This grant was to support a scientific meeting. Other than the on-line meeting report referred to elsewhere herein, no publications were planned or resulted.

CLOSTRIDIUM XIV

14th International Conference on the Genetics, Physiology and Synthetic Biology of Solvent- and Acid-forming Clostridia

August 28-31, 2016

Dartmouth College

Hanover, NH

Contents

Support is gratefully acknowledged from the meeting sponsors:

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- Green Biologics Ltd.
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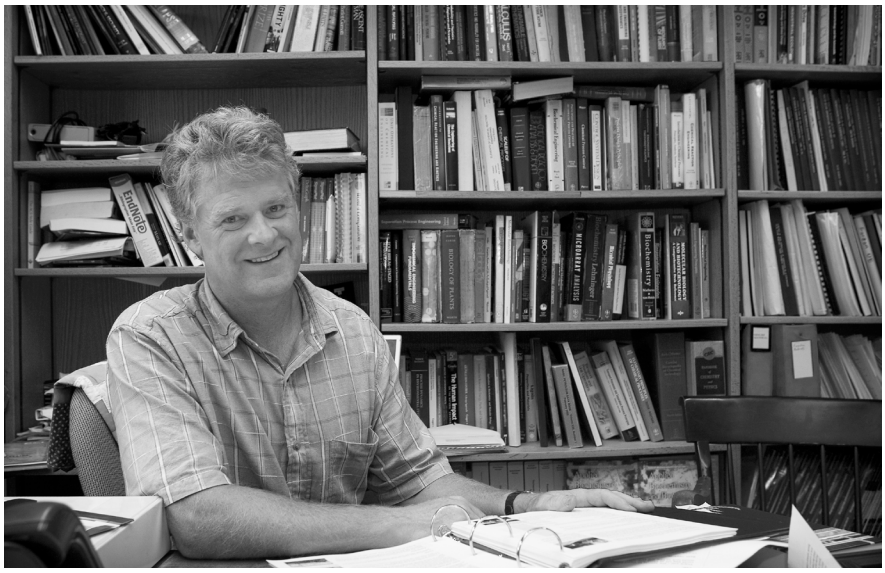


Welcome

We warmly welcome you to Hanover, New Hampshire, Dartmouth, and the Thayer School of Engineering for the 14th International Conference on the Genetics, Physiology, and Synthetic Biology of Solvent- and Acid-Forming *Clostridia*.

Held every other year since 1990, and most recently in Shanghai, the "Clostridium" conferences have become the leading international forum for presentation and discussion of new findings related to members of this genus with potential to be used for beneficial purposes. Within this scope, the meetings have spanned a broad range of topics including both fundamental and applied aspects, often in the same project. The success of prior meetings in this series has been due to the quality of the science presented combined with an informal atmosphere welcoming to scientists from around the world including both established experts and those new to the field. Clostridium XIV aims to continue in this tradition.

The program features two keynote lectures, 36 oral presentations – including five featured presentations and 6 presentations by young presenters – and 45 posters. In between full days of presentations on Monday and Wednesday, Tuesday afternoon and evening are devoted to opportunities for discussion and an introduction to the Northern New England region. About 120 people have registered for the meeting, indicative of a healthy ongoing interest in *Clostridium* biology and biotechnology.

With thanks for the support of our generous sponsors and appreciation to the meeting attendees for your participation and valuable contributions, we offer our best wishes for a productive meeting and an enjoyable visit.

Sincerely,

THAYER SCHOOL OF
ENGINEERING
AT DARTMOUTH

Clostridium XIV schedule of events

Sunday, August 28th

Welcome and reception		
19.00	Registration (Great Hall)	
19.30	**Ed Bayer** *Weizmann Institute*	Keynote speech
20.30	Reception and continued registration (Great Hall)	

Monday, August 29th

Session I: Physiology and Genetics (Chaired by Nigel Minton)		
07.00	Breakfast (Class of 1953 Commons)	
08.00	Registration (Great Hall)	
08.30	**Weihong Jiang** *Chinese Academy of Sciences, China*	Molecular Regulation of Sugar Utilization and Solvent Production in Solventogenic *Clostridia* (010)
09.10	**Ying Zhang** *University of Nottingham, UK*	Use of a Conditional RAM-less ClosTron to Identify Compensatory Mutations that Allow the Isolation of Mutations in Essential Genes in *Clostridia* (050)
09.40	**Tristan Cerisy*** *Genoscope/UMR Génomique métabolique/LGBM, France*	Transport of plant-derived sugars by *Clostridium phytofermentans* (069)
10.00	Tea and coffee break and continued registration (Great Hall)	
10.30	**Nicolaus Herman** *University of California Berkeley, USA*	Development of a High-Efficiency Transformation Method and Implementation of Rational Metabolic Engineering for an Industrial Strain of Solventogenic *Clostridium* (078)
11.00	**Daniela Heeg** *Chain Biotechnology Ltd, UK*	Accelerating the Understanding and Development of *Clostridium* for Biotechnology - the development of advanced modular vectors (115)
11.20	**Thao Nguyen*** *INSA Toulouse, France*	Cap0037, a Novel Global Regulator of *Clostridium acetobutylicum* Metabolism (076)
11.40	**Satya Dash*** *Pennsylvania State University, USA*	Kinetic Modeling of Metabolism in *Clostridia* (004)
12.00	Lunch (Class of 1953 Commons)	

Session II: Synthetic Biology, Genetic Tools, and Omics (Chaired by Weihong Jiang)		
13.00	**Daniel Amador-Noguez** *University of Wisconsin-Madison, USA*	Limited thermodynamic driving force in glycolysis of cellulolytic *Clostridia* (068)
13.40	**Chen Yang** *Ohio State University, USA*	Genomic Reconstruction of σ^{54} Regulons in *Clostridia* (030)

* young presenter

14.10	**Michael Pyne*** *Concordia University/U. Waterloo, Canada*	Development of a *Clostridium pasteurianum* Genetic Toolkit: Gene Disruption, CRISPR-Cas Genome Editing, and Genome Sequencing (016)
14.30	Tea and coffee break (Great Hall)	
15.00	**Yi Wang** *Auburn University, USA*	Efficient and precise genome editing and gene transcription repression in *Clostridium beijerinckii* using CRISPR-Cas9 system (027)
15.30	**Celine Foulquier** *INSA LISBP, France*	Markerless targeted and multiple gene modifications in *Clostridium acetobutylicum* (089)
16.00	**Steve Brown** *Oak Ridge National Laboratory, USA*	Dissecting industrial acetone-butanol-ethanol (ABE) fermentation by sequencing of largest existing collection of historic commercial solventogenic *clostridia* strains (060)
16.30	Short break	
16.45	**Lars Ljungdahl**	Keynote speech
17.30	Dinner (Class of 1953 Commons)	
19.30	Poster session and reception (GlycoFi Atrium)	

Tuesday, August 30th

Session III: Metabolic Engineering (Chaired by Philippe Soucaille)		
07.00	Breakfast (Class of 1953 Commons)	
08.30	**Peter Duerre** *University of Ulm, Germany*	Metabolism and metabolic engineering of autotrophic acetogens (033)
09.10	**Yang Gu** *Chinese Academy of Sciences, China*	Engineering Gas-Fermenting *Clostridia* for Production of Chemicals and Biofuels (007)
09.40	**Janet Westpheling** *University of Georgia, USA*	Genetic Methods to Enable Metabolic Engineering of *Clostridium themocellum* (028)
10.10	Tea and coffee break (Great Hall)	
10.40	**Daniel Olson** *Dartmouth College, USA*	Engineering *Clostridium thermocellum* to produce ethanol from cellulose at high yield and titer (048)
11.10	**Isabelle Meynial-Salles** *LISBP, INRA, France*	Identification and characterization of the Ferredoxin NAD(P)$^+$ Oxidoreductases of *Clostridium acetobutylicum* (106)
11.40	**Margaret Liu** *University of Alabama, USA*	Metabolic Cell-Process Engineering Guided by Systems Biology for High Production of Bioenergy (013)
12.10	Lunch (Class of 1953 Commons)	
13.15	Social program, busses leave from Hanover Inn	
18.00	Dinner (Dartmouth Skiway), busses leave from Hanover Inn	

* young presenter

Wednesday, August 31st

Session IV: Feedstock Utilization and Ecology (Chaired by Lee Lynd)		
07.00	Breakfast (Class of 1953 Commons)	
08.30	**Evert Holwerda** *Dartmouth College, USA*	Solubilization of Cellulosic Feedstocks by *Clostridium thermocellum* (049)
09.10	**Armin Ehrenreich** *Technical University of Munich, Germany*	Complete Hexose and Pentose Consumption by Catabolite Repression Mutants of *Clostridium acetobutylicum* ATCC 824 (038)
09.40	**George Bennett** *Rice University, USA*	Analysis of methanol utilization in acetogenic organisms (047)
10.10	**Ellinor Carlson (Schmidt)*** *University of Delaware, USA*	CO_2 Fixation for Increased Microbial Fermentation Yields (019)
10.30	Tea and coffee break (Great Hall)	
11.00	**Qi Xu** *National Renewable Energy Laboratory, USA*	A new cell-free cellulosome system in *Clostridium thermocellum* and new insights into the cellulosome system as a whole from systematic deletion of multiple scaffoldin genes. (074)
11.30	**Richard Sparling** *University of Manitoba, Canada*	Cofactor utilisation and interaction with core catalysis proteins in *R. thermocellum* and their prevalence in the genus *Ruminiclostridium* (*Clostridium* cluster III). (103)
12.00	**Alex Dumitrache** *Oak Ridge National Laboratory, USA*	Carbon spending and carbon scavenging: differential gene expression in planktonic and sessile cell populations of *Ruminiclostridium thermocellum* 27405. (055)
12.30	Lunch (Class of 1953 Commons)	

Session V: Bioprocessing and Industrial Applications (Chaired by Sean Simpson)		
13.30	**Torbjørn Jensen** *Technical University of Denmark* **Stephanie Redl** *The Novo Nordisk Foundation Center for Biosustainability, Denmark*	*Moorella thermoacetica*, a workhorse creating value from various gaseous substrates (018)
14.10	**Sean Simpson** *Lanza Tech, USA*	Gas fermentation: Waste to value at scale (125)
14.40	**Shawn Jones** *White Dog Labs, USA*	CO_2 fixation by anaerobic, non-photosynthetic mixotrophy for improved carbon conversion (032)
15.10	**Petra Patakova** *University of Chemistry and Technology Prague, Czech Republic*	*Clostridium pasteurianum* NRRL B-598, Physiology of No-Name Solventogenic *Clostridium* under Different Cultivation Conditions (015)
15.40	Tea and coffee break (Great Hall)	
16.10	**Nasib Qureshi** *USDA, NCAUR, USA*	Butanol Biorefineries: Simultaneous Product Removal & Process Integration for Conversion of Biomass & Food Waste to Biofuel (017)

* young presenter

16.40	**Wouter Van Hecke** *VITO NV, Belgium*	Biobutanol production integrated with organophilic pervaporation: experimental results and conceptual plant design (123)
17.10	**Stefan Pfluegl** *Vienna University of Technology, Austria*	Continuous butanol production from spent sulfite liquor with *Clostridium saccharoperbutylacetonicum* (036)
17.40	Adjourn	
19.30	Conference dinner (Canoe Club restaurant, 27 Main St, Hanover, NH)	

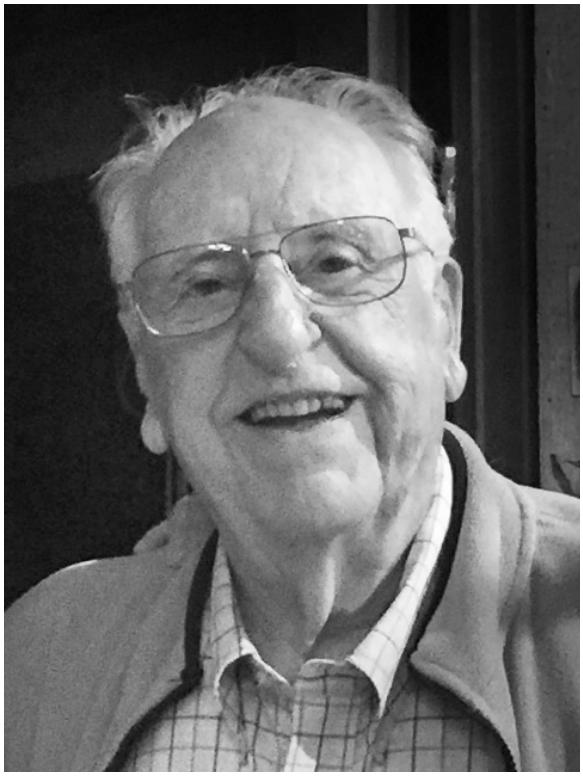
Thursday, September 1st

07.00	Breakfast (Class of 1953 Commons)
End of scheduled conference events	

* young presenter

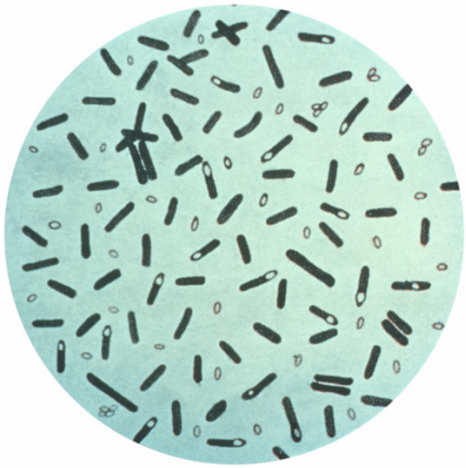
Ed Bayer

Ed Bayer is a professor in the Department of Biomolecular Sciences at the Weizmann Institute of Science, Rehovot, Israel. His early work focused on the early development of the avidin- and streptavidin-biotin system as a general tool in the biological sciences, and he received the Sarstedt Award (1990) for his contributions in this field. He is co-discoverer of the cellulosome concept and has founded and pioneered the development of designer cellulosomes for research and biotechnological applications. In 1999, he founded and chaired an ongoing Gordon Research Conferences on this subject. He is the recipient of The Ulitzky Prize (2006) from The Israel Society for Microbiology on his work in this area. Since 2008, he has served on the scientific advisory board of the US DOE BioEnergy Science Center (BESC). He has authored over 400 articles and reviews in both fields. He co-edited Methods in Enzymology Volume 184 on "Avidin-biotin technology", is editor-in-chief of Biotechnology Advances, section editor on Bacterial Genetics and Metabolic Engineering of Biotechnology for Biofuels, and serves on the editorial board of several other biotechnology- and microbiology-oriented journals, including Environmental Microbiology and Current Opinion in Biotechnology. He is an elected Fellow of both the American and the European Academies of Microbiology.



Lars Ljungdahl

Lars G. Ljundahl is retired from a distinguished career as a trailblazer in biochemistry and industrial microbiology. After receiving his BS in Chemical Engineering from the Stockholm Technical Institute in 1945, Lars worked for 11 years as a Research Chemist for the Stockholm Brewery. From 1958 to 1967, he worked with Harland Wood at Case Western University on CO_2 fixation and acetate formation in Clostridium thermaceticum, first as a graduate student and then as an Assistant Professor. From 1967 to the present, Lars has been a member of the Department of Biochemistry at the University of Georgia, Athens - progressing to full Professor and more recently Professor Emeritus. In recognition of Lars' pioneering work describing the Wood-Ljungdahl pathway for non-autotrophic CO_2 fixation, *Clostridium ljundahlii* was named in his honor. Lars also did pioneering work on thermophilic, saccharolytic bacteria as well as cellulolytic anaerobic fungi, and he described the first biological role for tungsten. As Chief Editor of Applied and Environmental Microbiology, Lars played a key role in shepherding it to its current prominent status. Recipient of many awards including the Humbolt Prize, Lars is a member of the Royal Swedish Academy of Engineering Sciences and Fellow of the American Academy of Microbiology.



Poster and presentation abstracts

001 - Sporulation in Solventogenic *Clostridium* sp.:Investigating the Link between Endospore Formation and Solvent Production in the Industrial ABE Fermentation

ATMADJAJA, ARETHA[1,2], AMANDA HARDING[1], ELIZABETH JENKINSON[1]

[1]*Green Biologics Ltd., 45a Western Avenue, Milton Park, Abingdon, Oxfordshire, OX14 4RU*

[2]*School of Life Sciences, University of Nottingham, Nottingham, NG7 UH*

Butanol (n-butanol or 1-butanol), a straight chain four-carbon alcohol with a molecular formula of C4H9OH, is an important bulk chemical widely used as a solvent and as a precursor in the production of monomers, polymeric emulsions, esters, and glycol ethers. It is also a promising candidate as a next generation biofuel, a potential replacement or supplement to the current fossil fuel. Although most of the n-butanol produced today is petrochemically derived through oxo synthesis, the main route for butanol production up to the 1960s was through acetone-butanol-ethanol (ABE) fermentation by solventogenic *Clostridium* sp. In recent years, an increased demand for a more sustainably produced butanol has driven renewed interest in the development of an industrially viable ABE fermentation process.

Green Biologics Ltd (GBL) is an industrial biotech company based in the UK and with a commercial plant currently being retrofitted in the US (CMR). Using a combination of proprietary clostridial genetic tools along with advanced fermentation processes, GBL are able to generate strains optimised for the production of bio-butanol and bio-acetone at economic yields and productivities. This project is focussed on overcoming challenges associated with sporulation. For example, in a prolonged cultivation the bacteria start to form non-reproductive, metabolically dormant endospores, making it prohibitive to use fed-batch or continuous bioprocessing techniques. As both endospore formation and solvent production are forms of stress response in solventogenic *Clostridium* sp., the regulation of the two pathways are tightly linked. Spo0A in particular is a master regulator which, in its active, phosphorylated form, controls the expression of various genes in both the sporulation network and the solventogenic pathway. Thus mutations which knock out sporulation have been shown to also impact negatively on solvent production. In contrast, through random mutagenesis GBL has developed an asporogenic *Clostridium* sp. which retains wild type solvent production characteristics. This project aims to investigate the effect of the resultant mutations on gene expression and protein activity, and how the interplay between them gives rise to the asporogenic phenotype.

