one of the benzastatin producers, *Streptomyces* sp. RI18, to elucidate the pathways for the biosynthesis of benzastatin derivatives. The benzastatin biosynthetic gene cluster (*bez* cluster) was identified in the *Streptomyces* sp. RI18 genome and the functions of six *bez*

genes (*bezA*, *bezB*, *bezC*, *bezE*, *bezG*, and *bezJ*) were analyzed by gene disruption in a heterologous expression system. Furthermore, the functions of six biosynthetic enzymes (BezA, BezB, BezC, BezE, BezF and BezG) were confirmed by *in vitro* assay systems. Based on the results of these experiments, we proposed the biosynthetic pathways for benzastatin derivatives. In these pathways, geranylated *p*-acetoxyaminobenzoic acid derivatives are key intermediates for synthesizing indoline and tetrahydroquinoline structures and BezE (cytochrome P450) catalyzes the heterocyclic ring formation. Detailed reaction mechanisms will be discussed.

9:00 AM S134: Divergent biosynthesis of indole alkaloids FR900452s and Maremycin E/F/G

S. Lin[*]*, Shanghai Jiao Tong University, Shanghai, China*

FR900452 and maremycinE/F/G feature natural indole diketopiperazine that are linked to oxocyclopentene moiety in distinct patterns. Although isolated from different *Streptomyces*, the closely related structures implied a common biosynthetic mechanism between FR900452 and maremycins. This work confirmed that *Streptomyces sp.* B9173, the reported producer of maremycins, could also generate FR900452 and its new analogues. Time course of the metabolic profile also showed that FR900452s and maremycins were synthesized successively. Disruption of *mar* gene cluster abolished both FR900452s and maremycin G, indicating that the biosynthesis of the skeleton of FR900452 and maremycin G used the same PKS/NRPS genes. Further cloning and heterologous expression of the *mar* gene cluster in *S. lividans* TK24 confirmed that production of maremycins and FR900452s shared the same biosynthetic machinery. Inactivation of *marP*, which was annotated as snoaL-like family protein, abolished the productivity of FR900452s and maremycin A, but remarkably accumulated the spiro maremycin E/F/G. Therefore, MarP, the snoaL-like family protein, played the determinant role in leading the pathway divergence.

9:30 AM Break

10:00 AM S135: Amide-bond forming enzymes in the biosynthesis of streptothricin group antibiotics

Y. Hamano[*]*, Fukui Prefectural University, Fukui, Japan*

Streptothricins (STs) produced by *Streptomyces* strains are broad-spectrum antibiotics and are characterized by a streptothrisamine core structure with the L-β-lysine (β-Lys) residue and its oligomeric side chains [oligo(β-Lys)]. In addition to the STs, it has been reported that *Streptomyces* strains produce ST-related compound, BD-12, which possess a glycine-derived side chain rather than the β-Lys residue. The amide bonds connecting the side chains in ST and BD-12 are formed *via* NRPS[1)] or tRNA-dependent[2)] pathways, respectively. Here, the biosynthetic pathway of SF-2111B, which contains two peptide derived side chains including the unique *O*-acyl peptide moiety, will be discussed.

References

1) C. Maruyama et. al, A stand-alone adenylation domain forms amide bonds in streptothricin biosynthesis, *Nat. Chem. Biol.,* 8, 791-797 (2012).

2) C. Maruyama et. al, tRNA-dependent aminoacylation of an amino-sugar intermediate in the biosynthesis of a streptothricin-related antibiotic, *Appl. Environ. Microbiol.*, 82, 3640-3648 (2016).

10:30 AM S136: The dynamic transcriptional and translational landscape of the model antibiotic producer *Streptomyces coelicolor* A3(2)

B.K. Cho[*]*, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South)*

Individual *Streptomyces* species have the genetic potential to produce a diverse array of natural products of considerable commercial, medical and veterinary importance. However, the genetic information flow of

their unique high G+C genomes by transcription and translation processes remains largely unexplored. In order to harness their full biosynthetic potential, it will be important to develop a detailed understanding of the regulatory networks that orchestrate their diverse metabolism. Here, we reveal extensive translational control of the secondary metabolic genes of the model streptomycete, *Streptomyces coelicolor* A3(2) through the genome-scale integration of transcriptome and translatome data. Our systematic study determined 3,570 transcription start sites and identified a high proportion (~21%) of leaderless mRNAs and 230 small RNAs; this enabled deduction of promoter architecture on a genome-scale. The comprehensive translational landscape was determined by using massively parallel sequencing of ribosome-protected mRNA fragments (Ribo-seq). Interestingly, our study reveals that the translation efficiency of secondary metabolic genes was negatively correlated with transcription and that several key antibiotic regulatory genes were translationally-induced at transition growth phase. These findings could lead to the design of new approaches to antibiotic discovery and development.

8:00 AM - 11:30 AM Session: 22: Computational Techniques for Studying the Microbiome

Conveners: **Chris Miller**, University of Colorado Denver, Denver, CO, USA and **Prof. Kostas Konstantinidis**, Georgia Institute of Technology, Atlanta, GA, USA

8:00 AM S119: Beyond ribosomal RNA genes: Specialized tools for analysis of microbial whole-genomes and metagenomes

K. Konstantinidis, Georgia Institute of Technology, Atlanta, GA, USA*

The small subunit ribosomal RNA gene (16S rRNA) has been successfully used to catalogue and study the diversity of microbial species and their communities to date. Nonetheless, several aspects of the rRNA gene-based studies remain problematic. Most importantly, how to better resolve microbial communities at the levels where the 16S rRNA gene provides inadequate resolution, namely the species and finer levels, and how to best catalogue whole-genome diversity and fluidity. Additionally, an explosion in culture-independent genomic approaches (aka metagenomics) has recently occurred. However, the tools to analyze metagenomic data are clearly lagging behind the developments in sequencing technologies (and data) and are typically limited to the assembly and gene annotation of the metagenomic sequences. To help closing these gaps, we have recently developed several whole-genome based tools for metagenomics/metatranscriptomics, including -but not limited to- Nonpareil, a reference-free algorithm to estimate the level of coverage of a microbial community achieved by the sequencing effort; and ROCker, a pipeline to identify metagenomic reads representing specific genes or organisms of interest that shows substantial improvements compared to the common practice of using e-value cut-offs. Examples from applying these tools to identify the etiological agent of foodborne diarrheal outbreaks, and predict the fate of crude oil spills will be presented. For further information and online access to the tools, please visit the lab website at: http://enve-omics.gatech.edu

8:30 AM S120: From metagenomes to population genomes and back: high-resolution surveys of microbial communities

T. Delmont and A.M. Eren, University of Chicago, Chicago, IL, USA*

High-throughput sequencing of environmental metagenomes enables scientists to characterize the genomic content of wide range of microbial populations and to link them back to the environment, providing new opportunities for biotechnological discoveries. The open source analysis and visualization platform anvi'o provides a comprehensive computational framework for characterizing and curating population genomes, recovering single-nucleotide variants, and for comparative genomics. Using anvi'o and the large collection of metagenomes released by the TARA Oceans Project, we will describe (1) a database of ~1,000 population genomes we characterized from the surface of four oceans and two seas,

(2) the concept of metapangenomics to link the genomic traits and niche partitioning of microbial populations, and (3) the genomic heterogeneity of a remarkably abundant and widespread marine microbial population at large-scale. These examples will set the stage for a discussion on the advantages and limitations of metagenomic assemblies to study the ecology of microbes, and the importance of going back from microbial genomes to metagenomes to better understand microbes in their natural habitat.

9:00 AM S121: Bioinformatics solutions towards the advancement of pathogen detection with metagenomics

P.E. Li, C.C. Lo and P. Chain, Los Alamos National Laboratory, Los Alamos, NM, USA; J. Russell and J. Jacobs, MRIGlobal, Gaithersburg, MD, USA*

Next-generation-sequencing (NGS) has great potential for use as an excellent tool for detecting and diagnosing infectious disease. As applied to metagenomics, NGS poses several challenges when geared toward general pathogen detection activities. Some of these challenges include the robust assignment of pathogens, given an incomplete database, short reads, and algorithms that focus only on easy use cases. General metagenomics taxonomy classifiers have been employed to help identify organisms within metagenomics samples. However, before NGS can be used as a routine procedure *in a clinical setting*, several hurdles must be overcome: (1) an easy-to-use environment that technicians or other non-bioinformatics experts can use, including reports and visualizations that can be interpreted by clinicians in a meaningful fashion; (2) rapid bioinformatics tools which run effectively on commodity hardware; (3) levels of confidence for reported organisms that is not tied solely to abundance. Here, we provide some examples of the issues surrounding the use of NGS as a method to robustly identify pathogens in complex samples. We also present a series of efforts designed to: (1) lower the barrier for non-experts to use NGS for routine bioinformatics applications by developing a user-friendly web-based suite of tools; (2) limit the number of organisms misidentified within samples, thereby improving positive predictive value; (3) provide the ability to fine-tune parameters to better assess what defaults should be used given specific questions that require different cutoffs. We also present a first attempt at developing confidence scoring algorithms that are not tied to abundance of identified organisms.

9:30 AM Break

10:00 AM S122: Exploring immune modulation by human gut bacteria in disease contexts with ‘OMICS data

10:30 AM S123: Targeted assembly of genes and genomes for a deeper view into archaeal populations in microbial communities

C. Miller, University of Colorado Denver, Denver, CO, USA*

In complex microbial communities, key ecosystem functions are often performed by low-abundance community members. Both shotgun metagenomics and 16S rRNA amplicon sequencing can provide insights into the metabolic potential and environmental distribution of these organisms. However, detection of rare sequences requires extensive sequencing effort, and relating shotgun sequencing data to 16S rRNA data is made challenging by the difficulties in assembling rRNA genes. To address these challenges, we have first developed and applied experimental and computational methods to sequence and assemble long 16S rRNA gene amplicons from short-read high throughput sequencing data. Via expanded primer choice offered by the ability to assemble long amplicons, we demonstrate deep sampling of the phylogenetic diversity of low-abundance but functionally important archaea in methane-producing freshwater wetland soils. This ability reveals that phylogenetic diversity within the archaea in these systems is surprisingly broad, both at the phylum and individual OTU level, and that fine-scale variation in environmental habitat preference exists for many understudied archaeal lineages, such as the Bathyarchaeota, Woesearchaeaota, and ANME-2d. We next targeted samples enriched in computationally-inferred rRNA archaeal phylogenetic novelty for shotgun metagenomic sequencing via

multiple sequencing technologies. Correct assembly of 16S rRNA from these shotgun metagenome datasets and publically available shotgun metagenome datasets allows for interpretation of genome bins within the context of much larger environmental distribution patterns. By integrating diverse shotgun sequencing data types, we provide improved genome assemblies and infer new metabolic and evolutionary insights for rare and poorly sampled archaeal lineages.