002 - Genetic Transformation of *Heliobacterium modesticaldum*: Progress and Challenges

BAKER, PATRICIA L., REDDING, KEVIN E.
Arizona State University, School of Molecular Sciences

Heliobacteria are members of the order Clostridiales and form the only group of phototrophs in the Firmicutes, making them an interesting subject of investigation. *Heliobacterium modesticaldum* is a choice candidate for genetic analysis because the genome of this organism has been sequenced and annotated. It is also a moderate thermophile and grows optimally at 52°C, facilitating protein isolation and biochemical studies. Although there is a great deal of interest in further studying the structure and function of proteins and the physiology of this organism, studies have been hampered by the lack of a genetic transformation system. We have succeeded in transforming this organism by cloning and expressing four DNA methyltransferase genes of *H. modesticaldum* in *E. coli*. This plasmid-bearing *E. coli* is used to conjugate pre-methylated plasmids into *H. modesticaldum*. That plasmids have been introduced and replicate is shown by recovery of plasmids from Heliobacteria. For this recovery, a modified plasmid mini-preparation procedure was developed to prevent DNAses present in the cell wall from destroying plasmid DNA. We have also attempted to make knockout mutants through homologous recombination by conjugating plasmids that do not have an origin of replication for gram-positive bacteria. In all of these attempts, antibiotic-resistant colonies have been observed and PCR confirms that the plasmids have integrated into the genome. In attempts to knock out the photosynthetic reaction center, a color change of the colony from brown to pink is seen. However, no stable double crossover mutants have been isolated, as has been seen previously in many species of *Clostridium*. PCR amplification of genomic DNA using primers that anneal outside of the region for homologous recombination show that wild-type, single integrant and double integrant genotypes may all be present in DNA from a single isolated colony. This result suggests that *H. modesticaldum* is functionally polyploid. Current attempts to delete genes focus on use of *mazF* as a negative selective marker and use of a targeted *cas9* gene. So far, the challenges we have faced are mirrored in the *clostridial* literature, demonstrating that they may be a general feature of the Clostridiales.

003 - Application of Flow Cytometry in *Clostridial* Spores Enumeration

BRANSKA, BARBORA, JAN KOLEK, MAREK DRAHOKOUPIL, PETRA PATAKOVA
University of Chemistry and Technology Prague, Department of Biotechnology, Technická 5, Prague 6, 166 28. barbora.branska@vscht.cz

Formation of highly resistant spores is one of the main characteristic of the genus *Clostridium*. Process of sporulation might be related to solvent production, however, this theory has been challenged both by existence of scarcely sporulating, solvent producing cells and later by implementing high throughput methods such as flow cytometry in morphological changes monitoring. Interconnection between spore formation and solvents production remains open-ended question. Clostridial spores formation can be monitored by phase contrast microscopy, but such methodology is very laborious enabling only limited number of particles counted. On the other hand, flow cytometry provides a tool for rapid quantification of spores within *clostridium* culture, however crucial task is unambiguous identification of spores. Recent literature suggests methodology based on light scatter characteristics for *C. acetobutylicum*. However, morphology of *Clostridium* spores is not uniform in different strains and in case of e.g. *C. pasteurianum* or *C. beijerinckii*, we found out, that light scatter characteristics did not enable clear spore identification. Therefore fluorescence staining strategies such as combination of Syto 9/propidium iodide or carboxyfluorescein diacetate/propidium iodide were tested. In case of carboxyfluorescein diacetate/propidium iodide combination, spore population was differentiated based on inability of both probes to penetrate spores envelop while vegetative cells were stained either red (dead) or green (alive). Syto 9 was found to stain both *C. pasteurianum* and *C. beijerinckii* mature spores with different fluorescence intensity so that it could be recognised from the vital cells. Both proposed methodologies are based on staining of fresh vital culture, which requires immediate measurement after sample collection, therefore Syto 9/propidium iodide staining strategy was tested on heat fixed culture. Division of culture into two sub-populations was expected: red - propidium iodide stained cells (heat fixed vegetative cells) and green - Syto 9 stained spores. Surprisingly, microscopic observation revealed vegetative rod shaped cells stained as vital even after 6 minutes at 95°C fixation. Simultaneously the fluorescence staining pattern of clostridial spores was not influenced by heat treatment.

Financial support from specific university research (MSMT No 20-SVV/2016)

004 - Kinetic Modeling of Metabolism in *Clostridia*

DASH, SATYAKAM[1], ALI KHODAYARI[1], EVERT K. HOLWERDA[2], DANIEL G. OLSON[2], M. AHSANUL ISLAM[3], MATTHEW THEISEN[4], JAMES C. LIAO[4], GREGORY STEPHANOPOULOS[3], LEE R. LYND[2], AND COSTAS D. MARANAS[1]

[1]The Pennsylvania State University; [2]Thayer School of Engineering at Dartmouth College; [3]Massachusetts Institute of Technology; [4]University of California, Los Angeles.

Clostridia have broad and flexible systems for substrate utilization such as *C. thermocellum* which metabolizes cellulose and *C. ljungdahlii* that metabolizes syngas to produce biofuels, but they remain poorly characterized with significant uncertainty in their metabolic repertoire. In this study, we develop dynamic metabolic models for the two organisms using the Ensemble Modeling (EM) paradigm which requires curated genome-scale metabolic (GSM) model of the organism as its foundation. For *C. thermocellum*, we constructed a second-generation GSM model (iCth446) with 446 genes, 598 metabolites and 660 reactions, along with gene-protein-reaction associations by updating cofactor dependencies, maintenance (GAM and NGAM) values and resolving elemental and charge imbalances. iCth446 model was subsequently used to develop k-ctherm119, a kinetic model of *C. thermocellum*'s central metabolism containing 119 reactions and 93 metabolites with cellobiose as the carbon source under anaerobic growth condition. k-ctherm119 encompasses the cellobiose degradation pathway, glycolysis/gluconeogenesis, the pentose phosphate (PP) pathway, the TCA cycle, pyruvate metabolism, anaplerotic reactions, alternative carbon metabolism, nucleotide salvage pathway, along with all biomass precursors and 22 substrate level regulatory interactions extracted from BRENDA. The kinetic model parameters were estimated by simultaneously imposing the mutant library data recently measured and provided by the Lynd group. This dataset includes 22 *C. thermocellum* mutants with genetic perturbations in lactate, malate, acetate, and hydrogen production pathways and combinations thereof with measured concentrations of various fermentation products such as acetate, lactate, formate, hydrogen, pyruvate, ethanol, and cellobiose (19 measured concentrations per mutant). The kinetic model accurately predicted metabolic phenotypes in multiple mutant strains not included in the model parametrization. Examples include cytosolic concentrations for fourteen out of eighteen metabolites in the Δ*ldh* mutant. The kinetic model also alludes to a systemic level effect of limiting nitrogen source resulting in increased yields for lactate, pyruvate and amino acids and an increase in ammonia and sugar phosphates concentrations due to down-regulation of fermentation pathways under ethanol stress. Robustness analysis of the kinetic model revealed the presence of secondary activity of ketol-acid reductoisomerase and its regulation by valine and leucine pool levels. A similar effort is also underway for *C. ljungdahlii*. A core metabolic model composed of 77 reactions and 63 metabolites was constructed with 41 substrate level regulatory interactions from BRENDA based on other *Clostridia* species. The network spans glycolysis, the Wood-Ljungdahl pathway, and TCA cycle. Experimental flux data for a wild-type and mutants will be used to estimate the core model parameters using the previously discussed framework. The constructed kinetic models will be ultimately used to explore metabolic drivers that underpin the over-production of iso-butanol in *C.thermocellum* and acetate in *C. ljungdahlii*.

005 - Nitrate reduction occurs simultaneously to growth of *Clostridium ljungdahlii* on CO_2 and H_2, and Acetogenesis

EMERSON, DAVID FREDERIC; BENJAMIN WOOLSTON; DEVIN CURRIE; AND GREG STEPHANOPOULOS
Massachusetts Institute of Technology

Syngas fermentation via the Wood-Ljungdahl pathway has received considerable attention as a possible platform for the renewable production of fuels and chemicals. However, this pathway operates near the thermodynamic limit of life, leading to low ATP production and long doubling times, and calling into question the feasibility of producing high-energy compounds at industrially relevant titers, yields and rates. In this work, we investigated the possibility of co-utilizing nitrate (NO_3^-) as an additional electron acceptor to enhance ATP production during autotrophic growth of *C. ljungdahlii*. In contrast to *M. thermoacetica*, which cannot grow autotrophically in the presence of NO_3^-, we were able to achieve robust growth of *C. ljungdahlii* in mixtures of H_2, CO_2, and NO_3^-. These results were confirmed by 1) monitoring $^{13}CO_2$ incorporation into acetate, which occurred simultaneously with nitrate reduction, and 2) an increased carbon yield of 0.261 ± 0.011 mol cell carbon per mol acetate, compared to 0.061 ± 0.004 for autotrophic growth in the absence of nitrate. Efforts are underway to understand the basis of the biochemical and transcriptional regulation of the ratio of electron partitioning between CO_2 and NO_3^-. This fundamental characterization will inform engineering strategies for controlling this ratio in order to maximize the production of non-native products.

006 - SYNPOL- Biopolymers from syngas Fermentation

C. ERZ, S. FLÜCHTER, S. HOFFMEISTER, AND P. DÜRRE

Expected depletion of oil and fossil resources urges the development of new alternative routes for production of bulk chemicals and fuels beyond petroleum resources. An alternative process is offered by 3rd generation of biotechnological fermentation which is based on utilization of synthesis gas (syngas; mainly consisting of CO and H_2). Acetogenic bacteria are known for their ability to use CO along with H_2 and CO_2 as energy and carbon source via Wood-Ljungdahl pathway (WLP). Thereby, main product of respective metabolic pathway is acetic acid, alongside with ethanol, 2,3-butanediol (2,3-BD), and butyrate as byproducts (Schiel-Bengelsdorf and Dürre, 2012; Bengelsdorf et al., 2013).

Aim of SYNPOL is to build a platform that integrates biopolymer production by modern processing technologies, with bacterial fermentation of syngas, and the pyrolysis of highly complex biowastes (e.g., municipal, commercial, sludge, agricultural). Focus of this work is establishment of 2,3-BD as well as 3-hydroxybutyrate (3-HB) production in *Clostridium ljungdahlii* and *C. coskatii*. Furthermore, 2,3-BD and 3-HB should serve as precursors for construction of new bioplastics.

C. ljungdahlii harbors genes for a natural 2,3-BD production pathway and can produce autotrophically approx. 4 mM 2,3-BD. Thereby, pyruvate is converted via the enzymes acetolactate synthase (AlsS), acetolactate decarboxylase (BudA), and 2,3-butanediol dehydrogenase (Bdh) into 2,3-BD. Respective genes were cloned into the *E. coli-Clostridium* sp. shuttle vector pJIR750, resulting in plasmid pJIR750_23BD. Autotrophic growth experiments using *C. ljungdahlii* [pJIR750_23BD] demonstrated that homologous overexpression of genes responsible for 2,3-BD production led only to a slight increase in 2,3-BD concentration (6.5 mM).

In case of 3-HB production, *C. coskatii* was chosen as a suitable acetogenic bacterium. Acetyl-CoA, central intermediate of WLP, serves as precursor of recombinant 3-HB production. Enzymes thiolase A (ThlA) and acetoacetyl-CoA:acetate/butyrate CoA transferase (CtfA/CtfB) from *C. acetobutylicum* and 3-hydroxybutyrate dehydrogenase (BdhA) from *Peptoclostridium difficile* convert acetyl-CoA into 3-HB. Respective genes were cloned into *E. coli-Clostridium* sp. shuttle vector pMTL83151, resulting in plasmid pMTL83151_tcb. After successful transformation of pMTL83151_tcb into *C. coskatii*, heterotrophic growth experiments (40 mM fructose) showed that *C. coskatii* [pMTL83151_tcb] produced 21 mM 3-HB. However, under autotrophic growth conditions 3-HB production was not observed.

007 - Engineering Gas-Fermenting *Clostridia* for Production of Chemicals and Biofuels

CHAI, CHANGSHENG, HE HUNG, NING LI, DECHEN, JIA, SHENG YANG, WEIHONG JIANG, YANG GU
Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China

The use of fossil fuels is no longer tenable and their use is damaging the environment through pollution and global warming. Alternatively, environmentally friendly sources of chemicals and fuels are required. One alternative is to directly capture carbon before incorporation into lignocellulosic biomass. Acetogenic bacteria, typified by *Clostridium ljungdahlii* and *Clostridium carboxidivorans*, are able to capture carbon (CO or CO_2) through the Wood-Ljungdahl pathway, allowing them to grow on a spectrum of waste gases from industry (e.g., steel manufacture and oil refining, coal and natural gas). They can also consume 'synthesis gas' (mixtures of CO, CO_2 and H_2) made from the gasification of renewable resources, such as biomass in the form of domestic or agricultural wastes. Here, we report the development of a highly efficient CRISPR/Cas9 system and TargeTron system for rapid genome editing of *Clostridium ljungdahlii*, as well as metabolic design and engineering of this anaerobe to produce a variety of important chemical and biofuels using C1 gases. Besides, through the optimization of medium and fermentation condition, the production of 1-butanol and hexanol by *Clostridium carboxidivorans* P7 were greatly improved using mixed CO and CO_2.

010 - Molecular Regulation of Sugar Utilization and Solvent Production in Solventogenic *Clostridia*

YANG, YUNPENG, SHENG YANG, CHEN YANG, YANG GU, WEIHONG JIANG
Key Laboratory of Synthetic Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

Clostridia-based fermentation is a traditional and recently reviving industrial-scale biological process for producing solvents and chemicals. To get ideal performance for co-utilizing multiple carbon sources from biomass and efficiently producing solvents, we need to better understand and further modify the metabolic and regulatory systems of solventogenic clostridia.

In our work, two representative strains, *Clostridium acetobutylicum* and *Clostridium beijerinckii*, were investigated for their transcriptional regulatory systems of sugar utilization and solvent production. A range of regulatory proteins were identified and their functional mechanisms were revealed detailedly, such as a three-component system XylFII-Lyts-YesN and the catabolite control protein A (CcpA) that are responsible for regulating sugar utilization; three AbrBs involved in acidogenesis, solventogenesis and a two-phase transition; an unknown protein Ups1 related to cell growth and solvent production, et al. In addition, metabolic engineering based on these regulatory elements was carried out and a series of modified strains with improved performance were derived from *C. acetobutylicum* and *C. beijerinckii*. These findings provide new insights into metabolic regulation and genetic modification for solventogenic clostridia.

012 - Influence of DNA Methylations on *C. pasteurianum* NRRL B-598 Transformation Efficiency and Development of Methods for Electrotransformation, Sonoporation and Sono/Electroporation

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The existence of methods for genetic manipulation of industrial microorganisms is generally essential for improving their properties like productivity or resistance against their own products. These methods are also very important for quicker and more effective research that could lead to the fast acquisition of important information useful in this process. First step of genetic manipulation is generally introduction of foreign DNA molecule into the bacterial cell and the most commonly used methods for introducing DNA molecule into bacterial cells are transformation (when exogenous molecule of DNA is introduced directly through the cell membrane), conjugation (provided by tight contact of cells and pili formation) and transduction (provided by viral particle).

We described step-by-step development of methods for genetic modification of *C. pasteurianum* NRRL B-598 which represents solventogenic bacterium producing butanol, acetone and negligible amount of ethanol and which is unique in its exceptional oxygen resistance. Because all initial attempts at plasmid transformation of our strain failed, we decided to perform a detailed bioinformatics analysis at first. The main purpose was to reveal genes encoding putative restriction-modification (R-M) systems that could present a problem during transformation of clostridia, and genes encoding putative DNA methyltransferases that could be connected with these R-M systems for protection of their own DNA. Potential restriction modification systems were investigated also by *in vitro* assay with protoplast extract and whole cell extract. It was obtained that Dam and Dcm methylation free DNA vector is necessary to use for good transformation efficiency.

Best conditions for conjugation and electrotransformation were described. Sonoporation, method based on the ultrasound-mediated cavitation of bacterial membrane, was revealed as new, very efficient transformation technique for *C. pasteurianum* NRRL B-598. This method does not require any special equipment and is very fast, which could be very important parameter for anaerobic bacteria transformation. It was also revealed that sonoporation could be used as step foregoing electroporation which provides much more transformation efficiency.

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013 - Metabolic Cell-Process Engineering Guided by Systems Biology for High Production of Bioenergy

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n-Biobutanol is a green, sustainable and environmentally benign biofuel that can be used to substitute for gasoline and generate bio-jet fuel. Extensive research has been performed to improve biobutanol production by metabolic cell engineering of clostridia, followed by a fermentation development, but the productivity of biobutanol is still relatively. In this study, we developed an entirely new approach, i.e. Metabolic Cell-Process Engineering (MCPE), to improve the biobutanol production by acidogenic *Clostridium tyrobutyricum via* understanding the cellular metabolism using proteomics and metabolite analysis. The key regulators of cell and process were identified and manipulated rationally. Our results showed that this approach led to significantly higher biobutanol production (titer >20 g/L and yield >0.4 g/g-sugar). In addition, we built a complete proteomics map (PeptideAtlas no. PASS00596), covering 2,374 intracellular proteins, 78.1% of open reading frames, and 95% of core enzymes in glycolysis, central metabolism and end-products formation. Currently we are applying this novel MCPE technology to other clostridia for n-butanol and other biochemicals production. We expect to accelerate the commercialization of n-butanol as a biofuel using this metabolic engineering platform in future.

015 - *Clostridium pasteurianum* NRRL B-598, Physiology of No-Name Solventogenic *Clostridium* under Different Cultivation Conditions

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Clostridium pasteurianum NRRL B-598, obtained from the ARS Culture Collection (USA), was selected for further extensive tests after screening of different solventogenic clostridia. The strain boasts unusual oxygen tolerance and stamina (the will to grow and recover even after adverse interventions to its environment) which were the main reasons for the selection. The genome of the strain consists of a single circular 6,186,879 bp chromosome and the structure of *sol* operon reminds that of *C.beijerinckii* NCIMB 8052, having *adhE* gene alternated with *ald.* Based on genomic and phenotypic traits, it seems the strain was not classified properly and should be re-classified as *C.beijerinckii* or *C.diolis*.

The production patterns of required metabolites, namely solvents, were studied using batch, fed-batch and continuous fermentation systems with glucose as a model substrate. The highest total solvent concentration (12.3 g/L) was reached in fed-batch process, while the highest solvent yield and productivity (32 % and 0.73 g/L/h) were achieved in packed-bed continuous fermentation at a dilution rate 0.12 1/h. For deeper insight into the population heterogeneity and recognition of cell subpopulations in different stages of sporulation cycle, flow cytometry with double staining using carboxy fluorescein diacetate and propidium iodide was used. The method enabled to enumerate mature spores (released from mother cells) and to estimate culture viability and metabolic activity.

Under standard and stress conditions (butanol addition), changes in membrane saturated fatty acids and plasmalogens were described by high resolution electrospray ionization tandem mass spectrometry. It was found that induced stress intervention (butanol addition) caused quick increase in both markers, saturated fatty acids and plasmalogens and thus it seems that syntheses of these compounds are significant components of the complex stress response of the strain.

In addition, an efficient, unique transformation method based on sono/electroporation has been developed *de novo* for the strain. This technique is extremely important for deeper understanding of the strain behavior on genomic level which is our future goal.

This work was supported by the project BIORAF No. TE 01020080 awarded by the Technological Agency of the Czech Republic and from specific university research (MSMT No 20-SVV/2016).

016 - Development of a *Clostridium pasteurianum* Genetic Toolkit: Gene Disruption, CRISPR-Cas Genome Editing, and Genome Sequencing

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Clostridium pasteurianum is an apathogenic, Gram-variable bacterium that can be cultivated in chemically defined medium, is not subject to strain degeneration, and is capable of utilizing electrons derived directly from electric current. The organism has emerged as a prospective host for the production of *n*-butanol owing to its capacity to ferment low-value industrial byproducts, such as biodiesel glycerol and thin stillage. It is clear that genetic and metabolic engineering will play a central role in the development of a bioprocess based on *C. pasteurianum*. Here, we recapitulate our 7-year effort to establish a versatile genetic toolkit for engineering the central metabolism of *C. pasteurianum*.

Beginning with the optimization of an electrotransformation methodology, we established techniques for gene disruption, knockdown, and overexpression in *C. pasteurianum*. Group II intron technology was employed to generate a mutant devoid of Type II restriction activity that can be efficiently transformed with unmethylated DNA. We also inactivated the 1,3-propanediol pathway, which led to a dramatic shift in end product distribution despite unaltered fermentative glycerol utilization. To further guide strain engineering efforts, we sequenced the *C. pasteurianum* genome, which was found to harbor an excisable prophage and a central Type I-B CRISPR-Cas locus. We show that the endogenous Cas machinery can be co-opted for genome editing and provides a superior platform to established strategies based on heterologous Cas9. We expect that the genetic engineering technologies resulting from these efforts will promote extensive metabolic engineering of *C. pasteurianum*.

017 - Butanol Biorefineries: Simultaneous Product Removal & Process Integration for Conversion of Biomass & Food Waste to Biofuel

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Butanol, a superior biofuel, packs 30% more energy than ethanol on a per gallon basis. It can be produced from various carbohydrates and lignocellulosic (biomass) feedstocks. For cost effective production of this renewable and high energy biofuel, inexpensive feedstocks and economical process technologies must be used. These feedstocks include corn stover (CS), switchgrass, miscanthus, sweet sorghum bagasse (SSB), sugarcane bagasse (SB), and wheat, barley, and rice straws. Because CS is inexpensive and readily available in the mid-western part of the United States, we have focused on production of butanol from this substrate. Food waste is another promising inexpensive substrate. According to the USEPA more than 33 million tons of food waste were generated in 2012 alone in the United States. Conversion of food waste to biofuel would potentially reduce environmental pollution and result in sustainable energy production. In addition, we focused on combining pretreatment (for CS), simultaneous saccharification, fermentation, and recovery technologies for butanol production from these substrates. The novel product recovery technique applied was vacuum fermentation, which offers high butanol removal rates. Prior to these studies, we were unable to ferment CS hydrolyzate due to the presence of toxic substances. The concepts presented in these studies are expected to become a reality for the economic production of this novel biofuel.

018 - *Moorella thermoacetica*, a workhorse creating value from various gaseous substrates

TORBJØRN ØLSHØJ JENSEN, STEPHANIE REDL, AND ALEX TOFTGAARD NIELSEN

The fermentation of waste gas streams to produce high value compounds is an attractive alternative to traditional biomass hydrolysate fermentation. Industrial waste gases as well as carbon- and energy-rich syngas obtained from gasification of organic-residues can serve as substrate for acetogenic bacteria, but are left unused to date.

Moorella thermoacetica is the model acetogenic bacterium and an ideal production organism for gas fermentation processes. Its ability to grow at elevated temperatures of 60°C allows recovery of chemical compounds that have a low boiling point (such as acetone) from the vapor phase.However, production of higher value compounds using *Moorella* requires a better understanding of its metabolism, as well as reliable tools that enable genetic modification.

In the pursuit for making *M. thermoacetica* into an industrially relevant production strain, we studied various relevant aspects: Assessing the cost-effectiveness of acetone production utilizing *M. thermoacetica* as production host, analyzing the response to different substrates by looking at variation in the expression profile (RNA-seq). Technology enabling reliable genetic engineering is very limited, and we have focused on overcoming this challenge through development of selection systems, and improving the transformation.

The gained expertise on cell-level and on process scale will help to make the former model organism an industrially relevant organism for converting waste gas streams into valuable compounds.

019 - CO_2 Fixation for Increased Microbial Fermentation Yields

ELLINOR D. SCHMIDT AND ELEFTHERIOS T. PAPOUTSAKIS

Atmospheric CO_2 concentrations have increased significantly in recent years due to human activities leading to a substantial threat to our climate on earth. Renewable transportation fuels generated from waste gases hold large promise in reducing greenhouse gases such as CO_2. One potential renewable fuel is the microbial fermentation product biobutanol. Biobutanol has many advantages over other potential fuels as it is compatible with our current transportation fuel infrastructure, has a high energy density and is immiscible with water. Currently, traditional fermentations employing carbohydrate substrates to produce butanol and other reduced products are limited by the decarboxylation reactions occurring during glycolysis. As a result, only a maximum of two-thirds of the carbon contained in the feedstock can be recovered in fermentation products. The other third of the feedstock is lost as undesirable CO_2. In order to increase butanol yield, we propose to re-assimilate the evolved CO_2 via the Wood-Ljungdahl pathway (WLP). In addition, exogenous CO_2 added to the fermentation provides an additional cheap and abundant feedstock. We have shown improved product mass yield in four microorganisms which natively contain the WLP and demonstrated the enhanced product yields when grown in the presence of carbohydrates and exogenous gases. We used carbon tracing experiments to show the carbon uptake was continuous and independent of the presence of higher-energy substrates. We show that this type of anaerobic, non-photosynthetic (ANP) mixotrophic fermentation is capable of overcoming the shortcomings in the current production of biofuels via traditional fermentations. To employ the concept of mixotrophy for the successful production of biobutanol in an industrially relevant strain, we over-expressed two essential genes found in the WLP in *C. acetobutylicum*, which is known for its high product titers of n-butanol and its diverse substrate utilization. With the overexpression of the carbon-monoxide dehydrogenase/acetyl-CoA enzyme system in *C. acetobutylicum* we show the reduction of CO_2 and interconversion of CO and CO_2, showing the potential of the water-gas shift reaction in an engineered biological host. With our engineered organism we investigated the activity of the enzymes to utilize CO_2 and achieve re-assimilation of CO_2 into the fermentation products of *C. acetobutylicum* during solventogenesis.

021 - Optimal Utilization Of Seaweeds In Integrated Biorefineries

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The green seaweed *Ulva lactuca* is a promising feedstock for biobased economies due to its unique composition. It contains a wide variety of carbohydrates and large amounts of protein and ash. It can therefore be used for the production of biofuels, chemicals or animal feeds in integrated biorefineries [1, 2]. In addition to its composition, *U. lactuca* possess other advantages compared to other first and second generation feedstocks, *U. lactuca* does not compete for arable land as it grows in the sea . Moreover, it is possible to produce large quantities of seaweeds in a short time due to their fast growth rates and high biomass densities. In a previous study performed in our institute, it was shown that rhamnose, the main sugar in *U. lactuca*, can be fermented to acetone, butanol, and ethanol (ABE) and 1,2-propanediol (1,2-PD) by *Clostridium beijerinckii* NCIMB 8052 [3]. The catabolic pathway of rhamnose, however, is not yet well studied in the organism. In the frame of the FP7 Marie Curie RENESENG project (grant number 607415), the catabolic route of rhamnose will be characterized in *C. beijerinckii* NCIMB 8052.

Bioinformatical studies have revealed that enzymes degrading rhamnose to (1,2-PD) are encoded in the genome of *C. beijerinckii* NCIMB 8052. Interestingly, enzymes disproportioning 1,2-PD to 1-propanol and propionate were also found. These are encoded in a gene cluster with bacterial microcompartment (BMC) proteins indicating that the degradation of 1,2-PD might occur in BMCs. As 1-propanol and propionate were only detected in cultures that were grown solely on rhamnose, we assume that rhamnose induces the formation of BMCs and the disproportioning of 1,2-PD. To confirm this hypothesis, fermentations will be performed with knock-out strains that were generated with the CRISPR-Cas9 system. Additionally, transmission electron microscopical images will be generated to show the presence of BMCs. Lastly, the enzymes involved in the rhamnose and 1,2-PD degradation will be characterized in more detail.

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022 - Thermophilic Anaerobic Bacteria for Bioenergy production by Consolidated Bioprocessing

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Lignocellulosic biomass derived from agricultural, municipal, and forestry operations are a renewable resource that can be used for the production of reliable forms of energy, such as biofuels and hydrogen. The benefits of using lignocellulosic biomass as raw material for biofuel production processes is attributed to abundance, relatively low expense and potential for global distribution. But the major challenge is effective breakdown of the recalcitrant cellulose structure, and efficient bioconversion to liquid fuels.

The conversion of biomass through biochemical techniques is typically achieved through unit operations like pretreatment, hydrolysis, and fermentation which are normally done in separate reactors and the process configuration is called Separate Saccharification and Fermentation (SF). While pretreatment process occurs in separate reactor, hydrolysis and fermentation can be combined in some process configuration known as Simultaneous Saccharification and Fermentation (SSF) that require exogenous addition of enzymes. Though this process reduces unit operations but faces the challenge of optimal performance as the optimal temperatures of enzymes for saccharification and yeast for fermentation differs widely.

A highly desirable process configuration that reduces the unit operations is consolidated bioprocessing (CBP), a conversion approach, that combines all steps of saccharification, and fermentation in a single reactor without addition of any external enzymes. Thermophilic anaerobes have received a renewed interest by the researchers all across the globe due to their wide range of substrate utilization and unique cellulolytic and hemicellulolytic system. The thermophilic anaerobes that can represent CBP mainly belong to genus *Clostridium, Caldicellulosiruptor, Thermoanaerobacter,Thermoanaerobacterium* and *Geobacillus* etc.

Amongst, them *Clostridium thermocellum* and other species has received increased attention as a high utility CBP candidate due to its highest growth rate on crystalline cellulose, presence of highly efficient cellulosome system, ability to produce ethanol directly from cellulose and availability of genetic and molecular tools. With this view, we have specifically screened sugar fermenting, cellulolytic and xylanolytic thermophilic anaerobic ethanol producing bacteria, from unexplored hot spring/s in India. Few isolated thermophiles have direct ethanol conversion ability from simple sugars as well as complex biomass with ~60-70% ethanol yield of theoretical maximum and with further improvement can be considered as a potential host for single step CBP process.

023 - Arginine boosts growth of the gas-fermenting bacterium *Clostridium autoethanogenum*

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Clostridium autoethanogenum is an attractive platform organism for the production of chemicals and fuels from inexpensive industrial waste gases (CO, CO_2 and H_2). Expanding its product spectrum is fundamentally limited by ATP availability. Although acetogens have evolved sophisticated strategies to conserve energy from potential differences between major redox couples, this coupling is sensitive to small changes in thermodynamic equilibriums, making acetogens favour synthesis of native products over heterologous ones. Thus to accelerate their engineering, alternative ATP generating pathways need to be explored. To this aim, a refined genome-scale metabolic model (GEM) of *C. autoethanogenum* was used in this work to analyse carbon, energy and redox balances, and for developing a chemically defined medium (CDM) that would favour ATP generation.

Shadow price analysis using our refined GEM revealed *C. autoethanogenum*'s preference for nine amino acids (AA). This prediction was experimentally confirmed for eight AAs with special preference for aspartate, glutamate, histidine and arginine. Subsequent *in silico* simulations showed growth on those AAs with similar rates (t_D of ~2-4h) to growth on 20 AAs. Indeed, substantially faster growth compared to yeast extract (t_D~9h) was measured in media containing those four AAs (t_D~3h) and only arginine (t_D~4h). The growth-boosting effect of arginine was confirmed in autotrophic growth. Metabolic modelling showed that the observed low synthesis of acetate and fast growth are realised by a three-fold increase of the total specific ATP production rate resulting from the activity of the arginine-deiminase (ADI) pathway. The involvement of the ADI pathway was experimentally confirmed by metabolomic and transcriptomic analyses. Notably, RNA-sequencing revealed a ~500-fold up-regulation of the ADI pathway genes. Interestingly, genes of the Wood-Ljungdahl pathway were down-regulated.

The data presented here offer a potential way of supplying cells with much-needed energy for the synthesis of ATP-intensive products. Our work also demonstrates the usefulness of metabolic modelling for designing a CDM and expanding the product spectrum of acetogens.

025 - Butyric Acid Production by *Clostridium thermobutyricum* at 50°C

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Butyric acid is a commodity chemical produced at industrial scale by chemical synthesis and by fermentation. Butyric acid is used as a feedstock for synthesis of plastics and it also severs as a precursor to produce butanol which is a next generation fuel with high energy content, less hygroscopic, less corrosive and high compatibility to current infrastructure. Production of butyrate from renewable biological feedstocks rather than from petroleum has the additional benefit of supporting rural economies.

Our recent research demonstrates that a moderate thermophile *C. thermobutyricum* can be an effective microbial biocatalyst for production of bio-based butyrate from renewable feedstocks. It is shown to ferment the sugars in sweet sorghum juice at 50 °C to 44 g L-1 butyrate at a calculated highest volumetric productivity of 1.45 g L-1 h-1. This volumetric productivity is among the highest reported for batch fermentations. Sugars from acid and enzyme-treated sweet sorghum bagasse were also fermented to butyrate by this organism with a molar yield of 0.81 (based on the amount of cellulose and hemicellulose). By combining the results from juice and bagasse, the calculated yield of butyric acid is approximately 90 kg per tonne of fresh sweet sorghum stalk.

027 - Efficient and precise genome editing and gene transcription repression in *Clostridium beijerinckii* using CRISPR-Cas9 system

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CRISPR-Cas9 has been demonstrated as a transformative genome engineering tool for many eukaryotic organisms; however, its utilization in bacteria remains limited and ineffective. Here we explored *Streptococcus pyogenes* CRISPR-Cas9 for genome editing in *Clostridium beijerinckii* (industrially significant but notorious for being difficult to metabolically engineer) as a representative attempt to explore CRISPR-Cas9 for genome editing in microorganisms previously lacked sufficient genetic tools. By combining inducible expression of Cas9 and plasmid-borne editing templates, we successfully achieved gene deletion and integration with high efficiency in single steps. We further achieved single nucleotide modification by applying innovative two-step approaches, which do not rely on availability of Protospacer Adjacent Motif sequences. Severe vector integration events were observed during the genome engineering process, which is likely difficult to avoid but has never been reported by other researchers for the bacterial genome engineering based on homologous recombination with plasmid-borne editing templates. We then further successfully employed CRISPR-Cas9 as an efficient tool for selecting desirable 'clean' mutants in this study. Besides, we also successfully applied CRISPR-dCas9 (dead Cas9) for efficient repressive control of gene expression. The approaches we developed are broadly applicable and will open the way for precise genome editing in diverse microorganisms.

028 - Genetic Methods to Enable Metabolic Engineering of *Clostridium themocellum*

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We recently developed a series of plasmid vectors for genetic manipulation of the cellulolytic thermophile *Caldicellulosiruptor bescii* and used them for the expression of homologous and heterologous genes for metabolic engineering. Surprisingly, they have also proven to be useful for clostridial species, notably *Clostridium thermocellum*, a cellulolytic thermophile that grows and produces ethanol from plant biomass at an optimal temperature of 60°C (Groom et al., Metabolic Engineering Communications 3: 30-38). Published genetic methods for *C. thermocellum* allow transformation of plasmid DNA, the generation of gene deletions and some methods for gene expression. A limitation to the engineering of this strain is the availability of stably replicating plasmid vectors for rapid homologous and heterologous expression of genes that provide improved or novel pathways for fuel production. Current vectors rely on replicons from mesophilic bacteria and are not stable at the optimum growth temperature of *C. thermocellum*. Phylogenetic analyses of the proteins contained on the cryptic thermophilic *C. bescii* plasmid pBAS2 revealed that the replication initiation protein RepL is unique among thermophiles. We also observed the requirement for the plasmid-encoded recombinase XerD. Vectors derived from pBAS2 transform *C. thermocellum* DSM 1313 at 60°C, with a transformation efficiency > 3000 colony forming units per μg of DNA and a copy number ~10 per chromosome. We used both homologous *C. thermocellum* and heterologous *C. bescii* promoters to express the heterologous butanol dehydrogenase enzyme BdhA from *Thermoanaerobacter pseudethanolicus*. Vectors with homologous promoters resulted in integration into the chromosome but still allowed expression. To facilitate autonomous plasmid replication we have generated a deletion of the *recA* gene to prevent plasmid integration into the chromosome.

029 - A short, linear oligopeptide regulates solvent formation and sporulation in *Clostridium acetobutylicum*

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Many bacteria are known to communicate via small, diffusible signal molecules to coordinate gene expression in response to cell population density (quorum sensing). While quorum sensing has been extensively studied in Gram-positive species of the genera *Bacillus*, *Staphylococcus* and *Streptococcus*, very little is known about its role in the class *Clostridia*.

Previously, we have shown that *Clostridium acetobutylicum* contains at least nine putative quorum sensing systems of the RNPP-type family, all of which rely on peptide-based signal molecules. Mutational analyses suggested that most of these, either directly or indirectly, influence solvent formation.

Here we report that one particular system, termed QssB, regulates early solvent formation and sporulation. QssB consists of the RNPP-type regulator QsrB and a linear signaling peptide derived from the precursor protein QspB. Inactivation of *qsrB* increased early solvent production, while its overexpression drastically reduced both solvent and spore formation. The inhibitory effects of *qsrB* overexpression could be overcome by the addition of synthetic peptides representing C-terminal sections of QspB. These findings are consistent with QsrB acting as a repressor, which is deactivated after binding to its cognate QspB-derived peptide signal

Using a series of sixteen oligopeptide variants an amino acid sequence essential for peptide activity was identified. A seven-amino acid oligopeptide containing this sequence was shown to fully restore solvent formation and sporulation in a *qsrB* over-expressing strain. Interestingly, however, addition of this and similar oligopeptides had little effect on the wildtype, suggesting that they could not be used to stimulate solvent production beyond wildtype levels. A QssB homologous system was also identified in the related species *Clostridium roseum*. However, the derived synthetic oligopeptide signal had neither activating or inhibiting effects when incubated with *C. acetobutylicum*, suggesting that there was no cross-talk between the two species.