11:00 AM S124: Modeling the dynamics of biogeochemical processes and bacterial diversity in anoxic aquatic environments

S. Preheim, Johns Hopkins University, Baltimore, MD, USA*

Microbial processes contribute to unfavorable conditions in polluted aquatic environment, resulting in oxygen-depleted dead-zones in natural water bodies. Understanding the dynamic chemical and microbial changes that occur in anoxic environments improve computational modeling efforts that guide remediation strategies, such as within the Chesapeake Bay. Here, we modified an existing multi-component transport model coupling major redox cycles in a seasonally stratified lake (Upper Mystic Lake). We found the model is sufficient to explain the distribution of the most abundant microorganisms observed in the lake, providing a mechanistic understanding of the factors that determine bacterial community structure. Water in the lake was sampled at approximately one- to two- meter intervals from the surface to 22 meters depth over the course of a season (spring to fall). The bacterial diversity at each depth was determined with high-resolution, next-generation sequencing and paired with the corresponding physical and chemical data. The distribution of microorganisms within the sample largely correspond to the modeled processes they are know to be involved in, demonstrating the applicability of this approach for modeling the dynamics of biogeochemical processes in the water column. Novel molecular biology techniques were used to identify organisms that carry genes involved in the modeled processes and groups of organisms that co-occur with these key bacterial species. We are applying this technique to other anoxic environments, such as the dead-zone within the Chesapeake Bay.

8:00 AM - 11:30 AM Session: 25: Dr. Lonnie Ingram: A Tribute to the Original Metabolic Pathway Engineer

Conveners: **Joy Doran Peterson**, University of Georgia, Athens, GA, USA and **Laura R. Jarboe**, Iowa State University, Ames, IA, USA

Plaza Ballroom A & B - Concourse Level

8:00 AM S137: Metabolism from A to Z (Animal microbiome to *Zymomonas*)

T. Conway, Oklahoma State University, Stillwater, KS, USA*

The pyruvate decarboxylase and alcohol dehydrogenase genes used to construct “Lonnie’s Bugs” originated from *Zymomonas mobilis*. Remarkably, 50% of protein in *Z. mobilis* consists of Entner-Doudoroff and ethanologenesis enzymes. The Entner-Doudoroff genes were characterized in the 1990’s, first in *Z. mobilis* and subsequently in *E. coli*. These organisms coordinate expression of their glycolytic and fermentation genes to achieve balanced metabolic flux. Ensuing characterization of the *E. coli* transcriptome revealed a genetic circuitry that dictates its cellular physiology and ecology. Unlike *Zymomonas*, an organism that lives in sugar-rich environments where maximum glycolytic flux is an advantage, *E. coli* is a colonizer of the intestine, a nutrient-rich yet extremely competitive environment. The Entner-Doudoroff pathway is key to *E. coli* colonization of the intestine, allowing it to utilize sugar acids that are not consumed by its competitors. The sugar acids are but a few of the many sugars that various *E. coli* strains use to colonize. Each *E. coli* strain behaves as a unique biotype in the intestine, relying on a different set of sugars to colonize. Stated another way, each *E. coli* biotype occupies a distinct niche, which allows multiple strains to colonize a single host, by utilizing different nutrients that are

available in its niche. Understanding how enteric pathogens compete for nutrients with the resident intestinal microbiota will lead to novel strategies to combat infection by manipulating the microbiota to consume nutrients needed by enteric pathogens to initiate infection.

8:30 AM S138: Ethanol and lactic acid production from sugarcane bagasse, corn stover and agave bagasse hydrolysates with metabolic engineered *Escherichia coli:* from feedstocks to fermentations, and Lonnie´s lessons

A. Martínez[*]*, J. Utrilla, M. Fernández-Sandoval, A.A. Vargas-Tah, E. Sierra, B. Trujillo-Martínez, C. Moss-Acosta, M. Rodríguez-Alegría and G. Gosset, Institute of Biotechnology - UNAM, Cuernavaca, Morelos, Mexico*

Ethanologenic and lactogenic *Escherichia coli*MG1655 derivative strains were developed using metabolic engineering and adaptive evolution tools to ferment xylose and glucose mixtures in the presence of acetate. Depending on the genetic background and inoculum development in pH-controlled batch fermenters, the strains showed sequential carbon source utilization and acetate production or simultaneous consumption of glucose and xylose and no acetic acid production using laboratory simulated hydrolysates. The strains have also been tested in actual diluted-acid lignocellulosic hydrolysates from different feedstocks, including sugarcane bagasse, corn stover, and agave bagasse. Ethanol or Lactic acid yields from laboratory sugar mixtures or lignocellulosic hydrolysates were close to 90% of the theoretical yield and volumetric productivities in the range of 0.6 to 1.1 g / L h in batch cultivations performed from 0.2 to 100-liter fermenter scale. Lessons taught by Professor Lonnie O. Ingram will be highlighted during the presentation.

Acknowledgement: Support by the Mexican National Council for Science and Technology (CONACYT), FONCICYT ERANet-LAC Grant C0013-248192: SMIBIO project is acknowledged.

9:00 AM S139: Engineering robust microbial cell factories: addressing product and substrate toxicity

L.R. Jarboe[*]*, Iowa State University, Ames, IA, USA*

Production of biorenewable fuels and chemicals by microbial cell factories is often limited by product toxicity or inhibitory components of the biomass-derived sugars. This toxicity can be addressed by detoxification of the feed stream, in situ removal of the product or increasing the resistance of the microbial biocatalyst. The Jarboe group has used evolutionary and rational strain improvement methods to increase the robustness of *Escherichia coli*. This talk will discuss our work to increase production of the bulk biorenewable chemicals styrene and fatty acids, and utilization of thermochemically depolymerized biomass, with a focus on engineering of the microbial cell membrane.

9:30 AM Break

10:00 AM S140: Experimental evolution reveals a novel avenue to release catabolite repression in *E. coli* via mutations in XylR

C. Sievert, L. Nieves, L. Panyon, T. Loeffler, C. Morris, R. Cartwright and X. Wang[*]*, Arizona State University, Tempe, AZ, USA*

Microbial production of fuels and chemicals from lignocellulosic biomass provides promising bio-renewable alternatives to the conventional petroleum-based products. However, heterogeneous sugar composition of lignocellulosic biomass hinders efficient microbial conversion due to carbon catabolite repression. The most abundant sugar monomers in lignocellulosic biomass materials are glucose and xylose. While industrial *Escherichia coli* strains efficiently utilize glucose, their ability to utilize xylose is often repressed in the presence of glucose. Here we independently evolved three *E. coli* strains from the

same ancestor to achieve high efficiency for xylose fermentation. Each evolved strain has a point mutation in a transcriptional activator for xylose catabolic operons, either CRP or XylR, and these mutations are demonstrated to enhance xylose fermentation by allelic replacements. Identified XylR variants (R121C and P363S) have a higher affinity to their DNA binding sites, leading to a xylose catabolic activation independent of catabolite repression control. Upon introducing these amino acid substitutions into the *E. coli* D-lactate producer TG114, 94 % of a glucose-xylose mixture (50 g/L each) was utilized in mineral salt media that led to a 50 % increase in product titer after 96 h of fermentation. The two amino acid substitutions in XylR enhance xylose utilization and release glucose-induced repression in different *E. coli* hosts, including wild-type, suggesting its potential wide application in industrial *E. coli* biocatalysts.

10:30 AM S141: Engineering an orthogonal and modular pathway for the energy and carbon-efficient synthesis of functionalized small molecules

R. Gonzalez, Rice University, Houston, TX, USA*

Anabolic metabolism can produce an array of small molecules, but yields and productivities are often limited by carbon and energy inefficiencies and slow kinetics. Catabolic and fermentative pathways, on the other hand, are carbon and energy efficient but support only a limited product range. To address these limitations, we engineered an orthogonal pathway for the synthesis of functionalized small molecules based on non-decarboxylative Claisen condensation reactions (and subsequent β-reductions) that uses functionalized primers and functionalized extender units and operates in an iterative manner. This carbon–carbon elongation mechanism was selected because of its ability to support iterative condensation reactions at high energy (ATP) efficiency, as previously demonstrated in our laboratory (Nature 476, 355-359, 2011). The orthogonality of the newly developed platform enables predictable, tunable, and programmable operation of a pathway that retains the high product diversity, modularity, and combinatorial capabilities of anabolism. Using different ω- and ω-1-functionalized primers and α-functionalized extender units in combination with various termination pathways, we engineered the synthesis of 18 products from 10 classes in Escherichia coli, including ω-phenylalkanoic, α,ω-dicarboxylic, ω-hydroxy, ω-1-oxo, ω-1-methyl, 2-methyl, 2-methyl-2-enolic and 2,3-dihydroxy acids, β-hydroxy-ω-lactones, and ω-1-methyl alcohols (Nature Biotechnology, 2016, 34 (5): doi:10.1038/nbt.3505)

11:00 AM S142: Increasing ethanol yield from *Saccharomyces cerevisiae* via metabolic engineering

G. Whited, DuPont Industrial Biosciences, Palo Alto, CA, USA*

The introduction of carbon rerouting pathways in *Saccharoyces cerevisiae* has led to the development of robust yeast strains that are capable of increasing the ethanol yield from grain. Our research has focused on the introduction of three different enzymes in yeast which are part of the phosphoketolase (PKL) pathway. The addition of the PKL pathway into the yeast metabolism facilitates the yeast to more efficient use of the available carbon to produce more ethanol and less glycerol than unmodified strains. The yeast strains, that are now available for use in commercial processes, operate at typical plant conditions and are capable of boosting the ethanol yield up to 3%. The yeast engineering strategy that has been used will be discussed and technical data will be provided to show performance at full production scale.

11:45 AM - 12:30 PM SIMB Annual Business Meeting Agenda: 2016 Annual Business Meeting minutes; Treasurer's Report; 2018 membership dues

discussion; President’s report; Installation of new officers

Plaza Ballroom A & B - Concourse Level

12:30 PM - 1:30 PM Student Mentoring Lunch - complimentary for students; sign up required at registration desk

Plaza Court 1 - Concourse Level

1:00 PM - 4:30 PM SIMB Outing - NREL Signed Waiver Form required prior to boarding the bus. Forms available at the registration desk.

1:30 PM - 2:30 PM 2018 Annual Meeting Program Committee Planning Meeting

Plaza Ballroom A & B - Concourse Level

Thursday, August 3

7:00 AM - 12:00 PM SIMB New Board of Directors Meeting

Plaza Court 7 - Concourse Level

7:30 AM - 3:30 PM Registration

Plaza Registration - Concourse Level

8:00 AM - 11:00 AM Session: 26: Microbiomes for Deconstruction of Plant Biomass

Conveners: **Steven W. Singer**, Lawrence Berkeley National Laboratory, Berkeley, CA, USA and **Sebastian Kolinko**, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Plaza Ballroom D - Concourse Level

8:00 AM S143: Identification and characterization of the components of a non-cellulosomal cellulase complex

S. Kolinko, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Cellulases have traditionally been identified and characterized from fungal and bacterial isolates. In natural environments, microbial consortia are responsible for lignocellulose deconstruction. However, these native communities are often too complex to assign functions to individual enzymes. By adapting complex communities from compost to grow aerobically with biomass substrates as the sole carbon source under defined conditions, we have obtained predictable consortia that generate highly active cellulases and can be grown at pilot scale. These enzymes have been used to perform a “one-pot” ionic pretreatment and saccharification of switchgrass. By a combination of meta-omics methods and biochemical techniques we have established that these community-derived cellulases are localized in a protein complex produced by an uncultivated thermophilic *Firmicutes* species. This complex was tolerant towards elevated temperatures and protease-resistant. It was purified from culture supernatants by a combination of affinity digestion with phosphoric acid-swollen cellulose as resin and anion-exchange chromatography. We identified three multi-domain proteins associated with the complex, CelA an endoglucanase, CelB a cellobiohydrolase, and CelC, which contained both exoglucanase (GH6) and endoglucanase (GH5) catalytic domains. Genes encoding these proteins were organized in a 17 kb putative operon together with two xylanases (XynA and XynB), and a lytic polysaccharide monooxygenase PmoA. Heterologous expression of CelABC demonstrated that CelA and CelC were active on both soluble and insoluble cellulose substrates and that CelA and CelB were heavily glycosylated. This glycosylation was critical for the formation and the stability of the complex.