030 - Genomic Reconstruction of σ^{54} Regulons in *Clostridia*

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The regulatory functions of σ^{54} and its interacting transcriptional activators in clostridia remain largely unexplored. I will first introduce our work on the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) regulation domain (PRD)-containing enhancer binding proteins (EBPs) that are an important class of σ^{54}-interacting transcriptional activators. The transcriptional regulons of about 50 PRD-containing EBPs are reconstructed by using a comparative genomic approach, which contain the genes associated with utilization of β-glucosides, fructose/levan, mannose/glucose, pentitols, and glucosamine/fructosamine. We then present experimental evidence that the *cel* operon involved in cellobiose utilization is directly regulated by CelR and σ^{54} (SigL) in *Clostridium acetobutylicum*. We show that CelR has an ATPase activity which is strongly stimulated by the presence of DNA containing the CelR-binding sites. Moreover, mutations in any one of the three CelR-binding sites significantly decreased the *cel* promoter activity probably due to the need for all three DNA sites for maximal ATPase activity of CelR. Our results suggest that CelR is regulated by PTS-mediated phosphorylation at His-551 and His-829, which exerts a positive effect and an inhibitory effect, respectively, on the CelR activity. In the second part of my talk, I will introduce our recent study on functions of another important type of σ^{54}-interacting transcriptional activators, which contain Per, ARNT, and Sim (PAS) domains.

032 - CO_2 fixation by anaerobic, non-photosynthetic mixotrophy for improved carbon conversion

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The major economic cost for fermentation processes is feedstock cultivation and conversion into fermentable carbohydrates. Therefore, it is vital to maximize the conversion of biomass into the product of interest. Unfortunately, for most fermentations, over one-third of the feedstock carbon is lost to CO_2 due to the decarboxylation of pyruvate to acetyl-CoA and limitations in the reducing power of the feedstock. Anaerobic, Non-Photosynthetic (ANP) mixotrophy, defined as the concurrent utilization of both organic (i.e., sugars) and inorganic (e.g., CO_2) substrates in a single microorganism, can overcome these constraints leading to increased product yields and reduction in overall CO_2 emissions. As a first demonstration of this technology, we have engineered a *Clostridium ljungdahlii* strain to produce acetone and have achieved mass yields 50% greater than the previous theoretical maximum using a high-cell density, continuous fermentation process. In addition, we show that when provided enough reductant (i.e., H_2), ANP mixotrophic fermentation processes will emit no CO_2, and that ANP mixotrophy is a general trait among acetogens.

033 - Metabolism and metabolic engineering of autotrophic acetogens

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Autotrophic acetogenic bacteria employ the so-called Wood-Ljungdahl pathway for growth, forming naturally acetate, ethanol, and/or 2,3-butanediol from gaseous substrates such as $CO_2 + H_2$ or syngas (mostly a mixture of $CO + H_2$). To date, different acetogens are used in industrial applications in pilot and demonstration plants aiming at ethanol formation from different syngas sources. A major challenge is to reengineer these bacteria metabolically for formation of other interesting chemicals, allowing fermentation with an abundant, cheap carbon source and, in parallel, even consumption of greenhouse gases.

Clostridium ljungdahlii is such an acetogen, able to ferment either organic compounds or $CO_2 + H_2$ and syngas ($CO + H_2$). The genome of *C. ljungdahlii* comprises 4,630,065 base pairs. Experimental data and *in silico* comparisons revealed differences in energy metabolism. Unlike *Moorella thermoacetica*, no cytochromes and quinones are involved in energy generation, but instead an H^+-dependent Rnf system is present, analogous to *Acetobacterium woodii* with a Na^+-dependent Rnf system. Electroporation of *C. ljungdahlii* with plasmids bearing heterologous genes for butanol production was successful and formation of the biofuel could be demonstrated. Thus, *C. ljungdahlii* can be used as a novel microbial production platform based on syngas.

As the organism does not grow well on $CO_2 + H_2$ mixtures, *Clostridium aceticum* was chosen for this type of gaseous substrate. Expression of both, heterologous butanol- and acetone-forming enzymes could be demonstrated. *C. aceticum* does contain cytochromes, but no quinones, as verified by genome sequencing. *C. aceticum* can also use syngas as a carbon source.

Finally, *A. woodii* became a model organism for autotrophic acetogens forming acetate from $CO_2 + H_2$. CO does inhibit hydrogenase and CO_2 reductase/formate dehydrogenase. *A. woodii* was metabolically engineered to produce acetone in addition to acetate by introducing and overexpressing respective clostridial genes from *C. acetobutylicum*.

034 - Physiological characterization of a degenerated variant of *Clostridium acetobutylicum* ATCC 824

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Microorganisms belonging to the *Clostridium* genus have been intensively investigated due to its butanol production capacity, a compound that has been proposed as potential replacement of fossil fuels. Despite the significant advances on this topic, the physiological changes associated to the onset of solventogenesis and sporulation are not completely understood. Moreover, solventogenic *Clostridium* spp. goes through a degeneration process characterized by the inability to produce solvents. In the case of *Clostridium acetobutylicum* ATCC824, this phenomenon is attributed to the loss of a megaplasmid (pSOL1), which carries the genes responsible for the production of solvents and sporulation [1]. According to Beveridge [2] and Jones, et al. [3], the transition from the acidogenic to the solventogenic phase in *Clostridium acetobutylicum* is associated to changes in the cell membrane that respond to GRAM(+) and GRAM(-). The aim of this research was to get a deeper knowledge about the physiological changes that are activated during the fermentation of a degenerated variant of *Clostridium acetobutylicum* ATCC 824 using the fluorescent dyes (Propidium iodine) PI and SYTO9. In this work, physiological responses commonly associated with the onset of solventogenesis and sporulation have been identified during the fermentation with the degenerated variant of *Clostridium acetobutylicum*. The monitoring of batch fermentations during 130 hours by fluorescent microscopy showed homogeneity of the culture evolution in terms of mobility, size and cell morphology, attributes that are commonly associated to an acidogenic phase, which in this case are maintained during all the culture. However, the application of the PI and SYTO9 dyes revealed physiological changes typically found in the initial stages of the solventogenesis, which are detected after 36 hours of culture. In addition, a redistribution of the cytoplasmic material can be detected after 72-hour culture with the staining procedure of SYTO9. In all cases, samples showed a double staining (PI/SYTO9), which according to Jones, et al. [3] and Tracy, et al. [4] corresponds to an acidogenic phase. Jones, et al. [3] proposed that the changes in the structure of the cell wall are consequence of unfavorable environmental conditions, mainly due to the toxicity of butanol. On the other hand, Lepage, et al. [5] concluded that the accumulation of solvents in the culture medium diminishes the thickness of the cell membrane, which could allow the entrance to the cell of SYTO9. Preliminary results obtained in this study showed that changes in the cell membrane occur in the absence of solvents and would allow to hypothesize that they are rather associated with the accumulation of acids. These changes were observed in the degenerated variant of *Clostridium acetobutylicum* at butyric acid concentrations above 5 [g L^{-1}]. However, cells produce up to 7 [g L^{-1}] of this compound, after which only lactic acid is produced up to a concentration of 12 [g L^{-1}] in a 130 hours' culture.

036 - Continuous butanol production from spent sulfite liquor with *Clostridium saccharoperbutylacetonicum*

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ABE fermentation has been known since the early 1900s. Low crude oil prices during the better part of the 20th century made biotechnological production of acetone, butanol and ethanol economically unattractive. However, with the prospect of ending crude oil reserves and the need to explore alternative liquid fuels, research efforts and attempts to optimize ABE fermentation with clostridial species have been increasing. The goal of this study was to use cheap substrates like spent sulfite liquor (SSL) as a residual stream during pulp production for conversion into value-added products. To that end, *Clostridium saccharoperbutylacetonicum* was used to develop a process for the conversion of the sugars and the acetate from SSL into acetone, butanol and ethanol. Continuous cultures turned out to be most efficient way for this transformation step, where a product yield of 0.23 Cmol/Cmol for butanol was obtained. This shows feasibility to use real industrial residual streams for production of butanol. With further development and optimization efforts, low product titers and productivities obtained so far could be improved and the process could eventually be of economic interest for pulp producers.

037 - Metabolic Engineering of *Clostridium acetobutylicum* ATCC 824 for the Production of 1,4-Butanediol

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Clostridium acetobutylicum was metabolically engineered to produce the non-natural compound 1,4-butanediol (1,4-BDO), which is currently manufactured entirely from petroleum-based feedstocks. For the new 1,4-BDO biosynthetic pathway, a 4-hydroxybutyryl-CoA dehydratase (4HBD) encoding gene either from *Clostridium aminobutyricum* (*abfD*), *Clostridium beijerinckii* (*cbei_2100*), or *Clostridium kluyveri* (*ckl_3020*), was introduced into *C. acetobutylicum*. The resulting strains were all able to produce 1,4-BDO, at concentrations of up to 41,4 mg/l using Cbei_2100. In addition to 1,4-BDO, γ-hydroxybutyrate (GHB) was also formed (up to 73,2 mg/l). Exchange of the thiolase A gene (*thlA*) against phosphotransbutyrylase (*ptb*) promoter sequences from *C. acetobutylicum* increased 1,4-BDO production by 34 % (55,8 mg/l). Furthermore, 1,4-BDO production was also possible from different carbon sources (arabinose, galactose, glucose, mannose and xylose), which are present in lignocellulosic material.

038 - Complete Hexose and Pentose Consumption by Catabolite Repression Mutants of *Clostridium acetobutylicum* ATCC 824.

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Introduction: The solventogenic bacterium *C. acetobutylicum* ATCC 824 is an organism that has been used for industrial acetone-butanol-ethanol (ABE) fermentation from renewable resources. However, these fermentations often compete with food production, so the usage of substrates that contain large amounts of pentoses such as hemicelluloses come into focus. Complete degradation of pentoses in mixtures of sugars also containing glucose is essential for the creation of new strains with improved butanol production from pentose feedstocks.

Objectives: Unfortunately, substrates that derive from hemicellulose materials also contain a certain amount of glucose that causes catabolite repression in *C. acetobutylicum*. Therefore, complete usage of pentoses is hindered. This phenomenon is well-studied in the Gram-positive model organism *B. subtilis* but little is known about catabolite repression in Clostridia. We developed a method for the isolation of numerous mutants that lack catabolite repression. Sequencing of these mutants revealed first insights in CCR of *C. acetobutylicum.*

Materials and Methods: Wild type *C. acetobutylicum* was cultivated in continuous cultures in a synthetic medium containing limiting glucose and excess xylose as carbon sources. This applied selective pressure on the cells, which promoted growth of CCR-negative mutants. Subsequent cultivation on xylose and the sugar analogue 2-deoxyglucose allowed us to isolate a couple of mutants that lacked catabolite repression when grown on a mixture of glucose, xylose, arabinose and galactose. Genome sequencing revealed the nature of the mutations.

Results: Altogether, 110 mutants were isolated. Sequencing revealed point mutations in nine genes of the PTS and frame shift mutations in signal transduction histidine kinases, in relevant permeases and in *sigD*/*whiG* sigma factor family. Several of these mutations led to new phenotypes that showed remarkable alterations in pentose metabolism comprising alleviation of catabolite repression and significantly increased consumption rates of xylose, arabinose and galactose and improved butanol production as compared to the wild type.

Conclusion: We developed a method that enabled us to create mutants of *C. acetobutylicum* with mutations that lead to the relief of catabolite repression. Genomic sequencing revealed the nature of these mutations. Next, strains with improved sugar consumption and butanol production rates will be created which could be of high biotechnological relevance.

040 - Heterologous Production of 3-Hydroxybutyric Acid in *C. ljungdahlii*

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Clostridium ljungdahlii has recently emerged as an attractive candidate for the bioconversion of synthesis gas (CO, CO2, H2) to a variety of fuels and chemicals through the Wood-Ljungdahl (WL) pathway. Here we examine another use of the WL pathway; namely the augmentation of fermentation yields on heterotrophic substrates by using reducing equivalents produced in glycolysis to fix the CO2 released, thus resulting in complete carbon conversion. As a proof of concept, we focused on 3-hydroxybutyric acid (3HB), a chiral compound that serves as feedstock in a variety of applications. First, we varied promoters, codon usage, and ribosome binding site spacing to optimize 3HB production, achieving titers of 1 g/L. Second, we implemented a CRISPRi approach to reduce flux to unwanted byproducts. Finally, we quantified the activity of the WL pathway during fermentation using isotopic tracers. The results demonstrate the ability to enhance fermentation yields above those that can be achieved in typical engineering hosts such as *E. coli* or *S. cerevisiae*.

042 - Metabolic inhibition of *Clostridium thermocellum* by pentose sugars

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Clostridium thermocellum possesses the ability to rapidly solubilize lignocellulosic biomass making this organism a potentially very effective biocatalyst for the direct conversion of dedicated energy crops to biofuels. Despite its ability to hydrolyze both cellulose and hemicellulose, the limited substrate utilization potential of the strain leaves hemicellulose-derived saccharides unutilized in culture fermentation broths. Of these hydrolysis products, xylooligomers are known to inhibit cellulase activity, but their effect on *C. thermocellum* metabolism has been relatively unexamined. Given the abundance of xylose-based polysaccharides in native and minimally pretreated biomass, understanding the degree to which they influence *C. thermocellum* metabolism and growth is important in the industrial development of the strain. In this study, we offer evidence that xylooligomers of varying size can significantly inhibit *C. thermocellum* growth, that the inhibition is isomer-specific and that xylose is a redox-reactive compound that can be metabolically transformed by the bacterium. Multiple omics-based strategies, mutational analysis and a variety of physiological studies have been employed in attempts to understand the mechanisms of inhibition. Further, we offer evidence of a quorum-sensing type signaling system in *C. thermocellum* based on a cyclic peptide which is upregulated in the presence of xylose. With a better understanding of pentose-induced metabolic inhibition in wild-type *C. thermocellum*, we also aim to generate improved host genotypes for introducing engineered C5 sugar catabolism in the near future.

045 - Solvent Production Using a CO_2 - Fixing, Synthetic, and Syntrophic *Clostridium* Co-culture

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The demand for energy is steadily growing, and despite the current low oil prices the stockpiles of petroleum reserves are quickly being depleted. As a result, new technologies capable of utilizing low value feedstocks and waste gasses, such as H_2, CO and CO_2, must be developed to meet our growing need for liquid fuels. One interesting solution is to utilize the capabilities of the organisms found in nature. As such, solventogenic *Clostridium acetobutylicum* can utilize a wide variety of sugar substrates, such as molasses, starch, and hemicellulose to produce acetone, butanol and ethanol, in a process known as the ABE fermentation. In this process, *C. acetobutylicum* releases CO_2 and H_2, which decreases the overall carbon balance of the system. On the other hand, acetogenic *C. ljungdahlii* is capable of sequestering CO_2 gas in the presence of an electron source, such as CO or H_2, to produce acetate and ethanol. In comparison to *C. acetobutylicum*, *C. ljungdahlii* is limited in its ability to consume a broad spectrum of sugars (notably glucose), and is a much slower growing organism. Many previous studies focused on genetic engineering, and the optimization of a single organism to carry out multiple reactions and produce commodity chemicals. In nature, most organism live in microbial consortia, where the metabolic burden is divided among multiple organism to produce the molecules necessary for survival. Therefore, this study examined a synthetic co-culture of *C. acetobutylicum* and *C. ljungdahlii*. In a co-culture system, *C. ljungdahlii* would provide additional acetate for *C. acetobutylicum* in order to increase the solvent production. At the same time, *C. ljungdahlii* would recapture the CO_2 released by *C. acetobutylicum* in order to improve the overall carbon balance of the process. We will present data from our efforts to engineer a *Clostridium* co-culture capable of utilizing glucose as a substrate together with inorganic waste gases in order to maximize alcohol (ethanol and butanol) production, while minimizing the production of acetate and butyrate.

046 - Complete Genome Sequence of the Isopropanol-Butanol Producer *Clostridium beijerinckii* DSM6423

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Solventogenic clostridia are gram-positive, strictly anaerobic bacteria capable of using various carbon sources to produce primary metabolites of interest. Most of them are producing a mixture of acetone, butanol and ethanol (ABE) as end products of fermentation. Few other strains, such as *Clostridium beijerinckii* DSM6423, can further reduce acetone into isopropanol, resulting in an IBE mixture. Isopropanol is a chemical of interest that can be used in cosmetics industry, dehydrated to propylene, or used together with butanol and ethanol for biofuel applications. Although the fermentation performances of *C. beijerinckii* DSM6423 are close to those of *C. acetobutylicum*, it has been poorly studied since its first identification as an isopropanol producer, especially because of the apparent inability to transform it. To overcome this issue, genomic analysis should contribute to identify genetic barriers. Here, we combined PacBio and MiSeq approaches to determine the complete genome sequence of the strain. Interestingly, this analysis revealed the presence of several extrachromosomal elements, providing new strategies for the successful transformation of this strain. These data pave the way for future targeted engineering approaches in this natural IBE producer.

047 - Analysis of methanol utilization in acetogenic organisms

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In recent years there has been renewed interest in biological production of commodity chemicals due to concerns regarding the sustainability and environmental impact of petrochemical methods. Acetogens are anaerobic bacteria that produce valuable short organic acids and alcohols from a variety of single-carbon compounds including methanol, a cheap and abundant potential feedstock. In this work we examine the methanol-utilizing *B. methylotrophicum* as a potential industrial acetogen. A transformation protocol for this organism was developed. A single clostridial shuttle vector was successfully transformed and used to investigate genetic engineering of carbon flow and acidogenesis by expressing well-studied clostridial genes. The genome was sequenced and analysis of genes encoding enzymes of key metabolic pathways shows the basic genes of acetogens and the arrangement of prospective methanol utilizing genes. The relationship of major metabolic genes with those of related organisms shows the distinctive features of the organism. Current experiments aim to further optimize product formation by up- and down-regulation of endogenous genes.

048 - Engineering *Clostridium thermocellum* to produce ethanol from cellulose at high yield and titer

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Ethanol is the biofuel produced in the largest amount worldwide. *Clostridium thermocellum* is a promising candidate for ethanol production from cellulose due to its native ability to consume cellulose, and thus engineering efforts have focused on methods for improving ethanol production. To date, we have created over 40 different strains that employ a variety of engineering strategies, however we have only been able to achieve an ethanol yield of 75% of the theoretical maximum and 25 g/l titer. To allow for further improvements, we suspected that we might need an exogenous ethanol production pathway. *Thermoanaerobacterium saccharolyticum* has been engineered to produce ethanol at 90% of the theoretical maximum yield and 70 g/l titer. We identified the genes responsible for the ethanol production pathway in this organism and transferred the pathway to *C. thermocellum*.

049 - Solubilization of Cellulosic Feedstocks by *Clostridium thermocellum*

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In extensive comparative studies, we find that fractional carbohydrate solubilization achieved by *C. thermocellum* in the absence of thermochemical pretreatment ranges from 40% for switchgrass to 80% for corn stover and 95% for corn fiber whereas solubilization achieved under controlled conditions with fungal cellulase is about lower by about 3-fold. The realization that nature offers biocatalysts that are more effective than industry-standard fungal cellulase invites rethinking biomass processing strategies. Motivated by the example of the rumen and data showing that *C. thermocellum* fermentation can proceed in the presence of aggressive milling, we are investigating milling of partially-fermented feedstocks - termed "cotreatment" - to enhance biologically-mediated solubilization in lieu of thermochemical pretreatment. Solubilization in excess of 90% has been achieved by *C. thermocellum* in the presence of milling for both switchgrass and *Populus,* consistent with the hypothesis that this microbe possesses the enzymatic capability to cleave the bonds found in cellulosic biomass given physical access to them. Economic analysis suggests potential for radically lower costs as compared to processing using the thermochemical pretreatment-fungal cellulase paradigm. Recent work with various milling technologies and higher solids loading will be presented, and outstanding issues for the further development of cotreatment-enhanced thermophilic conversion of cellulosic feedstocks identified.

050 - Use of a Conditional RAM-less ClosTron to Identify Compensatory Mutations that Allow the Isolation of Mutations in Essential Genes in *Clostridia*

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The branched metabolic pathway of *Clostridium acetobutylicum* has been studied in some detail, particularly through the isolation and analysis of mutants. This approach has indicated that null mutants of certain genes cannot be obtained using standard procedures. These include the genes encoding hydrogenase (*hydA*), thiolase (*thlA*), crotonase (*crt*) and butyryl CoA dehydrogenase (*bcd*). Moreover, the isolation of a ClosTron mutant in the *ptb* gene (encoding phosphotransbutyrylase) proved problematic, and the single mutant that was obtained [Cooksley *et al.,* Metab Eng 2012; **14**:630-641] could not be complemented and was shown by NGS to carry a frameshift in *thlA*. Whilst other studies have reported on the isolation of some of these genes they were either not complemented [Lehmann *et al*. Appl Microbiol Biotechnol 2012; **96:**1325-39], were uncharacterised by NGS or were made by the insertion of a RAM-less intron into the sense strand of a cell that retained the plasmid encoded *ltrA* gene [Jang *et al*. Metab Eng 2014; **23:**165-74]. The latter mutants were, therefore, not 'knock-outs' (KO) but 'knock-downs' (KD) due to LtrA-mediated splicing of the intron insertion from the mRNA.

We have recently shown the *ptb* mutant isolated by Lehmann *et al*. [Appl Microbiol Biotechnol 2012; **96:**1325-39] contains a SNP in *hbd*. We hypothesise that there are certain genes (eg., *ptb*) that cannot be inactivated unless a compensatory mutation (eg., *thlA* or *hbd*) arises. To test this hypothesis we have constructed a conditional, RAM-less ClosTron vector (encoding *catP*) that carries a negative selection marker, *codA*, which confers on the host sensitivity to 5-fluorocytosine (5-FC). Intron, KD mutants are isolated on thiamphenicol (Tm) supplemented media through PCR screening. Pure mutants are then plated on media lacking Tm but containing 5-FC. Only cells that both loose the plasmid (*codA*) and pick up a compensatory mutation, can grow. These may be identified by NGS. We have already used this system to identify different compensatory mutations that allow the isolation of *thlA, ptb* and *hbd* KO mutants, and are currently using the system to isolate *hydA, crt* and *bcd* mutants.

052 - Using *Clostridium acetobutylicum* to heterologously express enzymes from obligate anaerobes: the case of putative anaerobic benzene carboxylase

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Recent advances in DNA sequencing technology and metagenomics have resulted in a large number of gene sequences that lack functional annotation. The dependency on traditional heterologous expression hosts such as *E. coli* has confined the progress of functional annotation from phylogenetically distant species, since the physiological differences between organisms in distant phyla often fail to express the protein in a functional form. One such protein is a putative anaerobic benzene carboxylase that has been identified in a benzene-degrading *Peptococcaceae* sp., where heterologous expression using *E. coli* was attempted, but failed to yield a sufficiently soluble fraction for biochemical characterization. *Clostridium acetobutylicum* is proposed as an alternative expression host because of its robustness, rapid doubling time, and its phylogenetic proximity to *Peptococcaceae*, thus being more likely to contain the necessary molecular chaperones and cofactors for functional expression of the putative benzene carboxylase. Although systems for genetically modified *C. acetobuylicum* have been developed, work on functional expression of putative genes remains limited. Currently, the putative benzene carboxylase genes have been cloned into several *E. coli–Clostridium* shuttle vectors, and optimized protocols for *C. acetobuylicum* growth, transformation, and expression directed towards functional expression are being developed. Success in this work will provide a benzene carboxylase for functional characterization, and validate the gene as a biomarker in the monitoring of benzene-contaminated sites. The optimized *Clostridium* gene expression system can serve as a convenient expression platform for enzymes naturally expressed by obligate Gram-positive anaerobes and expand the functional annotation of metagenomes from anaerobic environments.

055 - Carbon spending and carbon scavenging: differential gene expression in planktonic and sessile cell populations of *Ruminiclostridium thermocellum* 27405

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Ruminiclostridium thermocellum employs cell-bound cellulosomes as its primary enzymatic toolset for cellulose and hemicellulose breakdown. Cellulosome binding to cellulosic substrates and, by extension, the bacterium's adherence to cellulose surfaces is required for efficient hydrolysis. The adherent bacteria (i.e., sessile or biofilm cells) may freely revert to the non-adherent form (i.e., planktonic cells) through generation of offspring cells or due to microenvironment constraints such as limited surface area. The two cell populations co-exist, are interdependent and have different contributions to the bioconversion of carbon substrates. We developed a novel bioreactor design to culture and rapidly harvest sessile and planktonic cell populations for omics studies. Distinct physiological changes within the different cell populations were identified through RNA-seq analyses, and 1,958 genes had a minimum of two-fold differential expression. Results suggest that sessile populations are metabolically active and fit for rapid proliferation while planktonic populations turn on persistence and survival mechanisms. This is unlike classic biofilms that are long-lasting slow-growing persistent communities. We illustrate the changes in the central carbon metabolism (from cellulose to dead-end metabolites), nutrient transport across membranes, expression of cellulosomal genes, activation of important biosynthetic pathways, oxidative stress, sporulation, flagellar motility, chemotaxis and more. For example, the MsrA (peptide methionine sulfoxide reductase) gene - a methionine repair mechanism in oxidative stress - had fifteen-fold higher expression in planktonic cells. Understanding these cellular responses will help the genetic engineering of the species for use in large scale conversion of cellulosic materials to liquid fuels.

058 - Metabolic Engineering of *Clostridium tyrobutyricum* for n-Butanol Production from Renewable Biomass

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Butanol is an important industrial solvent and potentially a better transportation fuel than ethanol. Conventional acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* or *Clostridium beijerinkii* is limited by its poor butanol yield (<0.25 g/g glucose), low productivity (<0.5 g/L·h), low final butanol concentration (<12 g/L), and poor process stability. Furthermore, solventogenic clostridia usually could not use cellulose directly and suffer from glucose-mediated catabolite repression (CCR), which limits their ability in using lignocuellulosic biomass as feedstock. To overcome these limitations, we have developed several novel *n*-butanol producing *Clostridium* strains by introducing the *n*-butanol biosynthesis pathway and blocking acid producing pathways in non-solventogenic acid-forming *C. tyrobutyricum*, which has relatively simple metabolic pathways with high flux toward butyryl-CoA, the precursor for *n*-butanol biosynthesis, and high butanol tolerance. A high butanol titer of >20 g/L with a high butanol yield of >0.30 g/g substrate were obtained in batch fermentation with glucose and xylose as carbon source. Butanol was the only major fermentation product and no acetone was produced by this mutant, allowing simplified downstream processing for product purification. We have also demonstrated butanol production from various lignocellulosic biomass hydrolysates using this mutant strain, which showed good tolerance to hydrolysate inhibitors. In addition, we have also engineered cellulolytic *Clostridium cellulovorans* to directly ferment cellulose to n-butanol. It is thus possible to produce butanol from biomass in a sustainable and consolidated bioprocess without requiring prior enzymatic hydrolysis, which is an expensive step in current biorefinery using lignocellulosic biomass. Several key technical issues and challenges in engineering clostridia will be discussed. Metabolic and process engineering strategies to increase butanol yield and production titer will also be highlighted.

060 - Dissecting industrial acetone-butanol-ethanol (ABE) fermentation by sequencing of largest existing collection of historic commercial solventogenic *clostridia* strains

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Last year was the centennial anniversary of the Chaim Weizmann Acetone-Butanol-Ethanol (ABE) fermentation. Solventogenic clostridia have been used for the production of solvents acetone and butanol since the early 1900s and the process played key roles in the both world wars. In most countries the process was replaced by chemicals from the petrochemical industry, except for politically isolated countries like Apartheid South Africa, where this process continued until the early 1980s. Recently new plants have been reopened in many countries around the globe such as China or Brazil and there is renewed interest solvent production from waste streams and renewable substrates.

The David T. Jones collection dates back to 1944 and contains over 300 unique strains used in various commercial operations, including the South African ABE fermentation process at National Chemical Products (NCP) of which Prof. David Jones was the last Post-Doc. The collection includes some of the earliest spore stocks sent from both Commercial Solvents Corporation (CSC, USA) and Commercial Solvents Great Britain (CS-GB, UK) and comprises of mainly *Clostridium acetobutylicum, C. beijerinckii, C. saccherobutylicum* and *C. saccheroperbutylacetonicum* strains, most of which are either original production stocks or direct descendants. The collection also includes strains from Japanese and Taiwanese commercial operations as well as strains for various research labs. Initially, the substrate for solvent production was maize and the process was later switched to molasses and over the years more efficient production strains were developed. The collection also includes a number of strains resulting from research into solving specific problems such as phage infections or lower solvent yield when the characteristics of the molasses changed on an annual basis.

In collaboration with the US Department of Energy Joint Genome Institute (JGI), the historic collection of commercial solventogenic that make up the David T. Jones collection of clostridia strains are having their genome sequences determined and analyzed. An overview of the project will be presented and initial sequence data will be discussed.

066 - Consolidated Bioprocessing of n-Butanol Production from Agricultural Wastes by Mesophilic *Clostridia*

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In order to reduce the carbon dioxide emissions that cause climate change, such as global warming, utilization of the cellulosic biomass that is inedible is necessary. In this study, aiming to biorefinery from the waste of mandarin lees and sugar beet pulp that are a by-product of juice maker and sugar production, direct fermented by co-cultivation of mesophilic Clostridia such as *Clostridium cellulovorans* 743B and C. *acetobutylicum* ATCC 824 or *C. beijerinckii* NCIMB 8052. The results of componential analysis of mandarin lees indicated that mandarin lees contained 81.0% moisture, 2.83% glucose, 3.10% fructose, 2.90% sucrose and any other trace sugars such as xylose, arabinose, mannose and galactose. In other words, mandarin lees included a lot of fermentable sugars. It was able to elute 42 g/L reducing sugars (as D-glucose equivalents) at the maximum to media from mandarin lees to wash them. *C. acetobutylicum* consumed sugars of 81.9% after 36h and produced 1.24 g/l n-butanol after 60h with productivity of 0.02 g/l/h. Butanol yield was 0.04 g/g, while *C. beijerinckii* consumed sugars of 91.7% after 60h and produced solvents; 4.07 g/l n-butanol, 0.81 g/l 2-propanol, 0.16 g/l ethanol and 0.08 g/l acetone. Butanol productivity and its yield were 0.11 g/l/h and 0.16 g/g, respectively (Figure 1). On the ther hands, it was revealed that *C. cellulovorans* degraded arabinan of SBP to arabinose that cannot be utilized by itself, and *C. beijerinckii* fermented the arabinose. Approximately 4.27 g/L of butyric acid and 0.276 g/L of n-butanol were obtained, respectively. Therefore, we succeeded to construct consolidated bioprocessing (CBP) for biobutanol production from agricultural wastes such asmandarin lees and sugar beet pulps by combining *C. cellulovorans* as a biomass degrader and C. *acetobutylicum* or *C. beijerinckii* as a sugar fermentor.

068 - Limited thermodynamic driving force in glycolysis of cellulolytic *clostridia*

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C. thermocellum and *C. cellulolyticum* are obligate anaerobes capable of converting cellulose into ethanol. The glycolytic pathways of these two microorganisms display unique cofactor utilization, resulting in an energy-efficient sugar catabolism that is thought to generate more usable energy in the form of high-energy phosphate bonds than canonical glycolytic pathways but at the cost of forward thermodynamic driving force.

Here, we have used a combination of ^{13}C-labeling and ^{2}H-labeling to measure absolute metabolite concentrations and fluxes in *C. thermocellum* and *C. cellulolyticum* and experimentally determine changes in free energy (ΔG) at each step in their glycolytic and ethanol fermentation pathways. Our experimental method relies on the fundamental principle that for any reaction, ΔG is log proportional both to a concentration ratio (reaction quotient to equilibrium constant) and to a flux ratio (backward to forward flux), which can be reliably estimated from steady-state labeling data.

We found that the glycolytic and fermentation pathways in these two cellulolytic clostridia are surprisingly fully reversible under normal growth conditions. The overall thermodynamic driving force in the glycolytic pathways of *C. thermocellum* and *C. cellulolyticum* is significantly limited compared to canonical glycolytic pathways in model organisms such as *E. coli* or *S. cerevisiae* or non-cellulolytic thermophilic bacteria such as *T. saccharolyticum*. We also found that forward driving force is dependent on environmental inputs; for example, as ethanol accumulates during fermentation the thermodynamic driving force in glycolysis of *C. thermocellum* becomes even more limited. The limited forward driving force that we observe in the glycolytic and fermentation pathways of cellulolytic clostridia may constitute an evolutionary adaptation to growth in cellulose and the need to produce as much usable energy as possible per glucose.

069 - Transport of plant-derived sugars by *Clostridium phytofermentans*

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Elucidating how bacteria uptake and metabolize plant-derived sugars is crucial to understand the function of soil and intestinal microbial communities and to develop improved strains for industrial processing of plant biomass. *Clostridium phytofermentans* is a model cellulolytic bacterium that degrades and metabolizes numerous plant polysaccharides that compose cellulose, hemicellulose, and pectin. In order to uptake these carbon sources, *C. phytofermentans* encodes a over 500 transporter proteins, including 158 genes for putative sugar ABC transport systems. While bioinformatics can make general predictions about transporter function, it cannot reliably associate a transporter with a substrate. Thus, the goal of this project is to apply functional genomics and targeted gene inactivation to identify the transporters responsible for the uptake of plant polysaccharides and sugars in *C. phytofermentans*.

C. phytofermentans genome-wide mRNA expression on 13 carbon sources revealed sugar-specific up-regulation of transporter genes. We used these gene expression changes to guide the selection of transporter genes to inactivate by group II intron insertion. We focused on glucose and galactose sugars and their associated polysaccharides as they are the preferred carbon sources of this bacterium. We isolated four *C. phytofermentans* mutants that metabolize most carbon sources normally, but have each lost the ability to grow on an individual substrate, either glucose, galactose, cellobiose, or galactan (Fig. 1). These results support that individual, non-redundant transporters are responsible for the uptake of these carbon sources. Moreover, our results support that distinct systems are used for the uptake of oligosaccharides versus the constituent sugars. We show that combining mRNA expression with targeted gene inactivation is a useful method to identify the transporters responsible for uptake of different sugars in clostridia. We discuss how these results can be applied to engineer novel strains with improved sugar uptake and metabolism.

074 - A new cell-free cellulosome system in *Clostridium thermocellum* and new insights into the cellulosome system as a whole from systematic deletion of multiple scaffoldin genes

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Clostridium thermocellum is the most efficient microorganism known to date for solubilizing lignocellulosic biomass. Its high cellulose-digestion capability is mainly attributed to a tethered cellulosomal system wherein carbohydrate active enzymes (CAZymes) are organized by primary and secondary scaffoldin proteins to generate large protein complexes attached to the bacterial cell wall (cell-bound cellulosome system), as has been described before. This study demonstrates that *C. thermocellum* also uses a type of cellulosomal system not bound to the bacterial cell wall, which we call the "cell-free" cellulosomal system. The cell-free cellulosome complex can be seen as a "long range cellulosome" because it can diffuse away from the cell and degrade polysaccharide substrates remotely from the bacterial cell. The contributions of these two types of cellulosomal systems in *C. thermocellum* are elucidated by characterization of mutants with different combinations of scaffoldin gene deletions. The primary scaffoldin, CipA, is found to play the most important role in cellulose degradation by *C. thermocellum*, whereas the secondary scaffoldins have less important roles. This new transcriptional and proteomic evidence shows that a functional primary scaffoldin plays a more important role, compared to secondary scaffoldins, in the proper regulation of CAZyme genes, cellodextrin transport, and other cellular functions.

076 - Cap0037, a Novel Global Regulator of *Clostridium acetobutylicum* Metabolism

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An operon comprising two genes, *CA_P0037* and *CA_P0036*, which encode proteins of unknown function that were previously shown to be highly expressed in acidogenic cells and repressed in solventogenic and alcohologenic cells, is located on the pSOL1 megaplasmid of *Clostridium acetobutylicum* upstream of *adhE2*. A *CA_P0037::int (189/190s)* mutant was successfully generated by the Targetron technique. The resultant mutant showed significantly different metabolic fluxes patterns in acidogenic (producing mainly lactate, butyrate and butanol) and alcohologenic (producing mainly butyrate, acetate and lactate) chemostat cultures but not in solventogenic or batch cultures. Transcriptomic investigation of the *CA_P0037::int (189/190s)* mutant showed that inactivation of *CA_P0037* significantly affected the expression of more than 258 genes under acidogenic conditions. Surprisingly, genes belonging to the Fur regulon, involved in iron transport (*CA_C1029-CA_C1032*), or coding for the main flavodoxin (*CA_C0587*) were the most significantly expressed genes under all conditions, whereas *fur* (coding for the ferric uptake regulator) gene expression remained unchanged. Furthermore, most of the genes of the Rex regulon, such as the *adhE2* or *ldhA* genes, and of the PerR regulon, such as *rbr3A-rbr3B* or *dfx* were overexpressed in the mutant. In addition, the whole *CA_P0037-CA_P0036* operon was highly expressed under all conditions in the *CA_P0037::int (189/190s)* mutant, suggesting a self-regulated expression mechanism. Cap0037 was shown to bind to the *CA_P0037-CA_P0036* operon, *sol* operon and *adc* promoters, and the binding sites were determined by DNA foot-printing. Finally, a putative Cap0037 regulon was generated using a bioinformatics approach.