8:30 AM S144: Exploration of hemicellulolityc enzymes in two wheat straw-degrading microbial consortia

D.J. Jiménez, M. Maruthamuthu and J.D. van Elsas, University of Groningen, Groningen, Netherlands*

Plant biomass (PB) is an important resource for biofuel production. However, the efficiency of PB saccharification is still an industrial bottleneck. The use of enzymes produced from PB-degrading microbial consortia is a promising approach to optimize this process. Here we performed an in-depth characterization of two microbial consortia, obtained by the dilution-to-stimulation approach, cultivated on untreated (RWS) and heat-treated (TWS) wheat straw. In both systems a reshaping of the bacterial communities was found, with reductions in overall richness and increases in the prevalence of particular members of the Enterobacteriales, Pseudomonadales, Flavobacteriales and Sphingobacteriales. The metagenomic analyses revealed an overrepresentation of diverse genes encoding enzymes of the glycosyl hydrolase families GH2, GH43, GH92 and GH95 in the two consortia, as compared to the forest soil inoculum. Moreover, two metagenomic libraries were constructed and screened for enzymes involved in hemicellulose deconstruction, using a novel mixture of different chromogenic substrates. Functional analyses unveiled two clones that were predicted to encode putative novel thermo-alkaline hemicellulases (an β-D-galactosidase and an β-D-xylosidase). In order to explore these consortia further, we performed a synthetic metagenomics approach. Thirteen large contigs - produced previously on the basis of shotgun sequencing of metagenomics DNA - were selected and screened for GH-encoding genes. Ten genes were codon-optimized, synthesized and expressed, after which they were further tested. We describe the full analysis, placing a focus on a gene for a key furan aldehyde-tolerant β-xylosidase/α-arabinosidase (CAZy family GH43) enzyme that is proposed for biorefining processes, especially the saccharification of pretreated plant biomass.

9:00 AM S145: Selective enrichment of syntrophic anaerobic communities enables rapid conversion of biomass

S. Gilmore, X. Peng, J. Henske, C. Swift, D. Valentine and M. O'Malley, University of California, Santa Barbara, Santa Barbara, CA, USA*

Anaerobic fungi in the hindgut of large herbivores are among the most robust organisms at degrading crude lignocellulose. Fungi have been characterized primarily in isolation, however they typically exist in nature with archaea, bacteria, and protozoa. In this work, we implemented a selective enrichment process to isolate a minimal consortium of fungi and methanogens capable of growth on unpretreated reed canary grass and other bioenergy crops. The consortium greatly exceeded the biomass-degrading capacity of fungi alone, and the consortia were supported on a wider range of substrates compared to isolated cultures, including pectin and xylan. The consortium also demonstrated remarkable stability, remaining together in consecutive batch culture for over 18 months and 150 consecutive transfers. The selective enrichment process was tuned to enrich for mixtures of bacteria and archaea, and expanded to include more substrates including bagasse, alfalfa stem, and xylan. Metagenomic sequencing identified the members of each community and allowed for the design of synthetic consortia that nearly equaled the stability and degradation of the native communities.

9:30 AM Break

10:00 AM S146: Teasing apart the roles of anaerobic bacterial lignin degradation and metabolism

G. Chaput and K.M. DeAngelis, University of Massachusetts Amherst, Amherst, MA, USA*

Currently, the paper industry uses hazardous chemicals in pulping to remove lignin from the hemicellulose and cellulose components of wood. This contributes to greenhouse gas emissions and water pollution. A sustainable alternative is to use bacteria that degrade lignin anaerobically and convert it to secondary chemicals or biofuels. To enrich for anaerobic bacteria capable of lignin degradation, microbial consortia were established from temperate forest soils on anoxic media containing lignin as the sole carbon source, with initial inoculations grown for 4 weeks, then transferred to fresh media with lignin as the sole carbon source every 4-9 weeks for two years. Using extinction-to-dilution with oligotrophic cultivation conditions, 152 bacteria were isolated, and screened for lignin degradation capabilities based on the lignolytic indicator dyes, malachite green and Congo red. Based on this screen, 26% (40 isolates) have lignin-degrading capabilities, being able to degrade both dyes anaerobically. Of the 40 lignin-degrading isolates, 7 species (97% 16S rRNA gene sequence identity) all belong to genus *Citrobacter*, *Serratia*, or *Bacillus*. These three groups have members previously shown to have aromatic degradation capabilities. Ongoing growth and physiology of the remaining 74% of isolates using lignin-associated monomers will determine if these consortia members can benefit from lignin-breakdown products. We hypothesize that mechanisms of lignin degradation and utilization are dispersed among different members of the consortia with metabolic intermediates being exchanged. By providing pathways and enzymes for anaerobic bacterial lignin degradation via metabolic engineering, we hope to improve the sustainability of the pulping process.

10:30 AM S147: Microbial and environmental arrangements in the gut of the wood-feeding beetle *Odontotaenius disjunctus* illustrate mechanisms for energy and nutrient extraction from lignocellulose

J.A. Ceja-Navarro, U. Karaoz, Z. Hao, M. Voltolini and E.L. Brodie, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; R. White, M. Lipton and J.N. Adkins, Pacific Northwest National Laboratory, Richland, WA, USA; T.R. Filley, Purdue University, West Lafayette, IN, USA; M. Blackwell, Louisiana State University, Baton Rouge, LA, USA; J. Pett-Ridge, Lawrence Livermore National Laboratory, Livermore, CA, USA*

Wood-feeding arthropods incorporated specific microbial groups into their gut to expedite woody biomass decomposition and may contain the keys for the development of energy-efficient strategies for second-generation biofuels production. The wood-feeding beetle *Odontotaenius disjunctus* is known for its ability to ingest large amounts of woody biomass (1 kg/year/beetle), and a morphologically differentiated gut with steep radial oxygen gradients. Infrared analyses, ^{13}C-thermochemolysis, and carbon and nitrogen

bulk chemistry analyses demonstrated that cellulose and xylan depolymerization occurs in the gut together with lignin side chain oxidation and nitrogen accumulation. Our multi-omics approaches show that microbial communities are segregated within *O. disjunctus'* gut regions, with the midgut (MG) and posterior hindgut (PHG) harboring similar microbiomes although separated by the anterior hindgut (AHG). We reconstructed 17 bacterial genomes from metagenomes and demonstrated that their localization within the gut corresponds with trait variation. Metagenomics and metaproteomics identified the MG as the key region for lignocellulose depolymerization and the AHG as the site for fermentation, methanogenesis, homoacetogenesis, and nitrogen fixation. Synchrotron X-Ray microCT imaging of intact beetles indicates that gut wall thickness likely plays an important role in driving the thermodynamics of these processes. Significant H_2 accumulation occurs in the AHG favoring acetogenesis over methanogenesis as a sink for hydrogen. Depolymerization continues in the PHG with a strong signal of further xylan depolymerization and xylose fermentation. Overall, these data illustrate how gut structural compartmentalization and assembly of microbial functional groups combine to enable lignocellulose deconstruction and the subsistence of this beetle on a low nutrient diet.

11:00 AM S148: Depth matters: Transitioning from big to deep data to facilitate enzyme discovery from complex microbial communities

M. Hess[*]*, University of California, Davis, Davis, CA, USA*

The unprecedented easy and extremely low cost at which sequence data can now be generated, resulted in an exponential growth of genomic and metagenomic information. Initial difficulties by the scientific community to handle large amounts of sequence data can today be addressed by analyzing sequence data via the cloud. The community is now tasked with converting large amounts of data (Big Data) into meaningful data (Deep Data). Once this transition has been achieved, meta-omics will have matured from a hypothesis-generating into hypothesis-testing approach and it will be possible to test complex hypotheses in Microbial Ecology. A first step into this direction is the ability to *i)* reconstruct full-length genes and *ii)* the ability to differentiate between genes that are only present and genes that are actively expressed and contribute to the phenotype of individual microorganisms and complex microbial communities.

We identified thousands of putative glycoside hydrolases expressed by anaerobic fungi during biomass-degradation in the cow's rumen. Anaerobic fungi are an essential component of many biomass-degrading ecosystems, but they are still poorly understood. This lack of understanding is mainly caused by the paucity of cultivation techniques that allow to isolate and grow anaerobic fungi under laboratory conditions.

We believe *i)* that the results from our study will contribute to a better understanding of the molecular machinery that is employed by anaerobic fungi to break down complex carbohydrates and *ii)* that the newly identified fungal enzymes will provide the opportunity to render current industrial biomass conversion processes more efficient.

8:00 AM - 11:00 AM Session: 27: Cultivation & Scaling of Fungal Fermentations

Conveners: **Peter J. Punt**, Dutch DNA Biotech, Utrecht, Netherlands and **Joshua Michel**, DuPont, Palo Alto, CA, USA

Plaza Ballroom F -Concourse Level

8:00 AM S149: Rational design of cell factories and novel pathways

S. Soares[*]*, SilicoLife, Braga, Portugal*

The rational design of cellular factories in the context of industrial biotechnology aims at the design of optimized organisms to work as biological factories to produce bulk chemicals, pharmaceuticals, food

ingredients and enzymes, among other products. Metabolic engineering (ME) is playing a key role in this process, supported by the latest advances in genetic engineering tools in combination with computational tools to define genetic targets for strain improvement.
SilicoLife builds computational models of microbial cells and develops proprietary state-of-the-art algorithms to find the most efficient pathways between raw-materials and end-products, enabling our clients to streamline the strain design process and explore non-intuitive pathway modifications, dramatically cutting down costs in their development programs and reducing laboratory experimentation. SilicoLife provides a complete package from genomic annotation to the validation of the optimized microbial strain as well as the evaluation of the incorporation of heterologous pathways into the host cell. The optimized strains enable increasing the productivity and yield of bioprocesses by maximizing the fluxes in the metabolic pathways linking raw-material uptake and product formation.

In this talk, the process from the organism reconstruction towards the design of optimized cellular factories by the application of *in silico* ME methods will be described, supported by examples from different projects.

8:30 AM S150: Strategies for scale up, textbook vs practice: Scale down!

R. Verlinden, *Bioprocess Pilot Facility, Delft, Netherlands*

Commonly, the scale up of a new bioprocess starts when the product of interest is found to be commercially interesting. The developed laboratory scale needs to be transferred to production scale (1-200m3). A full-scale fermentation plant is expensive to build and operate and requires hands on knowhow. The first scale up attempt can therefore be done at an open-access pilot plant like the BPF at intermediate scale. Next to the academic approaches to deal with scaling (up or down) you will also be confronted with more practical scale up issues such as legislation, strain stability, inoculum trajectory, raw material quality, raw material type, sterilization properties and logistics. To get a grip on the various factors that can affect the product formation and product yield, using a scale down approach when developing the lab process is very useful.