077 - Thiolase controls the phase transition from acidogenic to solventogenic in *Clostridium acetobutylicum*

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Clostridium acetobutylicum has been known to be the most efficient microorganism for the production of n-butanol, an important solvent and biofuel. It has been known that in anaerobic bacteria of the genus *Clostridium* n-butanol is synthesized from acetyl-CoA via six tightly regulated steps that are catalyzed by independent proteins. Thiolase is the first enzyme catalyzing the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA in a dedicated pathway towards the biosynthesis of n-butanol. Here we elucidate the crystal structure of *Clostridium acetobutylicum* thiolase (CaTHL) in its reduced/oxidized states. CaTHL, unlike those from other aerobic bacteria such as *Escherichia coli* and *Zoogloea ramegera*, is regulated by the redox-switch modulation through reversible disulfide bond formation between two catalytic cysteine residues, Cys88 and Cys378. When CaTHL is overexpressed in wild-type *C. acetobutylicum*, butanol production is reduced due to the disturbance of acidogenic-to-solventogenic shift. The $CaTHL^{V77Q/N153Y/A286K}$ mutant, which is not able to form disulfide bonds, exhibits higher activity than wild type CaTHL, and enhances butanol production upon overexpression. Based on these results, we suggest that CaTHL functions as a key enzyme in the regulation of the main metabolism of *C. acetobutylicum* through a redox-switch regulatory mechanism. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556) and by the C1 Gas Refinery Project (2015M3D3A1A01064918) funded by the MSIP through the NRF of Korea.]

078 - Development of a High-Efficiency Transformation Method and Implementation of Rational Metabolic Engineering for an Industrial Strain of Solventogenic *Clostridium*

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Since the discovery of an efficient gene transfer method over 20 years ago, *C. acetobutylicum* ATCC 824 has served as host for the vast majority of genetic engineering studies performed on solventogenic clostridia. This has largely been due to the well-studied physiology, fermentative metabolism, and industrial performance of this organism, dating back to the early 20th Century. While *C. acetobutylicum* has served as a useful host for the study and improvement of ABE fermentation, other species have also proved to be effective industrial ABE producers, primarily *C. beijerinkcii, C. saccharobutylicum,* and *C. saccharoperbutylacetonicum*. Despite their industrial utility, genetic manipulations of many non-model solventogenic strains have been extremely limited, particularly for *C. saccharobutylicum* and *C. saccharoperbutylacetonicum,* due to challenges surrounding gene transfer methods. Thus, there is great opportunity to discover the untapped potential of solventogenic *Clostridium* species which may serve as superior industrial hosts for ABE fermentation through the development and application of efficient gene transfer methods.

In this study, we present the development of a high-efficiency transformation method (approx. 1x10^6 cfu/μg DNA) for the industrial ABE-producer *C. saccharoperbutylacetonicum* strain N1-4 (*C. s.* N1-4). Following initial failures, we found the key to creating a successful transformation method was the identification of three distinct colony morphologies which displayed significant differences in transformability. Using our transformation methodology, we demonstrate, for the first time, targeted gene deletions in *C. s.* N1-4 using a homologous recombination-mediated allelic exchange method. Utilizing plasmid-based gene overexpression and targeted knock-outs of key genes in the native ABE metabolic pathway, we successfully implemented rational metabolic engineering strategies, yielding in the best case, an engineered strain displaying an 18% increase in butanol titers and 30% increase in total ABE (0.35 g ABE/g sucrose) in batch fermentations. Interestingly, two engineered strains overexpressing aldehyde/alcohol dehydrogenases displayed 8.5- and 11.8-fold increases in batch ethanol production with slight decreases in butanol production, while deletion of these genes severely diminished both ethanol and butanol production. Ultimately, this work serves as a foundation for future genetic manipulations of *C. s.* N1-4, not only for rational metabolic engineering efforts, but also for applications such as extending the range of accepted substrates, expanding the end-product profile to other valuable chemicals, and employing advanced genome editing tools (e.g. CRISPR/Cas9). Additionally, we believe that discussing the successes and failures encountered while developing this genetic transformation method will help others in approaching genetics in other historically intractable species of *Clostridium*.

079 - Identification and characterisation of the genes involved in N-acetylglucosamine metabolism in *Clostridium beijerinckii* NCIMB 8052

LAURA ANISHA RANA

Solventogenic *Clostridium* species have been used to ferment biomass into acetone butanol and ethanol (ABE), this was at one time the 2nd largest biotechnological process in the world – second only to yeast fermentation to produce potable ethanol (Jones et al 1986)

The ABE fermentation process was outcompeted by the petrochemical industry in the 1950s due to the availability of cheap oil (Ni et al 2009). Interest in this process has been rekindled as a search for a renewable, sustainable fuel becomes a top priority for many countries.

One of the largest costs of running the ABE process is purchase of the fermentation feedstock. Reintroduction of the ABE process will rely on securing renewable and economical feedstock which can be utilised and metabolised efficiently. Chitin is the second most abundant bio-polymer globally. It naturally occurs in a crystalline structure and is a major component of arthropods, (Herring 1979) fungi and molluscs, composing the exoskeleton as well as being a main component of the linings of interior tracts (Clark et al 1936). Chitin consists of around 5000 *N*-acetylglucosamine's (NAG), these are monosaccharide analogues of glucose which link with a β-linkage at 1,4.

This study found that *Clostridium beijerinckii* could utilise NAG as a sole carbon source. A putative pathway is proposed and metabolism genes identified.

This research demonstrates that the genes *cbe4564* and *cbe4562* in *C. beijerinkii* encode *N*-acetylglucosamine 6-phosphate deacetylase and glucosamine 6-phosphate deaminase for NAG metabolism. The genes were transformed into *E.coli* mutants which contained either the *cbe4564 gene* or *cbe4562* gene. The phenotype on MacConkey agar containing NAG as the only carbon source was observed. The mutants when transformed with the complimentary gene showed NAG metabolism whereas mutants lacking the complimentary gene were negative. The genes have been appropriately named, *cbe4564* as *nagA* and *cbe4562* as *nagB*. In order to support this conclusion enzyme assays are underway.

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081 - Cotreatment enhanced lignocellulosic fermentation with *C. thermocellum*

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Cotreatment is the concept of milling solids during fermentation as an alternative to conventional thermochemical pretreatment prior to fermentation. Current cellulosic ethanol schemes are limited by high cost of added enzymes and pretreatment necessary to achieve viable conversion. *C. thermocellum* is able to produce its own cellulase that solubilizes biomass more effectively than fungal cellulase, and is able to achieve high conversion without pretreatment. Milling has minimal effect on cellobiose consumption of *C. thermocellum*, but completely prevented glucose consumption by yeast. Adding cotreatment milling during fermentation of lignocellulosic feedstock gives yet better conversion, doubling solubilization achieved by *C. thermocellum* alone. Results are reported here for various lignocellulosic feedstocks for batch fermentation of *C. thermocellum* with and without continuous ball-milling.

083 - Important alcohol dehydrogenases for ethanol production in *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum*

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Clostridium thermocellum and *Thermoanaerobacterium saccharolyticum* are thermophilic bacteria that have been engineered to produce ethanol from the cellulose and hemicellulose fractions of biomass respectively. Although engineered strains of *T. saccharolyticum* produce ethanol with a yield of 90% of the theoretical maximum, engineered strains of *C. thermocellum* produce ethanol at lower yields. In the course of engineering these strains, a number of mutations have been discovered in their *adhE* gene, which encode both alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes and is necessary for ethanol production. Enzyme assays done with purified AdhE proteins showed a shift toward NADPH-linked activity in high-ethanol strains. In addition, deletions of multiple *adh* genes in a *T. saccharolyticum* engineered strain showed that *adhE* is not the only *adh* necessary for high ethanol production. Another alcohol dehydrogenase gene, *adhA*, is also needed: strains lacking *adhA* reduced ethanol yield by at least 50%. Interestingly, purified AdhA is also NADPH-linked in ADH activity. The study of alcohol dehydrogenases in *C. thermocellum* and *T. saccharolyticum* has important implications in engineering microorganisms for higher ethanol production.

086 - Identification And Characterization Of Ferredoxin:NAD$^+$ Oxidoreductase Enzyme In *Thermoanaerobacterium saccharolyticum*

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Ferredoxin:NAD$^+$ oxidoreductase (NADH-Fnor) catalyzes the transfer of electrons from reduced ferredoxin to NAD$^+$. This NADH-Fnor has been hypothesized to be the main enzyme for ferredoxin oxidization in NADH-based ethanol pathway in *Thermoanaerobacterium saccharolyticum*, however, this gene has not been identified. Here, we selected the candidates *tsac_1705* based on the protein function domains and its NADH-FNOR activity was confirmed by the ferredoxin based FNOR assay *in vitro*. The *tsac_1705* gene was deleted in different strains of *T. saccharolyticum* to determine its effect on end products distribution. In wild-type *T. saccharolyticum*, deletion of *tsac_1705* resulted in 75% loss of the activity of NADH-FNOR activity which indicated that Tsac_1705 is the main NADH-Fnor in *T. saccharolyticum*. When both NADH and NADPH linked Fnors were deleted, ethanol titer and the ratio of ethanol to acetate significantly decreased which demonstrated the important role of Fnor in ethanol pathway. We also heterologuesly overexpressed the gene of *tsac_1705* in *C. thermocellum* which improved both the titer and the yield of ethanol.

087 - Understanding the Role of *hfsB* in *Clostridium thermocellum* for Improved Ethanol Production

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The native hydrolytic and fermentative abilities of thermophilic anaerobic bacteria makes them an attractive research tools for consolidated bioprocessing (CBP) strategy to produce biofuels from cellulosic biomass conversion. However, in addition to ethanol, thermophilic anaerobes also synthesizes organic acids (acetate, lactate, fumarate, etc.) and H_2 that leads to decrease in ethanol yield due to the carbon and electron competition. The primary hydrogenase in *T. saccharolyticum* is called hfs, and it consists of 4 subunits, *hfsA*, *hfsB*, *hfsC* and *hfsD*. The HfsB contains a [FeFe] hydrogenase and PAS sensory domains, which could function as redox sensor and transcriptional regulator. In a recent study, we deleted the *hfsB* subunit in *Thermoanerobacterium saccharolyticum*, and we obtained increased ethanol yield to ≥87% of the theoretical maximum in *T.saccharolyticum ΔhfsB* strain. In *Clostridium thermocellum* the hfsABC genes are well conserved, and it has a *hfsB* gene cluster with 53.5% homology to the *T. saccarolyticum hfsB* gene. In order to see the effect of the *hfsB* homologues gene deletion in *C. thermocellum* we constructed a plasmid using the pDGO068 backbone for gene deletion with marker removal method. The construct pDGO068-hfsB was cloned in T7 Express lysY/Iq *E.coli* competent cells, by Gibbson assembly and will be transformed into *Clostridium thermocellum* with electroporation. After knockout of the corresponded gene, its effect on the hydrogen metabolism and ethanol production will be evaluated. In this work AE has been supported by TÜBİTAK with a scholarship.

088 - Development of a thermo-inducible promoter for *Clostridium acetobutylicum*

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Clostridium acetobutylicum is a microorganism of choice for the biological production of fuels and chemicals. However tightly control and inducible promoter for the expression of genes are limited and those that have been developed rely on expensive inducer and are more appropriate for laboratory study than for the use in large scale fermenters. The aim of this work is to develop a thermo-inducible expression system for *C. acetobutylicum*, similar to those described for *Escherichia coli* or *Bacillus subtilis*. For this purpose, we choose to develop a system based on a repressor/operator region from the R1T phage of *Lactococcus lactis*. A derivative of the transcriptional regulator Rro shown to exhibit a thermo-sensitive phenotype was selected. A specific vector for *C. acetobutylicum* containing the *rro_ts* gene with the *gusA* reporter gene has been developed and evaluated in this strain.

To conclude this inducible system will be a powerful tool for fundamental research and industrial applications to control protein production and/or metabolic pathways.

089 - Markerless targeted and multiple gene modifications in *Clostridium acetobutylicum*

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Clostridium acetobutylicum is a gram-positive, spore-forming, anaerobic bacterium able to utilize various carbon sources to produce solvents (acetone, butanol, and ethanol). To improve the industrial potential of this anaerobe, several genetic tools have been developed to facilitate its metabolic engineering.

In this work, we developed a new method allowing the modification of the genome from the introduction of a single point mutation, to the complete inactivation or deletion of target genes, in a *C. acetobutylicum* strain that lacks *upp* gene, which encodes uracil phosphoribosyl-transferase, for subsequent use as a counter-selectable marker.

This method is based on the integration, by single crossing-over, of a non-replicative plasmid possessing i) homologous region around the chromosomal regions to be deleted or modified, ii) an antibiotic resistance marker (ery^R or $thiam^R$) and iii) a *upp* gene that confers sensitivity to 5-FU.

Using this method, we thus succeed in the introduction of point mutations in different gene in different background*).* We also replaced the native promotor of the *pta-ack (ca_c1742-ca_c1743)* operon with a thiolase promoter (*pthl_pta-ack*). Finally, using two non-replicative plasmids with different antibiotic resistance markers, we were able to simultaneously inactivate two essential gene *hydA* & *thlA* to obtain a hydrogenase minus mutant of *C. acetobutylicum (Δupp Δcac1502ΔthlAΔhydA270).*

This technology will be useful for functional genomic studies and for the development of industrial strains for the production of biofuels and bulk chemicals.

090 - Genome edition in *Clostridium acetobutylicum*

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Clostridium acetobutylicum is a microorganism of choice for the biological production of fuels and chemicals. However, although a lot of genetic tools have been developed and are now available methods for genome edition are still missing.

We have developed a method involving RecT-mediated recombineering and type II CRISPR Cas9 for genome edition in *C. acetobutylicum*. This strategy couples *S. pyogenes* Cas9-mediated cleavage with homologous recombination repair, hence allowing an efficient genome edition method for *C. acetobutylicum*.

Recombineering is a genetic manipulation method based on the mechanism of phagenic RecT-mediated homologous recombination. A plasmid carrying the putative *recT* gene (*CPF0939*) from *Clostridium perfringens* was introduced under the control of an anhydro-tetracycline (aTc) inducible promoter in *C. acetobutylicum*. It has been shown to allow specific point mutation by simple introduction of a short synthetic oligonucleotide into the cell by electroporation. The recombineering efficiency was easily evaluated using an antibiotic resistance screening model.

Combined to the use of the single gRNA-directed Cas9 to select the edited *C. acetobutylicum* genome, we have been able to efficiently modify targeted genomic loci.

091 - Understanding Nitrogen Metabolism in *Clostridium thermocellum* through Genomic, Transcriptomic, and Metabolomic Platforms

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Clostridium thermocellum rapidly deconstructs cellulose and ferments resulting hydrolysis products into ethanol and other products, and is thus a promising platform organism for the development of cellulosic biofuel production via consolidated bioprocessing. While recent metabolic engineering strategies have targeted eliminating canonical fermentation products (acetate, lactate, formate, and H2), *C. thermocellum* also secretes amino acids (primarily valine and alanine) that has limited ethanol yields to approximately 70% of the theoretical maximum. To decrease amino acid secretion, we attempted to reduce ammonium assimilation by deleting either (i) NADPH-dependent glutamate dehydrogenase (gldh), (ii) glutamine-oxoglutarate aminotransferase/glutamate synthase (gogat) and/or (iii) Type I glutamine synthetase (glnA) in wild-type (Δhpt) *C. thermocellum*. While deletion of gogat had negligible results on growth or product yields, deletion of gldh significantly decreased growth rate and total carbon consumption, suggesting that it is the primary enzyme involved in nitrogen assimilation. Deletion of glnA reduced levels of secreted valine and total amino acids by 53% and 44% respectively, and increased ethanol yields by 53%. RNAseq analysis revealed that genes encoding the RNF-complex were more highly expressed in ΔglnA and may have a role in improving NADH-availability for ethanol production. While a significant upregulation of genes involved in nitrogen assimilation and urea uptake suggested that deletion of glnA induces a nitrogen starvation response, metabolomic analysis showed an increase in intracellular glutamine and α-ketoglutarate levels, indicative of nitrogen-rich conditions. Expression of glitamine synthetases (GS), gogat, and several urea metabolism genes also increased in Δgldh, however, expression of rnf or the ammonium transporter-PII fusion protein did not. Deletion of gogat had a minimal effect on transcript levels, whereas co-deletion of gogat and glnA resulted in transcriptional changes similar to that of the lone glnA deletion. We propose that deletion of glnA causes deregulation of nitrogen metabolism, leading to overexpression of nitrogen metabolism genes and, in turn, elevated glutamine/α-ketoglutarate levels. Here we demonstrate that perturbation of nitrogen assimilation is a promising strategy to redirect flux from the production of nitrogenous compounds towards biofuels in *C. thermocellum*.

092 - Identification of the main growth inhibitors to *C. sacharoperbutylacetonicum* metabolism during *n*-Butanol production using sugarcane C5 liquors

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Fermentative production of *n*-Butanol is nowadays considered less economically attractive compared to petrochemical route, mostly due to the high cost of the carbon sources used. In this context, second generation sugars from lignocellulosic biomass processing, such as C5 liquors, are potential alternative for this purpose. However, the composition of C5 liquors is known to strongly affect the fermentation due to the presence of inhibitors, such as organic acids, toxic products formed by sugars' dehydration and free-phenolic compounds from lignin degradation. To overcome this problem, optimized processes regarding carbon source and microorganism has been extensively pursued. In this work, we investigated the effect of inhibitors present in sugarcane C5-liquors on *Clostridium sacharoperbutylacetonicum* metabolism as well as the strain tolerance to the most toxic compounds. A Placket-Burman experimental design was performed to evaluate the effect of fourteen known inhibitors on the strain growth and butanol production. In the range of concentration studied, it was observed that acetic acid and hydroxymethilfurfural (HMF) presented the highest effect on the strain metabolism. A reduction of 46% on cell growth, in terms of final cell density, was found in the presence of a combination of acetic acid (2.6 g/L) and HMF (0.3 g/L). Following that, C5 liquors obtained by different pretreatment conditions were selected and evaluated based on its inhibitors composition and fermentation performance. Overall, the results allowed the identification of the most toxic compounds and maximum concentrations tolerated by *C. sacharoperbutylacetonicum* during butanol production and defined the most suitable C5 liquors to be used as the main carbon source.

093 - A Novel Heterologous Ethanol Producing Pathway Results In Higher Ethanol Yield In *Clostridium thermocellum*

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Clostridium thermocellum is a candidate organism for consolidated bioprocessing of lignocellulosic biomass to produce ethanol for us as a renewable fuel. As such, it has been the subject of several attempts at engineering it for high ethanol yield and titer, to varying degrees of success.

We recently have identified some of the key enzymes that enable another organism, *Thermoanaerobacterium saccharolyticum*, to produce ethanol at high yield and titer. We have introduced these genes into *C. thermocellum*, and have observed significant improvements to ethanol yield. Further strain engineering should allow us to create a high ethanol yield and titer strain of *C. thermocellum*.

094 - Engineering *Clostridium thermocellum* for improved Hydrogen yield

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Clostridium thermocellum is an anaerobic thermophile that can naturally breakdown and utilize plant biomass. While *C. thermocellum* can produce H2, it does so at yields and rates too low for commercial viability. The goal of this project is to understand metabolism in *C. thermocellum*, and genetically engineer it for higher H2 yield. Here, we use a variety of strategies to try and engineer strains for greater H2 yield and rate, including gene deletion and overexpression, targeting genes involved in electron metabolism.