9:00 AM S151: Fungal fermentation & upscaling protein production

J. Michel, A. Bedekar and K. Nose Crotty, DuPont, Palo Alto, CA, USA*

DuPont Industrial Biosciences discovers, develops, manufactures, and delivers enzyme product solutions in areas such as agricultural processing, industrial applications, and consumer products. Successful commercialization begins with a fundamental understanding of the metrics needed to properly evaluate microorganisms as production hosts. Traditionally, process development starts in shake flasks and then graduates to larger more industrially relevant conditions in a step wise fashion. Often this involves optimization experiments at each step that de-risk scaling but add to development timelines and cost. Fermentation development & scale-up strategies will be presented using some examples of enzymes from the industrial and consumer markets. In particular, the importance of mimicking manufacturing conditions at small scales in order to predict fermentation performance and process metrics will be discussed.

9:30 AM Break

10:00 AM S152: *Monascus ruber* as cell factory for lactic acid production at low pH

R. Weusthuis, Wageningen University, Wageningen, Netherlands*

A *Monascus ruber* strain was isolated that was able to grow on mineral medium at high sugar concentrations and 175 g/l lactic acid at pH 2.8. Its genome and transcriptomes were sequenced and annotated. Genes encoding lactate dehydrogenase (LDH) were introduced to accomplish lactic acid production and two genes encoding pyruvate decarboxylase (PDC) were knocked out to subdue ethanol

formation. The strain preferred lactic acid to glucose as carbon source, which hampered glucose consumption and therefore also lactic acid production. Lactic acid consumption was stopped by knocking out 4 cytochrome-dependent LDH (CLDH) genes, and evolutionary engineering was used to increase the glucose consumption rate. Application of this strain in a fed-batch fermentation resulted in a maximum lactic acid titer of 190 g/l at pH 3.8 and 129 g/l at pH 2.8, respectively 1.7 and 2.2 times higher than reported in literature before. Yield and productivity were on par with the best strains described in literature so far.

10:30 AM S153: Metabolic pathway engineering for organic acid production in Aspergillus

P.J. Punt, Dutch DNA Biotech BV, Zeist, Netherlands*

Among compounds listed as top building blocks chemicals in particular organic acids have gained industrial interest for biobased production. Many of these organic acids, traditionally also being food ingredients, are directly derived from the central metabolic pathway in every living cell. The largest biotechnologically produced organic acid is citric acid, which can be seen as a precursor for other organic acids. At the start of our research, using systems biology approaches we elucidated the genetic basis of the biochemical pathway for one of these other acids, itaconic acid. In doing so we did not only identify the biochemical pathway gene but also a mitochondrial and plasma membrane transporter, confirming that the pathway in the native host *A. terreus* is compartmentalized. The three identified genes form a unique gene cluster. Overexpression showed that all three were relevant for itaconic acid overproduction in *A. niger* (Li et al., 2011). Moreover molecular genetic and process technological research performed on the newly developed *A. niger* itaconic acid-producing strains resulted in the identification of several new leads for further improvement of the itaconic acid titres and the reduction of by-product formation. In particular several hitherto uncharacterised gene clusters in *A. niger* were shown to be related to itaconic acid production, which in *A. niger* is xenobiotic compound. Overexpression and deletion of genes from these gene clusters allowed further increase in itaconic acid titers.

References

Li, A. et al. (2011) Fungal Genetics and Biology 48, 602-611

Hossain et al. (2016) Microb Cell Fact. 2016 Jul 28;15(1):130.

11:00 AM S154: Fungal fermentation on anaerobic digestate for lipid-based biofuel production

Y. Zhong, Y. Liu and W. Liao, Michigan State University, East Lansing, MI, USA*

Anaerobic digestate, the effluent from anaerobic digestion (AD) of organic wastes, contains a significant number of nutrients and lignocellulosic materials, though AD consumed a large portion of organic matters in the wastes. Utilizing the nutrients and lignocellulosic materials in the digestate is critical to improve efficiency of AD technology and generate value-added chemicals and fuels from organic wastes. Therefore, this study focused on developing an integrated process that uses biogas energy to power fungal fermentation and converts remaining carbon sources, nutrients, and water in the digestate into biofuel precursor-lipid. The process contains two unit operations of AD and digestate utilization. The digestate utilization includes alkali treatment of the mixture feed of solid and liquid digestates, enzymatic hydrolysis for mono-sugar release, overliming detoxification, and fungal fermentation for lipid accumulation. The experimental results conclude that 5 hours and 30°C were the preferred conditions for the overliming detoxification regarding lipid accumulation of the following fungal cultivation. The repeated-batch fungal fermentation enhanced lipid accumulation, with a final lipid concentration of 3.16 g/L on the digestate (10% dry matter). The mass and energy balance analysis indicates the digestate had enough water for the process and the biogas energy could balance the needs of individual unit operations. A fresh-water-free and energy-positive process of lipid production from anaerobic digestate was achieved by integrating AD and fungal fermentation, which addresses the issues that both biofuel industry and waste management encounter—high water and energy demand of biofuel precursor production and few digestate utilization approaches of organic waste treatment.

8:00 AM - 11:30 AM Session: 28: Metabolic Engineering for Utilization of Alternative Feedstocks

Conveners: **Christopher W. Johnson**, National Renewable Energy Laboratory, Golden, CO, USA and **Jay Fitzgerald**, Department of Energy - Energy Efficiency & Renewable Energy, Washington, DC, USA

Plaza Ballroom A & B - Concourse Level

8:00 AM S155: A mixotrophic platform for valorizing multiple waste streams

P. Lammers, M. Seger, T. Selvaratnam and W. Park, Arizona State University, Mesa, AZ, USA; O. Holguin, O. Sujala, S. Henkanatte Gedara and N. Nirmalakhandan, New Mexico State University, Las Cruces, NM, USA; A. Weber, Heinrich Heine University, Dusseldorf, Germany*

Galdieria sulphuraria is an acidophilic microalga adapted to growth at elevated temperatures. The organism is capable of oxidative metabolism using a broad range of organic substrates under both light (mixotrophic) and dark (heterotrophic) conditions. A major benefit of mixotrophic metabolism is the potential for metabolic gas exchanges to reduce or eliminate the need for acquisition and supplementation of CO_2 for photosynthesis, which in turn supplements O_2 needed for oxidative metabolism while potentially reducing the cost and complexity of outdoor photobioreactor systems. We will show that outdoor cultivation on 35 mM glucose/xylose mixtures derived from corn stover yields 5-10 fold higher biomass producitiviy than autotrophy with simultaneous uptake of both sugars. The combination of low pH and elevated temperatures dramatically limits contamination of uni-algal outdoor cultures under xenic conditions. *G. sulphuraria* also grows directly on undiluted, acidified wastewater from anaerobic digester systems containing NH_4^+ concentrations as high as 1,400 PPM (78 mM), leading to effective nutrient and BOD recovery to meet water discharge standards. The strain has also been demonstrated to effectively treat primary, settled municipal wastewater. The resulting biomass has been processed via hydrothermal liquefaction, yielding ~40 wt% biocrude oil plus concentrated, sterile N & P (fertilizer) co-product. We will show multiple lines of evidence for cellulolytic activity in *G. sulphuraria* cultures. A combination of enzymatic and gene-expression approaches are being used to identify the genetic source of the activity. Three *G. suphuraria* genome sequences are available from strains adapted to different temperature optima to assist in the search.

8:30 AM S156: Development of a carbon-efficient methane biocatalysis platform

C.A. Henard, H. Smith and M.T. Guarnieri, National Renewable Energy Laboratory, Golden, CO, USA*

Microbial conversion of biogas- and remote or stranded natural gas-derived methane to high-value bio-based fuels, chemicals, and materials offers a path to mitigate GHG emissions and valorize this abundant-yet-underutilized carbon source. Techno-economic analyses have implicated yield from methane to products as a key cost driver in bioconversion processes. Rational methanotrophic bacterial strain and fermentation engineering offer a means to achieve industrially relevant carbon yields. Here we present a series of approaches to enhance carbon yield, including phosphoketolase pathway-mediated metabolic engineering and design of a novel reactor configuration. The resultant biocatalyst and gas fermentation configuration display greater than 2-fold enhancement in carbon conversion efficiency and volumetric gas mass transfer, respectively, and offer viable strategies to enhance the economics of an array of biological methane conversion processes. Additional techno-economic considerations and potential opportunities for enhanced methanotrophic bioproduction will also be presented.

9:00 AM S157: Commercial-scale production of fuels and chemicals from low cost feedstocks via an integrated, multi-scale anaerobic gas fermentation platform

M. Köpke, LanzaTech Inc., Skokie, IL, USA*

LanzaTech has developed a fully integrated platform for commercial-scale production of fuels and chemicals from sustainable biomass and waste feedstocks. At the heart of the process is an acetogenic microbe *Clostridium autoethanogenum* capable of autotrophic growth on a range of low cost C1 substrates such as carbon monoxide (CO) and/or CO_2. Using automated adaptive laboratory evolution, LanzaTech has developed a highly efficient platform strain.

Around this chassis, the company has developed an advanced strain engineering platform. Until a few years ago, acetogenic organisms were considered to be genetically inaccessible. LanzaTech has been able to overcome the challenges associated working with acetogens and rapidly developed a comprehensive genetic toolbox for acetogens comprising including advanced gene editing methods, an extensive library of genetic parts and high-throughput methods. To complement this effort, the company has developed a large metabolic knowledgebase, design algorithms and metabolic and process models. These models are highly integrated and validated against growth, metabolomics and transcriptomics data across hundreds of fermentation runs and strains.

Using this platform, production of over 40 new molecules has been demonstrated directly from a diverse range of feedstock options including waste gases from industrial sources (e.g., steel mills and processing plants) or syngas generated from any biomass resource (e.g., agricultural waste, municipal solid waste, or organic industrial waste). The process has been successfully scaled up from the laboratory bench through in-lab and in-field pilot plants to fully integrated 100,000-gallon/year pre-commercial demonstration plants and first full commercial units are under construction.

9:30 AM Break

10:00 AM S158: Improving microbial toxicity tolerance for valorization of thermochemical aqueous waste streams

L. Jayakody, M.A. Franden, B.A. Black, J.G. Linger, M.T. Guarnieri, A. Meyers, W. Michener, K.J. Ramirez, C.W. Johnson and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA; J.M. Whitham, S.D. Brown and A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA; R.J. Giannone, BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN, USA; R.L. Hettich, Biosciences Division and BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

Thermochemical processes such as fast pyrolysis and catalytic fast pyrolysis produce carbon-rich, aqueous waste streams that are currently slated for wastewater treatment, representing a process cost for the thermochemical biorefinery. Valorization of this biomass-derived waste carbon could ultimately provide a significant economic benefit to thermochemical biorefineries. To adapt the biological funneling concept for valorizing waste carbon in a thermochemical process, we first characterized a range of thermochemical aqueous waste streams. Around 200 compounds have been identified and quantified (aldehydes, ketones, acids, aromatics, and sugars) at near-complete mass closure. Based on the compositional analysis, we selected the robust and metabolically versatile microbe, *Pseudomonas putida* KT2440, as a biocatalyst for valorization of these streams. However, the extreme toxicity of these streams hampers microbial growth and carbon utilization. Proteomics and transcriptomics experiments in the presence of thermochemical waste streams suggest that protein damage is one of the key components of toxicity. Thus, we over-expressed the protein quality control machinery of *P. putida*, which resulted in a 200-fold improvement in toxicity tolerance. This strain will serve as the basis for further metabolic engineering to incorporate pathways for the catabolism of the major components of thermochemical aqueous waste streams, enabling the valorization of this waste carbon to produce value-added co-products.

10:30 AM S159: Directed combinatorial mutagenesis of Escherichia coli for complex phenotype engineering

R. Liu, L. Liang, A. Choudhury and R.T. Gill, University of Colorado, Boulder, CO, USA; A.D. Garst, Muse Biotechnology Inc., Boulder, CO, USA; V. Sànchez i Nogué and G. Beckham, National Renewable Energy Laboratory, Golden, CO, USA*

Engineering of strains for industrial production requires the targeted improvement of multiple complex traits ranging from pathway flux to tolerance to mixed sugar utilization. Here, we report the use of an iterative CRISPR EnAbled Trackable genome Engineering (iCREATE) method for generating targeted genomic modifications at high efficiency along with high throughput phenotypic screening and growth strategies to rapidly engineer multiple traits in *Escherichia coli*. Using iCREATE, we engineered rapid glucose and xylose co-consumption, tolerance to hydrolysate inhibitors, and 3-hydroxypropionate (3HP) production in *E. coli*. Deep mutagenesis libraries were rationally designed, constructed, and screened targeting ~40,000 mutations across 30 genes; including global and high level regulators that regulate the global gene expression, transcription factors that play important roles in genome level transcription, and enzymes that function in the sugar transport system, NAD(P)H metabolism, and the aldehyde reduction system. Specific mutants conferring increased growth in mixed sugars and hydrolysate tolerance conditions were isolated, confirmed, and evaluated for changes in genome-wide expression levels. The best producing quadruple mutant strain $BGHP_{ht}$ was tested under high furfural and high acetate hydrolysate fermentation, demonstrating a 6.3 and 7-fold increase in productivity relative to the parent strain, respectively. This technology enables a single researcher to iteratively generate hundreds of thousands of designer variants and to map each of these variants to a selected phenotype using the designed barcode.

11:00 AM S160: Methanol assimilation in *Escherichia coli* is improved by deletion of a global regulator

J. Gonzalez, K. Bennett, T. Papoutsakis and M. Antoniewicz, University of Delaware, Newark, DE, USA*

Methane, the main component of natural gas, can be used to produce methanol which can be further converted to other valuable products. There is increasing interest in using biological systems for the production of fuels and chemicals from methanol, termed methylotrophy. In this work, we have examined methanol assimilation metabolism in a synthetic methylotrophic *E. coli* strain. Specifically, we applied ^{13}C-tracers and evaluated 25 different co-substrates for methanol assimilation, including amino acids, sugars and organic acids. Through these investigations, we identified specific metabolic pathways that, when activated, correlated with increased methanol assimilation. These pathways are normally repressed by the leucine-responsive regulatory protein (Lrp), a global regulator of metabolism associated with the feast-or-famine response in *E. coli*. By deleting *lrp*, we were able to significantly enhance the methylotrophic ability of our synthetic strain, as demonstrated through improved growth on methanol and increased incorporation of ^{13}C carbon from ^{13}C-methanol into biomass. Overall, this work demonstrates a rational engineering approach for improving methylotrophy in *E. coli* and illustrates the application of the design-build-test cycle for strain design in metabolic engineering.

8:30 AM - 11:30 AM Session: 29: Mechanisms and Engineering of Peptidic Natural Product Biosynthesis

Conveners: **Yousong Ding**, University of Florida, Gainesville, FL, USA and **A. James Link**, Princeton University, Princeton, NJ, USA

Plaza Ballroom E - Concourse Level

8:30 AM S161: Versatility of the peptide macrocyclase POPB from amanitin-producing fungi

R.M. Sgambelluri[*] and J.D. Walton, Michigan State University, East Lansing, MI, USA

Species of *Amanita* and some other mushrooms produce a family of ribosomally biosynthesized bicyclic peptides, including alpha-amanitin and phalloidin. In the amanitin pathway, the first post-translational steps are removal of the leader sequence from a linear 35-amino acid precursor and internal trans-peptidation to create a monocyclic octapeptide. A specialized serine protease, prolyl oligopeptidase B (POPB), catalyzes both reactions. POPB from the amanitin-producing mushroom *Galerina marginata*, obtained by expression in yeast, is comparable in catalytic efficiency to the best known peptide macrocyclases. We have explored the versatility of GmPOPB as a general macrocyclase catalyst for peptide cyclization using a range of substrates produced either in *E. coli* or by chemical synthesis. Substrates produced in *E. coli* were expressed either individually or as mixtures by random mutagenesis of the core region. A total of 125 peptide substrates comprising different core regions were tested for cyclization by GmPOPB. Cyclization occurred for 97 of the substrates with yields of greater than 90%. Reduced yields were observed for substrates containing polar residues at position two of the core region and bulky residues at positions three or five. Substrates containing charged amino acids or a tyrosine residue at position one were also problematic for cyclization. GmPOPB could cyclize peptides with core domain lengths ranging from 8 to 16 residues. Synthetic substrates containing modified amino acids including D-amino acids, β-amino acids, and hydroxylated and N-methylated amino acids were also cyclized. GmPOPB should have broad applicability as a general macrocyclase for the production of small cyclic peptides.

9:00 AM S162: Biosynthesis and catabolism of lasso peptides

A.J. Link[*], Princeton University, Princeton, NJ, USA

Lasso peptides are defined by their unique rotaxane architecture and exhibit a wide range of bioactivities. This talk will focus on new insights into the biosynthesis of lasso peptides. In addition, we will describe the biochemical and structural characterization of lasso peptide isopeptidase, an enzyme that deconstructs lasso peptides, rendering them linear. The biological implications of lasso peptide catabolism will also be discussed.

9:30 AM Break

10:00 AM S163: Precursor protein-directed ribosomal peptide macrocyclization in microviridin biosynthetic pathways

S. Bruner[*], University of Florida, Gainesville, FL, USA

Macrocyclization is a common feature of natural product biosynthetic pathways including the diverse family of ribosomal peptides. Microviridins are a unique family of ribosomally synthesized and post-translationally modified peptides (RiPPs), with a complex tricyclic cage-like architecture. This scaffold is formed by ordered installation of two macrolactones and one macrolactam on the core region of a precursor peptide catalyzed by two ATP-grasp-family ligases. The pathway is completed by two gene products that cleave the leader peptide, acylate the N-terminus and export the mature product. Our group is interested in providing insight into structure and mechanism of the pathway. Described is the structural basis for the two enzyme-catalyzed macrocyclizations in the microviridin J pathway of *Microcystis aeruginosa*. Additionally, the interaction between the leader peptide and cyclases is unique motif along RiPPs. The results provide insight into the unique protein/protein interactions key to the chemistry, suggest an origin of the natural combinatorial synthesis of microviridin peptides and provide a framework for future engineering efforts to generate designed compounds.

10:30 AM S164: RiPPs from the symbiotic microbiota

E. Schmidt, University of Utah, Salt Lake City, UT, USA*

Many peptide natural products are made via the RiPP branch of biosynthesis. Here, I will describe the structures and biosynthesis of RiPPs and RiPP-like products synthesized by symbiotic bacteria living in marine animals. These compounds are major constituents of the animals, and based upon their biological activities they are potentially involved in chemical defensive interactions. Thus, RiPP peptides from the microbiota might represent a major source of new compounds with activity in animals.

11:00 AM S165: Biosynthesis and engineering of cyclic peptide antibiotics

W. van der Donk, Howard Hughes Medical Institute and the University of Illinois at Urbana-Champaign, Urbana, IL, USA*

The genome sequencing efforts of the first decade of the 21st century have revealed that ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a very large class of cyclic peptide natural products. These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Lanthionine-containing peptides (lanthipeptides) are examples of this growing class and many members are highly effective peptide-derived antimicrobial agents that display nanomolar minimal inhibitory concentrations (MICs) against pathogenic bacteria (lantibiotics). These peptides are post-translationally modified to install multiple thioether crosslinks. During their biosynthesis, a single enzyme typically breaks 8-16 chemical bonds and forms 6-10 new bonds with high control over regio- and chemoselectivity. This presentation will discuss investigations of the mechanisms of these remarkable catalysts as well as their use for the generation of non-natural cyclic peptides.

1:00 PM - 2:00 PM Charles Thom Award Lecture "Metabolite Valves: Dynamic Control of Metabolic Flux for Pathway Engineering" Kristala L. Jones Prather, MIT

Plaza Ballroom A & B - Concourse Level

2:00 PM - 5:30 PM Session: 30: Microbial Conversion Factories

Conveners: **Michael Resch**, National Renewable Energy Laboratory, Golden, CO, USA and **Kian Mau Goh**, University Teknologi Malaysia, Johor, Malaysia

Plaza Ballroom E - Concourse Level

2:00 PM S166: Energy and Carbon Efficiency Trade-offs in a Warming World: Engineering Advanced Bioconversion Pathways and Carbon Cycling Systems

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

Although rapid and large-scale deployment of renewable wind and solar is reducing carbon emissions from the power sector, technologies to decarbonize fuel and chemical reliant sectors, such as the

transportation sector, are less advanced and less readily deployable. Strategies to synthesize fuels, chemicals and industrially relevant intermediates from renewable biomass feedstock have long been examined, but to adequately address worsening climate realities, new biochemical pathways and carbon cycling systems need to be engineered that better optimize carbon conversion efficiency and utilization – perhaps at the expense of system energy efficiency. Instances in which system carbon efficiency could be prioritized relative to energy efficiency will occur more often as the deployment of cheaper and cleaner renewable power continues. Opportunities to leverage a low-carbon power sector to decarbonize the fuel and chemical sectors are being examined by the U.S. Department of Energy and its Bioenergy Technologies Office. Specifically, the viability of exploiting clean power to: 1.) improve the carbon efficiency of renewable feedstock conversion and upgrading, and to 2.) facilitate carbon reduction and organic product synthesis without photosynthetic steps.

This presentation will outline various specific system configurations that enable enhanced carbon conversion efficiency and non-photosynthetic carbon cycling, and will focus on the pathway engineering and system design strategies needed to establish appropriate microbial conversion factories for these purposes. Additionally, promising pathways and relevant organic intermediates, based on thermodynamic efficiencies and, as applicable, biological upgrading potential, will be examined and contextualized in terms system scalability.