095 - Enzyme activities of central metabolism in *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* in cellobiose-limited chemostat cultures

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A comparison was made of activities of key enzymes of sugar metabolism in *Clostridium thermocellum* and *Thermoanaerobacterium saccharolyticum* during carbon-limited growth on cellobiose in chemostat cultures. The activities detected were sufficient to explain the *in vivo* fluxes.

Major differences in both activity and cofactor specificity between the two organisms were found. *C. thermocellum* contained GTP-dependent glucokinase, pyrophosphate-dependent kinase activities with fructose-6-phosohate and sedoheptulose-7-phospate as substrates, phosphoglycerate kinase active with both ATP and GTP, GTP-dependent PEP carboxykinase and high activities of NADP-linked glutamate dehydrogenase. Transaldolase, pyruvate kinase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were not-detectable. In contrast, in *T. saccharolyticum* the latter four enzymes were present. In this organism, glucokinase, phosphofructokinase, phosphoglycerate kinase and PEP carboxykinase were all ATP dependent and the activity of NADP-linked glutamate dehydrogenase was absent. Instead, NADH-linked glutamate synthase was detected, indicative for ammonium fixation via the GS-GOGAT route as occurring in *Clostridium acetobutylicum*.

In *C. thermocellum*, the absence of pyruvate kinase can be compensated by pyruvate-phosphate dikinase and a transhydrogenating malate shunt consisting of PEP carboxykinase, malate dehydrogenase and an NADP-linked malic enzyme. Activities of the malate shunt enzymes in *T. saccharolyticum* were an order of magnitude lower. In both organisms a large part of the NADPH demand can be satisfied by isocitrate dehydrogenase that is specific for NADP. Glyceraldehyde-dehydrogenase was NAD-dependent in both organisms.

096 - Switchgrass solubilization by mixed methanogenic enrichments with comparison to pure cultures of *Clostridium thermocellum*

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Microbially-mediated fermentation of lignocellulose is a promising approach for biofuel production. Diverse, mixed microbial enrichment cultures provide an indication of the achievable extent of biomass solubilization. Yet there have been few fundamental studies of grass fermentation under the conditions anticipated for a high-rate, low cost industrial process, including very high solids concentration, short residence times and high temperatures. Motivated by this perspective, we have initiated study of thermophilic switchgrass fermentation using mixed culture anaerobic enrichments at decreasing residence times & increasing grass concentrations.

Triplicate thermophilic, semi-continuous, anaerobic digesters were operated at 30g/L mid-season switchgrass concentration for 214 days, and allowed to come to steady state at decreasing residence times (RT). Whereas we expected a shift from methanogenesis to acidogenesis as RT decreased to 5 days, surprisingly, even at RT=3.3 days stable methane production was still seen and acids inhibition was not observed. Total carbohydrate solubilization at RT = 20, 10, 5 and 3.3 days was 69.1%, 62.8%, 54.9% and 50.0% respectively. In addition to these continuous trials, batch studies compared the performance of mixed enrichment cultures to pure cultures of *Clostridium thermocellum*, where solubilization was similar despite the more limited enzyme diversity of the pure culture species. Perspectives on possible explanations for these observations will be offered, aided by characterization of microbial populations using 16S rDNA analysis.

101 - Unravelling new strategies for butanol production in *Clostridium acetobutylicum* using *in silico* approaches.

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For the last few years, the production of butanol has been the focus of researchers' attention when looking for alternatives to biofuels' production. Native producers from Clostridia group present the best alternative to succeed has they possess all the machinery required and evolutionarily were optimized to produce butanol. However, there are several limitations that need to be assessed in order to control the production of other unwanted end-products such as ethanol, acetone, lactate or succinate that may deviate the fluxes away from butanol.

Strategies of metabolic engineering have been on the table for over the last 15 years. However, the targets that seemed obvious at first, have proven not to increment significantly butanol titers showing that *C. acetobutylicum* metabolism is not as straightforward as it seemed. Going deep into understanding the solventogenic metabolism became therefore a key step into overcoming the difficulties to channel the metabolism towards butanol production.

In this work, we apply deep *in silico* analysis in order to learn and understand the peculiarities of this microorganism metabolism. Our study suggests a new *in silico* strategy to maximize butanol production.

102 - Development of a predictive model from a simulated artificial *clostridium* fermentation towards real-time culture monitoring

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In previous work, Raman spectroscopy together with statistical modeling has been shown to be effective for real-time data acquisition of consumable sugar (glucose) and accumulating products (butyric acid, acetic acid, and butanol) in a clostridial fermentation culture. The acquired data from the partial-least squares (PLS) models were applied to both agitated and static cultures with the former showing preferred modeling parameter values ($R^2Y = 0.99$ and $Q^2Y = 0.98$). Model outputs were shown to be comparable to off-line analyzed data from traditional HPLC for new clostridial experimental data through cross-validation. In this study, a bottom-up approach is employed where experimental data from HPLC analyzed data for reaction components is used to simulate an artificial fermentation culture devoid of cell activity. Raman spectra of corresponding reaction components; (i) glucose, (ii) butyric acid, (iii) acetic acid, and (iv) butanol, in specified proportions were acquired for known time points. The acquired spectra together with known concentrations of reaction components were used to build new sets of PLS models. These new models will be compared with original models created for the actual clostridial fermentation for model performance with both sets of models executed in real-time.

103 - Cofactor utilisation and interaction with core catalysis proteins in *R. thermocellum* and their prevalence in the genus *Ruminiclostridium* (*Clostridium* cluster III).

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The central catalysis of *R. thermocellum* includes alternative cofactor utilisation when compared to the traditional models of glycolysis. A deeper understanding of the regulatory mechanism controlling the metabolic flux through glycolysis and how glycolytic flux regulation modulates end product yields may reveal attractive targets for metabolic engineering. High-energy metabolites, such as ATP, GTP, and pyrophosphate, can directly interact with key catabolic enzymes modulating their activities as cofactors or allosteric activators/inhibitors. We describe the biochemical characterization of His-tagged purified enzymes predicted to be key contributors of catabolic flux in the central catalysis in *R. thermocellum* ATCC27405 such as glucokinase (Cthe_2938), phosphofructokinase (Cthe_0347), phosphoglycerate kinase (Cthe_0138) and pyruvate phosphate dikinase (Cthe_1308). Both glucokinase and phosphoglycerate kinase were capable of using GTP and ATP as phosphate donors while the phosphofructokinase was specific for PPi. All three enzymes exhibited substrate inhibition with increasing concentration of their respective phosphate group donors with maximum concentrations at approximately 1.5 mM. However, the variations in central catalysis as seen in *R. thermocellum* do not appear to be unique and are a broader feature of the *Ruminiclostridium* such as *R. termitidis* and *R. stercorarium*. Therefore, careful understanding of the fluctuations of high energy metabolites along with the regulatory mechanisms modulating flux through glycolysis will help elucidate the optimal internal conditions required for cellular growth and end product synthesis. Ultimately this could lead to the development of novel genetic engineering strategies for increased end product yield increasing the commercial viability of *Ruminiclostridium*.

106 - Identification and characterization of the Ferredoxin NAD(P)+ Oxidoreductases of *Clostridium acetobutylicum*

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In the strict anaerobe spore-forming bacterium *Clostridium acetobutylicum*, ferredoxin oxidoreductases enzymes play a key role in the regulation of the electron flow. In the solventogenic or the alcohologenic phases, the ferredoxin NAD(P)+ reductase enzyme acts in redirecting the electron flow from reduced ferredoxin toward the production of the NAD(P)H needed for ethanol and butanol production (1).

Although the presence of ferredoxin NAD(P)+ reductase activities in cell-free extracts from *C. acetobutylicum* were reported (2,3), the enzymes responsible of these activities have never been purified or identified.

First, an overall production, extraction and purification process was designed to purify both ferredoxin NAD+ reductases and ferredoxin NADP+ reductases enzymes from acidogenic, solventogenic and alcohologenic cultures of *C. acetobutylicum*. At each step of extraction or purification, ferredoxin NAD+ reductase or ferredoxin NADP+ reductase activities were evaluated to select positive fractions. Using this method different proteins or protein complexes were identified.

Second, to further characterize the putative candidates, the genes encoding these enzymes were cloned and the corresponding proteins purified and characterized for the NAD(P)H-ferredoxin reductases and ferredoxin NAD(P)+ reductases activities.

Finally, to evaluate the in vivo role of these candidates, the genes encoding each enzyme were separately disrupted in *C. acetobutylicum* ATTC824. The resulting mutant strains (if viable), were then characterized from a physiological point of view.

The identification of these key enzymes and encoding genes is of great interest as it opens new perspectives for heterologous n-Butanol production (4).

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108 - Calculating Energy Needs for Biofuel Fermentation by Acetogenic Bacteria

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Our modern world is currently facing a lot of challenges. Due to the excessive harvest of the earth's natural resources in the last centuries we now have to develop processes for the production of alternative energy sources to maintain our life standards. At the moment bacteria or yeast are mainly used for the fermentation of sugars from food crops to biofuels like ethanol or butanol. Nonetheless as sugars are already energy rich and also needed for the food industry, production of biofuels from low-energy sources is more desirable. Hence, using synthesis gas (syngas) for the fermentation of biofuels by acetogenic bacteria has attracted more and more interest over the last years. Syngas is a gas mixture consisting mainly of H_2, CO and CO_2 and can be derived from different sources, including the raw material lignocellulose. However, using acetogens for biofuel production is limited by two things: i) the lack of complete knowledge of their energy metabolism, and ii) the lack of sophisticated tools for the genetic modification of acetogens. Here, we present the complete enzymology and bioenergetics of acetate formation from H_2 + CO_2 in *Acetobacterium woodii*, which is the best understood acetogen in terms of energy conservation. In addition, syngas fermentation with *Clostridium autoethanogenum* will be discussed, since this organism is already used in industrial large-scale fermentation processes.

109 - Expression of Cellulolytic Genes From *Clostridium phytofermentans* DSM18823 In Non Cellulolytic *Clostridium acetobutylicum* DSM792

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Clostridium acetobutylicum is a well-studied organism for ABE production. It uses wide range of fermentable carbohydrates. Though its genome contains clusters of genes for cellulose hydrolysis, it is unable to degrade cellulose, a cheap and abundant substrate. *Clostridium phytofermentans*, an ethanol producing strain, efficiently degrades cellulose conferred by the genes GH9, GH48, GH5, GH26, GH12 and GH13 without complex cellulosome machinery. It was reported that *Cphy_3367* alone could efficiently degrade cellulose in *C. phytofermentans*. This property could be enhanced when *Cphy_3367* and other cellulose genes are overexpressed in *C. acetobutylicum* for efficient degradation of cellulolytic substrates. In our study, we have used *E. coli* -Clostridium shuttle vector pSOS952 carrying the *C. phytofermentans* cellulolytic genes with signal peptide. pSOS952 was used to repress the expression of cellulase genes in *E.coli* to avoid toxicity for cloning. We had successfully cloned *Cphy_2058* into pSOS952. However, *Cphy_3202* and *Cphy_1163* cloned in pSOS952, deleted thiolase promoter sequence (either completely or a part of the sequence) even the though it is under the regulation of lac operator flanking either sides of the promoter sequence. Hence, there is a need to find out a suitable way to clone these genes in the vector without deletion in promoter.

111 - Metabolic engineering of *Clostridium acetobutylicum* for butyric acid production

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A typical characteristic of the butyric acid-producing *Clostridium* is coproduction of both butyric and acetic acids. Increasing the butyric acid selectivity important for economical butyric acid production has been rather difficult in clostridia due to their complex metabolic pathways. In this work, *Clostridium acetobutylicum* was metabolically engineered for highly selective butyric acid production. For this purpose, the second butyrate kinase of *C. acetobutylicum* encoded by the bukII gene instead of butyrate kinase I encoded by the buk gene was employed. Furthermore, metabolic pathways were engineered to further enhance the NADH-driving force. Batch fermentation of the metabolically engineered *C. acetobutylicum* strain at pH 6.0 resulted in the production of 32.5 g/L of butyric acid with a butyric-to-acetic acid ratio of 31.3 g/g from 83.3 g/L of glucose. These results suggested that the *buk* gene knockout was essential to get a high butyric acid selectivity to acetic acid in *C. acetobutylicum*. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012-C1AAA001-2012M1A2A2026556) and by the C1 Gas Refinery Project (2015M3D3A1A01064918) funded by the MSIP through the NRF of Korea.]

115 - Accelerating the Understanding and Development of *Clostridium* for Biotechnology- the development of advanced modular vectors

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Efficient plasmid transformation and genetic modification of strains from the genus *Clostridium* has become increasingly important to study and exploit *Clostridia* for industrial and medical biotechnology. The *Clostridia* Research Group (CRG), based at the University of Nottingham, has previously developed a suite of molecular biology tools (CRG roadmap) that allows researchers to study and manipulate these important bacteria. This includes the modular pMTL80000 plasmids that support gene knockout, gene expression and chromosomal integration technologies including ClosTron, Allele Coupled Exchange (ACE) and mariner transposon mutagenesis. These tools have been demonstrated across a wide range of industrial and medically important species of *Clostridium*.

CHAIN Biotechnology Ltd. has partnered up with the researchers at the University of Nottingham to help disseminate and develop these important tools. Here, we report on transformation progress with new strains and also on improvements to the plasmid vectors including efficient plasmid assembly and on the development of plasmids for advanced gene editing. These important developments improve the efficiency and versatility of the vectors and should help accelerate research and development of *Clostridium*.

119 - NiFe Hydrogenase Importance during Solventogenisis in *Clostridium acetobutylicum*

GERMANE, KL; LIU, S; GERLACH, ES; SERVINSKY, MD; SUND, CJ

Clostridium acetobutylicum encodes genes for two FeFe hydrogenases and a NiFe hydrogenase. The FeFe hydrogenase oxidizes ferredoxin during glycolysis, producing H2, and is a necessary component to the central metabolism. The role of the NiFe hydrogenase has not been elucidated, though previous studies indicate it is involved in hydrogen uptake and NADH cycling. The NiFe hydrogenase genes are annotated to be encoded on the genome and the pSol megaplasmid, though growth of the pSol negative M5 strain indicates the NiFe is nonessential to acidogenic growth. In this study we show that interruption of the NiFe hydrogenase maturation factor gene *hypF*, *ca_c0810*, via type II transposon, reduces hydrogenase activity, demonstrating HypF is required for a functional hydrogenase. The Δ*hypF* mutant shows decreased ABE output and altered product ratios in early solventogenesis, which were consistent with a defect in hydrogen uptake.

121 - Metabolic Network Modeling of *Clostridium thermocellum* for Systems Biology and Metabolic Engineering.

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Clostridium *thermocellum* is a gram-positive thermophile that can directly convert lignocellulosic material into commercially relevant chemicals such as biofuels. Its metabolism contains many branches and redundancies, which limit the production of biofuels at industrially relevant yields and titers. In order to guide the experimental efforts required to overcome these barriers, we built two models of *C. thermocellum* metabolism. Through an extensive literature review, we first constructed a model of the core metabolism of C. *thermocellum*. This model was experimentally validated and served to investigate the range of phenotypes of *C. thermocellum* in response to significant perturbation of energy and redox pathways. The results revealed a complex, robust redox metabolism of *C. thermocellum*. By incorporating experimental data into this core model, we identified redox bottlenecks hindering high-yield ethanol production in *C. thermocellum*. With the recently published sequence of a genetically-tractable strain C. thermocellum DSM 1313, the KEGG database as a scaffold, and further literature review, we expanded the core model into a genome scale model (iAT601). This model constitutes a knowledge base for the organism, including detailed metabolic information, as well as gene protein reaction association. These features allow us to conduct studies on the impact of secondary metabolisms, isozymes, media composition, and provide a more solid basis for computational strain design. We used several sets of experimental data to train the model, e.g., estimation of the ATP requirement for growth-associated maintenance (13.5 mmol ATP/g DCW/hr) and cellulosome synthesis (57 mmol ATP/g cellulosome/hr). Using our tuned model, we predicted the experimentally observed differences in cell biomass yield based on which cellodextrin species is assimilated. We further employed our tuned model to analyze the experimentally quantified differences in fermentation profiles (i.e., the ethanol to acetate ratio) between cellobiose- and cellulose-grown cultures, for which we inferred potential regulatory mechanisms to explain the phenotypic differences. Finally, we used the model to design over 250 genetic modification strategies with the potential to optimize ethanol production, 6,155 for hydrogen production, and 28 for isobutanol production. Our developed genome-scale model iAT601 is capable of accurately predicting complex cellular phenotypes under a variety of conditions by integration of low- and high-throughput data, and serves as a high-quality platform for model-guided strain design to produce industrial biofuels and chemicals of interest.

123 - Biobutanol production integrated with organophilic pervaporation: experimental results and conceptual plant design

WOUTER VAN HECKE, PIETER VANDEZANDE, HERMAN BECKERS, KAROLIEN VANBROEKHOVEN, HELEEN DE WEVER

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In a conventional ABE fermentation, solvent concentrations are limited to 2 % (w/w) due to severe product inhibition. This leads to limited carbohydrate concentrations in the feed and a high energy consumption in the distillation section of the plant. An alleviation of product inhibition can be achieved by complementing the fermentation process with *in situ* product recovery (ISPR) technology. This presentation focuses on the use of organophilic pervaporation directly coupled to clostridial fermentations.

Major benefits of PDMS composite pervaporation membranes were experimentally demonstrated on lab-scale for long-term continuous fermentations: an increased volumetric productivity (up to 1.13 g/L.h), solvent enrichment in the permeate (up to 200 $g.kg^{-1}$) and an increased carbohydrate concentration (up to 15 % w/w) in the feed. The robustness of the technology was convincingly demonstrated by operating the integrated system during 654h at (total) membrane fluxes of ~0.6 $kg.m^{-2}.h^{-1}$ without any sign of membrane fouling.

The separation factors and long-term fluxes of several organophilic pervaporation membranes were tested in the integrated set-up as well. The flux and separation factor for PTMSP composite membranes decreased quickly when in contact with the fermentation broth. Evaluation of another type of composite pervaporation membrane lead to promising flux improvements in comparison with PDMS composite pervaporation membranes and similar separation factors. This type of pervaporation membrane was tested during prolonged periods of time without a sign of fouling.

Based on the experimental results, a continuous process integrated with organophilic pervaporation was conceptually designed and compared with a base-case for production of 99.75% (w/w) butanol. Rigorous calculations using chemcad as simulation software indicated energy savings of 47.4% for the plant integrated with organophilic pervaporation compared to the conventional biobutanol plant.

References:

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M. F. S. Dubreuil, P. Vandezande, W. Van Hecke, W. J. Porto-Carrero and C. T. E. Dotremont, *Journal of Membrane Science*, 2013, 447, 134-43.

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M. Uyttebroek, W. Van Hecke and K. Vanbroekhoven, *Catalysis today*, 2015, 239, 7-10.

W. Van Hecke, P. Vandezande, M. Dubreuil, M. Uyttebroek, H. Beckers and H. De Wever, *Journal of Industrial Microbiology & Biotechnology*, 2016, **43**, 25-36.

125 - Gas fermentation: Waste to value at scale

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The rise in atmospheric carbon dioxide drives global average temperatures up year by year, with some predictions indicating that we will breach the 2°C threshold as early as the mid 2030's. However, world energy demand is expected to increase by up to 40% and the global population is anticipated to reach 8billion people over the same timeframe. Thus a critical challenge facing the global community is to not only increase the sources of energy supply, but to also to minimize fossil carbon emissions to safeguard the environment while ensuring that food production and supply is not detrimentally impacted. In this regard, renewable sources of transport fuels will play an increasing role in the global primary energy supply. To address carbon emissions from this sector, governments have mandated the increased use of renewable transport fuels. Similarly, as a result of consumer driven demand, the global market for more environmentally sustainable alternatives to today's oil and coal-derived chemicals is anticipated to exceed $100 billion by 2020.

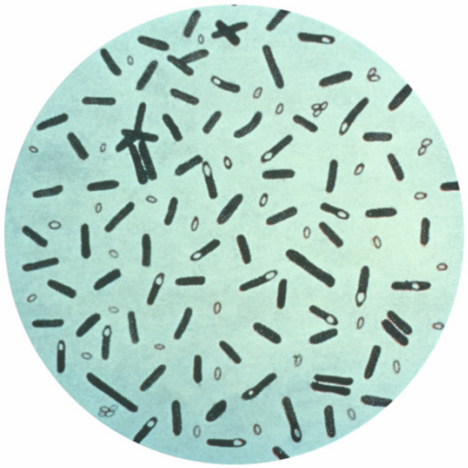
The production of biofuels and platform chemicals via gas fermentation is a rapidly developing technology for high volume, sustainable, production of fuels and chemicals that does not require food-based substrates as a feedstock. LanzaTech is commercializing a complete process platform to allow the continuous biological production of fuels and an array of chemicals intermediates from gases at scale. To date, this technology has been successfully demonstrated with such diverse gas streams as by-product gases from steel making, reformed natural gas, and syngas produced from gasified biomass and gasified municipal solid waste.

At the core of the LanzaTech gas fermentation process is a proprietary strain of an acetogenic clostridium. This strain, in combination with a novel reactor design, and optimized process chemistry allows efficient, single-pass gas conversion with a high selectivity to the product off interest. In order to maximise the value that can be added to the array of gas resources that the LanzaTech process can use, the company has developed a robust genetic toolbox to allow the carbon an energy consumed by its proprietary gas fermenting microbe to be channelled in to a spectrum of valuable chemicals. Gas fermentation offers an efficient route to add much greater value to gas streams than established technologies, while also reducing greenhouse emissions and providing a strategically important alternative to food or farmed resources for domestic production of sustainable fuels and chemicals at an impactful scale.

126 - Intensified Process for Consolidated n-Butanol Production from Cellulose by a Mesophilic Cellulosome-Producing *Clostridium cellulovorans* mutant

ZHAO[1], JINGBO, SHANG-TIAN YANG[1]
[1]*Ohio State University*

Lignocellulosic biomass is an abundant, inexpensive, and renewable source of sugars, and is thus a desired feedstock for sustainable production of fuels, chemicals, and polymers by biorefinery. However, enzymatic decomposition of the carbohydrate polymers (mainly cellulose and hemicelluloses) in lignocellulosic biomass into simple fermentable sugars is still one of the major bottlenecks for cost-effective biorefinery. Besides the traditional free cellulase systems produced by aerobic fungi, complexed cellulosome systems produced by anaerobic mesophilic bacterium, *Clostridium cellulovorans*, provide an alternative paradigm for efficient synergistic degradation of lignocellulosic biomass resulting from the combination of targeting the enzymatic complex to the substrate (target effect) and the spatial proximity of the different types of cellulases to each other (proximity effect). In our previous work, a *Clostridium cellulovorans* mutant wit*h* aldehyde/alcohol dehydrogenase (*adhE2*) overexpression was constructed, which can directly convert microcrystalline cellulose to n-butanol and ethanol. In this study, the consolidated process was further intensified to improve n-butanol production. During the static fermentation of soluble glucose in serum bottle, *Clostridium cellulovorans* cells tended to form cell aggregates and settled down at the bottom of the bottle. When inoculated into serum bottle with medium using solid cellulose as substrate, cells tended to attach to the solid cellulose and stayed at the bottom of the bottle. This characteristic allowed fast collection of *Clostridium cellulovorans* cells by removing supernatant, providing a simple way to realize high cell density fermentation. By concentrating cells/cellulose solids, n-butanol titer could be greatly improved from 1.42 g/L to 3.5 g/L, with little change of ethanol titer. However, acids (acetic and butyric acid) production also increased, which led to a low alcohol/acid ratio of ~0.7 g/g. To intensify alcohol production, the artificial electron carrier, methyl viologen (MV), was added to the concentrated cells/cellulose fermentation system. By using MV, ethanol titer was greatly improved from 1.4 g/L to 3.2 g/L and acid production was dramatically inhibited, with final alcohol/acid ratio as high as 6.3 g/g. However, n-butanol titer was not further improved, which suggested limited n-butanol tolerance of *Clostridium cellulovorans*. To further intensify n-butanol production, a naturally n-butanol-producing *Clostridium beijerinckii* with high n-butanol tolerance and good cellobiose utilizing ability was introduced to form a symbiotic system, which produced ~5 g/L n-butanol directly from microcrystalline cellulose with an alcohol/acid ratio of ~1.7 g/g. Higher n-butanol titer, yield, and productivity can be obtained after process optimization.



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Baker	Patricia	Arizona State University	002
Balch	Michael	Dartmouth College	081
Bayer	Ed	Weizmann Institute of Science	
Bennett	George	Rice University	047
Bird	Eve	Celtic Renewables	
Borovok	Ilya	Tel Aviv University	
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Ehrenreich	Armin	Technical University of Munich	038
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Gu	Yang	Chinese Academy of Sciences	007
Guss	Adam	Oak Ridge National Laboratory	091
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Heeg	Daniela	CHAIN Biotechnology Ltd.	115
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Holwerda	Evert	Dartmouth College	049
Hon	Shuen	Dartmouth College	093
Huang	Ching-Ning	Technical University of Munich	009
Jang	Yu-Sin	Gyeongsang National University	077
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Jensen	Torbjorn	Technical University of Denmark	018
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Leang	Ching	Lanza Tech Inc.	
Liang	Xiaoyu	Dartmouth College	096
Liu	Xiaoguang (Margaret)	University of Alabama	013
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Portela	Carla Andreia Freixo	CTBE	101
Pyne	Michael	Concordia University / University of Waterloo	016
Qureshi	Nasib	USDA NCAUR	017
Ramya	Mohandass	SRM University	109
Rana	Laura	Napier University	079
Redl	Stephanie	The Novo Nordisk Foundation Center for Biosustainability	018
Roussel	Celia	INSA LISBP	088
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Simpson	Sean	Lanza Tech Inc.	125
Singh	Nisha	Deakin University	022
Skinner	Kelly	Synata Bio	
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Soucaille	Philippe	INSA LISBP	
Sparling	Richard	University of Manitoba	103
Sund	Christian	U.S. Army Research Laboratory	
Tamaru	Yutaka	Mie University	066
Tangney	Martin	Celtic Renewables	
Tian	Liang	Dartmouth College	086
Valgepea	Kaspar	University of Queensland	023
van Hecke	Wouter	VITO NV	123
van Maris	Antonius	Delft University of Technology	

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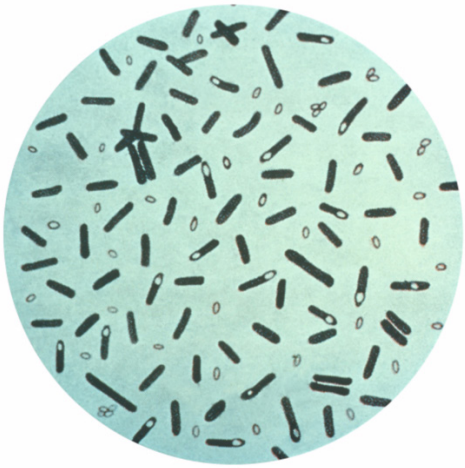
Wang	Liang	University of Florida	025
Wang	Faye	Auburn University	
Wang	Yi	Auburn University	027
Wasels	Francois	IFPEN	046
Westpheling	Janet	University of Georgia	028
Wiechmann	Anja	Goethe University Frankfurt	108
Winzer	Klaus	University of Nottingham	029
Woo	Ji Eun	Gyeongsang National University	111
Xiao	Johnny	University of Toronto	052
Xu	Qi	National Renewable Energy Laboratory	074
Yang	Chen	Chinese Academy of Sciences	030
Yang	Shang-Tian	Ohio State University	058
Zhang	Ying	University of Nottingham	050
Zhang	Min	National Renewable Energy Laboratory	
Zhang	Jie	Auburn University	
Zhao	Jingbo	Ohio State University	126
Zheng	Tianyong	Dartmouth College	083
Zhou	Jilai	Dartmouth College	095
Zu	Theresah	U.S. Army Research Laboratory	102



Maps and logistical information

General information

Venue

Dartmouth College is a private Ivy League research university in Hanover, New Hampshire, United States. Established in 1769 by Eleazar Wheelock, Dartmouth is one of the nine colonial colleges chartered before the American Revolution.

Thayer School of Engineering offers graduate and undergraduate education in engineering sciences at Dartmouth College. The school was established in 1867 with funds from Brig. Gen. Sylvanus Thayer, known for his work in establishing an engineering curriculum at the United States Military Academy at West Point, New York. Located in a two-building complex along the Connecticut River on the Dartmouth campus, the Thayer School today offers undergraduate, master's, and doctoral degrees. Over 500 students are currently enrolled at Thayer, overseen by a faculty of 56. In 2016 Thayer became the first US national research university with a graduating class of engineering undergraduates that was over 50% female.

Presentations

Oral presentations will take place in the Spanos auditorium, which is room C100 in the Cummings building at the Thayer School of Engineering

Posters will be displayed in the GlycoFi Atrium in the Maclean building and need to be removed before lunch on Tuesday.

Social events

On Tuesday afternoon, there will be a social excursion to Artisan's Park in Windsor, VT. Busses will leave from the Hanover Inn at 1:15 pm. There are a variety of activities within walking distance of Artisan's Park. See description on the "Artisan's Park" map (following page). For the **Silo Distillery tour**, **canoe trip** and **hiking trip**, you will need to sign up during registration.

The **canoe trip** will take approximately 2 hours. The **hiking trip** is 2.7 miles of moderately strenuous hiking.

Busses will leave Artisan's Park at 4:30 pm to return to Hanover, stop briefly at the hotels and then continue to the Dartmouth Skiway for dinner. At 10 pm, busses will take people back to the hotels in Hanover.

Meals

Unless otherwise noted in the conference schedule, mealswill be at the Class of 1953 Commons. When you register, you will be assigned a meal card. You use this card to enter the dining hall. Food is all-you-can eat. There are 8 distinct stations, each serving a different style of food, ranging from kosher to a meat-free kitchen serving vegetarian and vegan dishes. If you would like to take your food "to-go", you need to request a "to-go" container when you enter the dining hall.

Hours are Monday – Saturday
7:00 am – 10:30 am (breakfast)
11 am – 2:30 pm (lunch)

Sunday
7 am – 2:30 pm (breakfast/lunch)

Tuesday dinner will be at the Dartmouth Skiway in Lyme, NH.

Wednesday dinner will be at the Canoe Club restaurant on Main St. in Hanover, NH

Internet access

Wireless internet (wifi) access is available throughout the Dartmouth campus. Use the Dartmouth-Public SSID. No password is required. When you connect to the internet, you will have to click a button to agree to Dartmouth's internet access policy.

Transportation

The **Dartmouth Coach** bus service runs between Boston's Logan Airport and Hanover, NH. You can reach the Dartmouth Coach office at (603) 448-2800 (in New Hampshire) or (800) 637-0123 (outside New Hampshire). Normal business hours are 9:00 am to 5:00 pm EST, Monday through Friday. The answering service will provide limited information during non-business hours. Reservations are not accepted. Additional information is available at http://www.dartmouthcoach.com/.

Leaves Hanover, NH	Arrives at Boston Logan Airport
5:00AM	8:05AM
7:00AM	9:50AM
9:00AM	11:50AM
11:00AM	1:50PM
12:00PM	2:50PM
1:00PM	3:50PM
3:00PM	5:50PM
5:00PM	7:50PM

Upper Valley Ride (UVRide) offers a shuttle 5 times per day to/from Manchester-Regional Airport and Boston-Logan. Also, they offer car service to Boston-Logan, Manchester, Lebanon and Burlington Airports.

Call (603) 448.4004 or visit their websites: http://www.uvride.com/, https://www.facebook.com/UVRide

ARTISAN'S PARK
WINDSOR VERMONT

Vermont Farmstead Cheese - 1 -
Enjoy sampling from a wide array of their artisanal & specialty cheeses atop hand-cut Castleton Crackers all the while learning how they go from happy cows to fantastic cheese.

Sustainable Farmer - 2 -
Enjoy an incredible selection of Vermont cheese, maple and honey products—sourced from farmers committed to sustainability. Free tastings every day!

Harpoon Brewery - 3 -
See what's brewing at Harpoon! Take a tour, enjoy a fresh pint and a meal in the Harpoon Riverbend Taps and Beer Garden, or shop in our Brewery Store.

Great River Outfitters - 4 -
Float down the Connecticut River, camp in our tipi's or go dog-sledding in the Winter. Outfitting all ages and skill levels to enjoy outdoor activities in each of the four seasons.

SILO / American Crafted Spirits - 5 -
Take a tour of this beautiful new distillery and enjoy a free sample in our tasting room of flagship brand, SILO Vodka.

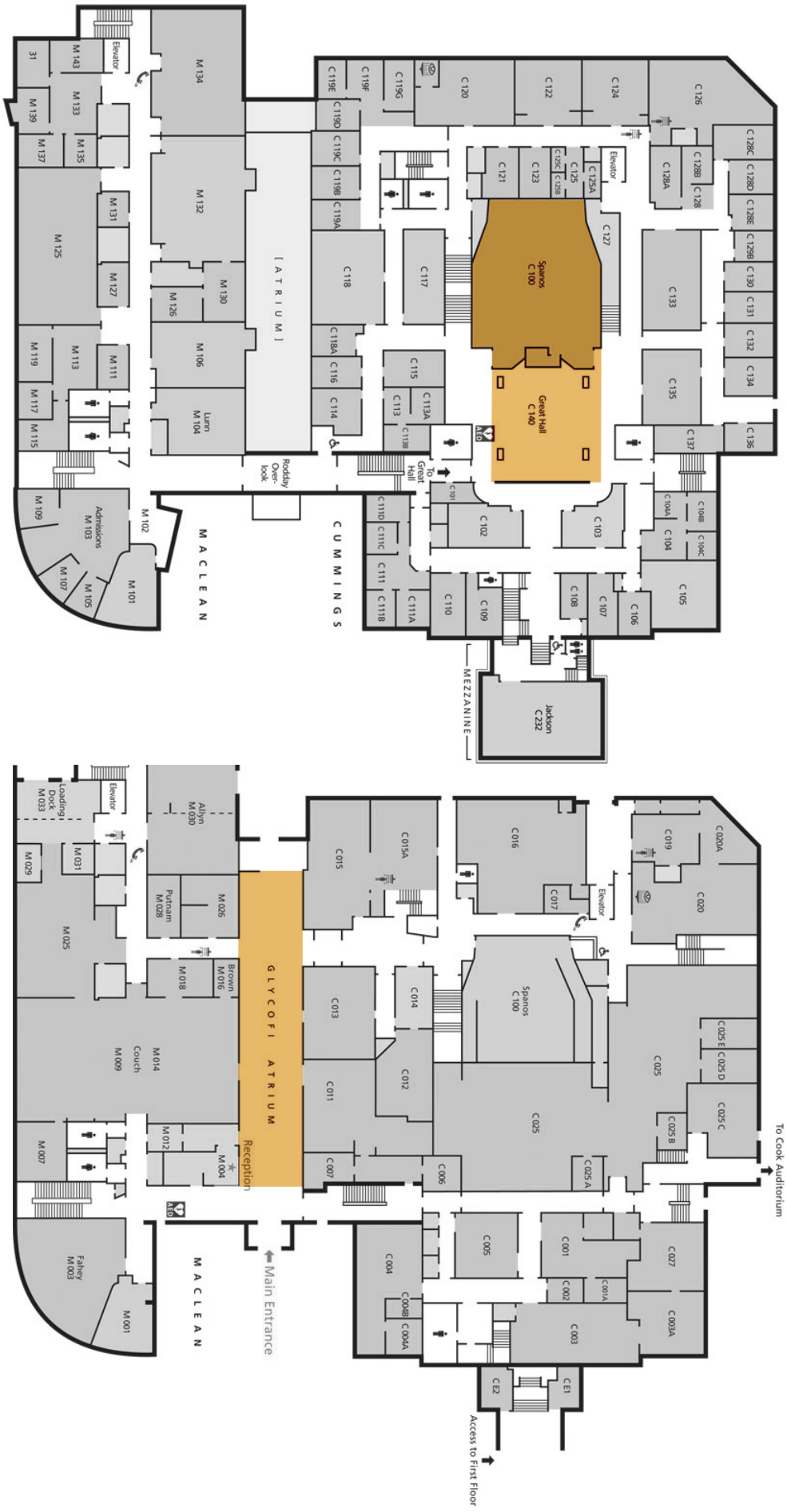
Path of Life Garden - 6 -
Walk through a 14-acre sculpture garden on the banks of the Connecticut River and explore large-scale works of art that tell the story of the Great Circle of Life.

Simon Pearce - 7 -
Take a self-guided tour through a glass factory and pottery and watch master artisans create glass and pottery, one piece at a time.

Minutes from I-91, the best of Vermont awaits on the banks of the Connecticut River.

www.ArtisansPark.net

Dartmouth campus


Ground floor
Spaans C 100
Great Hall C 140
Jackson C 232
MEZZANINE
[A T R I U M]
M A C L E A N
C U M M I N G S
Rockey Over-look
To Great Hall
Admissions M 103
Lunn M 104
First floor
Spaans C 100
G L Y C O F I A T R I U M
Reception
Main Entrance
To Cook Auditorium
Access to First Floor
Allyn M 030
Putnam M 028
Brown M 016
Couch M 009
Fahey M 003
Loading Dock M 033
Elevator