2:30 PM S167: Carboxylate platform: chemicals and fuels from biomass

M.T. Holtzapple[*]*, Texas A&M University, College Station, TX, USA*

In an anaerobic environment, mixed cultures of soil microorganisms hydrolyze biomass components (e.g., cellulose, hemicellulose, starch, pectin, protein) into monomers, which are subsequently fermented into short-chain carboxylate salts (e.g., acetate, propionate, and butyrate). Normally, methanogens convert these products to biogas (methane, carbon dioxide); however, by adding a methanogen inhibitor, the fermentation becomes “stuck” and the carboxylates accumulate. In this stuck fermentation, the short-chain carboxylates (C2, C3, C4) can elongate to medium-chain carboxylates (C5, C6, C7, C8). The carboxylates are recovered from the fermentation broth and become building blocks for the *carboxylate platform*. Using well-established chemistry, the carboxylates can be converted to a wide variety of chemicals (carboxylic acids, primary alcohols, secondary alcohols, ketones, aldehydes, esters, ethers, olefins, paraffins, cyclics, aromatics) and fuels (gasoline, jet fuel, diesel). Because the carboxylate platform does not require sterile operating conditions nor the addition of extraneous enzymes, processing costs are very modest. For example, from energy crops ($60/tonne), the selling price of gasoline and jet fuel is estimated to be $1.76 to $2.56/gal, depending on scale. Using wastes (e.g., municipal solid waste, sewage sludge, manure) as feedstock, the selling price of gasoline and jet fuel is estimated to be $1.25/gal.

3:00 PM Break

3:30 PM S168: Demonstration of Integrated Biorefinery for Hardwood to Ethanol Conversion (DOE Cooperative Agreement DE-FC36-08GO18103)

M. Ladisch[*]*, Purdue University, West Lafayette, IN, USA*

This paper, presented on behalf of the Mascoma / DOE team, communicates how the team, together with the States of Michigan, New York and partners, worked together from 2008 to 2014 to achieve what were, in retrospect, remarkable improvements in cellulose to ethanol technology when consolidated bioprocessing (CBP) was demonstrated at the pilot scale. Fundamental research was scaled up in the Rome facility, validated, and transformed into a plant design for producing 20 million gallons ethanol / year from 700 tons / day hardwood to be sited in Kinross, MI. Integration of biology with process technology decreased enzyme requirements while utilizing a sustainable and abundant hardwood feedstock. Yields of 62 to 67 gal/ton hardwood were obtained through a combination of liquid hot water pretreatment (no chemicals added), enzymes, and inhibitor resistant CBP yeasts capable of fermenting both cellulose and xylose to ethanol. Key technical parameters that affected scale-up included front-end

materials handling, lignin derived enzyme inhibitors, pretreatment parameters, and bioreactor design. Although the Kinross demonstration plant was not built, the results from the DOE sponsored cooperative agreement provided a framework for future efforts and contributed to a foundation of process knowledge for scale-up and plant design. This paper and the report on which it is based is intended to contribute to commercialization of cellulose ethanol by documenting the engineering, science, improvements, training and learnings obtained through the joint work and investments of Mascoma, DOE, Dartmouth, Purdue University and its partners.

4:00 PM S169: Genetically engineered yeast in the fuel ethanol industry: past, present and future

E. Koh[*]*, AB Biotek, St. Louis, MO, USA*

The U.S. produces almost 15 billion gallons of fuel ethanol annually, primarily from corn, using *Saccharomyces cerevisiae* as the ethanologen. Recent introduction of genetically engineered yeast into the fuel ethanol industry has disrupted the economics of fuel ethanol production due to expression of valuable traits engineered into the yeast. These traits include secretion of transgenic glucoamylase and modification of glycerol production pathways in yeast designed for production of ethanol from corn or grain. Secretion of glucoamylase by *Saccharomyces* reduces the requirement for exogenous glucoamylase addition during simultaneous saccharification and fermentation and decreasing the production of glycerol increases the metabolic ethanol yield on sugar. These genetically engineered yeasts thus acquire a value greatly exceeding its value as a simple ethanologen. Different protein secretion approaches used to facilitate enzyme secretion and the metabolic engineering approaches to glycerol reduction are presented and reviewed here, as well as insights as to what genetically engineered yeasts will bring to fuel ethanol industry in the future.

4:30 PM S170: Yeast extracts & peptones: optimal nutritional sources for culturing lactic acid bacteria

L. Jacob[*]*, Sensient Technologies, Hoffman Estates, IL, USA; D. Mehta and D. Antibus, SENSIENT TECHNOLOGIES, Hoffman Estates, IL, USA*

The increasing demand for the growth of dairy & probiotic cultures has led to a strong interest in establishing optimal fermentation media for lactic acid bacteria. This study highlights how yeast extracts serve as highly effective complex nitrogen sources. They are abundant in peptides, amino acids and various growth factors that meet the high nutritional demands and auxotrophies of these microorganisms. The supplementation of yeast extracts with peptones resulted in further increase in biomass production. M17 & MRS culture media were each modified using animal and allergen-free yeast extracts and peptones to enhance & optimize the growth of various lactic acid bacteria.

5:00 PM S171: Using a genome-scale model of metabolism and gene expression to highlight how the proteome drives performance in *Clostridium ljungdahlii* The acetogen *Clostridium ljungdahlii*

J. Liu[*]*, University of California San Diego, San Diego, CA, USA*

Using a genome-scale model of metabolism and gene expression to highlight how the proteome drives performance in Clostridium ljungdahlii
When grown autotrophically on carbon monoxide (CO), carbon dioxide / hydrogen (CO_2/H_2), or a mixture of these gases, the acetogen Clostridium ljungdahlii metabolizes the gases into multicarbon organics, an ability that can be redirected to produce biocommodities.
To advance towards this goal, a constraint-based modelling method was used to

systematize the biochemical, genetic, and genomic knowledge of C. ljungdahlii into a computable mathematical framework. This metabolic and gene expression model (MEmodel) accounts for 961 ORFs that are responsible for the production of transcriptional units, functional RNAs, and protein complexes that are necessary for major metabolic, amino acid, nucleotide, and lipid biosynthesis pathways. This ME-model is able to compute the transcriptome, proteome, and fluxome of C. ljungdahlii and is able to do so accurately, as the ME-model's in silico transcriptome reflects in vivo subsystem expression under CO, CO2/H2, and fructose growth.
Not only does the ME-model recapitulate results from standard laboratory conditions, but it can also calculate C. ljundahlii's phenotypic responses to gene knockouts, carbon sources, and metal availability. For example, a carbon CO dehydrogenase; acetyl-CoA synthesis knockout in the ME-model predicts that C. ljungdahlii will stop acetate production and increase ethanol production, similar to the findings in Liew et al. 2016. Additionally, the ME-model, unlike a metabolic model, can predict electron overflow resulting in ethanol and glycerol production. Finally, the MEmodel provides a systems biology approach to analyze unmetabolized media components, which was validated with the effects of nickel availability on heterotrophic and autotrophic growth rates and secretion profiles.

2:00 PM - 5:30 PM Session: 31: Bioremediation of Hazardous Chemicals

Conveners: **Raj Boopathy**, Nicholls State University, Thibodaux, LA, USA and **Kevin Finneran**, Clemson University, Anderson, SC, USA

Plaza Ballroom D - Concourse Level

2:00 PM S172: Synergistic treatment of 1,4-dioxane in mixed plumes by coupling electrochemical oxidation with aerobic biodegradation

J. Blotevogel and T. Borch, Colorado State University, Fort Collins, CO, USA; J. Jasmann, USGS, Boulder, CO, USA; P. Gedalanga and S. Mahendra, University of California, Los Angeles, Los Angeles, CO, USA*

Biodegradation of the persistent organic pollutant 1,4-dioxane in groundwater is often hindered by the absence of dissolved oxygen, and notoriously inhibited by frequently co-occurring chlorinated solvents. Electrolysis has the potential to mineralize persistent organic pollutants through direct anodic oxidation while generating molecular oxygen. We thus hypothesized that electrochemical oxidation will enhance aerobic biodegradation of 1,4-dioxane through electro-generation of O_2 while removing potentially inhibiting co-contaminants such as trichloroethene (TCE). In column experiments, we investigated the electrochemical oxidation of 1,4-dioxane in the presence of *Pseudonocardia dioxanivorans* CB1190, a microaerophilic bacterium that can grow on 1,4-dioxane as its sole electron donor. At 3.0 V applied, 169 mg 1,4-dioxane was oxidized per hour and per m^2 of mesh electrode surface, while at a higher potential of 8.0 V, only 100 $mg \cdot h^{-1} \cdot m^{-2}$ of 1,4-dioxane was removed. Quantitative real-time polymerase chain reaction (qPCR) counts revealed lower abundances of *P. dioxanivorans* CB 1190 at higher voltage, likely due to extreme conditions around the anode. In the presence of TCE, the 1,4-dioxane oxidation rate at 3.0 V decreased to 98 $mg \cdot h^{-1} \cdot m^{-2}$ while the rate at 8.0 V remained largely constant. In this case, application of a higher voltage led to more rapid removal of the inhibiting co-contaminant. In contrast, less than 15 $mg \cdot h^{-1} \cdot m^{-2}$ of 1,4-dioxane was oxidized at both 3.0 and 8.0 V in abiotic control columns. Our results suggest that coupling electrochemical oxidation with aerobic biodegradation may be a promising synergistic approach for the treatment of groundwater contaminated with persistent organic pollutants in mixed contaminant plumes.

2:30 PM S173: Little black houses, starch, and critters – a new direction

S. Noland, Remediation Products Inc, Golden, CO, USA*

Background. Products based on activated carbon have been offered by Remediation Products Inc. (RPI) for over fifteen years. There has been a recent interest in “Carbon Based Injectates” with many people and organizations offering opinions on applications and limitations. Several questions have come to the forefront of this technology.

1. Are compounds absorbed into the microporous structure of carbon bioavailable?
2. Is regeneration/reactivation of the carbon through biological mechanisms viable?
3. Do sorption limitations of activated carbon prevent its application to LNAPL?
4. Can a combination with enhanced reductive dichlorination (ERD) result in a more powerful and versatile technology?

The environmental industry at large has not widely embraced the in situ use of activated carbon because of skepticism and field data has not been adequate to dispel the questions. To squarely address these issues, a laboratory research program was undertaken at RPI to challenge widely held “rules of thumb” and carbon myths.

Activities. Two major directions of research were undertaken. The first looked at bioremediation of petroleum hydrocarbons and explored bioavailability and adsorption capacity as it relates to potential application to LNAPL sites. The question of biological regeneration of carbon was also explored. The second line of research involves RPI’s metallic iron impregnated carbon (BOS 100®) and its combination with ERD. Various substrates were evaluated along with numerous blends of microorganisms. The objective of this research was to find a combination that overcomes limitations of ERD and BOS 100®.

3:00 PM Break

3:30 PM S174: Bioelectrochemically enhanced remediation of petroleum-contaminated soil

Z. Ren and L. Lu, University of Colorado Boulder, Boulder, CO, USA; S. Jin and P. Fallgren, Advanced Environmental Technologies, Fort Collins, CO, USA; Y. Zuo, Chevron Energy Technology Company, San Ramon, CA, USA*

Petroleum hydrocarbon contamination in soil and groundwater is a widespread environmental problem. In this project we successfully developed a bioelectrochemical system (BES) for soil remediation and scaled the system from lab to pilot scale. Results show that not only soil BES is able to efficiently degrade hydrocarbons in situ, but also it generates electrical current that can be used as a non-intrusive monitoring tool. Since 2013 we have developed tubular BES reactors as a scalable configuration, characterized microbial community distribution, investigated system performance in different conditions, developed scaled reactors for field implementation, and integrated system monitoring with geophysical probes. We also scaled the tubular system to cubic meter scale and completed a field pilot in a hydrocarbon contaminated site. We will also discuss the progress and whether BES can be effective in different soil conditions. Previous researches showed that the diffusion of hydrocarbon towards electrode was highly affected by soil porosity and conductivity due to the hydrophobic feature of hydrocarbon, so this study expands the scope to understand how soil texture affects the BES performance. We found BES has a better performance for sandy soil remediation than clayey soil due to improved hydrocarbon transfer in sandy soil texture. High throughput sequencing also demonstrates distinct microbial community structure in different soil conditions. Geophysical monitoring showed that an increase of soil conductivity corresponds to a decrease of TPH content in sandy soil at the early period of the remediation, but not for clayey soil due to the high surface conductivity.

4:00 PM S175: Validation of cost-effective molecular probes to assess mercury methylation in the environment: an effort to link *hgcA* abundance to methyl- and total mercury concentrations

G. Christensen, A. Wymore, A. King, M. Podar, S.D. Brown, A.V. Palumbo, S. Brooks and D.A. Elias, Oak Ridge National Laboratory, Oak Ridge, TN, USA; C.C. Gilmour, Smithsonian Environmental Research Center, Edgewater, MD, USA; J.D. Wall, University of Missouri, Columbia, MO, USA*

Methylmercury (MeHg) is a common contaminant in many ecosystems but the relationship between the microorganism that produce MeHg and its concentration in the environment is poorly understood. Two genes, *hgcA* and *hgcB*, are essential for microbial mercury (Hg) methylation. We recently developed universal qualitative PCR probes for *hgcAB* and quantitative probes that select for *hgcA*$^{+}$ microorganisms from the three dominant Hg-methylating clades: *Deltaproteobacteria, Firmicutes,* and methanogenic *Archaea*. In an effort to link *hgcAB* diversity and abundance with MeHg concentrations, we analyzed sediments from eight diverse locations and compared our (q)PCR-based to 16S rRNA gene sequencing directly from the samples and after clone library constructions as well as to metagenomic shotgun sequencing. The *Deltaproteobacteria* dominated in both metagenome and amplicon sequencing of *hgcAB* diversity. The data collected from 16S pyrosequencing did not identify *hgcAB* microorganisms well. Furthermore, qPCR estimates of Hg-methylator abundance agreed well with metagenomics estimates and displayed similar correlations with sediment HgT and MeHg concentrations. Our cost-effective (q)PCR-based methods against *hgcAB* for Hg-methylator diversity and abundance is a valid means to study the relationship between Hg-methylators and soil Hg concentrations. Utilization of this validated technique could be performed on-site to provide rapid and accurate estimates of Hg-methylator abundance thereby quickly informing risk assessment and management as well as for remediation strategies.

4:30 PM S176: Peeling away “black box” processes in lignocellulose-based sulfate reducing bioreactors designed to remediate mining impacted water

J. Sharp, D. Drennan, R. Almstrand, L. Landkamer, L. Figueroa and J. Ladderud, Colorado School of Mines, Golden, CO, USA; I. Lee, Freeport McMoRan Inc., Oro Valley, AZ, USA; S. Webb, Stanford Synchrotron, Menlo Park, CA, USA*

Sulfate reducing bioreactors offer a sustainable and long-term option for bioimmobilization of metal(loids). These passively managed constructs have been applied to mitigate waters associated with legacy orphaned mining operations, which present challenges for human and ecological health and wellbeing with remediation costs estimated in the tens of billions of dollars for the ~half-million abandoned mines present in the US alone and similar challenges globally. While it is generally understood that these bioreactors rely on biogenic sulfide evolution to precipitate metals such as zinc and copper, their implementation is more “black box” in nature with limited spatial and temporal biogeochemical insights. To this end, we investigated microbial and geochemical interactions with a focus on the influence of organic substrate, inoculum and microbial assemblage on metal immobilization and stability across laboratory, pilot, and field scale scenarios. Our results reveal a robust microbial assemblage with respect to time and depth that relies on fermentative syntrophy in support of sulfate reducing bacteria. The production of and competition for secondary fermentation byproducts such as lactate further influences ecological composition and reactor efficiency. A combination of synchrotron-based analyses, electron microscopy, x-ray spectroscopy, and geochemical digests further revealed diffuse carbonate and more discrete sulfide-associated precipitation regimes that varied in crystalline structure. Collectively, these insights suggest interplay between inorganic ligand availability and biologically-mediated reactions that can be further optimized for more reliable and robust metal bioimmobilization processes.

5:00 PM S177: Bacterial spores as a robust protein display platform for heavy metal bioremediation

E. Drufva and K. Wu, University of New Hampshire, Durham, NH, USA*

Heavy metals from industries such as mining and electroplating cause serious contamination problems and need to be removed. Compared with physical and chemical methods, bioremediation provides an environmentally friendly for cleaning heavy metals from aqueous systems. Various microbes have been studied for their ability to detoxifying or absorbing heavy metals. However, the growth of these live

organisms is limited under harsh conditions such as high metal concentrations or extreme temperature and pH. By constrast, spores produced from bacteria are resistant to various harsh environments and can be preserved at room temperature for years. Some spores with metal oxidation activity have been expolored for the cycling of Mn and U. In this study, a lead binding protein PbrR and its mutants have been displayed on *Bacillus subtilis* spore surface and characterized for their binding affinity to lead ions. They are able to bind lead ions at a concentration as low as 0.01 uM. Moreover, the lead ion can be released by varying the incubation condition. The reversible and specific binding of lead ions by PbrRs on the spore surface allows the separation and recovery of lead ions from other metal ions. As part of the spore, they can be easily produced from sporulation and no further separation is needed. This provides a commercially viable method for lead remediation and recovery. The display of heavy metal binding proteins on bacterial spore surface, together with protein engineering for improved specificity, could be used for the recovery of other metals, particularly expensive noble metals.

2:00 PM - 5:30 PM Session: 32: Next Generation Technologies for Fermentation and Cell Culture

Conveners: **Swapnil Bhargava**, Seattle Genetics, Bothell, WA, USA and **Dr. Joel Sirois**, Universite de Sherbrooke, Sherbrooke, QC, Canada

Plaza Ballroom F -Concourse Level

2:00 PM S178: On-line control of glucose concentration in high-yielding mammalian cell cultures enabled through oxygen transfer rate measurements

S. Goldrick, University College London, London, United Kingdom; K. Lee, MedImmune, Gaithersburg, MD, USA; W. Holmes, M. Kuiper and R. Turner, MedImmune, London WC1E 6BT, United Kingdom; S. Farid, University College London, London WC1E 6BT, United Kingdom*

The primary method for glucose control during mammalian fed-batch fermentations, typically involves dynamic bolus glucose additions based on infrequent off-line daily samples. This glucose control strategy results in cells experiencing significant glucose concentration fluctuations which have been previously reported to influence product quality and growth. Current on-line methods to control glucose require expensive process analytical technology (PAT) devices that have proven to be difficult to validate and are not commonly incorporated in industrial biopharmaceutical facilities. This work proposes an on-line method to control and manipulate glucose utilising readily available process measurements. The method has been verified across multiple scales and used varied cell lines. The method generates a correlation between the cumulative oxygen transfer rate and the cumulative glucose consumed. The correlation generates an on-line prediction of glucose that has been successfully incorporated into a control algorithm manipulating the glucose feed-rate. This advanced process control (APC) strategy enables the glucose concentration to be maintained at an adjustable set-point and has been found to significantly reduce the deviation in glucose concentration in comparison to conventional operation. The method has been validated for production of various therapeutic proteins and successfully demonstrated on the micro-(15 mL), laboratory-(7 L) and pilot-(50 L) scale systems.

2:30 PM S179: Antibody fragment production faster and more cost-efficient than CHO: Applying *E. coli* secretion technology

M. Huber, Wacker Biotech GmbH, Jena, Germany and M. Berge, MedImmune, Gaithersburg, MD, USA*

WACKER's *E. coli* based secretion technology (ESETEC®) was applied to produce the target protein, a recombinant human antibody Fab fragment which was developed by MedImmune to rapidly and specifically reverse the effects of a drug. Administration of relatively high doses of the product made determining a more cost-efficient manufacturing a process objective for this project. While cost-of-goods based on mammalian CHO cells were not optimal, ESETEC® was the enabling technology for successful

production of the Fab. Following a two-month feasibility study, the entire USP and DSP process was developed within nine months and scaled up to WACKER's 300-L GMP production facility. The highly reproducible manufacturing process delivered 1.15 kg of non-GMP and 2.1 kg of GMP material from a 200-L fermentation scale just 18 months after initiation of the project. In parallel, a second-generation process was implemented with an improved ESETEC® strain to further increase product titers of the Fab, which was achieved using a definitive screening design approach in USP development.

3:00 PM Break

3:30 PM S180: Advances in adaptive feeding control – a closed loop system to monitor, control, and automate upstream mammalian and microbial feeding protocols

G. Emmerson, S. Watts and S. Saxby, Stratophase Ltd, Romsey, United Kingdom; G. Barringer, Stratophase Ltd (USA), W Groton, MA, USA*

An in-line process monitoring system, the Ranger™, and method for in-situ, real time monitoring and control of nutrient and carbon source feeding in upstream bioreactors and fermentors is described. The Ranger responds to the overall state of the metabolic environment of the process under observation and is highly sensitive to any molecular level perturbation in the process media, such as occurs when a biological process is fed nutrients and carbon sources. An automatic, closed loop adaptive feeding protocol that responds to these real time changes in nutrient concentration in the media then maintains proscribed optimum nutrient concentrations thus permitting faster process development cycles, time to clinic, and the promise of better product quality and titer. The system can actively and independently manage multiple feeds. The system is applicable to all scales of operation from process development to commercial production and is compatible with SUBs. This technology is applicable in microbial, fungal, and mammalian cultures.

4:00 PM S181: Fermentation and strain design for successful scale-up

M. Japs, Genomatica, Inc., San Diego, CA, USA*

Genomatica has established a track record of success in bioprocess development, technology transfer, and scale-up; delivering processes that work commercially the first time. In this presentation we'll share insights into our validated platform for bioprocess scale-up and bioreactor design. This includes how commercial scale conditions are taken into consideration in the earliest phases of strain design; how to choose fermentation and downstream processing approaches based on metabolic pathway and techno-economic analyses; and designing strains that optimize metabolism and product production under conditions compatible with "at-scale" constraints. The systematic evaluation of process robustness using these unique "scale-down" modeling and experimental methods minimizes scale-up risk and accelerates commercialization timelines for industrial fermentation processes.

4:30 PM S182: Real-time analysis of fermentation and cell culture enabling real-time optimization of profitability

J. Sirois, BioIntelligence Technologies inc., Sherbrooke, QC, Canada*

Biomanufacturing and bioprocess development suffer from a lack of probes to perform a valuable monitoring of product biosynthesis and its optimization. Many samples have to be taken and analyzed with instruments such as HPLC to have an idea of the kinetics and yields during fermentations and cell cultures. This constrain induces delays and losses. The BioAnalyst is a new instrument collecting data from any source, being probes on a bioreactor, datafile from a HPLC or user entries from manual analysis, and performing real-time calculations to unveil hidden information such as biokinetics. The embedded algorithms push information on personal sets of dashboards for each user enabling them to

accelerate decision making and implement real-time optimization. A layer of algorithms is dedicated to translate scientific information to financial information. Users can perform real-time optimization of profitability, identify bad batches and stop them early in the process and project culture behavior over time to anticipate problems and fix them before they happen.

5:00 PM S182A: Real-time *in-situ* fermentation monitoring using generalizable near-infrared assays

N. Agbonkonkon, M.D. Leavell, D. Yim, D. Abbott and S. Gaucher, Amyris, Emeryville, CA, USA*

Accurate measurement of feedstock consumption and product production is required for process development. Traditionally fermentation broth samples are taken and analyzed in batch. This approach is extremely resource intensive and results in a lengthy cycle time. For *in-situ* product titer monitoring, spectroscopic probes and multivariate data analysis are often used. Current thinking is that large data sets, in the 100's or 1000's of data points, are required to make calibration models and that models are not generalizable. This presentation will focus on the development of Near-IR assays for real-time *in-situ* measurement of product titers utilizing generalizable calibration models, and outline the cost and time savings realized from such an approach.

2:00 PM - 5:30 PM Session: 33: Photosynthetic and Non-Conventional Organisms in Metabolic Engineering

Conveners: **Kevin Solomon**, Purdue University, West Lafayette, IN, USA and **Dr. Michael Köpke**, LanzaTech, Inc., Skokie, IL, USA

Plaza Ballroom A & B - Concourse Level

2:00 PM S183: Isotopes, compartments and complex molecules: the application of isotope labeling and metabolic flux analysis to photosynthetic organisms

G. Sriram, University of Maryland, Baltimore, MD, USA*

Experimental and computational methods for metabolic pathway analysis are valuable in assessing carbon flow within an organism and in identifying of metabolic engineering targets. These methods principally include various versions of isotope-assisted metabolic flux analysis (isotope MFA) and flux balance analysis. In isotope MFA, isotopomers resulting from the feeding of 13C and 12C carbon sources are measured and interpreted computationally to develop a cellular or organismal flux model. This methodology is well-established in simple, model microbes. However, its application to non-conventional and especially photosynthetic, eukaryotic microorganisms is challenging for various reasons. First, isotope labeling for an organism wherein CO2 is the sole carbon source as this requires transient measurements of labeling and differential algebraic equations to process the ensuing measurements. Second, unknown or unexpected metabolic pathways and compartmentalization complicate the interpretation of the labeling patterns. Third, complex metabolites such as polyunsaturated and long-chain fatty acids routinely present in eukaryotes show labeling patterns that are difficult to parse. In this talk, we will present work from my laboratory on the application of isotope MFA to photosynthetic organisms. This chiefly includes the diatom (alga) Phaeodactylum tricornutum, but examples from other plant cells will also be illustrated. The results will include the development and mathematical modeling of isotope labeling experiments to probe the metabolism of these photosynthetic cell types, coupled with other molecular analyses to ultimately identify the molecular players and rate-limiting steps in key metabolic pathways.

2:30 PM P77: *Quid pro quo*: Engineering nitrogen self-sufficient cocultures

C. Diaz[*] and M. Antoniewicz, University of Delaware, Newark, DE, USA

Diazotrophs, or organisms capable of converting atmospheric nitrogen into ammonia, are attractive coculture partners, offering a sustainable alternative to the Haber-Bosch process as a source of fixed nitrogen. However, efforts to engineer synthetic consortia are currently impeded by difficulties in predicting metabolic compatibility and achieving long-term stability.

In this contribution, we investigated the performance of five, fully nitrogen self-sufficient cocultures involving an ammonium-secreting strain of the aerobic diazotroph, *Azotobacter vinelandii*. Coculture pairings were systematically designed to range from pure commensalism (*e.g.* with wild-type *E. coli*) to fully nitrogen and carbon self-sufficient, mutualist partnerships with engineered cyanobacteria.

^{13}C labeling studies and metabolic flux analysis (MFA) unexpectedly revealed that the diazotroph shared the majority of its fixed nitrogen, even in commensalist cocultures. For example, wild-type *E. coli* constituted more than 60-75% of a coculture population with a total OD of ~5, and was maintained as 30-40% of the population in later stages, up to a total OD of ~40. Furthermore, negative interactions could be overcome by introducing metabolic interdependence through genetic manipulation, *e.g.* by engineering the partner to secrete a carbon source suitable for the diazotroph. Preliminary adaptive evolution studies highlighted the surprising stability of such cocultures, where synthetic cross-feeding persisted over weeks of passaging. Ongoing work dually harnesses deep-sequencing and ^{13}C-MFA of evolved cocultures to identify additional genetic and metabolic traits that enable enhanced community stability and overall growth.

These results shed insight on how bioprocesses can benefit from the modularity, improved sustainability and potential cost reduction offered by diazotrophic cocultures.

3:00 PM S185: Thermophilic consolidated bioprocessing with cotreatment: A potentially disruptive paradigm for biological production of cellulosic biofuels

L.R. Lynd[*], Dartmouth College, Hanover, NH, USA

Data will be presented from a comprehensive recent study aimed at evaluating the cumulative and relative impact of "multiple levers" to overcome the lignocellulose recalcitrance barrier, including choice of biocatalyst and feedstock, genetically modified plants and less recalcitrant natural variants, and several non-biological approaches to assist the deconstruction process. Anaerobic thermophilic bacteria are found to be decisively more effective than industry-standard fungal cellulase at solubilizing cellulosic biomass under a broad range of conditions. However even the best plant cell wall-solubilizing biocatalysts require some assistance in order for lignocellulose to be processed with high yields in a reasonable amount of time. As an alternative to thermochemical pretreatment, we are investigating physical disruption once fermentation is initiated – termed cotreatment. Results presented include: a) demonstration of fermentation in the presence of physical disruption at an intensity sufficient to substantially increase lignocellulose solubilization, b) high extents of solubilization comparable to conventional pretreatment, c) lignin residues with less modification than result from thermochemical pretreatment. Taking advantage of the outstanding capability of thermophilic anaerobic bacteria to ferment cellulosic biomass without added enzymes requires that metabolic engineering tools be developed and applied to these organisms in order to bring product yields and titers to industrially acceptable levels. Recent progress will be described involving the cellulose-fermenting *Clostridium thermocellum* as well as hemicellulose-utilizing thermophiles such as *Thermoanaerobacterium saccharolyticum.* Technoeconomic analysis indicates potential for 8-fold shorter payback period and feasibility at several-fold smaller scale as compared to current technology using the fungal cellulase-thermochemical pretreatment paradigm.

3:30 PM Break

4:00 PM S186: Targeting cellular processes in methylotrophic bacteria to facilitate biologically-mediated recovery of lanthanides

E. Skovran, G. Subuyuj, H. Vu, R. Valentine, F. Yarza and C. Raghuraman, San Jose State University, San Jose, CA, USA; N. Martinez-Gomez, Michigan State University, East Lansing, MI, USA*

Lanthanides (Ln), are considered *critical metals* for the development of sustainable high efficiency electronics and energy technologies. However, extraction and purification of these metals is environmentally destructive, contributing to the closing of mines in the United States and reliance on foreign nations. Methylotrophic bacteria can acquire Ln for use as cofactors during methanol oxidation. This unique ability enables the identification of transporters, enzymes, and regulatory mechanisms as engineering targets for development of a biological Ln recovery platform. To identify genes required for Ln-dependent growth, ~500 transposon mutants were isolated in *Methylobacterium extorquens* and their insertion locations mapped. Over 40 null mutations were reconstructed in candidate genes followed by phenotypic and biochemical analyses to characterize the role of these genes in Ln acquisition and transport, and methanol oxidation. Among the genes identified are a Ln receptor and transporter. We have also identified putative regulators and cellular processes that control expression of the Ln receptor and the Ln-dependent methanol dehydrogenase, *xoxF*. Additionally, we have data which suggests that, like insoluble iron Fe, Ln acquisition and uptake is mediated by secreted siderophores; molecules that chelate metals in their oxidized state. Finally, we have shown that *M. extorquens* can grow using electronic-waste as a source of Nd^{3+} and mining ores as a source of mixed Ln. This work will further our understanding of how Ln function in biology and provide us with information to guide genetic engineering efforts toward increased recovery of Ln from recycled electronics, mining ores, and contaminated soils.

4:30 PM S187: Vmax™ – A next-generation microbial host for the biotech industry

M. Sturges, L. Wolf, S. Vasu and C. Chen, SGI-DNA, A Synthetic Genomics Company, La Jolla, CA, USA; C. Wilson, E. Hesek, M. Weinstock and D. Gibson, Synthetic Genomics Inc., La Jolla, CA, USA*

Typical cloning and protein expression projects using *E. coli* require several days to produce a plasmid construct or recombinant protein. Much of this time is spent waiting for the bacteria to grow to sufficient density. Because bacterial growth is a large portion of many biotech processes, a novel host with a rapid growth rate could streamline current molecular cloning and protein expression workflows and offer an alternative to current systems.

This presentation will focus on Vmax™, a novel prokaryotic host derived from the marine microorganism, *Vibrio natriegens*. This gram-negative, non-pathogenic bacterium exhibits the fastest growth rate of any known organism. With a doubling time more than twice that of *E. coli*., this newly developed host system promises to accelerate biotech R&D efforts on multiple fronts. We will describe the development of the platform, the advantages of using it in molecular cloning and protein expression applications, and ongoing large-scale genome engineering efforts to further enhance performance.

5:00 PM S188: Modifying carbon and nitrogen metabolism in *Clostridium thermocellum* to enhance cellulosic biofuel production

A.M. Guss, Oak Ridge National Laboratory, Oak Ridge, TN, USA*

A sustainable future will require the development of renewable alternatives to petroleum-derived fuels and chemicals, and one potential solution for the replacement of gasoline involves the conversion of plant biomass into liquid fuels. *Clostridium thermocellum* is a leading candidate organism for implementing a consolidated bioprocessing (CBP) strategy for biofuel production due to its native ability to rapidly consume cellulose and its existing ethanol production pathway. In addition to ethanol, *C. thermocellum* converts cellulose and soluble cellodextrins such as cellobiose to lactate, formate, acetate, H_2, amino acids, and other products. Therefore, metabolic engineering is required to optimize flux to a single product. A mutant strain of *C. thermocellum* was constructed to remove major side product formation,

resulting in *C. thermocellum* Δ*hydG* Δ*ldh* Δ*pfl* Δ*pta-ack*. This strain no longer produces formate, acetate and lactate; hydrogen production is decreased four-fold; and the ethanol yield is doubled compared with the wild type on cellobiose, crystalline cellulose Avicel, and pretreated biomass. Adaptive Laboratory Evolution further improved growth rate, yield, and titer, and genome resequencing revealed the mutations responsible for the improved phenotype. While this mutant exhibits higher ethanol yield, amino acids are still produced as end products. Therefore, genetic analysis of nitrogen metabolism is being investigated to understand the mechanism of ammonium assimilation and to devise strategies to prevent production of amino acids as fermentation products. Progress in understanding and altering nitrogen metabolism in *C. thermocellum* and the impact on product formation will be discussed.